Genetics of Well-Being

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General Introduction

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behavior-genetic studies of twins, siblings and other pairs of relatives are very useful to identify the heritable components of complex traits such as those related to well-being (WB). From these studies, we know that individual differences in well-being are accounted for by both genetic as well as environmental factors, although the range in estimates is large and varies between 0%[1] and 64%[2]. From a recent systematic review study, we know that the average heritability for WB tends to hover around 40%[3]. These heritability studies, based on both classical and extended twin designs, are valuable, as they allow us to gauge the relative influence of our genetic make-up on WB. Nevertheless, it is important to note that classical twin designs do not give insight into the specific genomic regions that may be involved in explaining the heritability of well-being. One of the most exciting directions for research in behavioral genetics is the combination of quantitative behavioral genetics and *molecular genetics* in an attempt to identify the specific genes underlying the substantial heritability estimates of complex behaviors. Although the GWAS Era is in full bloom in 2018, many obstacles has to be conquered to get to this point. Therefore, the present chapter can be seen as an overview of molecular genetic studies trying to associate genetic variants to WB up until 2014, the beginning of my PhD trajectory.

Linkage analysis

Traditionally, the search for *genes* involved in both normal behavior and behavioral disorders began with *linkage aannnalysis*, aimed at localizing genetic variants that regulate a trait of interest. It is well known that during *recombination*, *deoxyribonucleic acid* (DNA) is not copied straightforwardly from parents to offspring, but shuffled to create a "unique" DNA template. Linkage studies rely on the assumption that genes located closer to each other are more likely to be transmitted together (linked) than genes that are located separately on a *chromosome*. Therefore, within linkage analysis, the distance between a DNA marker (i.e., a landmark of known location in the genome) and a *locus* involved in a particular phenotype is analyzed in small numbers of large multigenerational *pedigrees*. For linkage analysis to succeed, markers that flank the trait of interest must co-segregate along familial lineages in extended pedigrees. This method has been very successful in *mapping* rare genetic diseases of large effect, such as DNA linkage for Huntington's Disease on chromosome 4[4].

Linkage analysis: complex traits

Although linkage analysis of large pedigrees has been very effective for locating genes for rare single-gene disorders, it is less powerful when many genes with small effect sizes are involved in the trait of interest. To overcome this problem, the "classical" linkage analysis has to be extended in a way to gain more power to detect genes with smaller effect sizes. With the development of the *quantitative trait loci* (QTL) linkage design, these problems could be (partly) addressed. Rather than studying large pedigrees in a few families, this method studies many families with a small number of relatives, usually siblings. QTL linkage examines *allele sharing* between two individuals of a sibling pair, also known as *identity-by-descent* (IBD). Full siblings receive each an allele from the father as well as an allele from the mother. The total IBD value of a sibling pair can therefore range between 0 and 2. QTL linkage is based on the assumption that linkage is supported if sibling pairs with two alleles IBD are significantly more alike on the (complex)-trait of interest than sibling pairs that share only 1 or 0 alleles IBD, respectively. The strength of linkage is denoted as a *logarithm of odds* (LOD) score, which compares the likelihood of whether two loci are indeed linked to the likelihood that the observed linkage is present purely by chance. The first success for QTL linkage studies came with the identification and replication of linkage for reading disability on chromosome 6 (6*p*21)[5], but QTL linkage has also been successful for complex traits. For instance, Cloninger et al.[6] found a significant linkage between the personality trait *Harm Avoidance*, a measure of anxiety proneness, and a locus on chromosome 8 which explained 38% of the trait variance.

Linkage analysis and well-being

To date, only one genome-wide linkage study has been performed to disentangle sources of individual differences in happiness[7]. Happiness was assessed with the four-item Subjective Happiness Scale[8], and a total of 1,157 offspring from 441 families were genotyped with an average of 371 micro-satellite markers per individual. Using QTL linkage, authors found suggestive linkage (LOD score of 2.73, $p = .10$) at the end of the long arm of chromosome 19 (q13.43) and at the short arm of chromosome 1 (LOD score of 2.37, $p = .21$). This result indicates that happiness might be positively associated with particular genes located in these genomic regions. However, after closer inspection, none of the genes (e.g. DUXA, AURKC, USP29, and several zinc finger protein genes) appeared to play a plausible role in happiness. For instance, DUXA genes are thought to be involved in early embryonic development, whereas USP29 is associated with Angelman Syndrome. In addition, zinc finger proteins are one of the most abundant proteins in the genome with extreme diverse functioning, including

DNA recognition, RNA packaging, and transcriptional activation[9].

Association studies

Candidate genes

Over the past few years, linkage analysis has lost its predominance in favor of *allelic association* analysis. In contrast to linkage studies, which are very systematic but often suffer from a lack of statistical power, association studies are more powerful but were initially less systematic. Association studies are more powerful because they do not rely on recombination within families as in linkage analysis, but simply compare allelic frequencies for groups such as low-scoring versus high-scoring individuals on a quantitative trait[10]. However, the strength of allelic association is also its biggest weakness, as allelic association can only be detected if a DNA marker is itself the functional genetic variant (i.e. *direct association*) or is located closely to the functional variant (i.e., indirect association or *linkage disequilibrium*). As a consequence, hundreds of thousands, or even millions of DNA markers must be *genotyped* in order to capture genetic variation across the genome. Therefore, for a long time, allelic association has been used primarily for fine-mapping of linkage regions and testing of potential candidate genes rather than scanning the genome in a systematic way. There are several strategies for the selection of candidate genes. For instance, the selection of genes based on their hypothesized involvement in physiological systems thought to influence the trait of interest or selection of chromosomal regions in animals that are known to influence the trait of interest as a starting point. To date, there are only two candidate gene studies that investigated subjective well-being (SWB), one involving the serotonin transporter gene (5-HTTLPR) and the other the Monoamine oxidase A (MAOA) gene which encodes the MAO-A enzyme.

Candidate genes: serotonin and life satisfaction

The neurotransmitter serotonin is believed to play an important role in human mental states and is therefore one of the most intensively studied neurotransmitters in the central nervous system (CNS). When serotonin is released in the synaptic cleft, serotonin transporters that are placed in the cell wall will recycle most of it. The 5-HTT gene that encodes for this transporter contains a VNTR in the promotor region (5-HTTLPR). As a result, the serotonin transporter gene promotor region exists as a “long” or “short” variant, although three additional but more rare variants have recently been discovered[11]. Although these alleles all produce the same protein, the long allele (L-allele) is associated with a three times higher

activity than the shorter allele (S-allele) and produces significantly more serotonin transporters[12]. The consequences of these different genetic variants are increasingly understood. For instance, it has been shown that in carriers of the S-allele, the amygdala was increasingly active to negative emotional stimuli[13,14]. In addition, another study found that individuals carrying the L-allele have a significant bias towards positive information and selectively avoid negative information[15]. Based on these studies, variation in the promotor region of 5-HTTLPR seems to be a promising candidate for individual differences in SWB. It is therefore no surprise that this gene was chosen in a study performed by De Neve and colleagues[16,17], who hypothesized that the L-allele of the 5-HTTLPR gene would be associated with a higher level of life satisfaction than the S-allele.

In their first study, genetic information was available from 2,574 individuals, including markers that identify alleles of the serotonin 5-HTT promotor gene. The included participants were asked to answer the following question: How satisfied are you with your life as a whole?, and response categories ranged from 1 ("very dissatisfied") to 7 ("very satisfied"). By comparing allelic frequencies between low-scoring and high-scoring individuals, it was found that participants carrying the L-allele (i.e., the more efficient variant of the serotonin transporter gene) reported significantly higher levels of life satisfaction. Two independent samples were used to replicate this interesting finding[17]. The first sample consisted of 3,460 individual's that were genotyped using the European HAPMAP sample, which contain an alternative marker (rs2020933) associated with the transcription of serotonin transporters[18–20]. Participants were asked to answer the following life-satisfaction question: Indicate where you think you belong between these two extremes: (1) satisfied with job or home life, or (2) ambitious, want change. Respondents could provide an answer on a seven-point scale and the coded scale was reversed so that higher values indicated greater life satisfaction. Although both, the DNA marker as the life satisfactory question were slightly different from the first study, a positive and statistically significant association between the more efficient A-allele of the rs2020933 marker and increased life satisfactory was found. The second sample extended the first discovery study, which was made possible due to a new release of genomic data (N = 10,163). Association models were identical to those described in the earlier mentioned discovery sample, but a significant association was no longer found between carriers of the L-allele and life satisfaction in this new sample nor in a pooled sample consisting of the discovery sample and the new released genomic data (N = 12,391).

Together, these results produce some mixed results indicating that more research is needed to test the hypothesis regarding the association between the 5-HTTLPR L-allele and life satisfaction. Moreover, the complete absence of any significant association between this gene variant and life satisfaction in the second replication study raises doubts whether this gene should be considered a suitable candidate gene for life satisfaction. Importantly, most molecular genetics studies have been studying this genetic polymorphism predominately in relation to depression and response to negative stimuli[13,14,21]. Given that the absence of depression of negative emotion is not the same as happiness, the role of 5-HTTLPR in processes related to well-being is currently unclear[22], although some studies suggest that the 5-HTTLPR short allele may increase the positive response to supportive exposures.

Candidate genes: MAOA and happiness in women

The second candidate gene study focused on a well-being phenotype, investigated the involvement of the monoamine oxidase A (MAOA) gene in modulating happiness[23]. The MAOA gene is located on the X chromosome[24] and encodes the MAOA enzyme, which metabolizes (i.e., rendering inactive) neurotransmitters such as norepinephrine (NE), serotonin (5-HT), and dopamine (DA)[25]. Just as the serotonin transporter gene (5-HTTPR), the MAOA gene possesses a VNTR polymorphism resulting in a short allele that is associated with lower-activity (L-allele) and a long allele which is associated with high activity (H-allele)[26]. In some studies, the L-allele has been associated with maladaptive outcomes such as alcoholism[27], aggression[28], and antisocial behavior[29]. Because of its putative involvement in mood regulation, it was hypothesized that the MAOA gene also plays a role in happiness. To test this hypothesis, 193 women and 152 men were assessed for the MAOA genotype. Happiness was assessed with the four-item Subjective Happiness Scale[8,30] and responses were combined and averaged to provide a single continuous score, ranging from 1 to 7. Since MAOA is an X-linked gene, women can be classified as having high (HH), intermediate (LH), or low (LL) MAOA activity, whereas men can only be classified by having high (H) or low (L) activity, since males only have one X chromosome.

Among male participants, no differences in level of happiness were observed between carriers of the L-allele and H-allele variant of the MAOA gene. Interestingly, females carrying two L-alleles reported significantly higher level of happiness (M = 5,83, SD = 0,75) compared to females carrying both the L- and H-allele (M = 5,50, SD = 1,00) or carrying two H-alleles (M = 5,30, SD = 0,97), respectively, indicating that there seems to be a gender difference in the

association between MAOA genotype and perceived happiness.

However, these results should be interpreted with caution. First, there is inconsistency in the literature about the role of L-allele in behavior. As mentioned, different studies associate this variant with stress, aggressiveness, and antisocial behavior[27–29], although there are also studies suggesting that L-allele carriers are more susceptible to positive experiences[31]. Therefore, more research is needed in order to understand the relation between the MAOA genotype and behavior more generally. Second, the main effect is based on a relatively small, probably underpowered sample size. In candidate gene studies, first findings often suggest a strong genetic effect that tends to be a lot weaker or no longer significant in replication studies. This is also known as the *winners-curse* and replication of this study is therefore warranted.

Genome complex trait analysis

In summary, while linkage analysis is systematic but not powerful, candidate gene allelic association studies are the opposite—able to detect genes with small effect sizes but only with a priori knowledge. So far, using these two approaches, no convincing evidence emerged for the identification of specific genes associated with well-being. A valid question that could arise from these studies is whether we are currently able to explain any genetic variance in well-being at all. GCTA, a recently developed software tool, might provide an answer to this question[32]. Rather than testing the association of any particular SNP, GCTA estimates the variance that is explained by multiple SNPs on a genome-wide basis. In other words, GCTA estimates how much of the variance in a trait can be accounted for by the genetic variance based on common SNPs, resulting in a heritability estimate based on molecular genetic data. Unlike twin studies, GCTA does not require a sample of related individuals and can therefore discard the assumption of environmental and genetic resemblance between relatives. Although the method is relatively new, it has already been successfully applied to several phenotypes, including height[33], intelligence[34,35], personality traits[36], and major depressive disorder[37].

Genome-wide complex trait analyses and well-being

Recently, GCTA has been applied in order to estimate how much of the variance in well-being could be explained by shared SNPs in a pooled sample of ~11.500 unrelated Swedish and Dutch individuals[38]. Well-being was measured using the positive affect items ("During the past week, I was happy" and "During the past week, I enjoyed life") from the Center for Epidemiology Studies Depression Scale (CES-D)[39]. In addition to separate analyses for each item, scores of the two items were combined to generate an overall well-being measurement.

Using GCTA, it was estimated that 5–10% of the variance in well-being was accounted for by shared SNPs of unrelated individuals when measured with single-item well-being questions. It is likely that this estimation is attenuated by measurement error given that well-being, as measured in this study, was based on only two questionnaire items. Hence, a correction for measurement error of the well-being measures was applied, which raised the point estimate to the range of 12–18%. This estimation is in line with a large twin study[40] yielding an additive genetic effect in the 10–20% range. Together, these results imply that SNPs, measured on existing platforms, do explain a significant proportion of the population variance in well-being. Therefore, future genome-wide large-scale efforts to search for SNPs associated with well-being are relevant and have potential for success.

Genome-wide association

The main reason why genetic association studies were not performed on a genome-wide scale—until recently—is easy to explain: they were simply too expensive. To illustrate this, genotyping of 500,000 SNPs in 1,000 individuals would require 500 million analyses. In the beginning of the allelic association era, such an effort would have cost tens of millions of dollars. This is why allelic association studies were initially restricted to candidate genomic regions. Nowadays, however, whole-genome SNP panels can be genotyped across many samples for relative low costs, and more than nine thousand studies for a great diversity of disease or traits have been published, which led to the discovery and replication of several novel gene loci for many phenotypes[41]. Such success was primarily restricted to medical and (some) psychiatric traits. However, a recent GWAS on educational attainment (N = 126,559) yielded three genome-wide significant SNPs which replicated Rietveld et al.[42].

GWAS are an extension of the allelic association model used in candidate gene studies. The ultimate aim of the GWAS design is to capture all common genetic variations across the genome and test for associations between this genetic variation and a trait of interest. In other words, GWAS is explicitly designed to detect genetic variants under the *common-disease common-variant* (CDCV) model for complex traits or diseases. The CDCV hypothesis postulates that a significant proportion of the phenotypic variation in a population is largely due to many common variants of small effects, suggesting that association with a given trait is largely due to common variants[43]. According to a recent review[44], GWAS have identified >100 loci for schizophrenia (SCZ), while only 20 loci for Alzheimer's Disease (AD), eight loci for bipolar disorders (BIP), one locus for autism disorder spectrum (ADS), and none for

attention deficit hyperactivity disorder (ADHD), anorexia nervosa (AN), major depressive disorder (MDD), obsessive compulsive disorder (OCD), and Tourette's syndrome (TS). The overt success for SCZ could be largely explained by the large sample size, which was achieved by the Psychiatric Genomic Consortium (PGC) which combined data from more than fifty studies (>35,000 cases compared with a maximum of ~17,000 cases for any other disorder). Importantly, sample size is only one of several factors to be considered in GWAS. Another significant influence is the distribution of the phenotype in the population, with studies focused on less frequent phenotypes having generally more power to detect SNPs[45]. Higher ratings of well-being are likely to be more prevalent in the population than most psychiatric disorders. As a consequence, the sample size to detect SNPs associated with well-being should be bigger than any existing GWAS of psychiatric illness.

GWAS and well-being

To accomplish such an enormous sample size, a large genome-wide association meta-analysis on SWB is currently under way within the Social Sciences Genetic Association Consortium (SSGAC) (<http://www.ssgac.org>). This study consists of at least 34 cohorts and will include ~150.000 individuals, which will yield a power of approximately 50% to detect SNPs with a *minor allele frequency* (MAF) of 3%. After the discovery and replication phase, meta-analyses on positive affect and life satisfaction will be conducted in order to explain individual differences in SWB caused by genetic factors.

The path between genes and behavior

As already mentioned, quantitative behavioral genetic research consistently reports that heritable factors contribute substantially to the population variance in well-being[3], but these studies do not give us insight into the specific genomic regions that are responsible for these differences. Molecular genetic research, which aims at identifying the specific genes responsible for the heritability estimates of well-being, are showing mixed results. However, although present efforts are somewhat disappointing, we should not forget that molecular research pertaining to SWB is still in its infancy. In summary, results of significant heritability estimates suggest that DNA variation is involved in behavioral variation and we (as behavioral genetics) need to puzzle out which genomic regions are associated with these complex traits in order to understand the molecular mechanisms, which requires not only the identification of genomic regions associated with well-being but also investigation of how these regions affect complex behavior.

Gene expression

A possible way to bridge this gap is the study of *gene expression* as a mechanism underlying genetics associations with complex traits. The genes that are expressed or transcribed from genomic DNA—sometimes referred to as the *transcriptome*—represent the major determinants of cellular phenotype and function. Transcription of genomic DNA to produce *messenger RNA* (mRNA) is the first step in the process of protein synthesis, and differences in gene expression are responsible for both morphological and phenotypic differences as well as being indicative of cellular responses to environmental stimuli and perturbation. Unlike the genome, the *transcriptome* is highly dynamic and changes rapidly and dramatically in response to perturbations, or even during normal cellular events such as DNA replication and cell division[46,47]. With the development of *micro-arrays*, expression of all genes in the genome can be assessed simultaneously using RNA transcripts as an outcome measurement.

Gene expression and well-being

To date, there is only one gene expression study involving SWB[48]. In their study, the conserved transcriptional response to adversity (CTRA) gene expression profile was used as a molecular reference space in which to map potentially distinct biological effects of *hedonic* and *eudaimonic* well-being. Hedonic well-being represents the sum of an individual's positive affective experience[49], whereas eudaimonic well-being results from striving toward meaning and a noble purpose beyond simple self-gratification[50]. Although eudaimonic well-being is sometimes explained as a "deeper" form of well-being, these two forms are highly correlated (r = .70) and tend to reciprocally influence one another[51,52]. CTRA is characterized by an increased expression of genes involved in inflammation (e.g., proinflammatory cytokines such as *IL1B*, *IL6*, *IL8*, and *TNF*), whereby genes involved in type I interferon antiviral responses (e.g., IF1-, OAS-, and MX-family genes) and IgG1 antibody synthesis (e.g., *IGJ*) are down-regulated. Activation of the CTRA response is associated with several pathological phenotypes, such as inflammatory mediated cardiovascular and neurodegenerative diseases, and impaired host resistance to viral infections[53,54]. Differential expression of the leukocyte CTRA was assessed in genome-wide transcriptional profiles of peripheral blood mononuclear cells (PBMCs) in 80 participants for whom hedonic and eudaimonic well-being was measured using the eight-item Short Flourishing Scale[55].

It was shown that hedonic and eudaimonic well-being, although strongly correlated with each other, have divergent gene transcriptional correlates in human immune cells. Eudaimonic

well-being was associated with decreased expression of the CTRA transcriptome (e.g., less antiviral responses and antibody synthesis), whereas CTRA gene expression was significantly up-regulated in association with increasing levels of hedonic well-being (e.g., increase in proinflammatory cytokines). Although high levels of hedonic and eudaimonic well-being seem to have a divergent gene-expression profile in human immune cells, these results should be interpreted with caution and are subject to debate[56–59]. The discussion focuses primarily on the fact that the complex analyses used in the study depend entirely on distinguishing the highly correlated constructs hedonic and eudaimonic well-being with a self-report measurement conducted in a small sample. The question that arises, therefore, is whether proper psychometric conditions could be met to see these two philosophic definitions of well-being as two independent constructs.

Strengths, weaknesses, and opportunities

Strengths and weaknesses

One of the most exciting directions for genetic research in well-being involves harnessing the power of molecular genetics to identify the specific genes responsible for the consistently reported, influence of genetics on well-being outcomes. The two major strategies for identifying genes associated with well-being are allelic association and linkage studies. Allelic association has a rather simple design and calculates the correlation between an allele and well-being. Linkage is—like association within families—tracing the co-inheritance of a DNA marker and well-being within families. A great strength of the linkage approach is that it systematically scans the genome with only a few hundred markers in order to test for violations of Mendel's law of independent assortment between well-being and a DNA marker. However, in most complex traits, like well-being, it is likely that many genes with small effect sizes are involved. Therefore, using linkage is like using the Hubble telescope: it can scan planets in our galaxy (large QTL effects), but will go out of focus when trying to detect the Apollo landing sites on the moon (small QTLs). Furthermore, linkage studies are difficult to replicate, which was demonstrated in a review study by Altmüller et al.[60], who found that many studies of the same disease were often showing inconsistency in their results.

In addition, if linkage can be compared to the Hubble telescope, candidate gene studies are more like a microscope with theoretically enough power to detect genes with small effect sizes. However, a major drawback of this approach is that these studies require the ability to predict functional candidate genes a priori—knowledge which is still limited despite our

increasing understanding of biochemical pathways and the etiology of quantitative traits. For instance, in existing candidate studies on well-being (5-HTTLPR and MAOA), the candidate gene of interest were chosen based on biological pathways that—at best—are only indirectly linked to well-being. Furthermore, just as with linkage, candidate genes studies are extremely difficult to replicate[61]. Most likely, the failure of replication is due to the fact that the largest effect sizes of genes involved in complex traits are still much smaller than initially expected. In other words, the existing candidate gene studies on well-being were most likely underpowered to defect any genetic effects in the first place. In contrast, GWAS are hypothesis free and provide a relatively unbiased screening of the human genome, thereby enabling the discovery of previously unsuspected genetic variants. At time of writing, the upcoming GWAS (~150 K) on SWB is still in the pipeline, but it will be exciting to see the first results in the near future, which will hopefully bring us a step closer to the identification of genes associated with SWB.

Opportunities

As mentioned, the field of molecular genetics and well-being is still in its infancy, meaning that there are many opportunities left to unravel the genetic architecture of this increasingly popular topic. An interesting approach that recently generated much interest is polygenic score analysis. Polygenic scores are created based on the weighted sum of multiple alleles associated with the outcome of interest in a discovery sample. It is then tested whether the same score predicts the outcome in an independent replication sample[62]. There is increasing evidence that a substantial proportion of the phenotypic variation might be better explained by a combination of multiple genetic variants rather than individual variants that often fail to reach significance in large GWAS studies. For instance, significant associations between polygenic scores and well-being would imply that a genetic signal is indeed present among the included markers. It would therefore be very interesting to construct such a polygenic risk score from the forthcoming GWAS meta-analysis on SWB.

Furthermore, and as mentioned at the very beginning of this chapter, well-being is influenced by both genetic and environmental factors. Despite a rich epidemiologic literature that is focused either on environmental and social influences or genetic factors, few studies to date have examined the dynamic interplay between genetics and environment in the prediction of well-being. It would therefore be very interesting to (1) investigate whether genetic factors (based on promising SNPs of the forthcoming GWAS on SWB) predict specific preferences

for particular (social) environment (gene–environment correlation), and (2) whether genetic factors predict different degrees of environmental sensitivity, including the sensitivity to positive exposures. To conclude, the field of molecular genetics is a scientific field that is constantly in development and changes very rapidly. Technical advances will ensure that we will be increasingly able to explain genetic variance that is associated with well-being.

Outline of this thesis

The overarching aim of this thesis is to increase knowledge on the causes of individual differences in well-being, and it relationship with related traits. This is achieved through a series of studies aimed at identifying genetic variants associated with well-being as well as identifying environmental influences through epigenetic measures.

Chapter 2 looks at the etiology of the association between well-being and depressive symptoms over the lifespan using data from over 43 thousand twins. It was shown that especially in adolescence and in young adults, the phenotypic correlation between well-being and depressive symptoms are explained by genetic effects, while in childhood genetic and environmental factors are equally important.

Chapter 3 reports the results of the first sufficiently powered GWAS of well-being, which identified three genome-wide significant associations. Additionally, we found the first two genetic variants associated with depressive symptoms and eleven genetic variants associated with neuroticism. Genetic correlations between these three traits revealed the existence of a large shared etiology.

Chapter 4 introduces two methods for multivariate genome-wide meta-analysis (GWAMA). We applied these methods to jointly analyze measures of well-being, depressive symptoms and neuroticism, collectively referred to as the well-being spectrum. We identified 319 genetic variants associated with this well-being spectrum. Moreover, extensive biological analyses including gene expression in brain tissue and single cells, showed that genes differentially expressed in the subiculum, the ventral tegmental area, and in GABAergic interneurons are enriched in their effect on the well-being spectrum.

Chapter 5 describes the results of the first epigenome-wide association study (EWAS) approach to identify differentially methylated sites with individual differences in well-being. Subjects in this study, were part of the longitudinal survey studies of the Netherlands Twin Register (NTR). We found that two CpG probes were genome-wide significant after Bonferroni correction. Gene ontology (GO) analysis highlighted enrichment of several central nervous system categories among higher-ranking methylation sites

C**hapter 6** examines epigenome-wide analyses of well-being through direct measurement (EWAS) and Mendelian Randomization (SMR). We found little correlation between the EWAS and SMR analyses suggesting that the associations we observed in our EWAS are mainly driven by processes other than pleiotropy or a direct causal effect of CpG methylation on well-being.

Chapter 7 provides a comprehensive review to investigate how much evidence there is for a conceptual overlap between subjective well-being (SWB) and psychological well-being (PWB). We found that SWB and PWB are related constructs that are likely domains of a general factor well-being. However, while the constructs are related, they are not interchangeable and can be distinguished both conceptually and biologically.

Chapter 8 reports the first two genetic variants associated with eudaimonic well-being as well as six genetic variants associated with hedonic well-being. Moreover, a large shared genetic etiology between both measures was observed indicated by the large genetic correlation and similar patterns of genetic correlations with related traits (e.g. depressive symptoms, personality, and loneliness).

Chapter 9 expands the interrelation among well-being, neuroticism, and depressive symptoms which we refer to as the 3-phenotype well-being spectrum (3-WBS). Based on polygenic scores and genetic correlation analyses of multiple related traits, we found that self-rated health as well as loneliness are important aspects influencing well-being.

Chapter 10 concludes with a reflection on the current state of the field of molecular genetics of well-being and provides a perspective on promising ways for future endeavors in the field.

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Table 10.1 Overview of molecular genetic studies involved in subjective well-being NA = not available; VNTR = variable-number tandem repeat; 5-HTT = serotonin transporter; 5-HTTPLR = serotonin-transporter-linked polymorphic region; MAOA = Monoamine oxidase A; α = Cronbach's alpha; GREML = genomic-relatedness-matrix restricted maximum likelihood; CES-D = Center for Epidemiologic Studies Depression scale; CTRA = conserved transcriptional response to adversity; PBMC = peripheral blood mononuclear cells

Author	**Sample**	**Design**	***N Markers***	**Gene of Interest**	**Measurement Well-being**	**Main Conclusion Study**
Bartels et al., 2010	3.412 subjects 711 families	Linkage Analysis	752 autosomal 22 X-linked Average: 371 (250–782) Average Space 4.78 cm	NA	*Subjective Happiness Scale* Items: 4 7-point scale α: .86	Two suggestive linkage peaks Chromosome 19: LOD: 2.73 (p = .095) Chromosome 1: LOD: 2.37 (*p* = 209)
De Neve, 2011	2.574 (Discovery sample)	Candidate Gene (serotonin transporter gene)	7 genetic markers (including 5-HTTLPR allele markers)	VNTR polymorphism marker for 5-HTT 528 bp (more efficient) 484 bp (less efficient)	*Single Item measuring Life Satisfaction* "How satisfied are you with your life as a whole?" 5-point scale α: NA	Individuals with the more efficient version of 5-HTTLPR respond significant higher levels of satisfaction (*p* = .001)

De Neve et al., 2012	2.843 (Replication 1) 10.163 (Replication 2)	Candidate Gene (serotonin transporter gene)	2.543.887 SNPS (European ancestry HaPMap sample) 7 genetic markers (including 5-HTTLPR allele markers)	Rs2020933 marker "A" allele more efficient VNTR polymorphism marker for 5-HTT 528 bp (more efficient) 484 bp (less efficient)	"Indicate where you belong between these two extremes. Satisfied with job or home life OR ambitious, want change" 7-point scale α: NA *Single Item measuring Life Satisfaction* "How satisfied are you with your life as a whole?" 5-point scale α: NA	Individuals carrying the more efficient "A" allele respond more significantly to satisfied with job or home (p = .05) No significant association between 5-HTTPLR polymorphism and increased levels of satisfaction (p = .823)
Chen et al., 2013	345 (193 women and 152 men)	Candidate Gene (MAOA gene)	NA	VNTR MAOA gene promoter (3.5–4 repeats: high activity) (3 repeats: low activity)	*Subjective Happiness Scale* Items: 4 7-point scale α: .86	Significant association between low expression of MAOA and greater happiness in women (p = .002). In contrast, no such association was found in men.

Rietveld et al., 2013	~11.500	GREML	>500.000 SNPS	NA	*CES-D Scale positive affect subscale* Items: 2 α: .85	Fraction of variance in SWB explained by common polymorphisms is 5–10% and 12–18% after correction for measurement error.
Fredrickson et al., 2013	84	Gene expression	NA	CTRA related gene set	*Short Flourishing Scale* Items 8 α: .87 *CES-D Scale* Items: 20 α: .85	Hedonic well-being was associated with increased expression of proinflammatory genes (*p* .0047). In contrast, eudaimonic well-being was associated with CTRA down-regulation (*p* = .0045).

02

Unraveling the genetic and environmental relationship between well-being and depressive symptoms throughout the lifespan

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Abstract

Whether well-being and depressive symptoms can be considered as two sides of the same coin is widely debated. The aim of this study was to gain insight into the etiology of the association between well-being and depressive symptoms across the lifespan. In a large twin-design, including data from 43,427 twins between age 7 and 99, we estimated the association between well-being and depressive symptoms throughout the lifespan and assessed genetic and environmental contributions to the observed overlap. For both well-being (range 31% –47%) and depressive symptoms (range 50%-61%), genetic factors explained a substantial part of the phenotypic variance across the lifespan. Phenotypic correlations between well-being and depressive symptoms across ages ranged from -.34 in childhood to -.49 in adulthood. In children, genetic effects explained 49% of the phenotypic correlation while in adolescents and young adults, genetic effects explained 60% to 77% of the phenotypic correlations. Moderate to high genetic correlations (ranging from -0.60 to -0.66) were observed in adolescence and adulthood, while in childhood environmental correlations were substantial but genetic correlations small. Our results suggest that in childhood genetic and environmental effects are about equally important in explaining the relationship between well-being and depressive symptoms. From adolescence onwards, the role of genetic effects increases compared to environmental effects. These results provided more insights into the etiological underpinnings of well-being and depressive symptoms, possibly allowing to articulate better strategies for health promotion and resource allocation in the future.

Well-being plays an important role in scientific disciplines such as psychology, medicine and public health policy[1–3]. Also, well-being is a topic of great interest in disciplines such as economics with increasing numbers of studies exploring the link between economics factors and well-being (e.g. "whether money buys happiness", see[4,5]). In contemporary sciences, well-being is often defined by a continuous spectrum of positive feelings and subjective life assessments, which is in line with the description of Diener and colleagues (1999), who explained well-being as a broad category of phenomena that includes people's emotional responses, and global judgments of life satisfaction. A considerable number of studies show that well-being is positively associated with physical health[7], success[8], and longevity[3,9]. Additionally, well-being is associated with less mental illness, notably mood disorders and depressive symptoms[10–12]. To this end, the world health organization (WHO) has recommended that national (mental)-health policies should actively promote well-being, rather than focusing exclusively on the prevention of (mental)-health disorders[13–15]. Crucial, however, to the possible use of well-being promotion to target depressive symptom is knowledge on the nature of the association between well-being and depressive symptoms throughout the lifespan.

Traditionally, well-being and depressive symptoms have been considered as opposite ends of a continuum: that low scores on depressive symptoms are considered to be indicative of high levels of well-being and vice versa [16]. However, measures of well-being and depressive symptoms are only moderately correlated between -0.40 and -0.55 in the general population[10–12]. This suggests that well-being and depressive symptoms belong to separable but correlated dimensions[16,17]. For example, it is possible to score low on psychiatric problems but not necessarily score high on well-being, or to score high on psychiatric problems and exhibit high levels of well-being[11,17,18]. Whether well-being and depressive symptoms are two sides of the same coin is still widely debated, and more research is needed to understand the commonalities and specificities underlying this association.

Twin-family studies are important in unraveling phenotypic associations. More specifically, twin studies can be used to examine the role of shared genetic and environmental influences in the relationship between traits[19], and have demonstrated that genetic factors play a substantial role in explaining the observed phenotypic correlation between well-being and depressive symptoms[10,20–23]. For example, genetic influences explain between 33% and 66% of the phenotypic association between well-being and depressive symptoms in adolescents[10,20]. Twin studies on the association between well-being and depressive symptoms in adult populations

yield similar bivariate heritability estimates ranging between 40% and 74%[21–23]. Additionally, these studies report genetic correlations (a quantification of the extent to which two traits are influenced by the same genes) between well-being and depression in the range of -0.55 to -0.79, which is consistent with a recent large scale genome-wide association study that reported a genetic correlation of -0.75[24].

While these findings extend our knowledge on the association between well-being and depressive symptoms, some limitations exist. First, the current literature is limited to adolescent[10,20] and adult samples[17,21–23]. Given the growing interest in well-being promotion across the lifespan[14,15], and the interest of policy makers in early-years interventions to reduce childhood risks[25], it is important to extend these studies to younger ages. Second, studies thus far focused on a specific age group, namely either adolescence or adulthood. As a result, the current literature lacks a perspective on the well-being - depressive symptom relationship throughout the lifespan. Given the complex development of depressive symptoms from childhood into adulthood[26], the changing genetic and environmental architecture of depressive symptoms over age[27], and the genetic stability of well-being[28,29], it is possible that contributions of genetic or environmental factors to the relationship between well-being and depressive symptoms vary over the lifespan. Therefore, a genetically informed study from childhood to adulthood is required to provide the necessary insights into the sources of phenotypic overlap between well-being and depression and thereby detect possible vulnerable but also malleable periods.

In sum, the aim of the present study is to evaluate the contributions of genetic and environmental factors to the association between well-being and depressive symptoms over the lifespan using an informative twin-design. Doing so, we provide better insight into the etiological underpinnings of the association, possibly articulating more targeted models of well-being promotion.

Method

Sample

Participants were registered with the Netherlands Twin Registry (NTR), which consists of the Young NTR (YNTR)[30] and the Adult NTR (ANTR)[31]. Subject recruitment is ongoing and is voluntary, for example, through the website of the register (https://www.tweelingenregister.org/) and through the "Dutch association for parents of multiples", NVOM (https://www.nvom.nl/). The YNTR twins were registered with the NTR as newborns and were followed throughout childhood and adolescence. Parents completed questionnaires concerning their children when the children were approximately 1, 2, 3, 5, 7, 9/10, and 12 years old. The parents were asked for consent to send their children self-report surveys from age 14 onwards. Given parental consent, twins and their non-twin-siblings received an online or a paper self-report survey when they were 14, 16, or 18 years old. When young twins reached the age of 18, they were enrolled in the ANTR. The ANTR includes adolescence and adults, who were recruited through city councils and by other means[31], and who receive self-report questionnaires every 2 to 3 years. Participants are allowed to unsubscribe at any moment.

The current study included all twins between the ages 7 and 99 years, with data on either or both well-being and depressive symptoms. Specifically, we included twins from the YNTR at age 7, 9/10, 12, 14, and 16 years old. Data from several age groups are collapsed, because of the relatively recent addition of well-being questions to the survey studies of the NTR. ANTR participants were divided in young adults (age-range 18-27 years) and adults (>27 years). We decided on the age bracket of 27 years for the following reasons. First, this cut-off is in line with earlier twin studies involving comparable traits[27]. Second, in the Dutch population, the late twenties are characterized by new "life events" such as fulltime working live, considering marriage and having children[32,33]. Third, in order to obtain reliable estimates a minimum sample size is essential. With a cut-off at age 27 reasonable sample sizes are obtained in each age group. The total dataset comprised 42,427 twins, including 16,089 monozygotic (MZ) and 26,338 same-sex and opposite-sex dizygotic (DZ) twins. The majority (54.6%) participated in more than one NTR survey study; with 16.9% taking part 3 times or more. Participants came from all regions of the Netherlands, both rural and urban areas, and were primarily Caucasian. For a detailed overview of included participants in the different age bins for well-being and depressive symptoms respectively see **Supplementary Table 1** and **Supplementary Figure 1**.

Measures

Maternal and self-report ratings based on the Cantril ladder[34] were analyzed for children and (young) adults. The ladder has 10 steps where step 10 indicates the best possible life, and step 1 indicates the worst possible life. Participants were asked to indicate well-being by choosing the step which corresponded to the evaluation of their general well-being (self-ratings, age 14 >) or the general well-being of their child (maternal ratings, age 7 – 12). In our previous work[35], we report on moderate to strong positive correlations between the Cantril ladder and other measures of well-being (see also Adamson 2013; Helliwell et al. 2017). This measure is frequently used (e.g. see[5]. Test-retest analyses showed test-retest correlations between 0.66 and 0.70;[37] as well as a substantial degree of concurrent validity with multi-item well-being scales (correlation between 0.62-0.64;[38]).

Depressive symptoms were assessed by the 'Anxious/Depressed' subscale of the age-appropriate survey of the Achenbach's System of Empirical Based Assessment (ASEBA). At ages 7, 9/10, and 12, maternal reports on the Child Behavior Checklist (CBCL/4-18;[39] were collected. Participants in the age range 14-16 years, completed the Youth Self Report (YSR;[40]) and adults completed the Adult Self Report (ASR;[41]). The instruments were designed to measure comparable constructs over the ages. All instruments collect symptom information on a 3-point scale, '*Not true'*, *'somewhat true or sometimes true'*, *'very or often* true'. Reliability and validity tests of the 'Anxious/Depressed' subscale revealed test-retest correlations in the range of 0.74-0.82 with a Cronbach's alpha of 0.84[39,41].

Strategy of Analyses

Descriptive statistics and phenotypic correlations

Descriptive statistics and phenotypic correlations between well-being and depressive symptoms were calculated in R[42]. Furthermore, we tested for main effects of sex and age on the two phenotypes.

Bivariate Genetic Modelling

We applied structural equation modelling to the twin data to estimate contributions of genetic and environmental factors to the phenotypic variance of well-being and depressive symptoms and to their phenotypic covariance. The classical twin design exploits the fact that monozygotic (MZ) twins are genetically identical and dizygotic twins (DZ) share on average

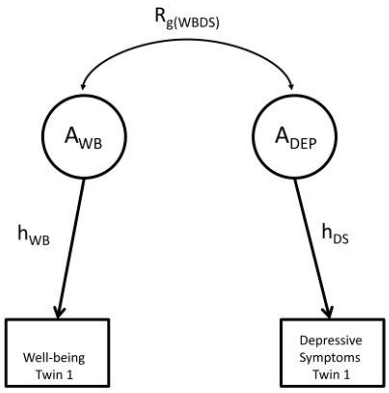
50% of their genetic material to estimate genetic, shared, and unshared environmental variance components. We can estimate additive genetic (A), shared environmental (C), non-shared environmental (E), and dominance genetic (D) components of variance and covariance. As C and D both increase the DZ correlation relative to the AE model they cannot be identified simultaneous in the presence of A and E and will therefore be modeled separately[43].

The depressive symptoms scores were strongly skewed (L-shaped distribution). Such non-normality may bias estimates of environmental influences on the phenotype[44]. Thus, we categorized the depressive symptoms data into three (low, middle, high) groups, and analyzed it as an ordinal variable, assuming an underlying liability with a normal distribution and with two thresholds[45]. The variance in the liability is subject to the decomposition into genetic and environmental components. The two-threshold model determines the prevalence of the low, middle, and high depressive symptoms scores. The well-being measure was modestly skewed to the right, but largely characterized by a bell-shaped curve and was analyzed as a continuous variable.

The bivariate genetic analyses were performed in OpenMx[46]. Within each age-group we estimated the summary statistics separately in the MZmales (MZM), DZmales (DZM), MZfemales (MZF), DZfemales (DZF) and Dizyogitic Opposite Sex (DOS) twin pairs. We estimated the thresholds of the ordinal variables separately in males and females. Sex differences in well-being mean scores and prevalence in depressive symptoms were analyzed by testing whether the means (well-being) or thresholds (depressive symptoms) of males and females could be constrained to be equal.

We used the log-likelihood ratio test to evaluate the significance of parameter estimates. This involves fitting the model with and without the constraints of interest, and basing the test statistic on difference in minus twice the differences in loglikelihood of the models, i.e., the log likelihood ratio (LLR). If the constraints of interest are tenable, this statistic follows a central χ^2 distribution with degrees of freedom (*df*) equal to difference in number of free parameters in the two models. The more parsimonious model (i.e., including the constraints of interest) is rejected if the LLR statistic exceeds the value of $p < 0.005$[47]. If this is not the case, the more parsimonious model is retained.

Figure I. The relationship between shared heritability and genetic correlation. $_{g}$ Represents genetic factors influencing either well-being or depressive symptoms; $r_{g(WBDS)}$ represents the genetic correlation between both phenotypes. Shared heritability equals the path $r_{g(WBDS)}h_{WB}h_{DS}$, where h_{WB} equals the square root of univariate heritability for well-being and h_{DS} equals the square root of the univariate heritability for depressive symptoms.



We estimated genetic and environmental contributions to the bivariate phenotypic covariance matrix by decomposing the phenotypic covariance matrix into (2x2) A, C, and E covariance matrices, or (2x2) A, D, and E covariance matrices. Bivariate heritability is a function of the heritability of the two traits and the genetic correlation (see figure 1). Bivariate heritability tell us what the contribution is of genetic factors to the phenotypic association of well-being and depressive symptoms. The genetic correlation quantifies the extent to which two traits are influenced by the same genes regardless of the magnitude of the contribution of genes (the bivariate heritability) to the phenotypic variance of the traits. We parameterized the covariance matrices using a bivariate Cholesky decomposition[46]. We first considered the full ACE or ADE bivariate models, and then fitted reduced models, in which we tested various parameters (e.g., the variance components due to shared environmental or dominance effects). Having established the best fitting bivariate models based on log likelihood tests, we calculated 95% confident intervals of all free parameters in the model of choice.

Results

Descriptives and phenotypic correlations

Means, standard deviations, and thresholds of males and females in all age-groups are provided in **Table 1.** The means of both maternal and self-reported ratings of depressive symptoms were significantly higher in females ($p < 0.005$), with largest effect size observed at age 16 (Cohen's $d = -0.62$). Sex differences in well-being scores were observed and strongest in adolescence at age 14. At this age, females reported lower levels of well-being ($p < 0.005$, Cohen's $d = 0.19$), but the effect was smaller compared to sex differences in depressive symptoms scores. In general, depressive symptoms scores tended to increase with age, whereas well-being scores were more stable, but it shows a decrease from adolescence onwards in both sexes (**Supplementary Table 1**). Well-being and depressive symptoms are significantly correlated and the correlation increases with age, ranging from -.34 during childhood to -.49 in adulthood (see Table 2).

Twin correlations

MZ and DZ twin correlations and cross-twin-cross trait correlations for each age-group are displayed in **Table 2**. In childhood (i.e., age < 14 years), the MZ correlations of both phenotypes were lower than twice the DZ correlations indicating the contribution of additive genetic (A) shared environment (C) and unique environmental (E) effects to the phenotypes. In adolescence and adulthood, both MZ and DZ correlations decreased, resulting in MZ correlations being larger than twice the DZ correlations, which suggests a role for dominant genetic effects (D) besides additive genetic effects. In these age-groups, an ADE model was fitted to the data to establish the presence of dominance genetic influences. Twin correlations and cross-twin cross trait correlations for each of the five zygosity and age-groups are summarized in **Supplementary Table 2**. The MZ correlations were always higher than the DZ correlations, indicating that genetic effects play a role in explaining individual differences in well-being and depressive symptoms. Sex differences were investigated by constraining the correlations of MZ males to MZ females and DZ males to DZ females. We observed sex differences in the correlation for well-being at ages 7, 14, and 18-27, and differences for depressive symptoms at age 14. The largest difference in twin correlations was observed at age 7 for well-being between MZ males ($r = 0.82$) and MZ females ($r = 0.89$). However, since the differences were relatively rare and small (largest Cohen's d is 0.3; **Supplementary Table 3**), we decided not to model sex specific effects in the variance decomposition. However, we did retain sex differences in the means and thresholds, allowing for a main effect of sex.

Table I. Mean and standard deviation for the raw data for all age groups, as well as the thresholds for the liability distribution and the percentages of twins in the three groups.

Males	**Age 7**	**Age 10**	**Age 12**	**Age 14**	**Age 16**	**Age 18-27**	**Age 27-99**
Mean	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Well-being	8.39 (0.98)	8.27 (1.05)	8.22 (1.12)	8.06 (1.03)	7.82 (1.03)	7.57 (1.10)	7.76 (1.01)
Depressive symptoms	2.19 (2.58)	2.26 (2.79)	1.99 (2.69)	2.57 (2.83)	2.63 (2.80)	3.54 (3.81)	2.77 (3.16)
Categories Depressive symptoms							
Threshold 1	0.05	0.07	-0.31	0.31	0.26	-0.46	-0.20
Threshold 2	0.65	0.69	0.42	0.90	0.63	0.38	0.42
Low	52.0%	52.9%	37.7%	62.3%	60.1%	32.3%	42.0%
Middle	25.8%	24.4%	33.7%	18.3%	26.6%	35.3%	33.6%
High	22.2%	22.7%	28.5%	19.4%	13.3%	32.4%	24.4%
Females							
Mean							
Well-being	8.42 (0.95)	8.37 (0.98)	8.27 (1.16)	7.85 (1.16)	7.63 (1.13)	7.51 (1.09)	7.67 (1.12)
Depressive symptoms	2.36 (2.61)	2.44 (2.90)	2.21 (2.76)	4.51 (3.90)	4.91 (4.08)	5.25 (4.55)	4.28 (3.86)
Categories Depressive symptoms							
Threshold 1	-0.04	-0.01	-0.45	-0.34	-0.46	-0.92	-0.71
Threshold 2	0.59	0.66	0.38	0.75	0.46	0.47	0.4
Low	48.2%	49.6%	32.7%	36.6%	32.3%	17.7%	23.6%
Middle	27.8%	25.6%	35.3%	22.5%	32.2%	32.0%	34.6%
High	24.0%	24.7%	32.0%	40.9%	35.5%	50.3%	41.8%

Bivariate Genetic analyses

The proportions of phenotypic variance of well-being and depressive symptoms attributable to genetic variance, the heritability (h^2), and environmental variance effects (i.e. c^2 and e^2) are displayed in **Table 3** and **Supplementary Figure 2**. For both well-being and depressive symptoms, a substantial amount of the phenotypic variances was due to additive genetic effects. For well-being, genetic effects explained 31% to 47% of the phenotypic variation, while for depressive symptoms estimates were between 50% and 61%. **Supplemental Table S4** shows the result of the Cholesky decompositions, illustrating the full model and sub-models that were tested. Models in bold are judged to provide the best model fit.

The bivariate Cholesky decomposition provide the decomposition of the variance of the two phenotypes and the decomposition of their covariance into genetic and environmental components (**Supplementary Table 4**). The results are presented in **Figure 2** and **Table 3.** In childhood, additive genetic and shared environmental effects contribute significantly to the phenotypic correlations. The bivariate heritability ranged from 41% to 49% and bivariate shared environmental effects ranged from 23% to 30%. In adolescence and young adults, additive genetic and non-shared environmental factors contribute largely to the phenotypic correlation, with genetic effects explaining a slightly larger proportion of the phenotypic correlation (range 60% - 77%). In adults over 27 years, non-shared environmental effects explained 54% of the phenotypic correlation, with the rest explained by additive genetic effects.

Figure 3 and **Table 4** shows the genetic correlations (r_G) and environmental (r_C/r_E) correlations between well-being and depressive symptoms for all age-groups. In childhood, we observe moderate genetic and environmental correlations, indicating that, while part of the genetic (correlations ranged between -0.34 to -0.37) and environmental (r_C ranged from -0.27 to -0.42 and r_E ranging from -0.35 to -0.5) susceptibility to well-being and depressive symptoms overlap, there are substantial trait specific genetic and environmental influences. With increasing age, from adolescence onwards, genetic correlations seems to become more important (range -0.59 to -.66), while the environmental correlations decrease and become limited to non-shared environmental overlap (range -0.20 to -0.48).

Table II: Phenotypic correlations, twin correlations and cross-twin cross-trait correlations for well-being and depressive symptoms

	Phenotypic	**MZ**		**DZ**	
	WB	**WB**	**DS**	**WB**	**DS**
WB y7	1	0.85 (0.83, 0.87)		0.66 (0.62, 0.69)	
DS y7	-0.34 (-0.38 - -0.28)	-0.30 (-0.37, -0.22)	0.71 (0.68, 0.73)	-0.36 (-0.41, -0,30)	0.46 (0.44, 0.48)
WB y10	1	0.79 (0.76, 0.81)		0.62 (0.59, 0.65)	
DS y10	-0.41 (-0.45 - -0.35)	-0.43 (-0.48, -0.35)	0.71 (0.68, 0.73)	-0.45 (-0.50, -0.41)	0.45 (0.42, 0.48)
WB y12	1	0.83 (0.81, 0.85)		0.63 (0.60, 0.65)	
DS y12	-0.39 (-0.43 - -0.34)	-0.34 (-0.40, -0.28)	0.70 (0.67, 0.72)	-0.39 (-0.43, -0.35)	0.46 (0.43, 0.48)
WB y14	1	0.46 (0.42, 0.50)		0.25 (0.21, 0.29)	
DS y14	-0.44 (-0.48 - -0.38)	-0.38 (-0.43, -0.33)	0.60 (0.55, 0.64)	-0.38 (-0.42, -0.34)	0.28 (0.22, 0.33)
WB y16	1	0.47 (0.42, 0.52)		0.21 (0.16, 0.26)	
DS y16	-0.47 (-0.50 - -0.40)	-0.44 (-0.50, -0.39)	0.52 (0.46, 0.57)	-0.40 (-0.44, -0.35)	0.23 (0.16, 0.29)
WB y18-27	1	0.42 (0.37, 0.48)		0.16 (0.11, 0.22)	
DS y18-27	-0.57 (-0.59 - -0.50)	-0.51 (-0.56, -0.45)	0.56 (0.50, 0.62)	-0.53 (-0.58, -0.49)	0.28 (0.21, 0.35)
WB >27y	1	0.30 (0.25, 0.35)		0.11 (0.04, 0.19)	
DS > 27y	-0.49 (-0.54 - -0.45)	-0.50 (-0.55, -0.45)	0.49 (0.43, 0.54)	-0.56 (-0.60, -0.50)	0.15 (0.06, 0.23)

Figure II. Dissection of phenotypic correlation between well-being and depressive symptoms over the lifespan by shared genetic -and environmental effects. A is the proportion of phenotypic correlation explained by shared genetic effects, C by shared environmental effects, and E by unique environmental effects.

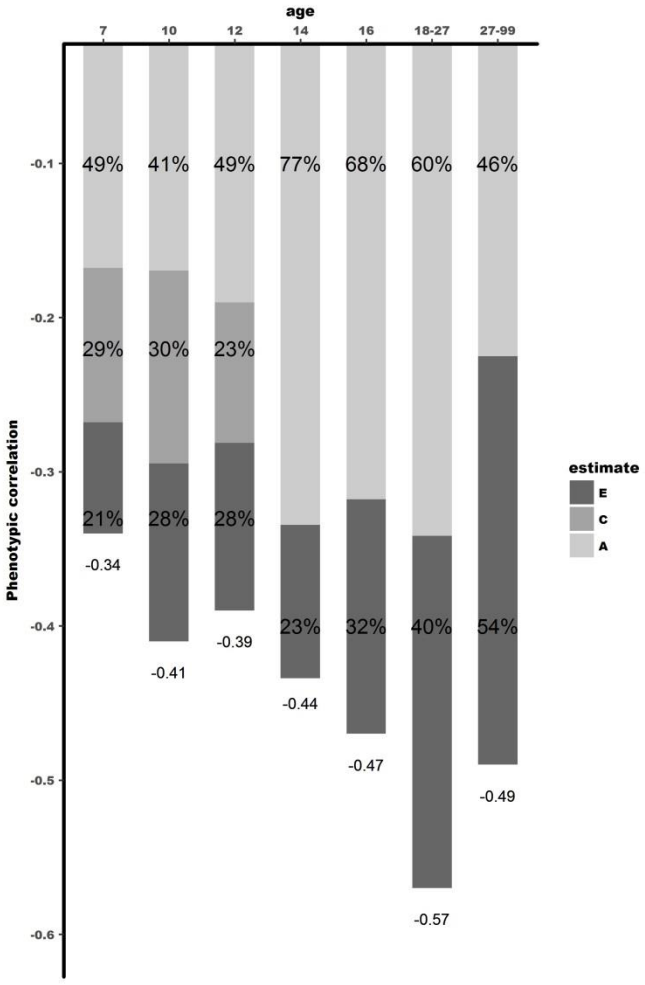


Table III: Standardized estimates (95 % CI) for additive genetic, shared and non-shared environmental influences on well-being and depressive symptoms and their covariance based on the best fitting model.

	A		**C**		**E**	
	WB	**DS**	**WB**	**DS**	**WB**	**DS**
WB y7	0.43 (0.37-0.49)		0.43 (0.37-0.49)		0.13 (0.12-0.16)	
DS y7	0.49 (0.29-0.70)	0.49 (0.29-0.70)	0.29 (0.10-0.48)	0.20 (0.15-0.25)	0.21 (0.16-0.27)	0.29 (0.27-0.31)
WB y10	0.40 (0.34-0.47)		0.41 (0.35-0.46)		0.20 (0.17-0.21)	
DS y10	0.41 (0.26-0.57)	0.53 (0.46-0.60)	0.30 (0.17-0.44)	0.18 (0.12-0.23)	0.28 (0.23 -0.34)	0.28 (0.27-0.31)
WB y12	0.36 (0.31-0.41)		0.46 (0.41-0.50)		0.18 (0.17-0.20)	
DS y12	0.49 (0.32-0.66)	0.50 (0.43-0.58)	0.23 (0.08-0.38)	0.19 (0.14-0.26)	0.28 (0.22-0.34)	0.30 (0.27-0.32)
WB y14	0.47 (0.43-0.50)		-		0.53 (0.50-0.57)	
DS y14	0.77 (0.70-0.84)	0.60 (0.57-0.65)	-	-	0.23 (0.16-0.30)	0.39 (0.35-0.43)
WB y16	0.45 (0.32-0.50)		-		0.55 (0.51-0.59)	
DS y16	0.68 (0.47-0.78)	0.53 (0.42-0.58)	-	-	0.32 (0.25-0.40)	0.47 (0.42-0.52)
WB y18-27	0.42 (0.37-0.47)		-		0.58 (0.53-0.63)	
DS y18-27	0.60 (0.52-0.67)	0.57 (0.52-0.62)	-	-	0.40 (0.33-0.48)	0.43 (0.38-0.48)
WB >27y	0.31 (0.18-0.36)		-		0.69 (0.64-0.75)	
DS > 27y	0.46 (0.36-0.58)	0.50 (0.42-0.55)	-	-	0.54 (0.46-0.62)	0.50 (0.45-0.55)

Figure III. Genetic and environmental correlations between well-being and depressive symptoms over the lifespan. (a) genetic correlation, **(b)** shared environmental correlation, and **(c)** unique environmental correlation.

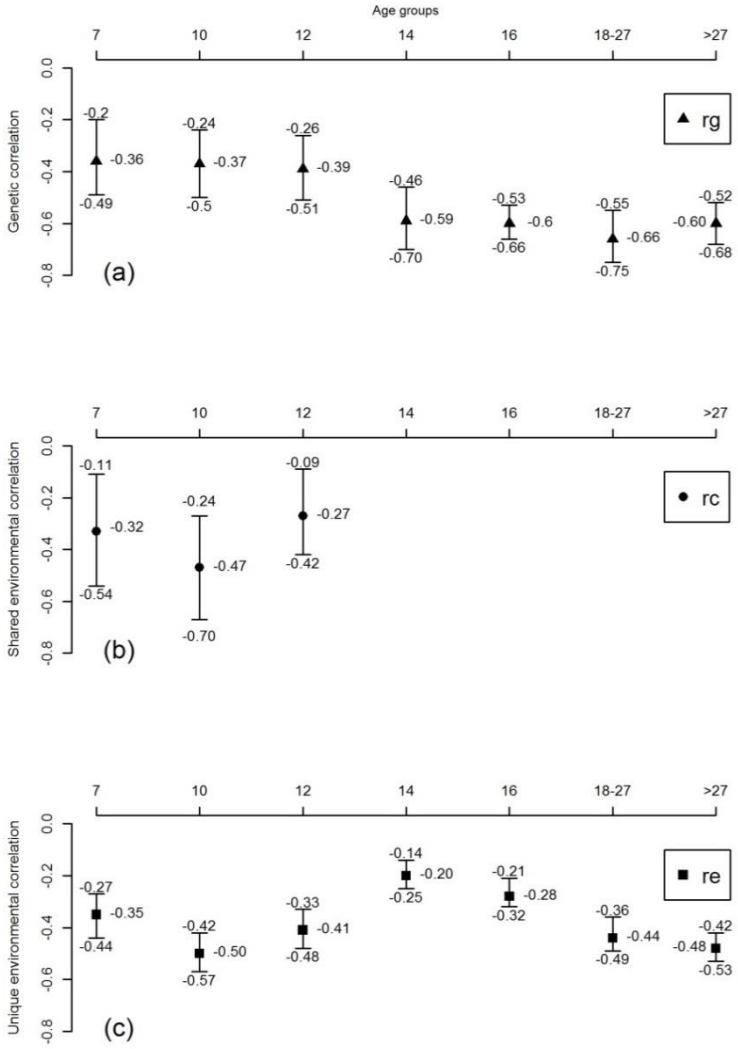


Table IV: Genetic (r_g), shared environmental (r_c) and unique environmental (r_e) correlations with their corresponding 95% confidence intervals.

Age	***rg***	***rc***	***re***
7	-0.36 (-0.49- -0.20)	-0.33 (-0.54 - -0.11)	-0.35 (-0.44- -0.27)
10	-0.37 (-0.50 - -0.24)	-0.47 (-0.67 - -0.27)	-0.50 (-0.57 - -0.42)
12	-0.39 (-0.51 - -0.26)	-0.27 (-0.42 - -0.09)	-0.41 (-0.48 - -0.33)
14	-0.59 (-0.70 - -0.46)	-	-0.20 (-0.25 - -0.14)
16	-0.60 (-0.66 - -0.53)	-	-0.28 (-0.32 - -0.21)
18-17	-0.66 (-0.75 - -0.55)	-	-0.44 (-0.49 - -0.36)
>27	-0.60 (-0.68 - -0.52)	-	-0.48 (-0.53 - -0.42)

Discussion

The aim of this study was to gain insight into the etiology of the association between well-being and depressive symptoms across the lifespan. Phenotypic correlations between well-being and depressive symptoms ranged from -.34 in childhood, to a correlation of -.49 in adulthood, with the highest correlations in young adults (-.57). Bivariate twin models revealed that shared environmental factors play an important role in explaining the relationship between well-being and depressive symptoms in childhood, while in adolescence and adulthood genetic factors become increasingly important.

The results of our study go beyond the available literature in several ways. First, the few twin studies carried out so far focused either on adolescents[10,20] or adults[17,21–23]. Our study is the first to extend these analyses to middle childhood, investigating the association between well-being and depressive symptoms in a cohort-sequential design for age 7, 9/10 and 12, respectively. Results showed that common environmental factors (ranging between 23% and 30%), unique environmental factors (ranging between 21% and 28%), and genetic factors (ranging between 41% and 49%), explain the phenotypic correlation between well-being and depressive symptoms in middle childhood.

Second, instead of focusing on a specific age group, this study examined the association between well-being and depressive symptoms across the lifespan. This study allowed us to shed light on contributions of genetic and environmental factors at different ages. Remarkably, from childhood to adolescence a stark increase was found in the contribution of genetic factors. In adolescence, and young adults, 60% to 77% of the phenotypic association was explained by genetic factors, with no influence of the shared environment. When looking at the genetic correlations, indicating to what extend the same group of genes influence different traits, moderate to high genetic correlations (between r_g =-0.59 and r_g =-0.66) were observed in adolescence, while in childhood environmental correlations are substantial, but genetic correlations small (ranging between r_g =-0.36 and r_g =-0.39). These results show that environmental factors are important in explaining the relationship between well-being and depressive symptoms in childhood, while in adolescence genetic factors play a more substantial role. In adulthood, unique environmental effects showed to be increasingly important, explaining 54% of the phenotypic correlation (with bivariate heritability of 46%). Genetic correlations were high in adulthood, with r_g = -0.60, showing overlap in genetic

factors influencing both well-being and depressive symptoms. These results were consistent with the results of Kendler *et al.* (2011), who reported similar genetic and phenotypic correlations. However, the proportion of the phenotypic correlation explained by genetic effects was larger in their study 86%, compared to 46% in our study. A possible explanation might be that the heritability of the latent factor mental well-being in Kendler *et al.* (2011) was substantially higher (72%) than the heritability of our measure of well-being (31%). This is attributable to their assessment of well-being which is modeled with a latent factor allowing to correct more explicitly for measurement error. In our design, not modelling well-being as a latent factor, part of the measurement error falls into the E component instead of the additive genetic component explaining the discrepancy in heritability estimates[49].

Overall, the moderate phenotypic correlations between well-being and depressive symptoms in the present study support the notion that well-being and depressive symptoms could belong to distinct, but correlated, dimensions. However, results of the genetic informative twin design shows that shared genetic effects explain a substantial part of this phenotypic correlation, especially from adolescence onwards. This finding raises the question whether different interventions are needed for promoting well-being and treating depressive symptoms, or whether we can use the promotion of well-being to reduce depressive symptoms. On the one hand, a growing body of literature suggests that, based on the unique environmental influences on both well-being and depressive symptoms, interventions targeting well-being may not necessarily have a direct impact on depressive symptoms[17,20]. On the other hand, empirical studies suggest that improved positive emotions enhance coping skills, weaken physiological effects of negative emotions and diminish relapses in depressed individuals[50–53]. Additionally, a recent meta-analysis on the effectiveness of positive psychology interventions, including 51 studies and 4,266 individuals, illustrate that, overall, enhancing well-being with positive psychology interventions significantly decrease depressive symptoms[54]. These findings, together with our results, suggest that well-being could be used in future studies as an index of mental health complementing other indices that focus
on mental illness.

Still, the question remains if these findings hold for the prevention of depressive symptoms by early screening and well-being promotion. Put differently, can we use measures of well-being to inform us about vulnerability to depression? Benefits of this approach include the low stigma associated with the content of well-being questionnaires compared to depressive

symptom screening (i.e., people are more willing to answer questions on their quality of life than on their depressive symptoms), and the possibility of screening those at risk in a timely manner. The relatively strong genetic correlation implies that we can identify individuals characterized by low well-being, and offer them suitable interventions to improve their well-being. Even stronger effect may be anticipated if we consider well-being promotion at a population level. Within epidemiology and somatic medicine, it has been proposed that larger benefits to overall public health are to be expected when the bell curve of mental health in the human population is shifted slightly to the healthy side, the so-called population strategy[55,56] . Specifically, a relative slight increase in the level of well-being of the majority of the population may have a larger preventive effect, than targeting the much smaller group of people at high risk or in the early stages of depressive symptoms.

Future studies should, however, focus on the direction of the relationship between well-being and depressive symptoms to use well-being as a possible candidate for novel approaches to reduce depressive symptoms. Recent methodological developments such as Mendelian randomization (MR) designs[57,58] together with the availability of large scale molecular genetic data provide additional opportunities to address the process underlying the correlation between well-being and depressive symptoms.

Limitations

This study has several strengths and weaknesses. First, well-being is a complex phenotype consisting of two well-recognized constructs: Subjective Well-being (SWB) and Psychological Well-being (PWB), shaped by the philosophical concepts of hedonism and eudaimonism, respectively[59]. Hedonic well-being is centered around pleasure, or how good a person feels about his or her life, whereas eudaimonic well-being is centered around living well or doing well and the fulfillment of human capacities[60]. We recognize that, by using the Cantril ladder, the present study does not capture the complete construct of well-being, but rather focuses on SWB. We are confident however, that our results are representative for SWB as the different questionnaires measuring SWB used in social and behavioral sciences correlate highly with the Cantril ladder, both phenotypically and genetically[35]. Second, it is important to keep in mind that high scores on the CBCL, YSR, and ASR ‘Anxiety and Depressive symptoms’ subscales are good predictors of depressive symptoms[61], but are not equivalent to a clinical diagnosis of depression[62]. Third, due to highly skewed scores, we analyzed the depressive symptoms data using a threshold model, resulting in lower statistical

power compared to an analysis of continuous data[44]. However, the parameter estimates in a threshold model are more accurate than in an analysis of continuous data characterized by large skewness[63] Fourth, in adolescence and adulthood, both MZ and DZ correlations decreased, resulting in MZ correlations being larger than twice the DZ correlations, suggesting a role for dominant genetic effects besides additive genetic effects. We recognize that this might be a methodological artifact as a result of the difference between parent rating (e.g. parents phenotype possibly contribute to similar ratings for both twins resulting in shared environmental influences) and self-rating scores (e.g., a twin's own genetic architecture contributing to their own behavior and therefore self-rating scores on well-being and depressive symptoms)[27,49,64,65]. However, while differences in parent reports and child reports exist, earlier studies have illustrated sufficient agreement between child and parent reports on children's quality of life[66]. Fifth, earlier research[67] has postulated that a U-shaped pattern of well-being mean-scores over time exist. It is important to note that the method applied in this study focuses on variance decomposition, rather than mean comparison. Additional analyses specifically exploring the mean of well-being over time did not yield a U-shaped pattern. Therefore we believe this does not influence the results presented in our paper.

Future recommendations

As the genetic and environmental factors explaining the relation between well-being and depressive symptoms differ between the included age-bins, future studies are needed to study the etiology of the relationship between well-being and depressive symptoms in more depth. Longitudinal twin designs such as the genetic simplex model or common factor model (with or without age specific influences) allow for estimation of the stability of the effects of genetic and environmental factors over age, and show to what extent genetic innovation come into play. Additionally, future studies are recommended to investigate key biological and environmental factors of relevance to well-being and depressive symptoms. For example, Routledge et al (2017) investigated the link between well-being , depression and cognitive functioning (and their genetic and environmental overlap). They illustrated some differentiation, with well-being in some cases related to specific cognitive functions independent of depression while for other cognitive functions they showed an overlap between well-being and depression. Additionally, the first genetic variants associated with both well-being and depressive symptoms are recently identified[24]. With the increasing availability of large-scale genetic data it would be interesting to study whether different

genetic variants are associated with well-being over age and whether these variants have a protective effect on the development of depressive symptoms. Finally, further research should not isolate genetic and environmental influences and preferably apply multi-layer designs incorporate both aspects explaining the underlying etiology of well-being and depressive symptoms. Finally, although this study is about variance decomposition with relatively small sex differences we observed larger sex differences in mean scores especially from adolescence onwards. Future studies should focus on the origin of these differences especially in (pre)-clinical settings. Furthermore, larger studies are needed to investigate sex-differences in variance components as the presence or absence is still inconclusive (see also review Bartels, 2015)

Conclusion

In the present study we dissected the association between well-being and depressive symptoms from childhood to adulthood. We confirmed that well-being and depressive symptoms correlate moderately across the lifespan. Importantly, shared environmental factors play an important role in explaining the relationship between well-being and depressive symptoms in childhood. However, from adolescence onward, we found evidence for the prominence of shared genetic effects, with genetic factors explaining a substantial part of the phenotypic correlation from adolescence onward. Therewith, this study provided more insights into the etiological underpinnings of well-being and depressive symptoms, possibly allowing to articulate better strategies for health promotion and resource allocation in the future.

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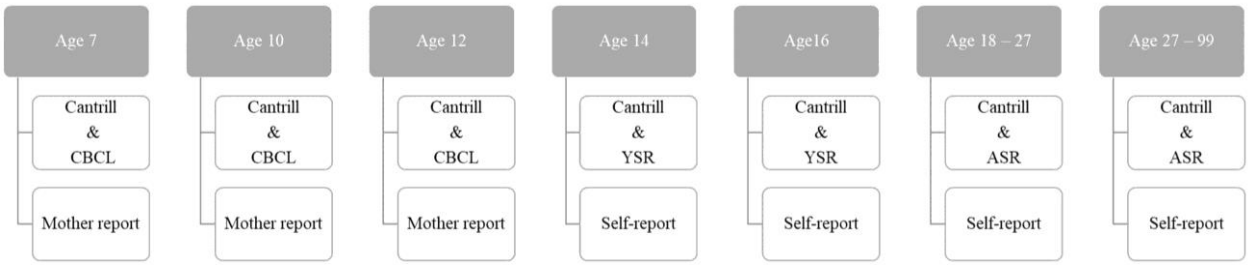
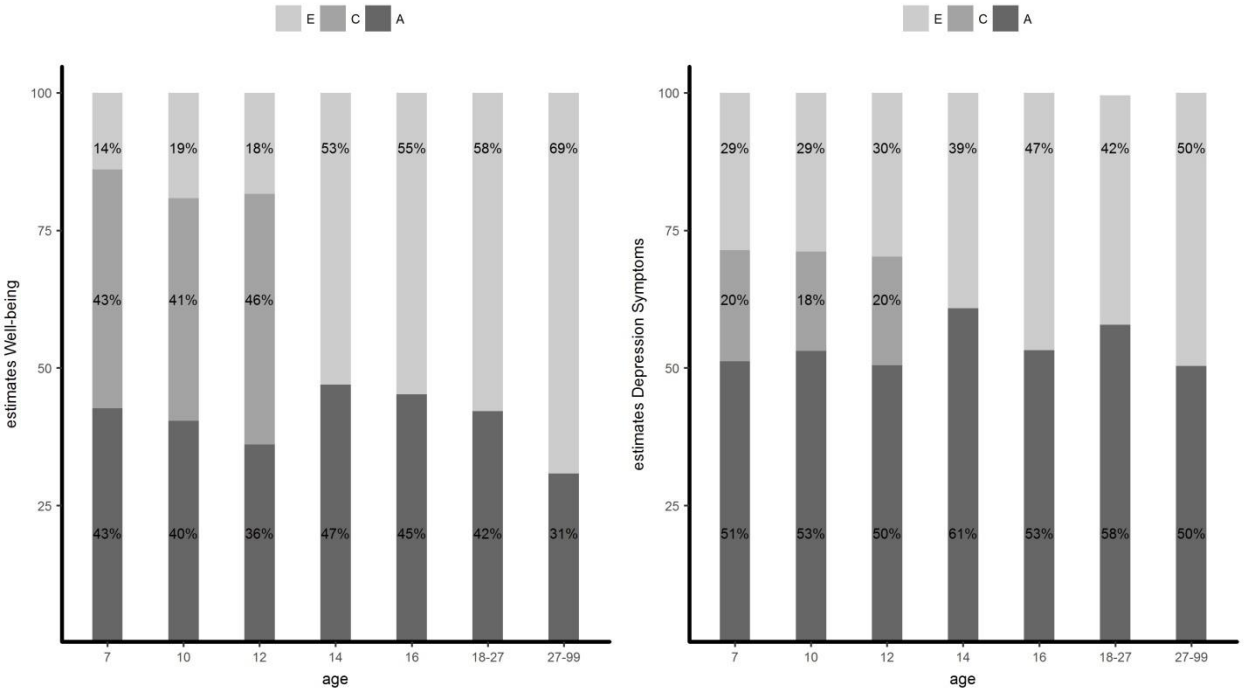
Supplementary Information

Supplementary Figure I. Overview of the survey collection.



Supplementary Figure II. Proportion of phenotypic variance of well-being and depressive symptoms explained over the lifespan by genetic, shared environmental, and unique environmental effects. h^2 represents the heritability, c^2 represents shared environmental influences, and e^2 represents unique environmental influences.



SI. Sample size, mean scores and standard deviations for well-being and depression over the lifespan separated by age bin and gender. Significance (*p*) and effect size (cohen's D) of mean differences are provided. ♂ = male and ♀ = female

Well-being								**Depressive Symptoms**						
Age	***N***	***Mean***	***SD***	**df**	***t***	***p***	**cohen's d**	***N***	***Mean***	***SD***	**df**	***t***	***p***	**cohen's d**
7 ♂	2072	8.39	0.98	4035	-0.74	0.09	-0.02	12235	2.19	2.58	24572	-5.124	0.014	-0.07
7 ♀	1965	8.42	0.95					12339	2.36	2.61				
10 ♂	2699	8.27	1.05	5244	-3.53	0.12	-0.11	9652	2.26	2.79	19526	-4.316	0.002	-0.06
10 ♀	2547	8.37	0.98					9876	2.44	2.9				
12 ♂	3236	8.22	1.12	6438	-1.75	0.704	-0.04	8186	1.99	2.69	16596	-5.325	< 0.01	-0.08
12 ♀	3204	8.27	1.16					8412	2.21	2.76				
14 ♂	3759	8.06	1.03	8820	8.923	< 0.01	0.19	3892	2.57	2.83	9141	-26.388	< 0.01	-0.55
14 ♀	5063	7.85	1.16					5251	4.51	3.9				
16 ♂	2488	7.82	1.03	6103	6.58	< 0.01	0.17	2696	2.63	2.8	6612	-25.33	< 0.01	-0.62
16 ♀	3617	7.63	1.13					3918	4.91	4.08				
18-27 ♂	2852	7.57	1.1	8256	2.313	0.316	0.05	3050	3.54	3.81	8779	-17.777	< 0.01	-0.38
18-27 ♀	5406	7.51	1.09					5731	5.25	4.55				
27-99 ♂	7100	7.76	1.01	17968	5.172	< 0.01	0.08	7991	2.77	3.16	19790	-28.894	< 0.01	-0.41
27-99 ♀	10870	7.67	1.12					11801	4.28	3.86				

SII. Twin correlations and cross-trait-cross-twin correlations for well-being and depression over the lifespan for all five zygosity.

Age 7					**Age 10**			
Males			Females		Males		Females	
MZM			MZF		MZM		MZF	
	WB	DEP	WB	DEP	WB	DEP	WB	DEP
WB	0.81 (0.77, 0.84)		0.89 (0.86-0.91)		0.79 (0.76, -0.83)		0.79 (0.75, 0.82)	
DEP	-0.30 (-0.39, -0.19)	0.69 (0.66, 0.73)	-0.30 (-0.41, -0.19)	0.72 (0.68, 0.74)	-0.38 (-0.47, -0.28)	0.71 (0.67, 0.74)	-0.45 (-0.53, -0.35)	0.71 (0.67, 0.74)
DZM			DZF		DZM		DZF	
	WB	DEP	WB	DEP	WB	DEP	WB	DEP
WB	0.61 (0.55, 0.68)		0.67 (0.60, 0.73)		0.60 (0.52, 0.65)		0.64 (0.58, 0.69)	
DEP	-0.38 (-0.48, -0.26)	0.43 (0.38, 0.48)	-0.37 (-0.48, -0.25)	0.44 (0.39, 0.49)	-0.42 (-0.51, -0.33)	0.42 (0.36, 0.48)	-0.45 (-0.54, -0.34)	0.47 (0.41, 0.53)
Dos/males females					Dos/males females			
	WB	DEP			WB	DEP		
WB	0.68 (0.63, 0.72)				0.63 (0.59, 0.67)			
DEP	-0.33 (-0.42, -0.25)	0.49 (0.45-0.52)			-0.46 (-0.52, -0.40)	0.45 (0.41, 0.49)		

Age 12					**Age 14**			
Males			Females		Males		Females	
MZM			MZF		MZM		MZF	
	WB	DEP	WB	DEP	WB	DEP	WB	DEP
WB	0.83 (0.80, 0.85)		0.83 (0.80, 0.85)		0.37 (0.29, 0.43)		0.50 (0.45, 0.55)	
DEP	-0.36 (-0.44, -0.27)	0.71 (0.67, 0.75)	-0.33 (-0.41, -0.24)	0.69 (0.65, 0.72)	-0.33 (-0.41, -0.24)	0.47 (0.37, 0.56)	-0.41 (-0.47, -0.34)	0.66 (0.60, 0.71)
DZM			DZF		DZM		DZF	
	WB	DEP	WB	DEP	WB	DEP	WB	DEP
WB	0.63 (0.57, 0.67)		0.68 (0.63, 0.72)		0.13 (0.04, 0.21)		0.36 (0.28, 0.42)	
DEP	-0.45 (-0.52, -0.37)	0.40 (0.34, 0.45)	-0.31 (-0.39, -0.21	0.51 (0.46, 0.57)	-0.35 (-0.44, -0.27)	0.28 (0.16, 0.40)	-0.43 (-0.50, -0.35)	0.30 (0.20, 0.39)

Dos/males females			Dos/males females	
	WB	DEP	WB	DEP
WB	0.60 (0.56, 0.64)		0.25 (0.20, 0.31)	
DEP	-0.41 (-0.47, -0.35)	0.46 (0.42, 0.50)	-0.35 (-0.41, -0.29)	0.26 (0.18, 0.33)

Age 16					**Age 18-27**			
Males			Females		Males		Females	
MZM			MZF		MZM		MZF	
	WB	DEP	WB	DEP	WB	DEP	WB	DEP
WB	0.48 (0.40, 0.54)		0.46 (0.39, 0.52)		0.55 (0.46, 0.63)		0.37 (0.30, 0.43)	
DEP	-0.39, (-0.48, -0.29)	0.51 (0.40, 0.60)	-0.48 (-0.55, -0.41)	0.52 (0.44, 0.59)	-0.49 (-0.58, -0.38)	0.57 (0.46, 0.66)	-0.52 (-0.58, -0.45)	0.55 (0.47, 0.62)
DZM			DZF		DZM		DZF	
	WB	DEP	WB	DEP	WB	DEP	WB	DEP
WB	0.14 (0.02, 0.26)		0.25 (0.15, 0.34)		0.07 (0, 0.21)		0.26 (0.17, 0.34)	
DEP	-0.46 (-0.56, -0.37)	0.20 (0.04, 0.34)	-0.23 (-0.33, -0.12)	0.28 (0.17, 0.39)	-0.50 (-0.59, -0.39)	0.24 (0.07, 0.40)	-0.56 (-0.63, -0.48)	0.35 (0.24, 0.45)
Dos/males females					Dos/males females			
	WB	DEP			WB	DEP		
WB	0.22 (0.14, 0.29)				0.12 (0.03, 0.20)			
DEP	-0.14 (-0.22, -0.05)	0.20 (0.10, 0.29)			-0.52 (-0.59, -0.45)	0.24 (0.14, 0.34)		

Age 27-99				
Males				
MZM			MZF	
	WB	DEP	WB	DEP
WB	0.35 (0.24, 0.44)		0.29 (0.22, 0.35)	
DEP	-0.48 (-0.56, -0.38)	0.52 (0.41, 0.62)	-0.50 (-0.56, -0.54)	0.48 (-0.41, 0.54)
DZM			DZF	
	WB	DEP	WB	DEP
WB	0.12 (0-0.28)		0.14 (0.03, 0.25)	
DEP	-0.57 (-0.68, -0.44)	0.11 (0, 0.32)	-0.54 (-0.61, -0.46)	0.14 (0.02, 0.26)
	Dos/males females			
	WB	DEP		
WB	0.07 (0, 0.18)			
DEP	-0.56 (-0.63, -0.47)	0.17 (0.04, 0.30)		

SIII. MZmale/MZfemale and DZmale/DZfemale twin correlation differences and corresponding effect sizes.

Well-being

Age	MZM	N	MZF	N	Fishers'Z	Cohen's D	DZM	N	DZF	N	Fishers'Z	Cohen's D
7	0.81	769	0.89	762	-5.76	**-0.29**	0.61	697	0.67	598	0.16	0.01
10	0.79	937	0.79	912	0	0	0.6	887	0.64	760	-1.31	-0.06
12	0.83	1074	0.83	1115	0	0	0.63	1110	0.68	1033	-2.03	-0.09
14	0.37	1234	0.5	1932	-4.41	-0.16	0.13	1064	0.36	1391	-6.04	-0.25
16	0.48	904	0.46	1377	0.6	0.03	0.14	650	0.25	930	-2.23	-0.11
18-27	0.55	748	0.37	1662	5.21	0.21	0.07	549	0.26	1082	-3.73	-0.18
27plus	0.35	718	0.29	1960	1.53	0.06	0.12	323	0.14	835	-0.31	-0.02

Depression

Age	MZM	N	MZF	N	Fishers'Z	Cohen's D	DZM	N	DZF	N	Fishers'Z	Cohen's D
7	0.69	4206	0.72	4673	-2.81	-0.059	0.43	4142	0.44	3778	-0.55	-0.01
10	0.71	3354	0.71	3819	0	0	0.42	3122	0.47	2875	-2.41	-0.06
12	0.71	2870	0.69	3245	1.53	0.04	0.4	2640	0.51	2497	-4.98	-0.14
14	0.47	1284	0.66	2003	-7.9	-0.28	0.28	1115	0.3	1471	-0.55	-0.02
16	0.51	970	0.52	1511	-0.33	-0.01	0.2	706	0.28	1021	-1.73	-0.08
18-27	0.57	791	0.55	1770	0.68	0.03	0.24	547	0.35	1127	-2.31	-0.11
27plus	0.52	777	0.48	2097	1.27	0.05	0.11	391	0.14	985	-0.51	-0.03

SIV. Model fitting results explaining the relationship between well-being and depression. Bold represents the best fitting model.

Age7					
Model	-2LL	df	χ^2	df	*p*
ACE	**57449.441**	**28598**		**14**	
AE	57636.112	28601	186.67057	11	<0.001
Age10					
Model	-2LL	df	χ^2	df	*p*
ACE	**50859.769**	**24761**		**14**	
AE	51031.227	24764	171.45803	11	<0.001
Age12					
Model	-2LL	df	χ^2	df	*p*
ACE	**51464.688**	**23025**		**14**	
AE	51730.210	23028	265.52208	11	<0.001
Age14					
Model	-2LL	df	χ^2	df	*p*
ADE	43854.645	17952		14	
AE	**43856.907**	**17955**	**2.26**	**11**	**0.52**
Age16					
Model	-2LL	df	χ^2	df	*p*
ADE	31113.213	12706		14	
AE	**31114.154**	**12709**	**0.94**	**11**	**0.82**
Age18-27					
Model	-2LL	df	χ^2	df	*p*
ADE	33260.502	13618		14	
AE	**33265.100**	**13621**	**4.6**	**11**	**0.20**
Age27-99					
Model	-2LL	df	χ^2	df	*p*
ACE	28508.713	11739		14	
AE	**28517.726**	**11742**	**9.01**	**11**	**0.03**

03

Genetics variants associated with subjective well-being, depressive symptoms and neuroticism identified through genome-wide analyses

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Abstract

We conducted genome-wide association studies of three phenotypes: subjective well-being (*N* = 298,420), depressive symptoms (*N* = 161,460), and neuroticism (*N* = 170,910). We identified three variants associated with subjective well-being, two with depressive symptoms, and eleven with neuroticism, including two inversion polymorphisms. The two depressive symptoms loci replicate in an independent depression sample. Joint analyses that exploit the high genetic correlations between the phenotypes ($|\hat{\rho}| \approx 0.8$) strengthen the overall credibility of the findings, and allow us to identify additional variants. Across our phenotypes, loci regulating expression in central nervous system and adrenal/pancreas tissues are strongly enriched for association.

Introduction

Subjective well-being—as measured by survey questions on life satisfaction, positive affect, or happiness—is a major topic of research within psychology, economics, and epidemiology. Twin studies have found that subjective well-being is genetically correlated with depression (characterized by negative affect, anxiety, low energy, bodily aches and pains, pessimism, and other symptoms) and neuroticism (a personality trait characterized by easily experiencing negative emotions such as anxiety and fear)[1–3]. Depression and neuroticism have received much more attention than subjective well-being in genetic-association studies, but the discovery of associated genetic variants with either of them has proven elusive[4,5].

In this paper, we report a series of separate and joint analyses of subjective well-being, depressive symptoms, and neuroticism. Our primary analysis is a genome-wide association study (GWAS) of subjective well-being based on data from 59 cohorts (N = 298,420). This GWAS identifies three loci associated with subjective well-being at genome-wide significance ($p < 5\times10^{-8}$). We supplement this primary analysis with auxiliary GWAS meta-analyses of depressive symptoms (N = 180,866) and neuroticism (N = 170,910), performed by combining publicly available summary statistics from published studies with new genome-wide analyses of additional data. In these auxiliary analyses we identify two loci associated with depressive symptoms and eleven with neuroticism, including two inversion polymorphisms. In depression data from an independent sample (N = 368,890), both depressive symptoms associations replicate (p = 0.004 and p = 0.015).

In our two joint analyses, we exploit the high genetic correlation between subjective well-being, depressive symptoms, and neuroticism (i) to evaluate the credibility of the 16 genome-wide significant associations across the three phenotypes, and (ii) to identify novel associations (beyond those identified by the GWAS). For (i), we investigate whether our three subjective well-being-associated SNPs "quasi-replicate" by testing them for association with depressive symptoms and neuroticism. We similarly examine the quasi-replication record of the depressive symptoms and neuroticism loci by testing them for association with subjective well-being. We find that the quasi-replication record closely matches what would be expected given our statistical power if none of the genome-wide significant associations were chance findings. These results strengthen the credibility of (most of) the original associations. For (ii), we use a "proxy phenotype" approach[6]: we treat the set of loci associated with subjective well-being at $p < 10^{-4}$ as candidates, and we test them for association with depressive symptoms and neuroticism. At the Bonferroni-adjusted 0.05 significance threshold, we

identify two loci associated with both depressive symptoms and neuroticism and another two associated with neuroticism.

In designing our study, we faced a tradeoff between analyzing a smaller sample with a homogeneous phenotype measure versus attaining a larger sample by jointly analyzing data from multiple cohorts with heterogeneous measures. For example, in our analysis of subjective well-being, we included measures of both life satisfaction and positive affect, even though these constructs are conceptually distinct[7]. In **Supplementary Note** and **Supplementary Figure 1**, we present a theoretical framework for evaluating the costs and benefits of pooling heterogeneous measures. In our context, given the high genetic correlation across measures, the framework predicts that pooling increases statistical power to detect variants. This prediction is supported by our results.

Results

GWAS of subjective well-being

Following a pre-specified analysis plan, we conducted a sample-size-weighted meta-analysis (N = 298,420) of cohort-level GWAS summary statistics. The phenotype measure was life satisfaction, positive affect, or (in some cohorts) a measure combining life satisfaction and positive affect. We confirmed previous findings[9] of high pairwise genetic correlation between life satisfaction and positive affect using bivariate LD Score regression[10] ($\hat{\rho}$ = 0.981 (SE = 0.065); **Supplementary Table 1**). Details on the 59 participating cohorts, their phenotype measures, genotyping, quality-control filters, and association models are provided in Online Methods, **Supplementary Note**, and **Supplementary Tables 2-6**.

As expected under polygenicity[11], we observe inflation of the median test statistic (λ_{GC} = 1.206). The estimated intercept from LD Score regression (1.012) suggests that nearly all of the inflation is due to polygenic signal rather than bias. We also performed family-based analyses that similarly suggest minimal confounding due to population stratification (Online Methods). Using a clumping procedure (**Supplementary Note**), we identified three approximately independent SNPs reaching genome-wide significance ("lead SNPs"). These three lead SNPs are indicated in the Manhattan plot (**Figure 1a**) and listed in **Table 1**. The SNPs have estimated effects in the range 0.015 to 0.018 standard deviations (SDs) per allele (each $R^2 \approx 0.01\%$).

We also conducted separate meta-analyses of the components of our subjective well-being measure, life satisfaction (N = 166,205) and positive affect (N = 180,281) (Online Methods).

Consistent with our theoretical conclusion that pooling heterogeneous measures increased power in our context, the life satisfaction and positive affect analyses yielded fewer signals across a range of *p*-value thresholds than our meta-analysis of subjective well-being (**Supplementary Table 7**).

GWAS of depressive symptoms and neuroticism

We conducted auxiliary GWAS of depressive symptoms and neuroticism (see Online Methods, **Supplementary Note**, and **Supplementary Tables 8-12** for details on cohorts, phenotype measures, genotyping, association models, and quality-control filters). For depressive symptoms (N = 180,866), we meta-analyzed publicly available results from a study performed by the Psychiatric Genomics Consortium (PGC)[12] together with new results from analyses of the initial release of the UK Biobank data (UKB)[13] and the Resource for Genetic Epidemiology Research on Aging (GERA) Cohort[14]. In UKB (N = 105,739), we constructed a continuous phenotype measure by combining responses to two questions, which ask about the frequency in the past two weeks with which the respondent experienced feelings of unenthusiasm/disinterest and depression/hopelessness. The other cohorts had ascertained case-control data on major depressive disorder (GERA: N_{cases} = 7,231, $N_{controls}$ = 49,316; PGC: N_{cases} = 9,240, $N_{controls}$ = 9,519).

For neuroticism (N = 170,910), we pooled summary statistics from a published study by the Genetics of Personality Consortium (GPC)[4] with results from a new analysis of UKB data. The GPC (N = 63,661) harmonized different neuroticism batteries. In UKB (N = 107,245), our measure was the respondent's score on a 12-item version of the Eysenck Personality Inventory Neuroticism scale[15].

In both the depressive symptoms and neuroticism GWAS, the heterogeneous phenotypic measures are highly genetically correlated (**Supplementary Table 1**). As in our subjective well-being analyses, there is substantial inflation of the median test statistics (λ_{GC} = 1.168 for depressive symptoms, λ_{GC} = 1.317 for neuroticism), but the estimated LD Score intercepts (1.008 and 0.998, respectively) suggest that bias accounts for little or none of the inflation.

For depressive symptoms, we identified two lead SNPs, indicated in the Manhattan plot (**Fig. 1b**). For neuroticism, our meta-analysis yielded 16 loci that are independent according to our locus definition (**Fig. 1c**). However, 6 of these reside within a well-known inversion polymorphism[16] on chromosome 8. We established that all genome-wide significant signals in the inversion region are attributable to the inversion, and we confirmed that the inversion is

associated with neuroticism in both of our neuroticism datasets, the GPC and the UKB (Online Methods and **Supplementary Note**). In our list of lead SNPs (**Table 1**), we only retain the most strongly associated SNP from these 6 loci to tag the chromosome 8 inversion.

Fig. 1. Manhattan plots of GWAS results. (a) Subjective well-being (N = 298,420), **(b)** Depressive symptoms (N = 180,866), **(c)** Neuroticism (N = 170,911). The x-axis is chromosomal position, and the y-axis is the significance on a $-\log_{10}$ scale. The upper dashed line marks the threshold for genome-wide significance ($p = 5\times10^{-8}$); the lower line marks the threshold for nominal significance ($p = 10^{-5}$). Each approximately independent genome-wide significant association ("lead SNP") is marked by ×. Each lead SNP is the lowest p-value SNP within the locus, as defined by our clumping algorithm (**Supplementary Note**).

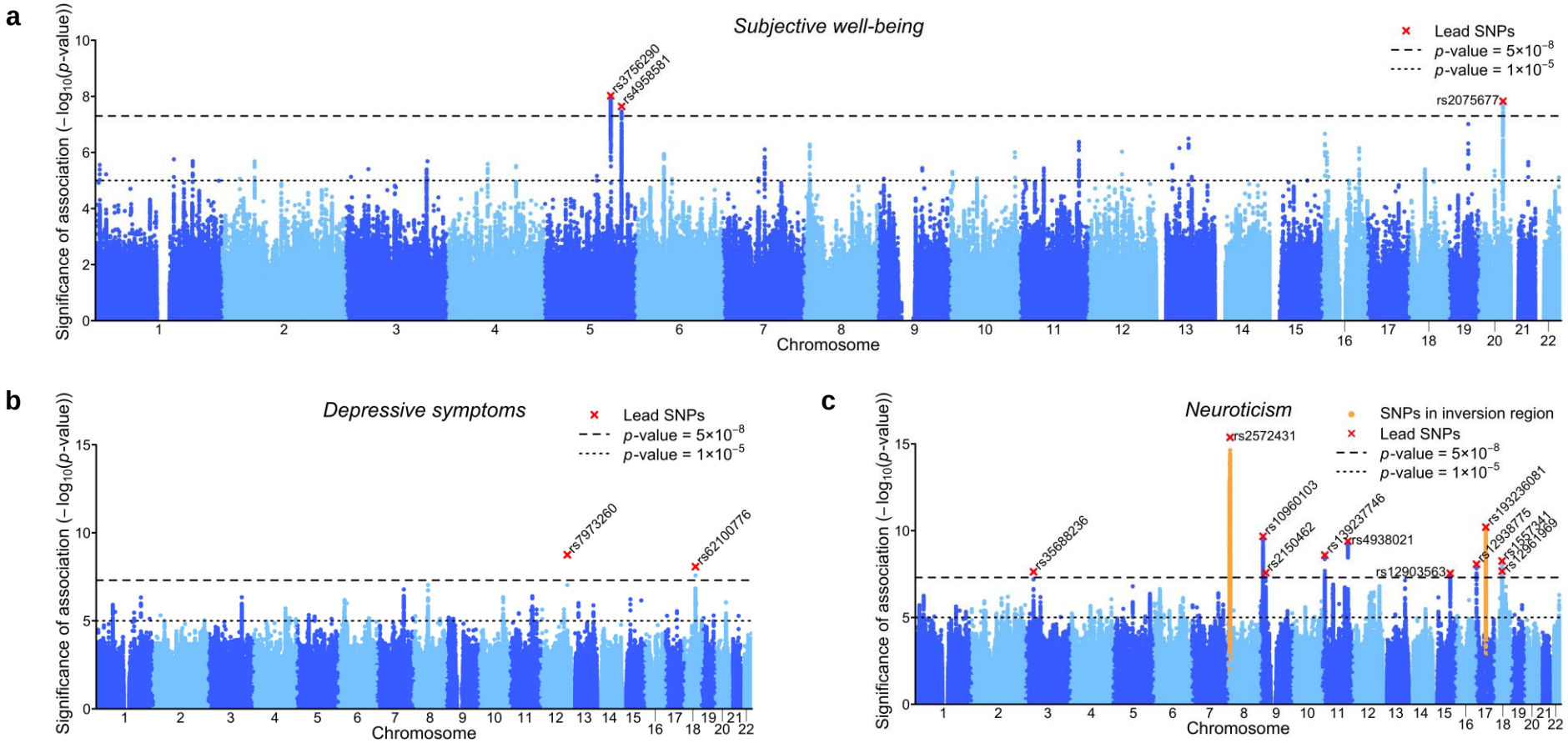


Table 1. Summary of polymorphisms identified across analyses. EA: effect allele. EAF: effect allele frequency. All effect sizes are reported in units of SDs per allele. "Quasi-Repl.": phenotypes for which SNP was found to be nominally associated in quasi-replication analyses conducted in independent samples. *significant at the 5%-level, **significant at the 1%-level, ***significant at the 0.1%-level. #inversion-tagging polymorphism on chromosome 8. ##inversion-tagging polymorphism on chromosome 17. †proxy for rs6904596 (R^2 = 0.98).

Panel A. Genome-Wide Significant Associations

Subjective Well-Being (SWB, N = 298,420)

SNPID	CHR	BP	EA	EAF	Beta	SE	R^2	p-value	N	Quasi-Repl
rs3756290	5	130,951,750	A	0.24	-0.0177	0.0031	0.011%	9.6×10^{-9}	286,851	
rs2075677	20	47,701,024	A	0.76	0.0175	0.0031	0.011%	1.5×10^{-8}	288,454	DS**
rs4958581	5	152,187,729	T	0.66	0.0153	0.0027	0.011%	2.3×10^{-8}	294,043	DS***

Neuroticism (N = 170,908)

SNPID	CHR	BP	EA	EAF	Beta	SE	R^2	p-value	N	Quasi-Repl
rs2572431#	8	11,105,077	T	0.41	0.0283	0.0035	0.039%	4.2×10^{-16}	170,908	SWB*
rs193236081##	17	44,142,332	T	0.77	-0.0284	0.0045	0.028%	6.3×10^{-11}	151,297	
rs10960103	9	11,699,270	C	0.77	0.0264	0.0038	0.024%	2.1×10^{-10}	165,380	$D^{*}_{23andMe}$
rs4938021	11	113,364,803	T	0.34	0.0233	0.0037	0.024%	4.0×10^{-10}	159,900	$D^{***}_{23andMe}$, SWB*
rs139237746	11	10,253,183	T	0.51	-0.0204	0.0034	0.021%	2.6×10^{-9}	170,908	
rs1557341	18	35,127,427	A	0.34	0.0213	0.0036	0.021%	5.6×10^{-9}	165,579	$D^{**}_{23andMe}$
rs12938775	17	2,574,821	A	0.47	-0.0202	0.0035	0.020%	8.5×10^{-9}	163,283	SWB*
rs12961969	18	35,364,098	A	0.2	0.0250	0.0045	0.020%	2.2×10^{-8}	156,758	
rs35688236	3	34,582,993	A	0.69	0.0213	0.0037	0.019%	2.4×10^{-8}	161,636	
rs2150462	9	23,316,330	C	0.26	-0.0217	0.0038	0.018%	2.7×10^{-8}	170,907	
rs12903563	15	78,033,735	T	0.50	0.0198	0.0036	0.020%	2.9×10^{-8}	157,562	$D^{*}_{23andMe}$,SWB*

Depressive Symptoms (DS, N = 180,866)

SNPID	CHR	BP	EA	EAF	Beta	SE	R^2	p-value	N	Quasi-Repl/Repl
rs7973260	12	118,375,486	A	0.19	0.0306	0.0051	0.029%	1.8×10^{-9}	124,498	$D^{*}_{23andMe}$
rs62100776	18	50,754,633	A	0.56	-0.0252	0.0044	0.031%	8.5×10^{-9}	105,739	$D^{**}_{23andMe}$,SWB*

Panel B. SNPs Identified via Proxy-Phenotype Analyses of SWB Loci with p-value<10^{-4}

Depressive Symptoms in Non-Overlapping Cohorts

SNPID	CHR	BP	EA	EAF	$Beta_{DS}$	SE_{DS}	R^2	p_{DS}	Bonferroni	N_{DS}
rs4346787†	6	27,491,299	A	0.113	-0.023	0.0059	0.011%	9.8×10^{-5}	0.0160	142,265
rs4481363	5	164,483,794	A	0.524	0.014	0.0038	0.009%	3.1×10^{-4}	0.0499	142,265

Neuroticism in Non-Overlapping Cohorts

SNPID	CHR	BP	EA	EAF	$Beta_{neuro}$	SE_{neuro}	R^2	p_{neuro}	Bonferroni	N_{neuro}
rs10838738	11	47,663,049	A	0.49	0.0178	0.0039	0.016%	5.0×10^{-6}	0.0009	131,864
rs10774909	12	117,674,129	C	0.52	-0.0150	0.0039	0.011%	1.2×10^{-4}	0.0203	131,235
rs6904596	6	27,491,299	A	0.09	-0.0264	0.0072	0.012%	2.5×10^{-4}	0.0423	116,335
rs4481363	5	164,474,719	A	0.49	0.0151	0.0040	0.011%	1.9×10^{-4}	0.0316	122,592

Another lead SNP associated with neuroticism, rs193236081, is located within a well-known inversion polymorphism on chromosome 17. We established that this association is attributable to the inversion polymorphism (Online Methods and **Supplementary Note**). Because this inversion yields only one significant locus and is genetically complex[17], we hereafter simply use its lead SNP as its proxy. Our neuroticism GWAS therefore identified 11 lead SNPs, two of which tag inversion polymorphisms. A concurrent neuroticism GWAS using a subset of our sample reports similar findings[18].

As shown in **Table 1**, the estimated effects of all lead SNPs associated with depressive symptoms and neuroticism are in the range 0.020 to 0.031 SDs per allele ($R^2 \approx 0.02\%$ to 0.04%). In the UKB cohort we estimated the effect of an additional allele of the chromosome 8 inversion polymorphism itself on neuroticism to be 0.035 SDs (**Supplementary Table 13**). The inversion explains 0.06% of the variance in neuroticism (roughly the same as the total variance explained jointly by the 6 SNPs in the inversion region).

Genetic overlap across subjective well-being, depressive symptoms, and neuroticism

Figure 2a shows that the three pairwise genetic correlations between our phenotypes, estimated using bivariate LD Score regression[10], are substantial: -0.81 (SE = 0.046) between subjective well-being and depressive symptoms, -0.75 (SE = 0.034) between subjective well-being and neuroticism, and 0.75 (SE = 0.027) between depressive symptoms and neuroticism. Using height as a negative control, we also examined pairwise genetic correlations between each of our phenotypes and height and, as expected, found all three to be modest, e.g., 0.07 with subjective well-being (**Supplementary Table 1**). The high genetic correlations between subjective well-being, depressive symptoms, and neuroticism may suggest that the genetic influences on these phenotypes are predominantly related to processes common across the phenotypes, such as mood, rather than being phenotype-specific.

Quasi-replication and Bayesian credibility analyses

We assessed the credibility of our findings using a standard Bayesian framework[19,20] in which a positive fraction of SNPs have null effects and a positive fraction have non-null effects (Online Methods). For each phenotype, the non-null effect sizes are assumed to be drawn from a normal distribution whose variance is estimated from the GWAS summary statistics. As a first analysis, for each lead SNP's association with its phenotype, we calculated the posterior probability of null association after having observed the GWAS results. We found that, for any assumption about the fraction of non-null SNPs in the range 1% to 99%, the

probability of true association always exceeds 95% for all 16 loci (and always exceeds 98% for 14 of them).

To further probe the credibility of the findings, we performed "quasi-replication" exercises (Online Methods) in which we tested the subjective well-being lead-SNPs for association with depressive symptoms and neuroticism. We similarly tested the depressive symptoms lead-SNPs and the neuroticism lead-SNPs for association with subjective well-being. Below, we refer to the phenotype for which the lead SNP was identified as the first-stage phenotype and the phenotype used for the quasi-replication as the second-stage phenotype. To avoid sample overlap, for each quasi-replication analysis we omitted any cohorts that contributed to the GWAS of the first-stage phenotype.

Results of the quasi-replication of the three subjective well-being lead-SNPs are shown in **Figure 3a**. For ease of interpretation, the reference allele for each association in the figure is chosen such that the predicted sign of the second-stage estimate is positive. We find that two out of the three subjective well-being lead-SNPs are significantly associated with depressive symptoms (p = 0.004 and p = 0.001) in the predicted direction. For neuroticism, where the second-stage sample size (N = 68,201) is about half as large, the subjective well-being-increasing allele has the predicted sign for all three SNPs, but none reach significance.

Figures 3b and 3c show the results for the depressive symptoms and neuroticism lead-SNPs, respectively. In each panel, the blue crosses depict results from the quasi-replications where subjective well-being is the second-stage phenotype. We find that the two depressive symptoms lead-SNPs have the predicted sign for subjective well-being, and one is nominally significant (p = 0.04). Finally, of the eleven neuroticism lead-SNPs, nine have the predicted sign for subjective well-being. Four of the eleven are nominally significantly associated with subjective well-being, all with the predicted sign. One of the four is the SNP tagging the inversion on chromosome 8[16]. That SNP's association with neuroticism (and likely with subjective well-being) is driven by its correlation with the inversion (**Supplementary Fig. 2**).

To evaluate what these quasi-replication results imply about the credibility of the 16 GWAS associations, we compared the observed quasi-replication record to the quasi-replication record expected given our statistical power. We calculated statistical power using our Bayesian framework, under the hypothesis that each lead SNP has a non-null effect on both the first- and second-stage phenotypes. Our calculations take into account both the imperfect genetic correlation between the first- and second-stage phenotypes and inflation of the first-stage estimates due to the well-known problem of winner's curse (Online Methods). Of the 19

quasi-replication tests, our calculations imply that 16.7 would be expected to yield the anticipated sign and 6.9 would be significant at the 5% level. The observed numbers are 16 and 7. Our quasi-replication results are thus consistent with the hypothesis that none of the 16 genome-wide significant associations are chance findings, and in fact strengthen the credibility of our GWAS results (**Supplementary Table 14**).

Fig. 2. Genetic correlations with bars representing 95% confidence intervals. The correlations are estimated using bivariate LD Score (LDSC) regression. (**a**) Genetic correlations between subjective well-being, depressive symptoms, and neuroticism ("our three phenotypes"), as well as between our three phenotypes and height. (**b**) Genetic correlations between our three phenotypes and selected neuropsychiatric phenotypes. (**c**) Genetic correlations between our three phenotypes and selected physical health phenotypes. In (**b**) and (**c**), we report the negative of the estimated correlation with depressive symptoms and neuroticism (but not subjective well-being).

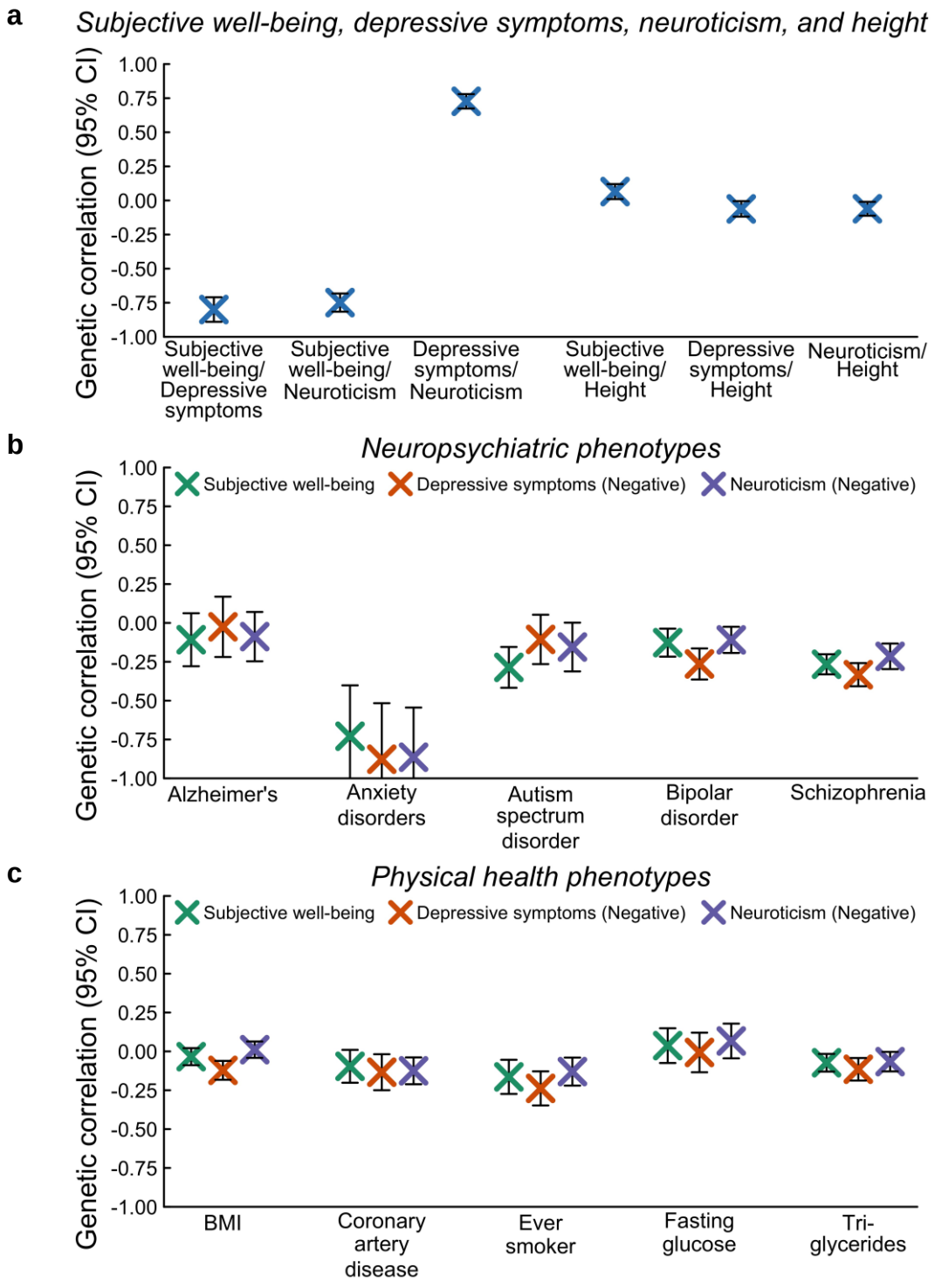
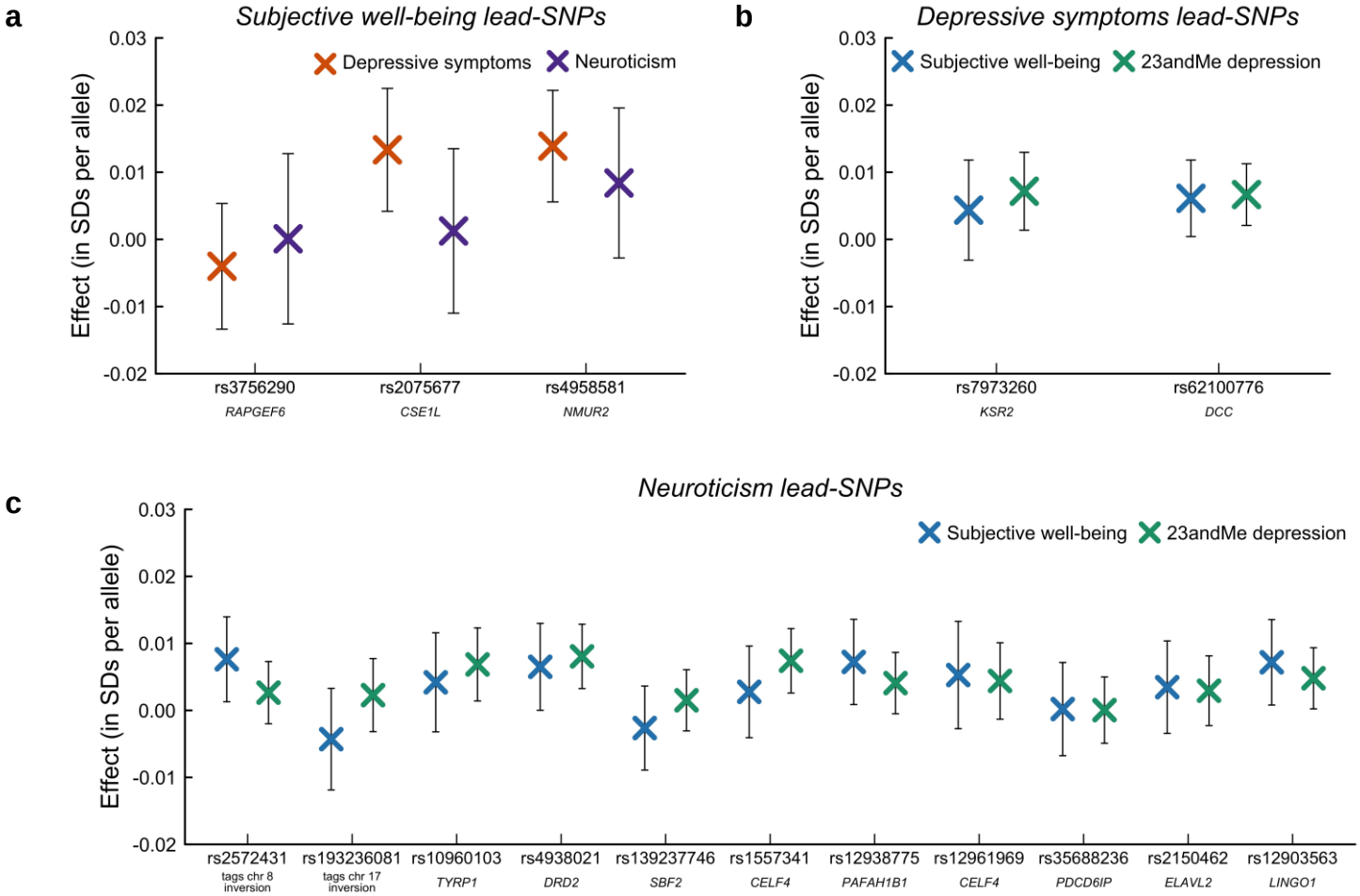


Fig. 3. Quasi-replication and lookup of lead SNPs. In quasi-replication analyses, we examined whether (**a**) lead SNPs identified in the subjective well-being meta-analyses are associated with depressive symptoms or neuroticism, (**b**) lead SNPs identified in the analyses of depressive symptoms are associated with subjective well-being, and (**c**) lead SNPs identified in the analyses of neuroticism are associated with subjective well-being. The quasi-replication sample is always restricted to non-overlapping cohorts. In a separate lookup exercise, we examined whether lead SNPs for depressive symptoms and neuroticism are associated with depression in an independent sample of 23andMe customers (N = 368,890). The results from this lookup are depicted as green crosses in (**b**) and (**c**). Bars represent 95% CIs (not adjusted for multiple testing). For interpretational ease, we choose the reference allele so that positive coefficients imply that the estimated effect is in the predicted direction. Listed below each lead SNP is the nearest gene.



Lookup of depressive symptoms and neuroticism lead-SNPs

Investigators of an ongoing large-scale GWAS of major depressive disorder (N = 368,890) in the 23andMe cohort shared association results for the loci identified in our depressive symptoms and neuroticism analyses (Online Methods and **Supplementary Table 15**)[21]. Because the depression sample overlaps with our subjective well-being sample, we did not request a lookup of the subjective well-being-associated SNPs.

In **Figures 3b** and **3c**, the results are depicted as green crosses. For interpretational ease, we chose the reference allele so that positive coefficients imply that the estimated effect is in the predicted direction. All 13 associations have the predicted sign. Of the 11 neuroticism polymorphisms, four are significantly associated with depression at the 5% level. Both of the depressive symptoms lead-SNPs replicate (p = 0.004 and p = 0.015), with effect sizes (0.007 and -0.007 SDs per allele), close to those predicted by our Bayesian framework (0.008 and -0.006) (**Supplementary Table 14** and **Supplementary Table 15**).

Panel A of **Table 1** summarizes the results for the 16 lead SNPs identified across our separate GWA analyses of the three phenotypes. The right-most column summarizes the statistical significance of the quasi-replication and depression lookup analyses of each SNP.

Proxy-phenotype analyses

To identify additional SNPs associated with depressive symptoms, we conducted a two-stage "proxy phenotype" analysis (Online Methods). In the first stage, we ran a new GWAS of subjective well-being to identify a set of candidate SNPs. Specifically, from each locus exhibiting suggestive evidence of association ($p < 10^{-4}$) with subjective well-being, we retained the SNP with the lowest p-value as a candidate. In the second stage, we tested these candidates for association with depressive symptoms at the 5% significance threshold, Bonferroni-adjusted for the number of candidates. We used an analogous two-stage procedure to identify additional SNPs associated with neuroticism. The first-stage subjective well-being sample differs across the two proxy-phenotype analyses (and from the primary subjective well-being GWAS sample) because we assigned cohorts across the first and second stages so as to maximize statistical power for the overall procedure.

For depressive symptoms, there are 163 candidate SNPs. 115 of them (71%) have the predicted direction of effect on depressive symptoms, 20 are significantly associated at the 5% significance level (19 in the predicted direction), and two remain significant after Bonferroni adjustment. For neuroticism, there are 170 candidate SNPs. 129 of them (76%) have the

predicted direction of effect, all 28 SNPs significant at the 5% level have the predicted sign, and four of these remain significant after Bonferroni adjustment (**Supplementary Fig. 3** and **Supplementary Tables 16** and **17**). Two of the four are the SNPs identified in the proxy-phenotype analysis for depressive symptoms.

Table 1 lists the four SNPs in total identified by the proxy-phenotype analyses.

Biological analyses

To shed some light on possible biological mechanisms underlying our findings, we conducted several analyses.

We began by using bivariate LD Score regression[10] to quantify the amount of genetic overlap between each of our three phenotypes and ten neuropsychiatric and physical health phenotypes. **Figures 2b** and **c** display the estimates for subjective well-being and the *negative* of the estimates for depressive symptoms and neuroticism (since subjective well-being is negatively genetically correlated with depressive symptoms and neuroticism). Subjective well-being, depressive symptoms, and neuroticism have strikingly similar patterns of pairwise genetic correlation with the other phenotypes.

Figure 2b shows the results for the five neuropsychiatric phenotypes we examined: Alzheimer's disease, anxiety disorders, autism spectrum disorder, bipolar disorder, and schizophrenia. For four of these phenotypes, genetic correlations with depression (but not neuroticism or subjective well-being) were reported in Bulik-Sullivan et al.[10]. For schizophrenia and bipolar disorder, our estimated correlations with depressive symptoms, 0.33 and 0.26, are substantially lower than Bulik-Sullivan et al.'s point estimates but contained within their 95% confidence intervals. By far the largest genetic correlations we estimate are with anxiety disorders: −0.73 with subjective well-being, 0.88 with depressive symptoms, and 0.86 with neuroticism. Genetic correlations estimated from GWAS data have not been previously reported for anxiety disorders.

Figure 2c shows the results for five physical health phenotypes that are known or believed to be risk factors for various adverse health outcomes: body mass index (BMI), ever-smoker status, coronary artery disease, fasting glucose, and triglycerides. The estimated genetic correlations are all small in magnitude, consistent with earlier work, although the greater precision of our estimates allows us to reject null effects in most cases. The signs are generally consistent with those of the phenotypic correlations reported in earlier work

between our phenotypes and outcomes such as obesity[22], smoking[23,24], and cardiovascular health[25].

Next, to investigate whether our GWAS results are enriched in particular functional categories, we applied stratified LD Score regression[26] to our meta-analysis results. In our first analysis, we report estimates for all 53 functional categories included in the "baseline model"; the results for subjective well-being, depressive symptoms, and neuroticism are broadly similar (**Supplementary Tables 18-20**) and are in line with what has been found for other phenotypes[26]. In our second analysis, the categories are groupings of SNPs likely to regulate gene expression in cells of a specific tissue. The estimates for subjective well-being, depressive symptoms, and neuroticism are shown in **Figure 4a**, alongside height, which is again included as a benchmark[27] (see also **Supplementary Table 21**).

We found significant enrichment of CENTRAL NERVOUS SYSTEM for all three phenotypes and, perhaps more surprisingly, enrichment of ADRENAL/PANCREAS for subjective well-being and depressive symptoms. The cause of the ADRENAL/PANCREAS enrichment is unclear, but we note that the adrenal glands produce several hormones, including cortisol, epinephrine, and norepinephrine, known to play important roles in the bodily regulation of mood and stress. It has been robustly found that blood serum levels of cortisol in patients afflicted by depression are elevated relative to controls[28].

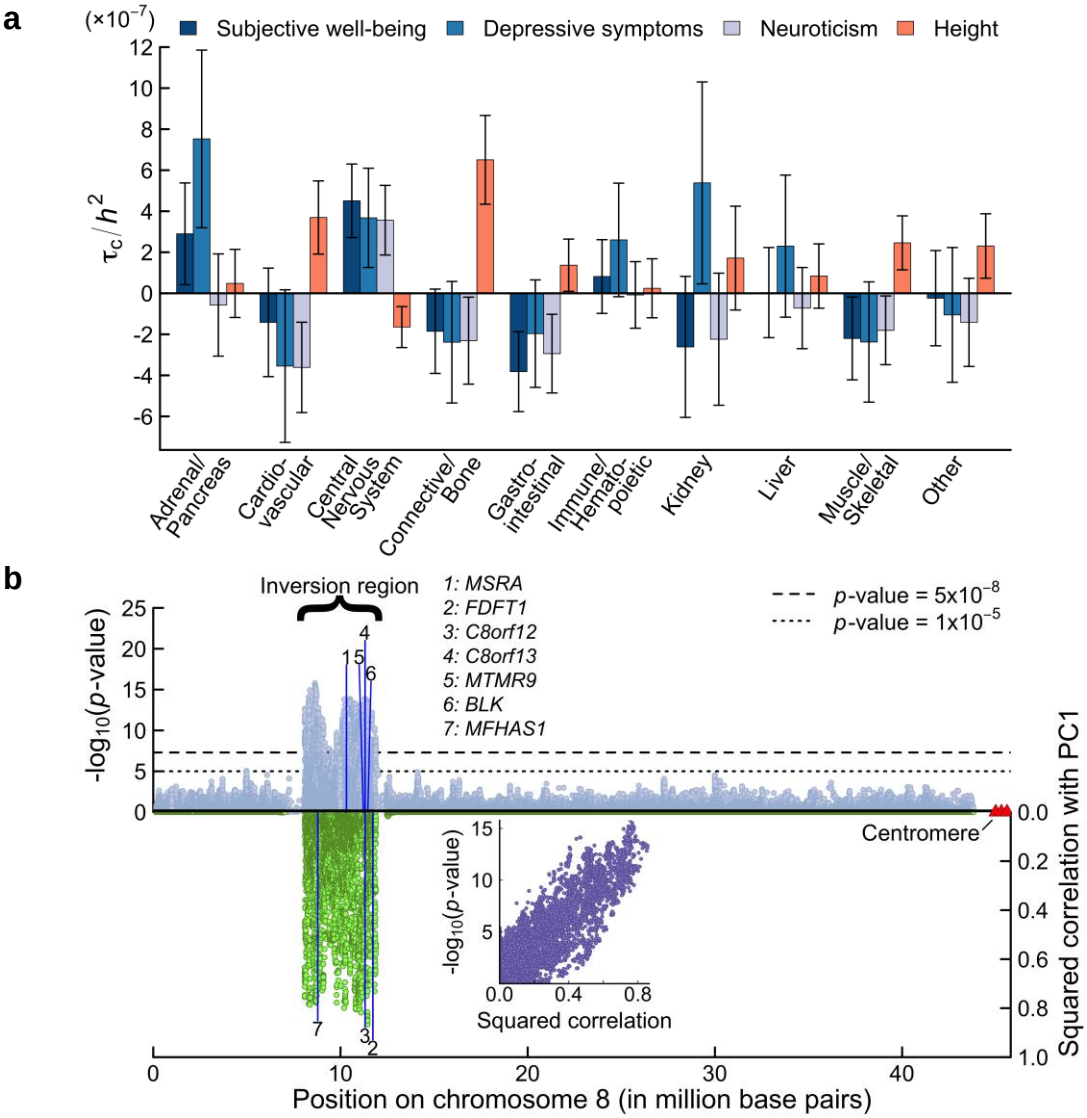
While the above analyses utilize the genome-wide data, we also conducted three analyses (Online Methods) restricted to the 16 GWAS and four proxy-phenotype SNPs in **Table 1**. In brief, we ascertained whether each SNP (or a variant in strong linkage disequilibrium (LD) with it) falls into any of the following three classes: (i) resides in a locus for which genome-wide significant associations with other phenotypes have been reported (**Supplementary Table 22**), (ii) is nonsynonymous (**Supplementary Table 23**), and (iii) is an eQTL in blood or in one of 14 other tissues (although the non-blood analyses are based on smaller samples) (**Supplementary Table 24**). Here we highlight a few particularly interesting results.

We found that five of the 20 SNPs are in loci in which genome-wide significant associations have previously been reported. Two of these five are schizophrenia loci. Interestingly, one of them harbors the gene *DRD2*, which encodes the D_2 subtype of the dopamine receptor, a target for antipsychotic drugs[29] that is also known to play a key role in neural reward pathways[30]. Motivated by these findings, as well as by the modest genetic correlations with schizophrenia reported in **Figure 2b**, we examined whether the SNPs identified in a recent study of schizophrenia[31] are enriched for association with neuroticism in our non-overlapping

UKB sample (N = 107,245). We conducted several tests and found strong evidence of such enrichment (**Supplementary Note**). For example, we found that the p-values of the schizophrenia SNPs tend to be much lower than the p-values of a randomly selected set of SNPs matched on allele frequency ($p = 6.50 \times 10^{-71}$).

Perhaps the most notable pattern that emerges from our biological analyses is that the inversions on chromosomes 8 and 17 are implicated consistently across all analyses. The inversion-tagging SNP on chromosome 8 is in LD with SNPs that have previously been found to be associated with BMI[32] and triglycerides[33] (**Supplementary Table 22**). We also conducted eQTL analyses in blood for the inversion itself and found that it is a significant *cis*-eQTL for 7 genes (**Supplementary Table 24**). As shown in **Figure 4b**, all 7 genes are positioned in close proximity to the inversion breakpoints, suggesting that the molecular mechanism underlying the inversion's effect on neuroticism could involve the relocation of regulatory sequences. Two of the genes (*MSRA*, *MTMR9*) are known to be highly expressed in tissues and cell types that belong to the nervous system, and two (*BLK*, *MFHAS1*) in the immune system. In the tissue-specific analyses, we found that the SNP tagging the inversion is a significant eQTL for two genes, *AF131215.9* (in tibial nerve and thyroid tissue analyses) and *NEIL2* (tibial nerve tissue), both of which are also located near the inversion breakpoint.

Fig. 4. Results from selected biological analyses. (**a**) Estimates of the expected increase in the phenotypic variance accounted for by a SNP due to the SNP's being in a given category (τ_c), divided by the LD Score heritability of the phenotype (h^2). Each estimate of τ_c comes from a separate stratified LD Score regression, controlling for the 52 functional annotation categories in the "baseline model." The bars represent 95% CIs (not adjusted for multiple testing). To benchmark the estimates, we compare them to those obtained from a recent study of height[27]. (**b**) Inversion polymorphism on chromosome 8 and the 7 genes for which the inversion is a significant *cis*-eQTL at FDR < 0.05. The upper half of the figure shows the Manhattan plot for neuroticism for the inversion and surrounding regions. The bottom half shows the squared correlation between the SNPs and the principal component that captures the inversion. The inlay plots the relationship, for each SNP in the inversion region, between the SNP's significance and its squared correlation with the principal component that captures the inversion.



The SNP tagging the chromosome 17 inversion is a significant *cis*-eQTL for five genes in blood and is an eQTL in all 14 other tissues (**Supplementary Table 24**). It alone accounts for 151 out of the 169 significant associations identified in the 14 tissue-specific analyses. Additionally, the SNP is in near-perfect LD (R^2 > 0.97) with 11 missense variants (**Supplementary Table 23**) in three different genes, one of which is *MAPT*. *MAPT*, which is also implicated in both the blood and the other tissue-specific analyses, encodes a protein important in the stabilization of microtubules in neurons. Associations have been previously reported between SNPs in *MAPT* (all of which are in strong LD with our inversion-tagging SNP) and neurodegenerative disorders, including Parkinson's disease[34] and progressive supranuclear palsy[35], a rare disease whose symptoms include depression and apathy.

Discussion

The discovery of genetic loci associated with subjective well-being, depression, and neuroticism has proven elusive. Our study identified several credible associations for two main reasons. First, our analyses had greater statistical power than prior studies because ours were conducted in larger samples. Our GWAS findings—three loci associated with subjective well-being, two with depressive symptoms, and eleven with neuroticism—support the view that GWAS can successfully identify genetic associations with highly polygenic phenotypes in sufficiently large samples[5,36]. A striking finding is that two of our identified associations are with inversion polymorphisms.

Second, our proxy-phenotype analyses further boosted power by exploiting the strong genetic overlap between our three phenotypes. These analyses identified two additional loci associated with neuroticism and two with both depressive symptoms and neuroticism. Through our quasi-replication tests, we also demonstrated how studying genetically overlapping phenotypes in concert can provide evidence on the credibility of GWAS findings. Our direct replication of the two genome-wide significant associations with depressive symptoms in an independent depression sample provides further confirmation of those findings (**Fig. 2b** and **Supplementary Table 15**).

We were able to assemble much larger samples than prior work in part because we combined data across heterogeneous phenotype measures. Our results reinforce the conclusions from our theoretical analysis that doing so increased our statistical power, but our strategy also has drawbacks. One is that mixing different measures may make any discovered associations more difficult to interpret. Research studying higher quality measures of the various facets of subjective well-being, depressive symptoms, and neuroticism is a critical next step. Our

results can help facilitate such work because if the variants we identify are used as candidates, studies conducted in the smaller samples in which more fine-grained phenotype measures are available can be well powered.

Another limitation of mixing different measures is that doing so may reduce the heritability of the resulting phenotype, if the measures are influenced by different genetic factors. Indeed, our estimates of SNP-based heritability[10] for our three phenotypes are quite low: 0.040 (SE = 0.002) for subjective well-being, 0.047 (SE = 0.004) for depressive symptoms, and 0.091 (SE = 0.007) for neuroticism. We correspondingly find that polygenic scores constructed from all measured SNPs explain a low fraction of variance in independent samples: ~0.9% for subjective well-being, ~0.5% for depressive symptoms, and ~0.7% for neuroticism (Online Methods). The low heritabilities imply that even when polygenic scores can be estimated using much larger samples than ours, they are unlikely to attain enough predictive power to be clinically useful.

According to our Bayesian calculations, the true explanatory power (corrected for winner's curse) of the SNP with the largest posterior R^2 is 0.003% for subjective well-being, 0.002% for depressive symptoms, and 0.011% for neuroticism (**Supplementary Table 14**). These effect sizes imply that in order to account for even a moderate share of the heritability, hundreds or (more likely) thousands of variants will be required. They also imply that our study's power to detect variants of these effect sizes was not high—for example, our statistical power to detect the lead SNP with largest posterior R^2 was only ~13%—which in turn means it is likely that there exist many variants with effect sizes comparable to our identified SNPs that evaded detection. These estimates suggest that many more loci will be found in studies with sample sizes realistically attainable in the near future. Consistent with this projection, when we meta-analyze the 54 SNPs reaching $p < 10^{-5}$ in our analyses of depressive symptoms together with the 23andMe replication sample for depression, the number of genome-wide significant associations rises from 2 to 5 (**Supplementary Table 15**).

References

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ONLINE METHODS

This article is accompanied by a **Supplementary Note** with further details.

GWAS of subjective well-being. Genome-wide association analyses were performed at the cohort level according to a pre-specified analysis plan. Genotyping was performed using a range of common, commercially available genotyping arrays. The analysis plan instructed cohorts to upload results imputed using the HapMap2 CEU (r22.b36) reference sample[37]. We meta-analyzed summary association statistics from 59 contributing cohorts with a combined sample size of 298,420 individuals. Before meta-analysis, a uniform set of quality-control (QC) procedures were applied to the cohort-level summary statistics, including but not limited to the EasyQC[38] protocol. All analyses were restricted to European-ancestry individuals.

We performed a sample-size-weighted meta-analysis of the cohort-level summary statistics. To adjust standard errors for non-independence, we inflated them using the square root of the estimated intercept from a LD Score regression[10]. We also performed secondary, separate meta-analyses of positive affect (N = 180,281) and life satisfaction (N = 166,205) and a post hoc genome-wide analysis of subjective well-being in cohorts with 1000G-imputed data (N = 229,883); see **Supplementary Figures 4-6.**

Detailed cohort descriptions, information about cohort-level genotyping and imputation procedures, cohort-level measures, and quality-control filters are shown in **Supplementary Tables 2-6**. **Supplementary Table 7** reports association results from the following four meta-analyses: the primary subjective well-being analysis, the life satisfaction analysis, the positive affect analysis, and the post hoc subjective well-being analysis. For each phenotype, we provide association results for the set of approximately independent SNPs that attained a p-value smaller than 10^{-5}. We identify these SNP using the same clumping algorithm as for the lead SNPs, but with the p-value threshold set at 10^{-5} instead of genome-wide significance.

GWAS of depressive symptoms and neuroticism. Our auxiliary genome-wide association studies of DS and neuroticism were conducted in 1000G-imputed data, combining new genome-wide association analyses with publicly available summary statistics from previously published studies. We applied a similar QC protocol to that used in our primary subjective well-being analysis. In the DS meta-analysis (N = 180,866), we weighted the UKB analysis by sample size and the two case-control studies by effective sample size. In the neuroticism

meta-analysis, we performed a sample-size-weighted fixed-effects meta-analysis of the UKB data and the publicly available summary statistics from a previous GWAS of neuroticism.

Detailed cohort descriptions, information about cohort-level genotyping and imputation procedures and quality-control filters are provided in **Supplementary Tables 8-12**. See **Supplementary Figure 7** for quantile-quantile plots of the neuroticism and DS meta-analysis results. Association results for the set of approximately independent set of SNPs that attained a *p*-value smaller than 10^{-5} are supplied in **Supplementary Table 25**.

Population stratification. To quantify the fraction of the observed inflation of the mean test statistic that is due to bias, we used LD Score regression[10]. The estimated LD Score regression intercepts were all close to 1, suggesting no appreciable inflation of the test statistics attributable to population stratification in any of our subjective well-being, depressive symptoms, or neuroticism meta-analyses (**Supplementary Fig. 8**). For all three phenotypes, our estimates suggest that less than 2% of the observed inflation of the mean test statistic was accounted for by bias.

In our primary GWAS of subjective well-being, we also used two family-based analyses to test for and quantify stratification biases. These analyses used within-family (WF) estimates, the coefficients from regressing the difference in phenotype across siblings on the difference in siblings' genotype (and controls). These WF estimates are not biased by population stratification because siblings share their ancestry entirely, and therefore differences in siblings' genotypes cannot be due to the siblings being from different population groups. We meta-analyzed association statistics from WF analyses conducted in four cohorts.

In the first analysis, we estimated the fraction of SNPs for which the signs of the WF estimates were concordant with the signs of the estimates obtained from a GWAS identical to our primary subjective well-being GWAS except with the four family cohorts excluded. For the 112,884 approximately independent SNPs considered, we found a sign concordance of 50.83%, which is significantly greater than 50% ($p = 1.04 \times 10^{-8}$). Under the null hypothesis of no population stratification, the observed sign concordance matches the expected rate after winner's curse adjustment nearly perfectly, 50.83% (**Supplementary Fig 9**).

The second analysis utilized the WF regression coefficient estimates (i.e., not only their signs) to estimate the amount of stratification bias. For each SNP j, let $\hat{\beta}_j$ denote the GWAS estimate, and let $\hat{\beta}_{WF,j}$ denote the WF estimate. Under the assumption that the causal effect of

each SNP is the same within families as in the population, we can decompose the estimates as:

$$\hat{\beta}_j = \beta_j + s_j + U_j$$
$$\hat{\beta}_{WF,j} = \beta_j + V_j,$$

where β_j is the true underlying GWAS parameter for SNP j, s_j is the bias due to stratification (defined to be orthogonal to β_j and U_j), and U_j and V_j are the sampling variances of the estimates with $\mathrm{E}(U_j) = \mathrm{E}(V_j) = 0$. Whenever $s_j \neq 0$, the GWAS estimate of $\hat{\beta}_j$ is biased away from the population parameter β_j. The proportion of variance in the GWAS coefficients accounted for by true genetic signals can be written as:

$$\frac{\mathrm{Var}(\beta_j)}{\mathrm{Var}(\beta_j) + \mathrm{Var}(s_j)}.$$

In **Supplementary Note**, we show that with estimates $\hat{\beta}_j$ and $\hat{\beta}_{WF,j}$ (and their standard errors) from independent samples, it is possible to consistently estimate the above ratio. The 95% confidence interval for the ratio implies that between 72% and 100% of the signal in the GWAS estimates is a result of true genetic effects on subjective well-being rather than stratification.

Analyses of inversion polymorphisms. Two genome-wide significant SNPs for the neuroticism analysis are located within well-known inversion polymorphisms, on chromosomes 8 and 17. Using the genotypic data available for UKB participants, we called the inversion genotypes for UKB participants using a PCA-mixture method. For both inversions, the method clearly distinguishes 3 clusters of genotypes, corresponding to inversion genotypes (**Supplementary Fig. 10**). We validated the PCA-mixture procedure using existing methods designed to call inversion genotypes[39] (**Supplementary Table 26**).

For both inversions, we established that the inversion-tagging SNPs were always located in close proximity of the inversion region (**Fig. 3b** and **Supplementary Figs. 10-11**). **Supplementary Tables 27-28** list the twenty variants that most strongly correlate with the PCs that capture the inversion polymorphisms on chromosome 8 and 17, respectively. In additional analyses, we confirmed that the inversion is associated with neuroticism and subjective well-being in independent cohorts (**Supplementary Tables 29-30** and **Supplementary Fig. 12-13**).

Proxy-phenotype analyses. In these analyses, we used a two-stage approach that has been successfully applied in other contexts[6]. In the first stage, we conducted a meta-analysis of our first-stage "proxy phenotype" and used our clumping procedure to identify the set of approximately independent SNPs at the *p*-value threshold of 10^{-4}. In the second stage, we tested SNPs identified in stage 1 (or high-LD proxies for them) for association with a second-stage phenotype in an independent (non-overlapping) sample. In our analyses, we used our primary phenotype of subjective well-being as the proxy-phenotype. We conducted one analysis with depressive symptoms as the second-stage phenotype, and one analysis with neuroticism as the second-stage phenotype. In the analyses, we omit cohorts from the first-stage or second-stage as needed to ensure that the samples in the two stages are non-overlapping. **Supplementary Table 31** lists the cohort restrictions imposed. These cohort restrictions, as well as the *p*-value threshold of 10^{-4}, were chosen before the data were analyzed on the basis of statistical power calculations.

To test for cross-phenotype enrichment, we used a non-parametric procedure that tests whether the lead SNPs are more strongly associated with the second-stage phenotype than randomly chosen sets of SNPs with a similar distribution of allele frequencies (**Supplementary Note**).

To test the individual lead SNPs for experiment-wide significance, we examined whether any of the lead SNPs (or their high-LD proxies) are significantly associated with the second-stage phenotype at the Bonferroni-adjusted significance level of $0.05/Y$.

Genetic correlations. We used bivariate LD Score regression[10] to quantify the amount of genetic heterogeneity among the phenotypic measures pooled in each of our three separate meta-analyses. For subjective well-being, we estimated a pairwise correlation of 0.981 (SE = 0.065) between life satisfaction and positive affect, 0.897 (SE = 0.017) between "wellbeing" (our measure that combines life satisfaction and positive affect) and life satisfaction, and 1.031 (SE = 0.019) between positive affect and wellbeing. For depressive symptoms, we estimated a genetic correlation of 0.588 (SE = 0.242) between GERA and PGC, 0.972 (SE = 0.216) between GERA and UKB, and 0.797 (SE = 0.108) between UKB and PGC. Finally, we estimated a genetic correlation of 1.11 (SE = 0.14) between the measures of neuroticism in the UKB analyses and the summary statistics from a previously published meta-analysis[4].

Bayesian credibility analyses. To evaluate the credibility of our findings, we use a standard Bayesian framework[19] in which our prior distribution for any SNP's effect is:

$$\beta \sim \begin{cases} N(0, \tau_j^2) & \text{with probability } \pi \\ 0 & \text{otherwise.} \end{cases}.$$

Here, π is the fraction of non-null SNPs, and τ_j^2 is the variance of the non-null SNPs for trait $j \in \{\text{subjective well-being, depressive symptoms, neuroticism}\}$. In this framework, credibility is defined as the probability that a given SNP is non-null.

We begin with univariate analyses of the GWAS results that do not incorporate the additional information from the quasi-replication analyses of the 16 lead SNPs reported in **Table 1**. We use the three subjective well-being-associated SNPs to illustrate our approach, but we use analogous procedures when analyzing depressive symptoms and neuroticism. We calculate credibility for each value $\pi \in \{0.01, 0.02, \ldots, 0.99\}$. For each assumed value of π, we estimate τ_{SWB}^2 by maximum likelihood (**Supplementary Note**). For each SNP, we use Bayes' rule to obtain a posterior estimate of credibility for each of the assumed values of π. **Supplementary Figure 14** shows that for all considered values of π and all three SNPs, the posterior probability that the SNP is null is below 1%. Similar analyses of the depressive symptoms and neuroticism SNPs show that the posterior probability never exceeds 5%.

In our joint analyses, we consider two phenotypes with genetic correlation r_g. We make the simplifying assumption that the set of null SNPs is the same for both phenotypes. The joint distribution of a SNP's effect on the two phenotypes is then given by

$$\begin{bmatrix} \beta_1 \\ \beta_2 \end{bmatrix} \sim \begin{cases} N\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \tau_1^2 & \tau_1\tau_2 r_g \\ \tau_1\tau_2 r_g & \tau_2^2 \end{bmatrix}\right) & \text{with probability } \pi \\ \begin{bmatrix} 0 \\ 0 \end{bmatrix} & \text{otherwise.} \end{cases}$$

With coefficient estimates, $\hat{\beta}_1$ and $\hat{\beta}_2$, obtained from non-overlapping samples, the variance-covariance matrix of the estimation error will be diagonal. We denote the diagonal entries of this matrix, which represent the variances of the estimation error in the two samples, by σ_1^2 and σ_2^2. This gives us the joint prior distribution

$$\begin{bmatrix} \hat{\beta}_1 \\ \hat{\beta}_2 \end{bmatrix} \sim \begin{cases} N\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \tau_1^2 & \tau_1\tau_2 r_g \\ \tau_1\tau_2 r_g & \tau_2^2 \end{bmatrix} + \begin{bmatrix} \sigma_1^2 & 0 \\ 0 & \sigma_2^2 \end{bmatrix}\right) & \text{with probability } \pi \\ N\left(\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} \sigma_1^2 & 0 \\ 0 & \sigma_2^2 \end{bmatrix}\right). & \text{otherwise.} \end{cases}$$

To select parameter values for the prior, we use the estimates of r_g reported in **Supplementary Table 1**, and we estimate the parameters π, τ_1^2, and τ_2^2 from GWAS

summary statistics using a maximum likelihood procedure. For this procedure, we make the standard assumption[10,40] that the variance of a SNP's effect size is inversely proportional to the variance of its genotype, $2 \times \text{MAF} \times (1 - \text{MAF})$.

The credibility estimates follow from applying Bayes' Rule to calculate either the probability that the SNP is non-null (an event denoted C) given only the first-stage estimate, $P(C \mid \hat{\beta}_1)$, or the probability that the SNP is non-null conditional on the results of both the first-stage GWAS and the quasi-replication analysis, $P(C \mid \hat{\beta}_1, \hat{\beta}_2)$. Credibility estimates for our lead SNPs are in **Supplementary Table 14**.

To calculate the expected record of a replication or quasi-replication study, we assume that the SNP is non-null for both phenotypes. (This is analogous to a standard power calculation for a single phenotype, in which the SNP is assumed to be non-null.) Under this assumption, $\hat{\beta}_1$ and $\hat{\beta}_2$ are jointly normally distributed, implying that the conditional distribution of $\hat{\beta}_2$ given $\hat{\beta}_1$ is

$$(\hat{\beta}_2 \mid \hat{\beta}_1, C) \sim N\left[\frac{\tau_1 \tau_2 r_g}{\tau_1^2 + \sigma_1^2}\hat{\beta}_1, \frac{(\tau_1^2 + \sigma_1^2)(\tau_2^2 + \sigma_2^2) - \tau_1^2 \tau_2^2 r_g^2}{\tau_1^2 + \sigma_1^2}\right].$$

Using this equation, we can calculate the probability that the GWAS estimates will have concordant signs across the two phenotypes, or that the GWAS estimate of the second-stage phenotype will reach some level of significance. These probabilities can be summed over the set of lead SNPs to generate the expected number of SNPs meeting the criterion.

To obtain effect-size estimates for a SNP that are adjusted for the winner's curse (**Supplementary Table 32**), we use the mean of the posterior distribution of the SNP's effect, conditional on the quasi-replication result and the SNP being non-null. We derive the posterior distribution and expected R^2 in the **Supplementary Note**.

Lookup of depressive symptoms and neuroticism-associated SNPs in an independent depression study. We partnered with the investigators of an ongoing large-scale GWAS of major depressive symptoms (N = 368,890) to follow up on the associations identified in the depressive symptoms and neuroticism analyses. The participants of the study were all European-ancestry customers of 23andMe, a personal genomics company, who responded to online survey questions about mental health. We did not request results for the SNPs identified in the subjective well-being or proxy-phenotype analyses, since these were both conducted in samples that overlap with 23andMe's depression sample. For details on

association models, quality-control filters, and the ascertainment of depression status, we refer to the companion study[21]. The *p*-values we report are based on standard errors that have been inflated by the square by the intercept from an LD score regression[10].

Polygenic prediction. To evaluate the predictive power of a polygenic score derived from the subjective well-being meta-analysis results, we used two independent hold-out cohorts: the Health and Retirement Study (HRS[41]) and the Netherlands Twin Register (NTR[42,43]). To generate the weights for the polygenic score, we performed meta-analyses of the pooled subjective well-being phenotype excluding each of the holdout cohorts, applying a minimum-sample-size filter of 100,000 individuals (**Supplementary Note**). The results from these analyses are reported in **Supplementary Table 33** and depicted in **Supplementary Figure 15**.

Biological annotation. For the biological annotation of the 20 SNPs in **Table 1**, we generated a list of LD partners for each of the original SNPs. A SNP was considered an LD partner for the original SNP if (i) its pairwise LD with the original SNP exceeded $R^2 = 0.6$ and (ii) it was located within 250kb of the original SNP. We also generated a list of genes residing within loci tagged by our lead SNPs (**Supplementary Table 34**).

We used the NHGRI GWAS catalog[44] to determine which of our 20 SNPs (and their LD partners) were in LD with SNPs for which genome-wide significant associations have been previously reported. Since the GWAS catalog does not always include the most recent GWAS results available, we included additional recent GWAS studies. We used the tool HaploReg[45] to identify nonsynonymous variants in LD with any of the 20 SNPs or their LD partners.

We examined whether the 20 polymorphisms in **Table 1** were associated with gene expression levels (**Supplementary Table 24** and **Supplementary Note**). The c*is*-eQTL associations were performed in 4,896 peripheral-blood gene expression and genome-wide SNP samples from two Dutch cohorts measured on the Affymetrix U219 platform[42,43,46]. We also performed eQTL lookups of our 20 SNPs in the Genotype-Tissue Expression Portal[47,48]. We restricted the search to the following trait-relevant tissues: hippocampus, hypothalamus, anterior cingulate cortex (BA24), putamen (basal ganglia), frontal cortex (BA9), nucleus accumbens (basal ganglia), caudate (basal ganglia), cortex, cerebellar hemisphere, cerebellum, tibial nerve, thyroid, adrenal gland, and pituitary.

Finally, using a gene co-expression database[49], we explored the predicted functions of genes co-locating with the 20 SNPs in Table 1 (**Supplementary Table 35**).

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04

Multivariate Genome-Wide Analyses of the Well-being Spectrum

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Abstract

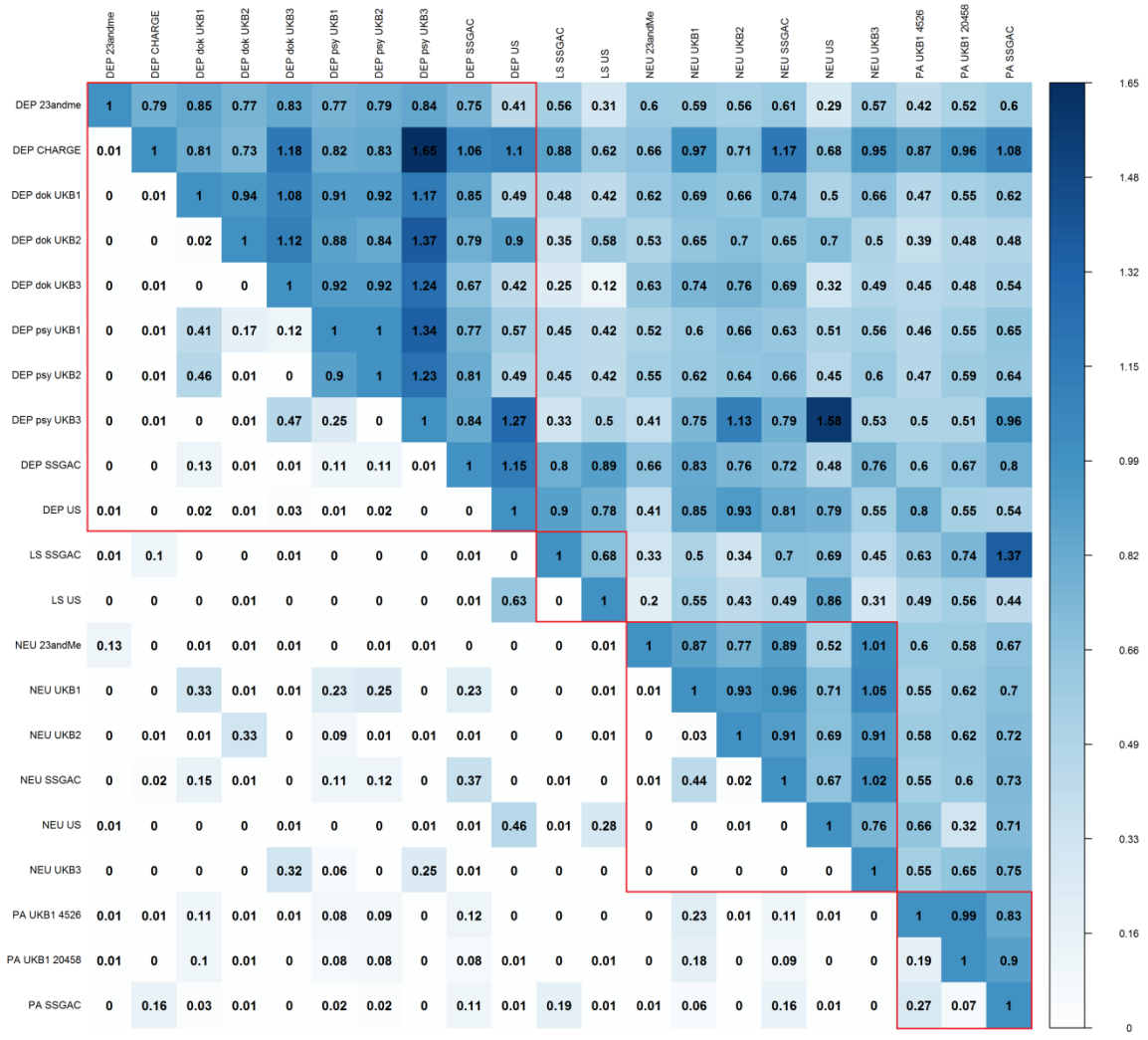
Traits related to well-being (life satisfaction, positive affect, neuroticism, and depressive symptoms), are genetically highly correlated ($r_g > 0.70$). We introduce two novel methods for multivariate genome-wide meta-analysis (GWAMA) of related traits that also correct for sample overlap. We applied these methods to the abovementioned traits, collectively referred to as the well-being spectrum (N_{obs} = 2,370,390), and found 319 significant independent signals. This 32% increase over the 241 independent signals found in the four univariate GWAS analyses was paired to an increase (~38%) in the predictive power of polygenic risk scores. A broad range of simulation scenarios supports the added value of our multivariate methods relative to univariate GWASs. Bioinformatic analyses based on the multivariate GWAMA, including gene expression in brain tissue and single cells, showed that genes differentially expressed in the subiculum, the ventral tegmental area, and in GABAergic interneurons are enriched in their effect on the well-being spectrum.

Main

Well-being plays an important role in psychology and medicine, as well as in economics[1,2]. Well-being owes its interdisciplinary prominence to its associations with physical and mental health, and its role as a desired socio-economic outcome and index of economic development[3]. Most existing research on the genetics of well-being is characterized by a focus on separate traits including life satisfaction[4], positive affect[4], neuroticism[5], and depressive symptoms[6], despite the high phenotypic and genetic correlations between these traits. This overlap is strongly suggestive of a common underlying biology. Acknowledging this, we performed two multivariate genome-wide meta-analyses (N_{obs}= 2,370,390) of these four traits to increase the power to identify associated genetic variants (**Supplementary Table 1)**.

Our analyses leveraged published univariate GWAMA of life satisfaction[4,7] (N_{obs} = 80,852; 2 studies), positive affect[4,7,8] (N_{obs} = 410,603; 3 studies), neuroticism[4,7–9] (N_{obs} = 582,989; 6 studies), and depressive symptoms[4,7,8,10,11] (N_{obs} = 1,295,946; 10 studies), which show strong genetic correlations (**Fig. 1** (upper triangle)). Overall, the mean genetic correlations between different measures of the same trait were higher (LS, r_g = 0.68, PA, r_g = 0.9, NEU, r_g = 0.84, and DS, r_g = 0.89) than the mean genetic correlations between measures of different traits (r_g = 0.7). This justifies our two-stage approach by first meta-analyze the datasets measuring the same traits (LS, PA, NEU, and DS). Secondly, we meta-analyze these four datasets into what we refer to as the well-being spectrum (N_{obs} = 2,370,390; **Supplementary Fig. 1).** For the purpose of the multivariate GWAMA, we reversed the estimated SNP effects on neuroticism and depressive symptoms to ensure a positive correlation with life satisfaction and positive affect. The dependence between effect sizes (error correlation) induced by sample overlap was estimated from the genome-wide summary statistics obtained from the univariate GWAMA analyses using LD score regression[12,13] (see online methods and **Fig. 1** (lower triangle)). Knowledge of the error correlation between univariate meta-analyses allowed dependent samples to be meta-analyzed, providing a gain in power while guarding against inflated type 1 error rates (see online methods).

Fig 1: Genetic correlations and error correlations (cross-trait intercepts) between the included GWAMA data sets. Upper triangle: genetic correlations. Lower triangle: error correlation representing the magnitude of inflation due to population stratification. Red boxes indicate trait-specific genetic correlations and error correlation. Note, **UKB1** represents Caucasian UK Biobank participants living in the UK. **UKB2** represents Caucasian UK Biobank participants living in the UK that are relatives from **UKB1**, and **UKB3** represents Caucasian UK Biobank participants not living in the UKB.



We recognize that the measures included in the well-being spectrum are not necessarily interchangeable. Therefore, we performed two types of multivariate analyses; 1) N-weighted multivariate GWAMA (N-GWAMA), which assumes a single underlying construct with a unitary effect of the SNP on all traits (see online methods); 2) model averaging GWAMA (MA-GWAMA), where we relaxed the assumption of a unitary effect of the SNP on all traits. The latter resulting in a separate estimate of effect for the four traits of interest for each SNP, allowing for a certain degree of heterogeneity (see online methods). We performed simulations to elucidate in which scenarios multivariate N-GWAMA and MA-GWAMA outperform univariate GWAMA, in which scenarios N-GWAMA outperforms MA-GWAMA and in which scenarios the reverse is true. Using the N-GWAMA results we proceeded with biological annotation to elucidate the etiology of the well-being spectrum through transcriptome-wide (TWAS) and methylome-wide (MWAS) association, and stratified LD score regression based on histone modification, brain region differential gene expression, and nerve cell-type specific gene expression.

Validation of multivariate methods

Simulations

To validate the two multivariate methods we simulated GWAS summary statistics for a range of different scenarios. For each scenario we simulated four heritable traits (h_{SNP}^2 = 30%) affected by 80K SNPS using data for 100,000 individuals sampled from the UK Biobank [8]. We choose parameters that far exceed the reported SNP h^2 for these traits[4–6], which allows us to simulate at smaller sample sizes (100K) and reduce the computational burden. The genetic correlation between the four traits varied between .1 and .9 (see online methods). We found that in the presence of genetic correlations equal or higher than .5, both N-GWAMA and MA-GWAMA outperform univariate GWAS (**Supplementary Table 2**). The added value of multivariate analyses disappears when traits showed lower genetic correlations (≤ 0.4) (**Supplementary Fig. 2**). To validate MA-GWAMA, we simulated data where the assumption of a unitary effects of the SNP on all traits was relaxed (see online methods). We found that, in the scenario where a SNP has an effect on at least three out of four traits, N-GWAMA and MA-GWAMA perform equally. However, when a SNP has an effect on two out of four traits or one out of four traits, MA-GWAMA outperforms N-GWAMA (**Supplementary Table 3**).

GWAMA results

In our N-GWAMA, we identified 222 independent (250kb window LD > 0.1) loci associated with the well-being spectrum (**Fig. 2A, Supplementary Table 4**), whereas MA-GWAMA identified 103 (LS), 149 (PA), 234 (NEU), and 201 (DS) loci (**Fig. 2B-E**), some of which overlap, resulting in 277 independent signals (**Supplementary Table 5 -8**). Of these independent MA-GWAMA signals, 154 were within a 50kb window of the independent signals present in the N-GWAMA analysis (69,4%). Considering both multivariate methods, we found 319 independent genome-wide signals associated with the well-being spectrum. This is a 32 % increase over the independent signals found in univariate GWAMAs (LS, PA, NEU and DS (**Supplementary Table 9** and **Supplementary Fig. 3A-D**). The low LD-score intercepts for all our analyses, confirmed that the inflation in test statistics was due to an increase in signal, rather than population stratification or inaccurate accounting for sample overlap (see online methods, **Supplementary Table 10**).

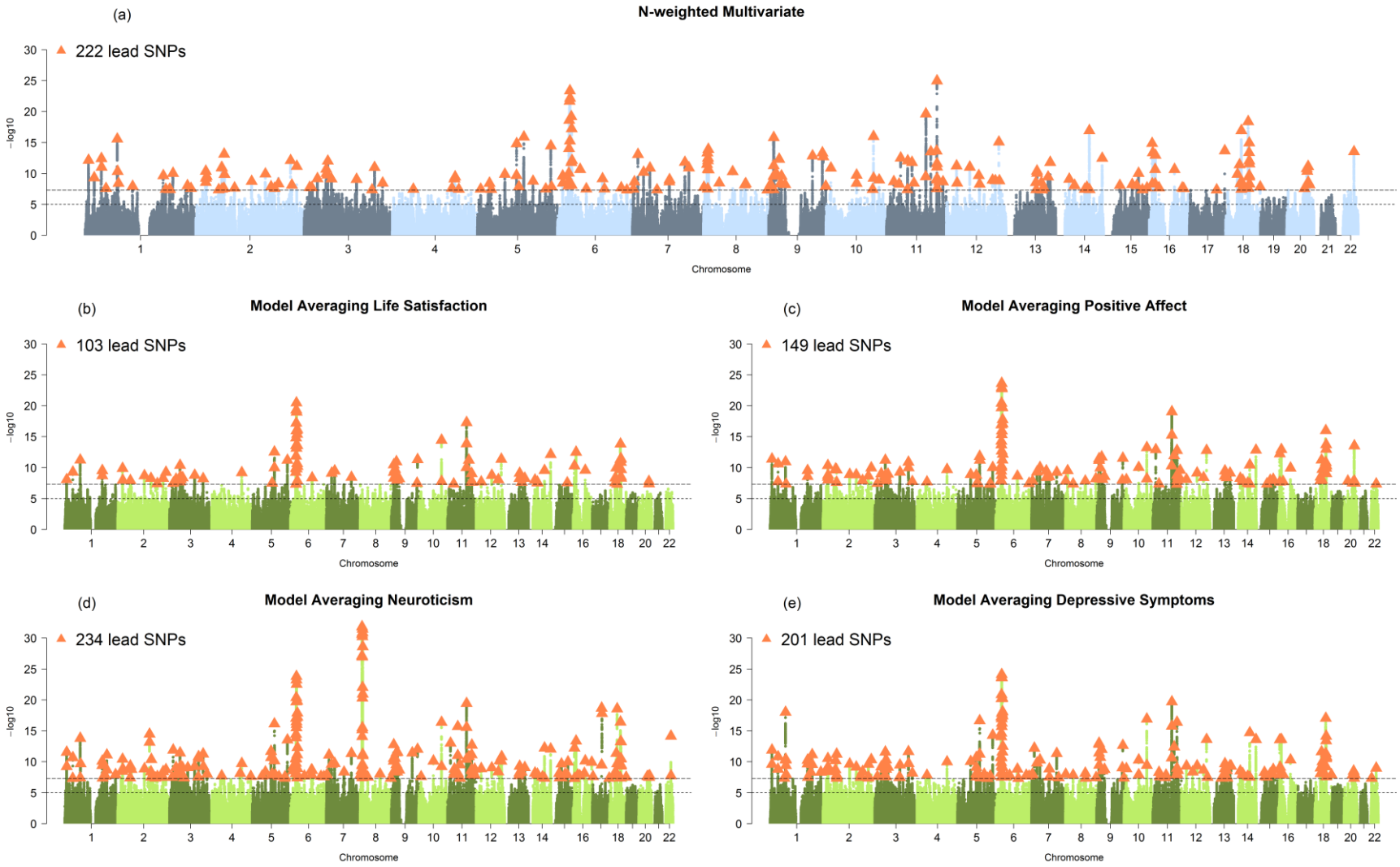
We performed a lookup for the genome-wide significant loci reported in published studies of related traits. We identified 27 loci in close proximity (< 250 kb) to the 44 genome wide significant loci (61%) Wray et al reported for the recent major depressive disorder MDD[6]. In addition, we identified 62 loci in close proximity to the 79 loci identified using an alternative multivariate method considering the same traits (78.4%) in a subset of the data we used[14]. Using height as a negative control, we identified 38 loci in close proximity to the 697 loci associated with height (5,5%)[15].

Polygenic prediction

We compared the predictive power of polygenic scores constructed from univariate GWAMA against N-GWAMA and MA-GWAMA to confirm the gain in power. Prediction of measures of LS, PA, NEU, and DS was performed in samples of the Netherlands Twin Register (NTR: $_{mean}N$ = > 8,100) and Understanding Society (US: $_{mean}N$ > 8,846) [7,16]. We evaluated the predictive power of each polygenic score by its incremental R^2 value, defined as the increase in R^2 of the regression including the polygenic score as independent variable together with a set of controls (age, age^2, sex, and ten principal components) over a regression omitting the polygenic score. Univariate GWAMA polygenic scores had an incremental R^2 value of 0.13% for LS, 0.49% for PA, 1.53% for NEU, and 1.22% for DS. The corresponding N-GWAMA and MA-GWAMA had larger incremental R^2 for LS: 0.87% and 0.88%, for PA: 0.88% and 0.93%, and 1.53% and 1.45% for DS. For NEU, prediction was comparable for N-GWAMA

(1.52%) and lower for MA-GWAMA (1.27%). On average, N-GWAMA improved prediction by 42% and MA-GWAMA improved prediction by 34% (**Supplementary Fig 4** and **Supplementary Table 11**).

Fig. 2. Manhattan plots of N-weighted and model averaging GWAMA. (**a**) N-weighted GWAMA. Model averaging GWAMA of (**b**) life satisfaction, (**c**) positive affect, (**d**) neuroticism, (**e**) depressive symptoms. All plots in all panels are based on the same set of SNPs. The *x*-axis represents the chromosomal position, and the *y*-axis represents the significance on a $-\log_{10}$ scale. Each approximately independent genome-wide significant association ("lead SNP") is marked by Δ.



Transcriptome -and Methylome-wide Analyses

Both flavors of multivariate GWAMA aggregate the effect of a single SNP across multiple traits, informed by prior knowledge of the genetic correlation between these traits. We next proceeded to aggregate the effect across multiple SNPs based on prior knowledge that some of these SNPs influence the expression level of a gene transcript or the methylation level at a CpG site (mQTL) measured in whole blood. Applying these methods (known as TWAS/MWAS) can identify genes involved in complex traits [17–19]. Given the equal performance of both multivariate GWAMAs and to avoid multiple testing, we used the results of the N- GWAMA for TWAS and MWAS and further analyses. In TWAS, we uncovered 87 transcript-trait associations (45 loci) at a Bonferroni corrected significance level ($p < 4.29 \times 10^{-6}$). For 23 TWAS hits (18 loci), the corresponding locus (1000 kb around the transcript) did not contain a significant N- GWAMA SNP. For 46 out of the 87 transcripts (13 loci), the maximum LD between the TWAS model SNPs and N-GWAMA top SNP in the corresponding locus is larger than 0.8 (**Supplementary Table 12**). Furthermore, we found 852 CpG methylation-trait associations mapping to 131 loci at a Bonferroni corrected significance level. For 72 out of 852 CpG methylation-trait associations, the corresponding locus did not contain a N-GWAMA significant signal. For 381 CpG methylation-trait associations (76 loci), the maximum LD between the MWAS model SNPs and a N-GWAMA top SNP is larger than 0.8 (**Supplementary Table 13**).

A locus of particular interest was found within the major histocompatibility complex. Recent work has identified 3 individual signals related to schizophrenia in the MHC region, one of which is linked to complement 4 (C4A) gene expression and synapse elimination during puberty[20]. The genome-wide significant signal for the well-being spectrum in the MHC region is not in strong LD with lead eQTL's for C4A gene expression. Rather, a second independent signal tagged by rs13194504 is associated with both schizophrenia and well-being. TWAS results for the MHC region implicate the expression of *TRIM38* in the etiology of well-being (**Supplementary Fig. 5**).

Bioinformatics: Stratified LD Score Regression

We performed further biological annotation using stratified LD score regression[12,13]. Our first analysis aimed to confirm the involvement of the central nervous system (CNS) in the etiology of the well-being spectrum. Our second analysis aimed to pinpoint specific locations

in the brain. Our final analysis used single cell sequencing data to confirm the specific cell type involvement.

We considered the enrichment in the N-GWAMA derived SNP set of 220 genomic annotations (33 brain and 187 non-brain annotations), which reflected the locations of four specific histone marks (H3K4me1, H3K4me3, H3K27ac, or H3K9ac) in 54 tissues in their effect on the well-being spectrum[21]. This allow detection of, for example, enrichment of regions of the genome which are histone modified in the prefrontal cortex. Such enrichment would suggest the involvement of processes in the prefrontal cortex in the etiology of the wellbeing spectrum.

Our analyses revealed significant enrichment of 68 annotations characterized by 32 histone marks in 10 brain tissues (**Supplementary Table 14 and Supplementary Figure 6**). Note that the top 15 significant annotations involved brain tissues. Among these brain tissues were the mid-frontal and inferior-temporal lobe, fetal brain, cingulate and angular gyrus, germinal matrix (a highly cellular and highly vascularized region in the brain from which cells migrate out during brain development), hippocampus anterior caudate, substantia nigra, and the neurosphere.

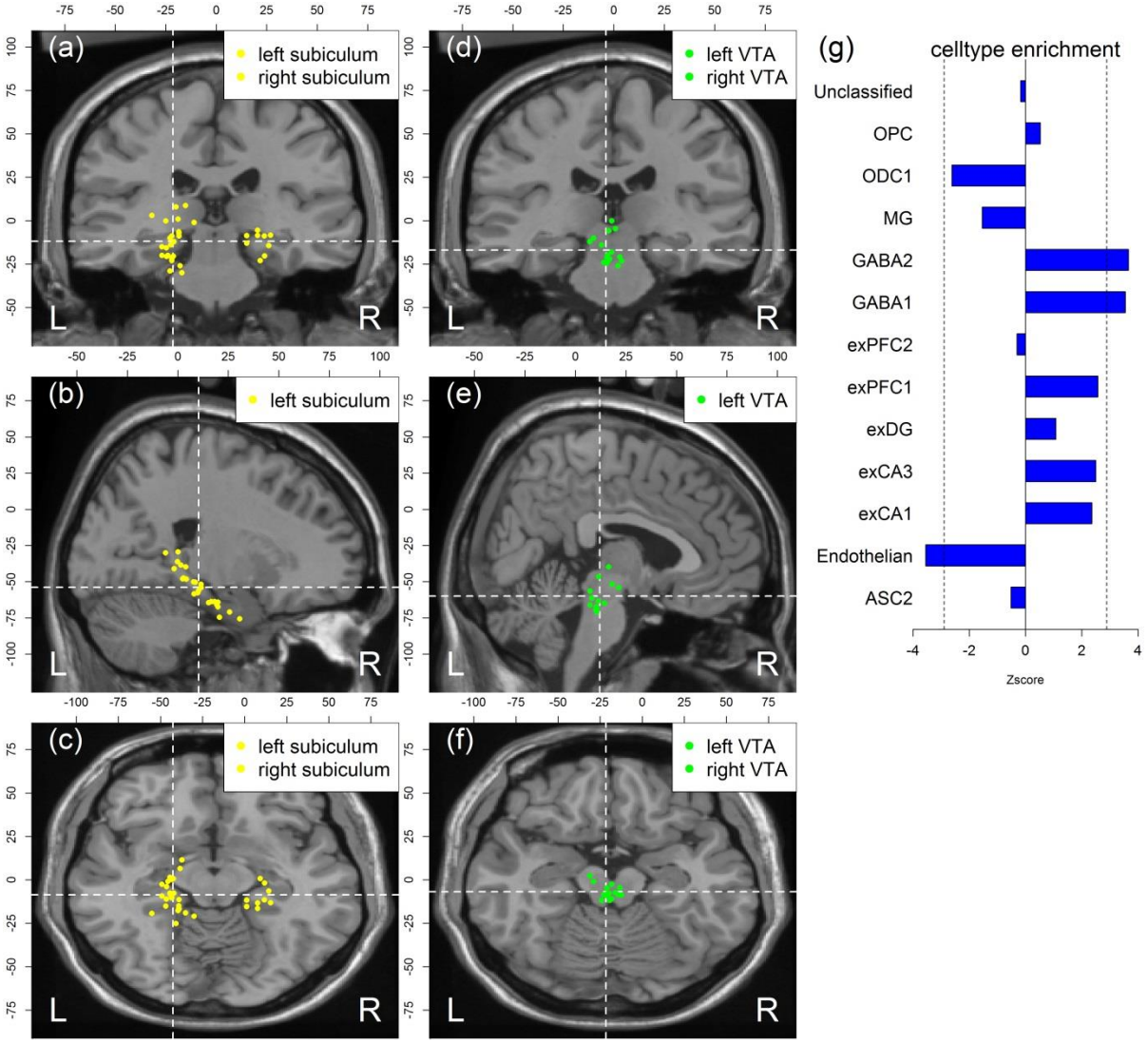
In order to more accurately pinpoint brain regions where genes relevant to the well-being spectrum are differentially expressed, we computed stratified LD scores based on differential gene expression in an anatomically comprehensive set of 210 brain regions, based on 3707 measurement in 6 human brains[22]. For each brain region, genes were selected that showed higher expression compared to all other regions (global differential gene expression). The LD scores were significantly enriched at FDR < 0.05 at multiple gyri in the cortex (**Supplementary Table 15**). Differential gene expression appeared driven mainly by transcriptional differences between gross anatomical structures in the brain (cortex, sub-cortical structures, brainstem, and cerebellum). To reveal regions related to the well-being spectrum within these structures, we divided the 210 regions into four sets (brain stem, cortex, sub cortex, and cerebellum) based on their locations and computed differential gene expression across the regions *within* each structure (local differential gene expression). Our results showed a significant enrichment (Bonferroni corrected) of N-GWAMA signal for genes specifically expressed in the in the subiculum ($Z = 3.60$, $p < 0.001$; **Fig. 3A-C**).The subiculum is considered part of the hippocampal formation and plays a key role in hippocampal-cortical interaction[23] in the inhibition of the Hypothalamic-Pituitary-Adrenal-

axis and the human response to stress[24]. Additionally, we identified enrichment of N-GWAMA signal for genes specifically expressed in the Ventral Tegmental Area (VTA; Z = 3.34, p < 0.001; **Fig. 3D-F**, **Supplementary Table 16-19**). The VTA is a group of neurons located close to the midline on the floor of the midbrain. The VTA is the origin of the dopaminergic cell bodies of the mesocortico-limbic dopamine system and has a central role in reward-related and goal-directed behaviors[25].

We repeated the analyses using GWAMA summary statistics of educational attainment (EA)[26] and schizophrenia[27], two traits in which the CNS has been implicated in their etiologies before. In particular, we wanted to see whether the signal observed in the subiculum and the VTA were specific to the well-being spectrum. As a negative control, we considered the enrichment of genes differentially expressed in all brain regions using height GWAMA summary statistics[28]. We found no enrichment of genes differentially expressed in the subiculum on EA (Z=1.251; *p*=0.105), but did found an effect on schizophrenia (*Z*=2.938; *p*=0.002). Genes differentially expressed in the VTA showed no enrichment on EA (Z=0.016; *p*=0.494), but were nominal significant in their effect on schizophrenia (Z=2.121; *p*=0.017). No region was significantly enriched in their effect on height, both when considering global and local differential gene expression (all p > 0.05). All results of the differential gene expression analysis were mapped to the MNI coordinates at which the tissue samples were obtained, allowing future integration of our findings and other neuroimaging modalities (**Supplementary Table 20**).

Finally, we obtained the publicly available matrix of gene counts generated based on single nuclei (*N* = 14,963) from the prefrontal cortex and hippocampus of multiple human donors by Habib et al (2017)[29]. We divided these nuclei into 7 types of neurons, 2 subtypes of astrocytes, oligodendrocytes, oligodendrocyte precursors cells, microglia, endothelial cells and unclassified cells (hippocampus and prefrontal cortex) and computed cell type specific genes for the different types of neurons (see online methods). Using LD Score regression we then tested the enrichment in the N-GWAMA and found enrichment in the CA1 (*Z* = 2.36; *p* = 0.005*)* and CA3 (*Z* = 2.5; *p* = 0.003*)* parts of the hippocampus as well as in the prefrontal cortex for glutamatergic neurons (*Z* = 2.58; *p* = 0.002*)*. Additionally, we found enrichment for GABAergic interneurons expressed in the hippocampus (GABA1; *Z* = 3.55; *p* = 2.41 X 10^{-6} and GABA2 *Z* = 3.7; *p* = 8.52 X 10^{-7}*;* **Supplementary Table 21 and Fig 3G**).

Fig. 3 Local differential gene expression between subcortical structures identifies enrichment of genes specifically expressed in the subiculum ($Z = 3.60$, $p < 0.001$) and ventral tegmental area ($Z = 3.34$, $p < 0.001$), in their effect on the well-being spectrum. **(a,d)** coronial view (**b,e**) sagittal view (**c,f**) axial view. The location of the samples of brain tissues which were used to measure gene expression by Hawrylycz et al. (2012) is projected to a standard MNI template brain ("Colin27"). The figure is centered on the averaged MNI coordinates of brain samples which are part of the annotation "left Subiculum" (x = 77, y = 90 and z = 60).(**g**) bar graph representing the cell-type specific enrichment of mainly glutamatergic and GABAergic neurons. Dashed line indicate Bonferroni corrected significance. (OPC = oligodendrocyte precursors cells, ODC1 = oligodendrocytes, MG = microglia, GABA = GABAergic interneurons, exPFC = glutamatergic neurons from the prefrontal cortex, exDG = granule neurons from the hip denate gyrus, exCA1/3 = pyramidal neurons from the hippocampus CA region, endothelian = endothelian cells, ASC = astrocytes.



Discussion

We have introduced N-GWAMA and MA-GWAMA, two novel methods for conducting meta-analysis of GWAS summary statistics for related traits that are robust to sample overlap. While previous univariate analyses of traits in the well-being spectrum were moderately successful, we gained power by the use of multivariate analyses. MA-GWAMA identified many additional loci associated with some, but not all, traits in the well-being spectrum, and provides flexibility in terms of model specification. Model averaging can in fact incorporate *any* multivariate GWAMA or GWAS model for which the per SNP model fit can be expressed in terms of an AICc fit statistic. The averaging procedure is done per locus, allowing for heterogeneity across traits and loci. Both N-GWAMA and MA-GWAMA are complementary of each other and can be used together to identify genetic variants associated with clusters of genetically correlated traits. Given the equal performance between our two methods and to lower the multiple testing burden, we used the N-GWAMA analyses for our follow-up analyses. We further confirm that TWAS and MWAS can further increase the identified pool of loci related to variation in complex traits, like well-being by aggregating the effect across multiple SNPs based on prior knowledge that some of these SNPs influence the expression level of a gene transcript or the methylation level at a CpG site.

By leveraging the genome-wide results, LD score regression, and an atlas of brain gene expression we were able to pinpoint brain regions where region specific gene expression exists for genes enriched in their effect on well-being, and we report evidence for enrichment of genes differentially expressed in the VTA, as well as in the subiculum. Furthermore, we find enrichment for glutamatergic neurons in the CA1 and CA3 of the hippocampus and in the prefrontal cortex as well as enrichment for GABAergic interneurons.

In the regions for which we have cell types available (hippocampus and prefrontal cortex) we find specific cell type enrichment for the wellbeing spectrum. However, it stands to reason that the same cell type specific enrichment in other regions exists, which we now missed. Gene expression is known to vary systematically between cell-types within the brain[30] (e.g neurons, microglia, astrocytes) and developmental phases[31] (prenatally, childhood, adulthood and old age), and likely even between sub-types of a single cell type. Differences in gene expression across or within cell types may induce differences between regions as cell type composition might differ between regions. This limitation needs to be addressed in future well-being research, capitalizing on ongoing efforts to categorize gene expression across the human brain at increased (single cell) resolution. Single cell sequencing (e.g. drop-seq based anatomically comprehensive survey of the brain), based on donors deceased at different ages,

could disentangle cell type specific from region specific differential gene expression as well as age specific gene expression[32].

Our study showed, through simulations, that multivariate GWAMA of traits with genetic correlation higher than 0.5 always outperform univariate GWAMA. With summary statistics from large-scale GWAS publicly accessible for an even-increasing number of traits, it is becoming increasingly feasible to detect clusters of genetically correlated traits. Both N-GWAMA and MA GWAMA can be used to detect genetic variants associated with the shared etiology of these genetically correlated traits, while simultaneously correcting for population stratification. The results of our new multivariate GWAMA methods could be meaningfully mapped to brain regions based on a coordinate system used within multiple other neuroscientific disciplines, facilitating future integration of genetic and neuroscientific research on the well-being spectrum.

Online Methods

N-weighted multivariate GWAMA.

We obtained summary statistics from previous published studies[4,7–11] , where multiple cohorts contributed to the univariate GWAMAs of life satisfaction, positive affect, neuroticism and depressive symptoms http://www.thessgac.org/. To quantify the dependence between the univariate GWAMAs, we estimated the cross trait LD score intercept (CTI)[12,13]:

$$CTI = \frac{N_s * r_p}{\sqrt{N_1 N_2}}$$

Where N_s equals the sample overlap, N_1 the sample size for trait 1 and N_2 the sample size for trait 2, r_p equals the phenotypic correlation between trait one and two. The CTI is approximately equal to the covariance between the test statistics obtained in a GWAMA of trait 1 and trait 2. We assume that the estimated CTI is equal to the true CTI, though note the uncertainty in the estimated CTI is generally low. Given the estimated covariance between effect sizes, we can meta-analyse the four dependent GWAMAs and obtain a multivariate test statistic per SNP:

$$Z_k = \frac{\sum_{i=1}^{4} w_{ik} * Z_{ik}}{\sqrt{\sum_{i=1}^{4} w_{ik} * V_{ik} + \sum_{i=1}^{4}\sum_{j=1}^{4} \sqrt{w_{ik} * w_{jk}} * C_{i,j,k}\ (j \neq i)}}$$

Where w_{ik} is the square root of the sample size for SNP k in the GWAMA of trait i, Z_{ik} is the test statistic of SNP k in the GWAMA of trait i; V_{ik} is the variance of the test statistic for SNP k in the GWAMA of trait i (i.e 1 given that Z is a standardized test statistic) and $C_{i,j,k}$ is the covariance between test statistics for SNP k between GWAMA of trait i and trait j (where C equals CTI obtained from cross trait LD score regression between trait i and trait j). The multivariate test statistic Z_k, is a standardized sum of tests statistics, all of which follow a normal distribution under their respective null distributions. The statistic Z_k follows a standard normal distribution under the null hypothesis of no effect.

Model averaging GWAMA

Consider the following model:

$$\beta = MVN(\gamma X + e, V)$$

Where β (1xn) is a multivariate normal vector of effect sizes obtained from the regression of n standardized traits on a standardized genotype (SNP). The matrix V (nxn) is the variance-covariance matrix of effect sizes, matrix X a design matrix (pxn), and γ the corresponding vector of parameters (1xp). The indexed p denotes the number of variables included in the means model of the response vector β.

In this context, a regular GWAMA restricts the design matrix X to a unit vector (i.e. we model a single genetic effect, which is assumed identical across cohorts, and any observed variation is attributed to sample fluctuation). Generally, matrix V is diagonal, and contains the squared standard errors of elements in β. A regular GWAMA is the most restricted model one can consider. However, when considering multivariate GWAMA (i.e. the elements in β reflect SNP effects on separate yet correlated traits), this model might be too restrictive. Even when traits have a substantial genetic correlation, not all genetic effects need to be shared between traits or be identical in magnitude. The least restrictive model is to consider the SNP effects in β independent (i.e. run univariate GWAMA of the correlated traits). In between the most restrictive and least restrictive model, a manifold of models can be specified, equating the effects in y across combinations of traits, while allowing it to differ between other combinations of traits. These models can be specified by ways of the design matrix X.

One could consider a manifold (z) of models (m), each with a different design matrix X.

$$\beta_1 = MVN(\gamma_1 X_1 + e, V)$$

$$\beta_2 = MVN(\gamma_2 X_2 + e, V)$$

$$\beta_z = MVN(\gamma_z X_z + e, V)$$

When considering i correlated traits, a simple expansion of X is to allow for 2 vectors (p =2), a unit vector and a second vector which is coded dichotomously (0,1), where the coding varies over each of the m models. Other codings, based on analysis of the genetic correlation between traits (i.e. PCA or Cholesky decomposition), can be applied to summary statistics and included in the average. Practically, this allows for the existence of 2 distinct genetic effect. This procedure results in $.5 * k^2$ models. The 1df model with a unit vector for X and $(.5 * k^2 - 1)$ 2-df models with a unit vector and a second vector which codes for all possible combinations of pairs of k traits. However, simply considering m models for all SNPs across the genome results in a prohibitive increase of the already substantial multiple testing burden. Given m possible models, each of which predict a different vector γ, and uncertainty for the predicted elements in γ, a possible way forward is to average the model predictions. The models are weighted by the relative proportion of evidence for each model. Specifically, the weights can be based on the AICc[33] information criteria. The AICc for model m equals:

$$AICc_m = -\ln(LogLik_m) + 2k_m + \frac{2k_m(k_m+1)}{n-k_m-1}$$

For each AICc we compute the delta (Δ_m) to the best (i.e lowest) AICc value, and from these we compute the model weights (g) for the k models as:

$$g_m = \frac{\exp(-\frac{1}{2}\Delta_m)}{\sum_{m=1}^{z}\exp(-\frac{1}{2}\Delta_m)}$$

We predict the vector β using each of the models

$$\hat{\beta}_m = \gamma_m X_m$$

One can aggregate the prediction over all models as:

$$\beta_a = \sum_{m=1}^{z} \frac{\hat{\beta}_m * g_m}{\sum_{m=1}^{z} g_m}$$

And we aggregate the uncertainty within and between models to obtain $var\ (\beta_a)$:

$$var(\beta_a) = \left[\sum_{\mathrm{m=1}}^{\mathrm{z}} \mathrm{g_m} \sqrt{\mathrm{var}(\hat{\beta}_m) + (\hat{\beta}_m - \hat{\bar{\beta}})^2} \right]^2$$

The resulting vector $\beta_a$contains the model averaged effect sizes for the effect of a particular SNP on the traits subjected to multivariate analysis. Note how the variance estimate contains a variance component which reflects within model variability ($\mathrm{var}(\hat{\beta}_m)$) which equals the square of the standard error, and a variance component between model variability ($(\hat{\beta}_m - \hat{\bar{\beta}})^2$) in estimate, which ensures no overfitting occurs.

Our procedure boosts power if the SNP effect is concordant between traits, while retaining strongly discordant SNP effects if the model favors these. Model averaging offers several avenues for extension. One can constrain the SNP effects across multiple SNPs based on biological knowledge of the relation between the SNPs and gene expression, or CpG methylation (analog to TWAS). Alternatively, it might be beneficial to average the AICc weights across regions of the genome. Model averaging can in principle accommodate *any model* for which the AICc information criterion can be expressed. These models should result in a vector of SNP effects (β) and an asymptotic variance for the SNP effects. In the current application, models per SNP are estimated in R using the "metafor" package and models are averaged using the "AICcmodavg" package[34,35].

Simulations

To assess N-GWAMA, which assumes a single underlying construct, we used ten different scenarios. For each scenario we simulated four heritable traits (h_{SNP}^2 = 30%) effected by 80K SNPS. The genetic correlation between the four traits varied between .1 and .9. Using real genotypes and simulated effects, we simulated phenotypic data for 100,000 individuals sampled from the UK Biobank[8]. In all these analyses, we included the 656,284 genotyped SNPs (MAF > 0.01). From these 100K individuals we sample 40K individuals to conduct univariate GWASs. This introduced partial sample overlap between the univariate GWASs. Next, we performed N-GWAMA and MA-GWAMA analyses and correlated the true SNP effects with the estimated SNP effects obtained from the univariate GWASs, N-GWAMA and MA-GWAMA.

To validate MA-GWAMA, we simulated data where the assumption of a unitary effects of the SNP on all traits was relaxed. We again simulated four traits, which were affected by 80K SNPs. The SNP effects are perfectly correlated, however, we replaced true effects with zero in a way that guarantees that 10K SNPs have a true effect on only one trait, 10K SNPs have a true effect on two traits, and 10K SNPs have a true effect on three traits. Based on these effect sizes and genotypes we simulated traits for 100K individuals and performed univariate GWAS, N-GWAMA, and MA-GWAMA analyses as described above.

Polygenic Prediction

To confirm the gain in power of our multivariate GWAMA results, we performed polygenic score prediction (PRS) in two independent samples; 1) the Netherlands Twin Register (NTR)[1616,36] and 2) Understanding Society (UKHLS)[7]. We predicted the traits in the well-being spectrum (life satisfaction, positive affect, neuroticism, and depressive symptoms). In NTR, LS and PA data is available in 9,143 and 6,836 genotyped participants, respectively. LS was measured longitudinally using the Satisfaction with Life Scale consisting of five items (e.g., “My life is going more or less as I wanted”) with responses given on a seven-point scale, resulting in a minimum score of five and a maximum score of 35[37]. PA is also measured longitudinally using four questions that were adapted from the Subjective Happiness Scale[38] (e.g., “On the whole, I am a happy person”) with responses on a seven-point scale, resulting in a minimum score of four and a maximum score of 28. Neuroticism data is available for 8,527 genotyped participants. The Big Five personality traits (including neuroticism) were measured by using the NEO-FFI[39], a sixty-item personality questionnaire consisting of five subscales: neuroticism, extraversion, openness, agreeableness and conscientiousness. The responses

were given on a five-point scale (0-4). Subscale scores were constructed for each time point by taking the sum across the twelve subscale-specific items (after recoding opposite-stated items), and were set to missing if ten or more items of the total scale were unanswered. When subjects had fewer than ten missing items, missing items were scored at two (which is neutral given the 0-4 scale). Depressive symptoms were obtained from the DSM-oriented Depression subscale of the age-appropriate survey from the ASEBA taxonomy[40] and were available for 7,898 participants. To measure depressive symptoms, fourteen questions are used (e.g., "Enjoys little ") and responses were given on a three-point scale ranging from zero ("not true") to two ("very true"). The DSM-oriented subscale was constructed for each time point by taking the sum across the fourteen subscale-specific items and was set to missing if more than twenty percent of the total survey items were unanswered. When less than twenty percent of items were missing for a participant, the missing items were replaced by the participant's mean score.

In UKHLS data were available for 9,944 participants. LS was measured longitudinally (waves 1-6). Participants were asked how satisfied they were "with life overall" with responses given on a seven-point scale, resulting in a minimum score of one and a maximum score of seven. PA was also measured longitudinally (waves 1 and 4 only) using The Warwick-Edinburgh Mental well-being scale (WEMWBS). SWEMWBS is a shortened version of WEMWBS. This 7-item short version (see Tennant et al., 2007) is scored on a 5-point Likert scale, from "none of the time" to "all of the time", and summed to give a total score, ranging from 7 to 35. Neuroticism data were available for 8,198 genotyped participants from wave 3. The Big Five personality traits (including neuroticism) were measured using The Big Five Inventory (BFI), a 44-item personality questionnaire consisting of five subscales: neuroticism, extraversion, openness, agreeableness and conscientiousness. The responses were given on a seven-point scale (1-7). The neuroticism score combines three items on the neuroticism subdomain. Component scores were calculated as the average item response if no more than one of the three input responses was missing. Depressive symptoms (DS) were measured longitudinally (waves 1-6) and was obtained from The General Health Questionnaire (GHQ), which was available for 9,203 participants. The 12 question GHQ was used containing questions relating to concentration, loss of sleep and general happiness. The 12 questions are scored on a four-point scale (1-4). Valid answers to the 12 questions of the GHQ-12 were converted to a single scale by recoding 1 and 2 values on individual variables to 0, and 3 and 4 values to 1, and then summing, giving a scale running from 0 (the least distressed) to 12 (the most distressed).

The weights used for the polygenic scores were based on the four univariate GWAMAs as well as our two flavors of multivariate GWAMAs. Scores were based on the intersection of SNPs available in any of these GWAMAs. In the NTR, SNPs were imputed to a common reference. SNPs with MAF < 0.005, Hardy-Weinberg Equilibrium (HWE) with $p < 10^{-12}$, and call rate < 0.95 were removed. Individuals were excluded from the analyses if the genotyping call rate was < 0.90, the inbreeding coefficient as computed in PLINK[48] (F) was < -0.075 or > 0.075), the Affymetrix Contrast QC metric was < .40, if the Mendelian error rate was > 5 standard deviations (SDs) from the mean, or if the gender and Identity-by-State (IBS) status did not agree with known relationship status and genotypic assessment. In UKHLS, SNPs were imputed to a common reference (1000 Genomes project March 2012 version 3). SNPs with MAF < 0.01, HWE $p < 10^{-4}$ and call rate < 0.98 were removed. In NTR 1,224,793 SNPs passed QC and were used to construct polygenic scores and in UKHLS, 955,441 SNPs passed QC and were used to construct polygenic scores. The traits were regressed on sex and age as well as principal components, which were included to correct for ancestry, and the polygenic scores. Results can be found in **Supplemental Table 11.**

Summary-Based transcriptome wide (TWAS) and methylome wide (MWAS) association studies

We used the tool DIST[41] to impute the HapMap reference based results for the N-weighted GWAMA to the 1000Genomes Phase1 reference. We aggregated SNP effects informed by their common effect on expression level of gene or CpG methylation, as proposed by Gusev et al.[19] We used the BIOS eQTL resource as eQTL reference set to build imputation models that predict gene expression using multiple eQTL SNPs[42]. Models were built per gene (gene models) by identifying independent eQTL SNPs based on stepwise conditional regression.[42] The z-score for each eQTL SNP was used in TWAS as a weight (q). The eQTLs used are available at http://genenetwork.nl/biosqtlbrowser/. Based on the gene models, N-weighted GWAMA summary statistics and LD based on the GONL reference[43], TWAS was performed. That is, for each gene- prediction-model containing eQTLs S_1- S_N with weights $q=q_1,q_2,...,q_n$, the corresponding GWAMA z-scores $z=z_1,z_2,...,z_n$ and LD, an n-by-n correlation matrix for eQTLs S_1- S_N, were used to construct a test statistic:

$$Z_{twas} = \frac{\sum_{i=1}^{n} q_i z_i}{\sqrt{q * LD * q}}$$

MWAS was performed following the same procedure to build imputation models to predict CpG site methylation of the DNA strand using multiple mQTL SNPs. The methylation site specific weights were obtained from the BIOS mQTL study[44].

Stratified LD score regression

To determine whether specific genomic regions are enriched for genetic effects on the well-being spectrum traits, we used LD Score regression[12,13]. We were specifically interested in regions of the genome which are histone modified in a specific tissue. For example, regions of the genome which are histone modified in the prefrontal cortex can be transcribed more frequently in prefrontal tissue. The enrichment of these genomic regions in their effect on the well-being spectrum suggest the involvement of processes in the prefrontal cortex in the etiology of the wellbeing spectrum.

LD Score regression is based on the relationship between the observed chi-square of a SNP and the degree of LD between a SNP and its neighbor. SNPs in strong LD are more likely to tag causal effects on complex traits and therefore have a higher expected chi-square. The procedure can be extended to *stratified* LD score regression, where multiple LD scores are created, each of which captures the LD for a SNP with other SNPs of a specific category of interest, for example SNPs in a histone modified region of the genome.

We followed the exact procedure described by Finucane et al.[21], and estimated stratified LD Score regression for the "baseline" model, which contains 53 categories. The model consists of a category containing all SNPs, 24 categories corresponding to main annotations of interest, 24 categories corresponding to 500-bp windows around the main annotations, and categories corresponding to 100-bp windows around ChIP-seq peaks (i.e. regions that are Sensitive to DNase1 or associated with histones bearing the modification marks H3K4me1, H3K4me3, H3K27ac or H3K9ac). In addition to the analyses of the baseline model, we performed analyses using cell type-specific annotations for the four histone marks, which correspond to specific chemical modifications of the histone protein, which in turn packages and orders the DNA molecule. Epigenetic modifications of histones, specifically histones bearing the marks H3K4me1, H3K4me3, H3K27ac or H3K9ac, are associated with increased transcription of DNA into RNA. Each cell type-specific annotation corresponds to a histone mark in a specific cell obtained from distinct human tissue, for example H3K27ac in Fetal Brain cells, generating 220 combinations of histone modification by tissue. When generating estimates of enrichment for the 220 Histone marks(?) by tissue annotations, we controlled for overlap with

the functional categories in the full baseline model, but not for overlap with the 219 other cell type specific annotations. Then for our well-being trait, we ran LD Score regression on each of the 220 models (one for each histone by tissue combination) and ranked the histone by tissue annotations by P-value derived from the Z-values of the coefficient. Results are displayed in **Supplementary Table 14.**

Stratified LD score regression of local gene expression across the human brain.

We downloaded the normalized and QC'ed gene expression measured in an anatomically comprehensive set of brain regions from http://www.brain-map.org/. The data contains 3707 measurements across 6 adult human brains. The procedures used to measure standardized gene expression across the brains are described in Hawrylycz et al[22]. Based on these data we computed differential gene expression for 48154 probes which map to 20724 unique genes (probes which did not map to genes were omitted). We considered differential gene expression across 210 regions for which at least 3 measurements were available. As Hawrylycz et al.[22] found little evidence for lateral difference in gene expression, regions in the left and right hemisphere were collapsed into a single region. For each gene in each region a t-test was performed, testing the difference in standardized expression between the region in question and all other brain regions. The top 10% of probes ranked in terms of t-statistic per region were retained. The unique genes mapped to this set of probes were extracted (mapping ~2900-3500 genes to each region). The correlation between t-statistics for the 48154 probes revealed fairly strong differential expression between the cortex, brainstem, and cerebellum and clustering of differential expression within these regions.

A partitioned LD score with respect to the genomic regions spanned by these genes (using gencode v19 as a reference), and a 100 kilobase window around each gene, was computed. The heritability of well-being was partitioned across the 54 baseline annotations developed by Finucane et al[21] and each of the 210 brain regions (the regions are considered separately). The substantial differences in gene expression between gross anatomical brain regions (cerebellum, cortex, sub-cortical regions and brainstem) dominated the results (**Supplementary Table 15)**. We therefore proceeded to compute differential gene expression *within* the cerebellum, cortex, sub cortical regions, and brainstem. In this analysis we omitted the fibre bundles as these are anatomically distinct from both the cortex and the sub cortical regions, yet not measured densely enough to warrant the computation of differential expression within the fibre bundle tissues. The procedure to compute differentially expressed

genes is identical to the procedure used to compute differential expression across the whole brain, but considers the gross anatomical regions separately. New LD scores were computed based on the local differential gene expression analyses (**Supplementary Table 16-19**). All analyses were repeated using height as a negative control trait. The genomic regions spanning genes differentially expressed in these 210 brain regions were not significantly enriched with SNP effects on height.

Stratified LD score regression of Single nuclei for 7 types of neurons

We obtained the publicly available matrix of gene counts generated based on single nuclei from the prefrontal cortex and hippocampus of multiple human donors by Habib et al (2017)[29]. To compute differential enrichment we deviated from the procedure outlined for regional brain expression as the zero inflated nature of single nuclei expression violates assumptions off the t-test. The matrix contained counts for 32111 genes measured in 14964 nuclei. The nuclei were divided into 7 types of neurons, 2 subtypes of astrocytes, oligodendrocytes, oligodendrocyte precursors cells, microglia, endothelial cells and unclassified cells. We omitted genes for which the total count across cells < 150, or for which less than 30 cells have a count above 0, retaining 11719 genes for analysis. For each gene we computed the ratio of count per nuclei type over the total number of nuclei measured of the specific type (generating the average gene count in each nuclei type). We next computed the ratio of the average count per nuclei type over the average count of the gene across all nuclei (generating the nucleic type specific fold change in average expression). We then defined, for each nuclei type, the nuclei type specifically expressed set of genes as the 1600 genes with the highest nucleic type specific fold change in average expression. For each of the gene sets we constructed an LD score with respect to genes in the set, in order to compute the gene set specific enrichment in h2 in our multivariate GWAMA.

Our method to determine cell-type specific expression purely relies on the relative expression in one cell type over the other, whereas others have developed different statistics to assess differential expression[45]. Application of both methods to the same gene expression dataset (GTEX) and subsequent differential enrichment analysis for wellbeing yielded highly concordant test statistics (r=.83).

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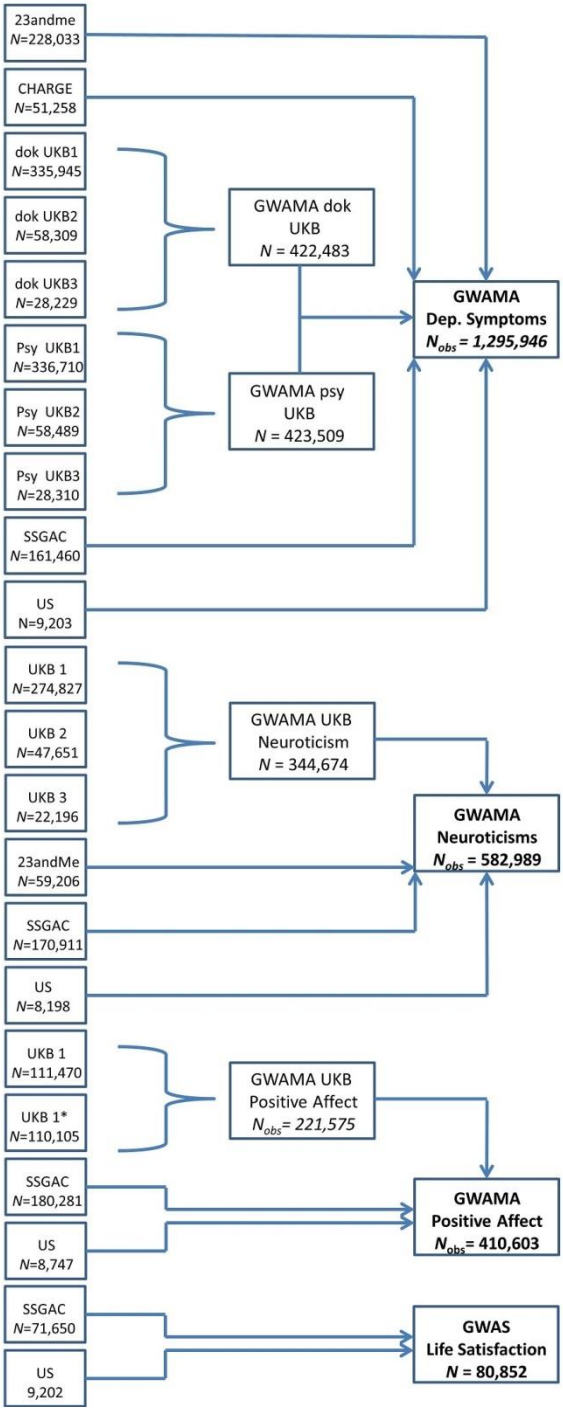
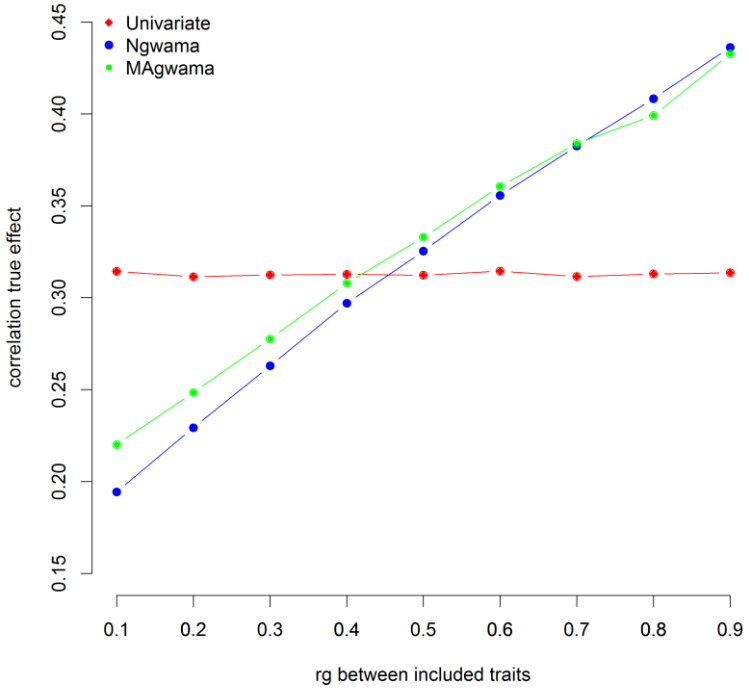
Supplementary Information

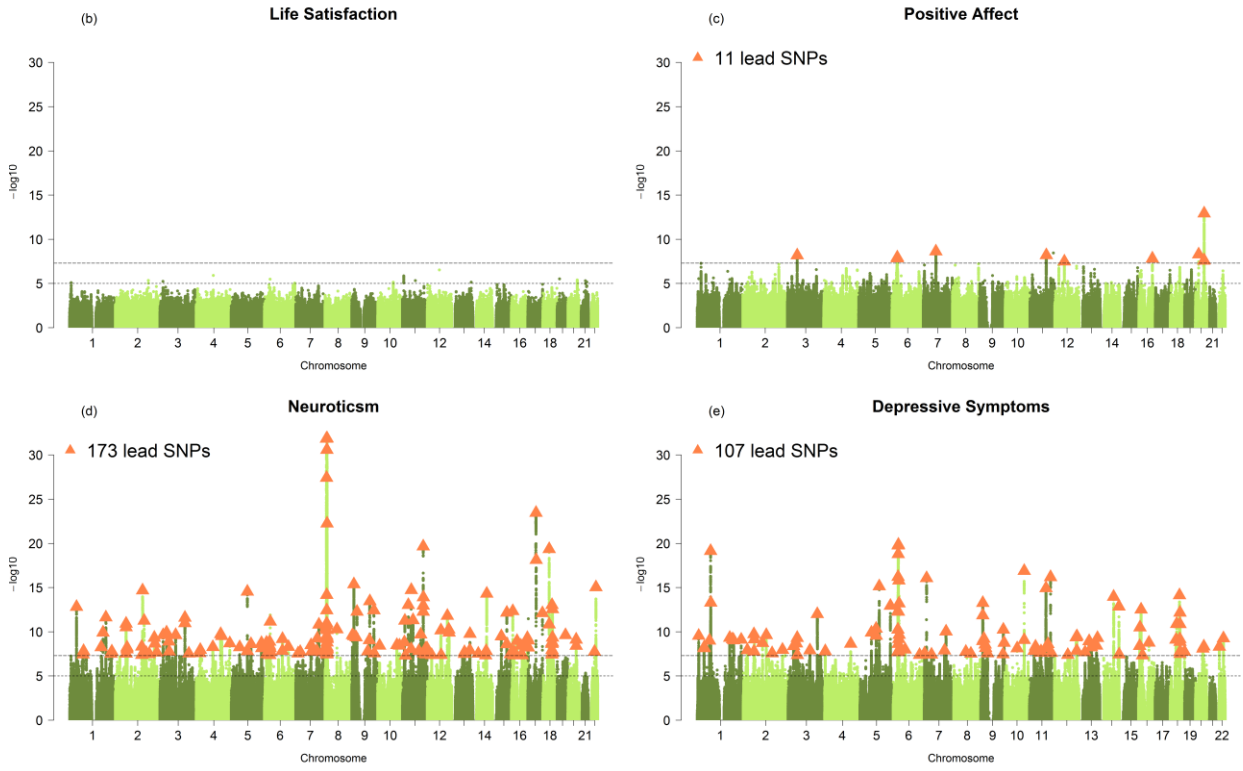
Supplementary Figure 1: Flowchart of the study design showing the trait-specific studies that were combined in the four univariate GWAMA's: Life Satisfaction, Positive Affect, Neuroticism, and Depressive Symptoms.



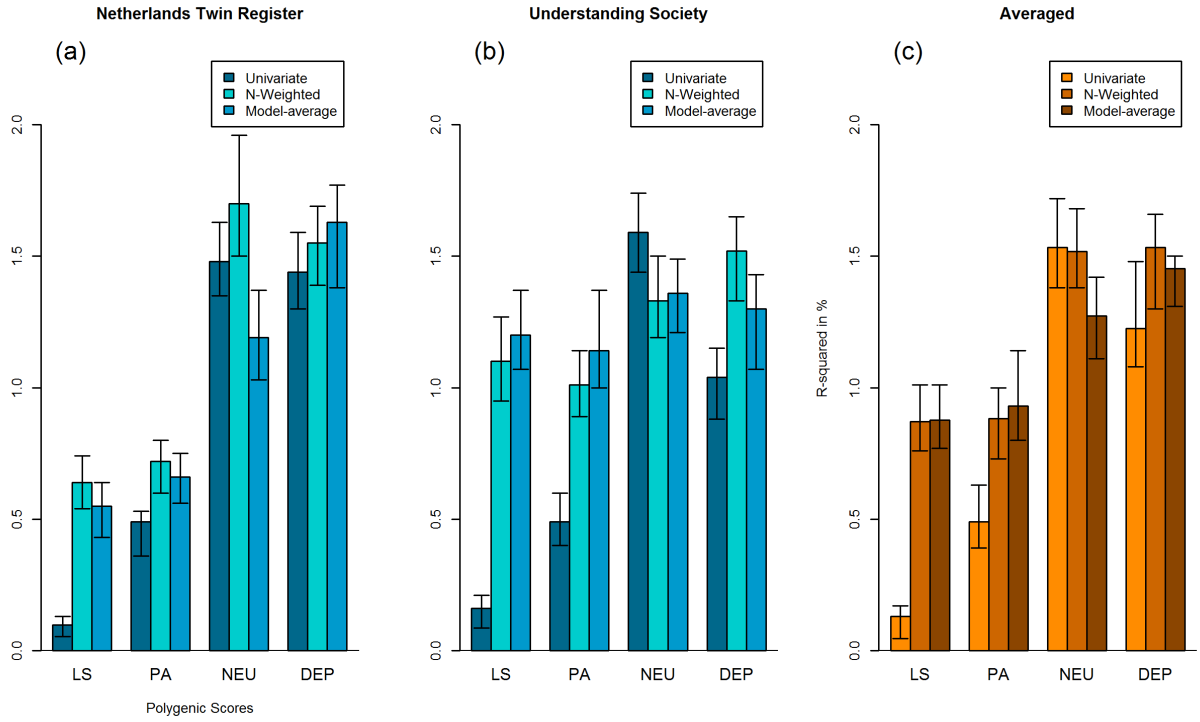
Supplementary Figure 2: Barplot of nine simulation scenarios in which the *rg* between the four traits varied between .9 and .1. The red line represents the mean correlation (of the four traits) between the Beta's of the univariate GWAS and the true effects. Blue represents the correlation of the beta's obtained from the N-GWAMA with the true effects. Green represents the correlation of the beta's obtained from the MA-GWAMA and the true effects.



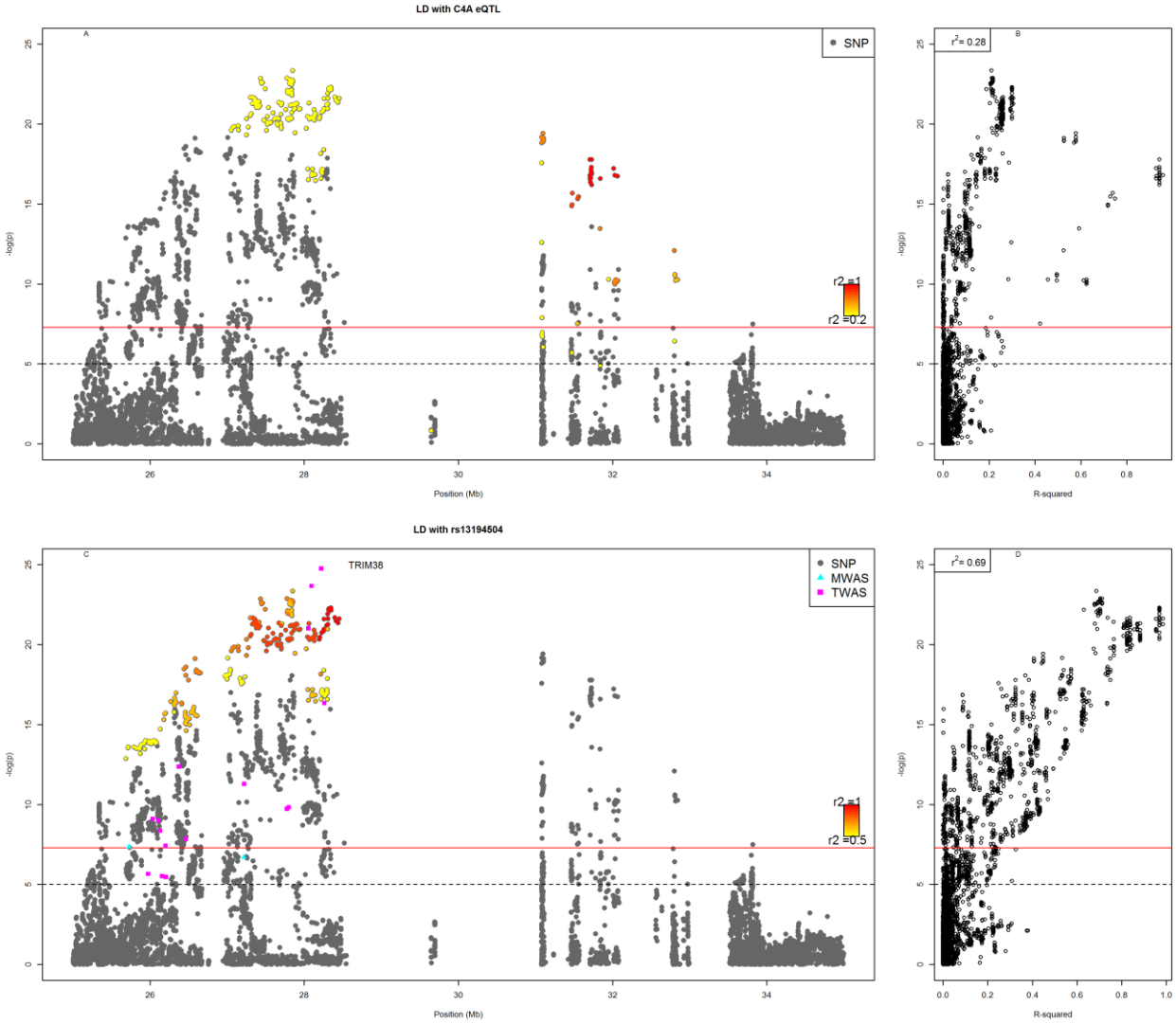
Supplementary Figure 3. Manhattan plots of univariate GWAMA. (**a**) life satisfaction, (**b**) positive affect, (**c**) neuroticism, (**d**) depressive symptoms. The *x*-axis represents the chromosomal position, and the *y*-axis represents the significance on a $-\log_{10}$ scale. Each approximately independent genome-wide significant association ("lead SNP") is marked by Δ.



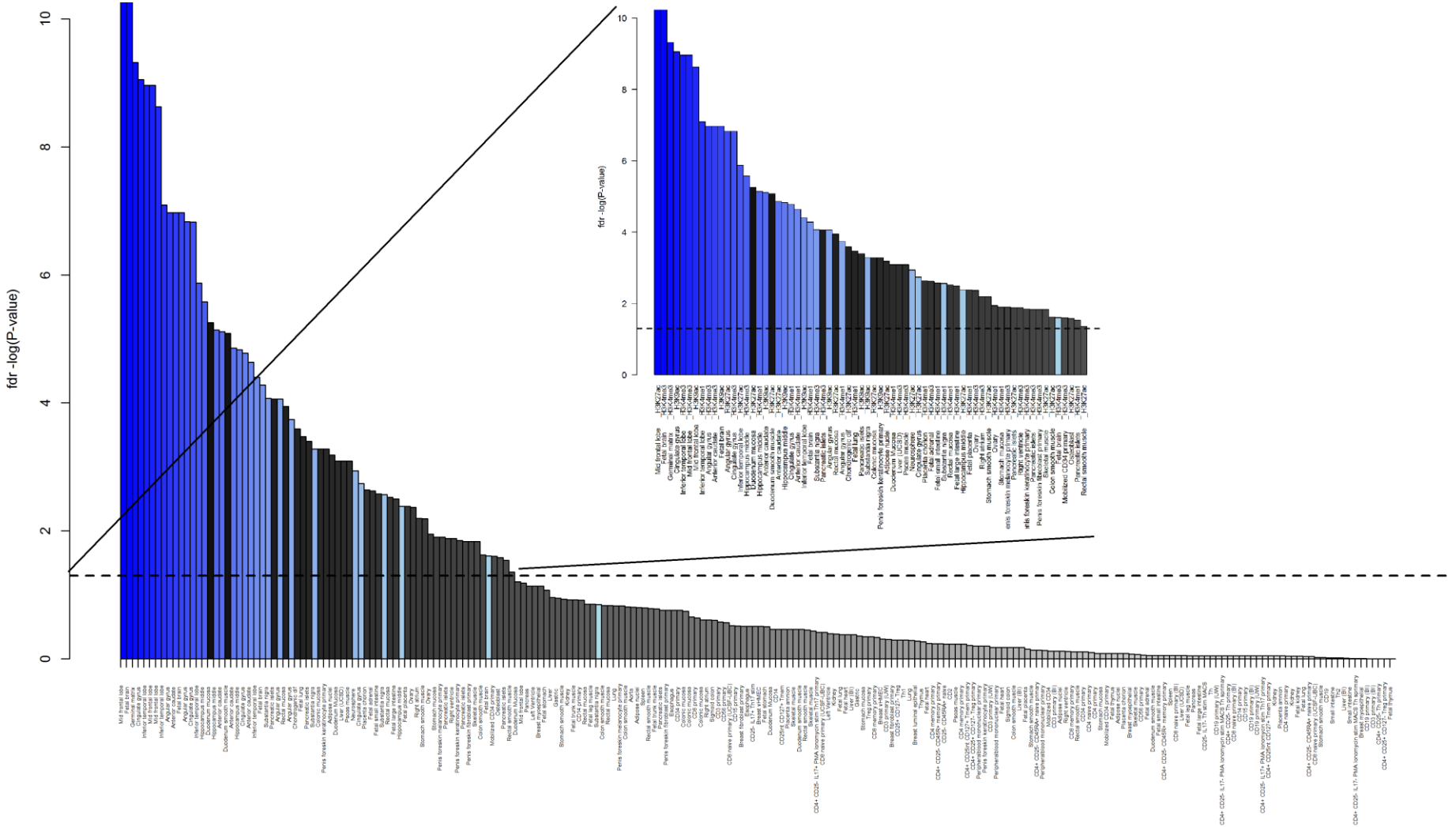
Supplementary Figure 4. The result of polygenic risk prediction based on univariate discovery GWAMA, N-weighted discovery GWAMA or model averaging discovery GWAMA. The unit on the Y-axis is the R-squared in percentage, obtained from a regression of the trait on the PRS, age, sex and 10 principle components (**a**) displays the polygenic prediction results from the Netherlands Twin Register and (**b**) displays the polygenic results from Understanding Society, and (**c**) displays the combined N-weighted polygenic score results. . LS is life satisfaction, PA is positive affect, NEU is neuroticism, and DS is depressive symptoms.



Supplementary Figure 5. Local association in the MHC region. (a) provides a local Manhattan plot for the MHC region with interposed on top the LD with a strong eQTL for the C4 gene linked to neuronal pruning in adolescence and schizophrenia by Sekar et al[27]. (b) is a scatter plot for the $-\log_{10}(p)$ against the R2 with the C4 eQTL. (c) provides a local Manhattan plot for the MHC region with interposed on top the LD with SNP rs13194504, the strongest MHC signal found for schizophrenia. (d) is a scatter plot of the $-\log_{10}(p)$ against the R2 with rs13194504. Round symbols represent SNPs, square symbols represent gene transcripts and triangle symbols represent CpG sites.



Supplementary Figure 6. 220 Cell specific histone modified region enrichment. The bar plot is reflecting the FDR adjusted p-value for tissue specific histone modified regions of the genome, as estimated using partitioned LD-score regression. Blue bars represent brain regions, black bars represent non-brain regions.



05

Epigenome-wide association study of well-being

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Abstract

Well-being (WB) is a major topic of research across several scientific disciplines, partly driven by its strong association with psychological and mental health. Twin-family studies have found that both genotype and environment play an important role in explaining the variance in WB. Epigenetic mechanisms, such as DNA methylation, regulate gene expression and may mediate genetic and environmental effects on WB. Here, for the first time, we apply an epigenome-wide association study (EWAS) approach to identify differentially methylated sites associated with individual differences in WB. Subjects were part of the longitudinal survey studies of the Netherlands Twin Register (NTR) and participated in the NTR biobank project between 2002 and 2011. WB was assessed by a short inventory that measures satisfaction with life (SAT). DNA methylation was measured in whole blood by the Illumina Infinium HumanMethylation450 BeadChip (HM450k array) and the association between WB and DNA methylation level was tested at 411,169 autosomal sites. Two sites (cg10845147, $p = 1.51 * 10^{-8}$ and cg01940273, $p = 2.34 * 10^{-8}$) reached genome-wide significance following Bonferroni correction. Four more sites (cg03329539, $p = 2.76* 10^{-7}$; cg09716613, $p = 3.23 * 10^{-7}$; cg04387347, $p = 3.95 * 10^{-7}$ and cg02290168, $p = 5.23 * 10^{-7}$) were considered to be genome-wide significant when applying the widely used criterion of a FDR q-value < 0.05. Gene ontology (GO) analysis highlighted enrichment of several central nervous system categories among higher-ranking methylation sites. Overall, these results provide a first insight into the epigenetic mechanisms associated with well-being and lay the foundations for future work aiming to unravel the biological mechanisms underlying a complex trait like WB.

Introduction

Because of its strong associations, in individuals and in society, with physical and mental health as well as economic development, well-being (WB) has become a topic of interest across different scientific disciplines[1–3]. In general, WB is conceptualized to include a continuous spectrum of positive feelings and subjective life assessment that can be assessed with a series of measures, such as satisfaction with life (SAT), happiness (HAP) and quality of life (QoL).

Twin-family studies report that in the general population, a substantial part of the variation in the different measures of WB is explained by genetic differences between individuals[4]. A large meta-analysis[5] showed that the weighted average heritability for WB was 36% (95% CI: 34-38, and for SAT 32% (95% CI: 29-35). A multivariate twin-sibling study exploring the etiology of the covariance among multiple WB indices revealed that the genetic variance in the different measures was explained by one underlying set of genes[6]. These results also emphasize the importance of environmental factors in the variation of WB, and a dynamic interplay between genes and environment is to be expected for a complex trait like WB.

Epigenetic regulation of gene expression by mechanisms such as DNA methylation may mediate the interplay between the genetic make-up of individuals and their exposure to the environment[7–9]. Methylation changes can be caused by external conditions, such as long-term stress-exposure[10,11], (prenatal, maternal) smoking exposure [12,13] and dietary modifications at conception[14]. There are no epigenetic studies of the association between DNA methylation and WB, but some epigenetic studies have been performed involving complex traits related to WB. From twin studies, it is known that there is a negative association between psychopathology and WB, with the strongest association between WB and anxiety/depression[15]. Epigenetic differences in candidate genes related to major depressive disorder (MDD) have been reported in multiple studies[16,17]. Additionally, a DNA methylation EWAS of monozygotic twins discordant for adolescent depression [18] found two reproducible differentially-methylated probes (DMPs) that were located within *STK32C,* which encodes a serine/threonine kinase of unknown function.

Here we describe the first EWAS for well-being performed in a population-based sample from the Netherlands Twin Register (NTR). Our aim was to identify genomic locations where differences in DNA methylation in blood level are associated with differences in well-being.

Methods

Subjects and samples

The subjects in this EWAS participated in longitudinal survey studies conducted by the Netherlands Twin Register [19,20] and in the NTR biobank project [21]. Peripheral blood samples were drawn from the NTR participants in the morning after an overnight fast, and for biomarkers studies and for DNA and RNA isolation (see [20]). In 3264 peripheral blood samples from 3221 participants genome -wide methylation probes were assessed. After quality control (QC) of the methylation data, 3089 samples from 3057 participants were retained (for a detailed description of the QC procedures, see van Dongen 2015; *this issue*). For the present study, we included participants if the following information was available: Satisfaction with Life score, good quality methylation data and data on white blood cell counts leaving 2519 samples from 2456 subjects for the final analyses. The dataset included 606 complete MZ and 291 complete DZ pairs, 102 fathers of twins, 112 mothers of twins, 15 siblings and 2 spouses of twins.

All subjects provided written informed consent and study protocols were approved by the Central Ethics Committee on Research, involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the US Office of Human research Protections (IRB-2991 under Federal wide Assurance-3703: IRB/institute codes, NTR 03-180).

Well-being

Well-being was assessed by a short inventory that measures satisfaction with life (SAT)[20]. Data on satisfaction with life were collected in multiple NTR surveys. For the current study, data from surveys 6 (2002), 8 (2009) and 10 (2013) were analyzed. The SAT scale consists of 5 items which have to be answered on a 7-point scale ranging from 1 = *'strongly disagree'* to 7 = *'strongly agree'*. A typical question that is included in this questionnaire is "If I could live my life over, I would change almost nothing" (for all items see Table 1). Internal consistency of the scale was good with a Chronbach's Alpha of 0.86 and test-retest scores in the range of 0.24 (over 16 years), to 0.54 (over 4 years) to 0.84 for a period of two weeks to 1 month[22]. Within this NTR sample the test-retest scores are 0.53 between survey 6 and 8 (7 year interval), 0.48 between survey 6 and 10 (11 year interval) and 0.63 between survey 8 and 10 (4 year interval) and the phenotypic correlation of satisfaction with life with an overall well-

being factor score is 0.97. For individuals who completed survey 6, 8 and 10, the WB score closest to the moment of blood draw was selected.

Table 1: Satisfaction with life scale [50]

Item
SAT item 1 In most ways my life is close to ideal
SAT item 2 The conditions of my life are excellent
SAT item 3 I am satisfied with my life
SAT item 4 So far I have gotten the most important things I want in life
SAT item 5 If I could live my life over , I would change almost nothing

Infinium HumanMethylation450 BeadChip data

DNA methylation was assessed with the Infinium HumanMethylation450 BeadChip Kit (Illumina, Inc.) [23]. 500ng of genomic DNA from whole blood was treated by bisulfite using the ZymoResearch EZ DNA Methylation kit (Zymo Research Corp, Irvine, CA, USA) following the standard protocol for Illumina 450K micro-arrays, by the department of Molecular Epidemiology from the Leiden University Medical Center (LUMC), The Netherlands. Subsequent steps (i.e. sample hybridization, staining, scanning) were performed by the Erasmus Medical Center micro-array facility, Rotterdam, The Netherlands. Quality control and processing of the blood methylation dataset has been described in detail previously[24]. A number of sample-level and probe-level quality checks were performed. Sample-level QC was performed using MethylAid[26]. Probes were set to missing in a sample if they had an intensity value of exactly zero, or a detection P-value > 0.01, or a bead count < 3. After these steps, probes that failed based on the above criteria in > 5 % of the samples were excluded from all samples (only probes with a success rate >= 0.95 were retained). Probes were also excluded from all samples if they mapped to multiple locations in the genome[27], or if they had a SNP within the CpG site (at the C or G position) irrespective of minor allele frequency in the Dutch population (Genome of the Netherlands Consortium 2014). Only autosomal methylation sites were analyzed in the EWAS. The methylation data were normalized with Functional Normalization[28], and normalized intensity values were converted into beta (β)-values. The β-value represents the methylation level at a site, ranging from 0 to 1 and is calculated as:

$$\beta = \frac{M}{M+U+100}$$

where M = Methylated signal, U=Unmethylated signal, and 100 represents a correction term to control the β-value of probes with very low overall signal intensity. After QC and normalization, Principal component analysis was conducted on genome-wide methylation sites.

Covariates

White blood cell percentages were included as covariates in the EWAS to account for variation in cellular composition between whole blood samples. The following subtypes of white blood cells were counted in blood cells: neutrophils, lymphocytes, monocytes, eosinophils, and basophils[20]. Because of its strong correlation with neutrophil counts (r= -0.93), lymphocyte counts were not included in the model, while basophil counts were not included because they showed little variation between subjects (many subjects having 0% of basophils in their blood). Inspection of the PCs that were computed on the genome-wide methylation data indicated that PC1 reflected, as expected, sex (r = 0.99), PC 2 showed a strong correlation with lymphocyte percentage (r = -0.8) and neutrophil percentage (r = 0.79). Additionally, PC3 showed a modest correlation with age (r = -0.41) and a weak correlation with white blood cell percentages (absolute r ~0.1). However, it is possible that this PC is reflective of unmeasured white blood cell subtypes and was therefore included in the model. Because of their correlation with several lab procedures, such as sample plate and order of processing, PC4 and PC5 were included to account for technical variability (For a graphical representation of the included PC's, see Supplemental figure S1).

Epigenome-wide Association analysis

Using generalized estimation equation (GEE) models, we tested whether DNA methylation was associated with WB for each methylation site, with DNA methylation β-value as outcome variable and the following predictors: WB score, sex, age at blood sampling, age squared, monocyte percentage, eosinophil percentage, neutrophil percentage, HM450k array row, and Principal Components (PCs) 3, 4 and 5 from the methylation data. Age squared was included as a covariate as several studies suggest a U-shaped relationship between WB and age, with the lowest point approximately in midlife[29,30]. GEE models were fitted with the R package gee, with the following specifications: Gaussian link function (for continuous data), 100 iterations, and the "exchangeable" option to account for the correlation structure within families and within persons.

FDR q-value was computed with the R package q-value with default settings. The genomic inflation factor (λ) was calculated with the default regression method from the R package GenABEL. In all analyses, an FDR q-value <0.05 was considered statistically significant[31]. Additionally, a more stringent Bonferonni correction was applied by dividing 0.05 by the number of observations (N = 411169). Follow-up analyses, including a test for enrichment of genomic locations and gene ontologies, were performed based on the output from the EWAS.

Genomic Annotation

As described in detail by Slieker et al. (2013), methylation sites were mapped to genomic features and DNase I hypersensitive sites (DHS). These genomic features consists of five gene-centric regions: (1) intergenic regions (>10kb to -1.5 kb from the nearest transcription start site (TSS)), (2) proximal promotor (-1.5 kb to +500kb form the nearest TSS), (3) distal promotor (-10 kb to -1.5 kb from the nearest TSS), (4) gene body (+500bp to 3' end of the gene) and (5) downstream region (3'end to +5 kb from 3'end). Additionally, CpG were mapped to CG island (CGIs) (CG content > 50% length > 200bp and observed/expected ratio of CpGs > 0.6), CGI shore (2kb region flanking CGI), CGI shelf (2kb region flanking CGI shore), or non-CGI regions. Locates were obtained from the UCSC genome browser[33]. DHS locations, mapped by the ENCODE project[34] were also downloaded from the UCSC genome browser[33].

Enrichment of genomic locations

To test whether specific genomic locations showed a stronger association between DNA methylation and WB, eight categories were tested for being enriched using the EWAS test statistics for all genome-wide methylation sites The locations tested are: (1) gene body, (2) proximal promoter, (3) distal promoter, (4) downstream region, (5) CGI, (6) CGI shore, (7) CGI shelf, and (8) DHS. To account for differences in variability between methylation sites we also included the mean and standard deviation of DNA methylation level in the model as covariates. For a detailed description of the method used for this analysis, see also Van Dongen et al. (2015), published in this same special issue of TRHG.

Enrichment of the Gene Ontology terms

Methylation sites with a stronger association with WB were tested for enrichment of Gene Ontology (GO) terms. To do so, all methylation sites that were tested were ranked by EWAS p-value and the resulting ranked gene list was supplied to the online software tool GOrilla [36]. GOrilla performs GO enrichment analyses based on gene rank, and therefore no p-value cut-off for defining the input list of genes is required. The background in this analysis consists of all genes for which methylation sites were analysed in the EWAS. In all analyses, we accounted for multiple testing by controlling the false discovery rate (FDR). An FDR q-value < 0.05 was considered statistically significant.

Results

Table 2: characteristics of the well-being data

		Well-being					Age at survey			Age at blood sampling		
Data	N	Mean	Median	Sd	Min	Max	Mean	Median	Sd	Mean	Median	Sd
Survey 6	10087	26.62	28	5.26	5	35	39.44	34.00	14.35			
Survey 8	19746	27.22	29	5.32	5	35	40.41	39.94	16.32			
Survey 10	11604	26.80	28	5.24	5	35	44.42	47.00	17.49			
EWAS[A]	2519	27.02	29	5.46	5	35	38.30	35.33	13.53	36.79	33.10	13.01

[A]Includes individuals with 450k methylation data and data on white blood cell counts.

Characteristics of the study sample

Data on well-being were available for 1747 individuals who filled out survey 6, 2056 individuals who filled out survey 8 and 1059 individuals who filled out survey 10. The EWAS was performed on 2519 blood samples from 2456 subjects (mean age at blood sampling = 36.8 years, SD = 13, % male = 31.1), for which the well-being score closest to the moment of blood draw was selected: For 1799 samples, WB was assessed after blood draw (mean 3.1 years) and for 720 samples, WB was assessed before blood drawn (mean 2.5 years). Table 2 summarizes the characteristics of the WB data and EWAS study sample. The average WB score of the EWAS study sample was comparable to the averages of the different survey waves (mean EWAS study = 27.0, mean survey 6 = 26.8, mean survey 8 = 27.5, mean survey 10 = 27.0), and was also comparable to the mean of the overall NTR survey database satisfaction with life score (26.9, n= 38,740) (For a distribution of the well-being data, see Supplementary figure S1).

EWAS

After QC, we tested 411,169 autosomal sites in the genome for their association between DNA methylation and WB score, while correcting for white blood cells counts, age at blood sampling, age squared, sex, array row and 3 PCs from the methylation data. Figure 1 shows the Quantile-Quantile (QQ) plot. The genomic inflation factor (λ) was 1.227.

Figure 1 Quantile-Quantile (QQ) plot from the EWAS of well-being. The observed p-values (y-axis) are plotted against the p-values expected under the null hypothesis (x-axis). The straight diagonal line denotes the pattern that is expected under the null hypothesis, with 95% confidence intervals indicated by the shaded grey area.

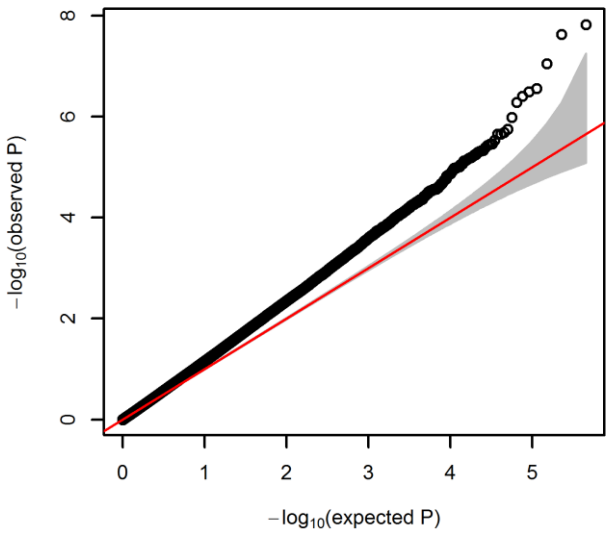
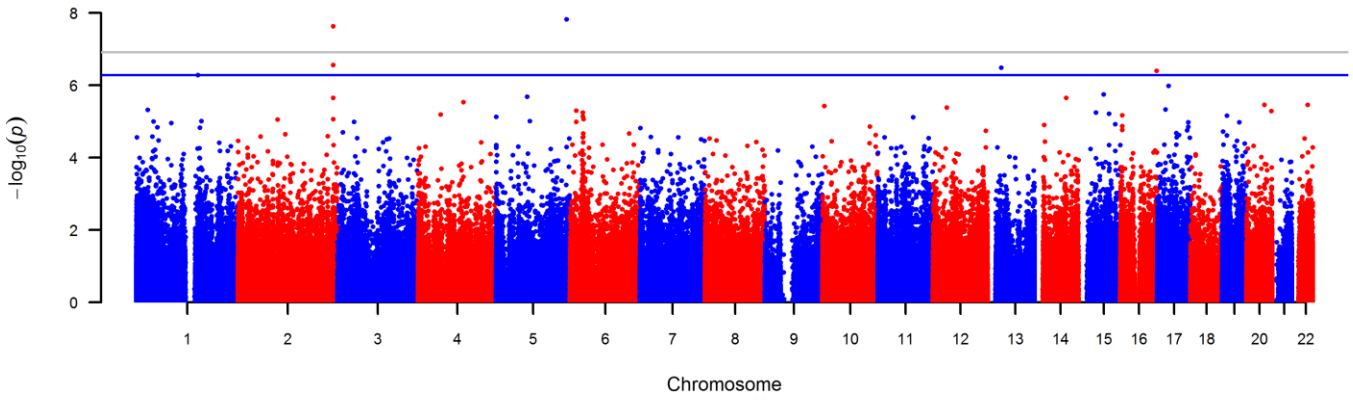


Figure 2 Manhattan plot showing the P-values for the association between well-being and DNA methylation level at genome-wide autosomal sites. The horizontal grey line represents the bonferroni-adjusted p-value threshold. The blue horizontal line represents the FDR q-value <0.05.



Two of the methylation sites reached the genome wide significant threshold of $p = 1.22 * 10^{-7}$ when Bonferroni corrected and six of the methylation sites reached genome significance when using a threshold of FDR q-value < 0.05 ($p = 5.23 * 10^{-7}$) (figure 2). The highest ranking methylation site was cg10845147 ($p = 1.51 * 10^{-8}$), located on chromosome 5: 172149624, which was negatively associated with WB (figure 3A). The nearest gene associated with this site is the *DKFZP761M1511* gene (synonym is *NEURL1B*). This gene spans 50274 base pairs (bps) of chromosome 5 and ranges from 172641266 to 172691540. The other site reaching Bonferroni genome-wide significance is cg01940273 ($p = 2.34 * 10^{-8}$), located on chromosome 2:233284934, which showed a positive relationship between DNA methylation and WB (figure 3B). The gene closest located to this site is the *ALPPL2* gene, which ranges from chromosome 2:232406843 to 2: 232410714 (3871 bps) (Supplementary Figure S2). The four additional CpG sites that were genome-wide significant using FDR q-value < 0.05 are cg03329539 ($p = 2.76* 10^{-7}$, chromosome 2), cg09716613 ($p = 3.23 * 10^{-7}$, chromosome 13), cg04387347 ($p = 3.95 * 10^{-7}$, chromosome 16), and cg02290168 ($p = 5.23 * 10^{-7}$, chromosome 1). The significant CpG sites located on chromosome 1 and 2 were positively associated with WB, whereas the CpG sites located on chromosome 13 and 16 were negative associated with WB. Characteristics of genome wide significant methylation sites as well as the location of the nearest genes are provided in table 3.

Table 3: Top-ranking CpG sites from the EWAS of well-being

CpG site	Chr	Position (hg19)	(Nearest) Gene name	Distance gene from each site (bps)	Mean Methylation level [A]	SD Methylation level [A]	Estimate[B]	Robust SE[C]	p-value
cg10845147	5	172149624	*DKFZP761M1511*	491642	0.69	0.04	-0.00072	1.27 E-04	1.51 E-08
cg01940273	2	233284934	*ALPPL2*	874220	0.66	0.05	0.00101	1.80 E-04	2.33 E-08
cg03329539	2	233283329	*ALPPL2*	874220	0.45	0.05	0.00065	1.27 E-04	2.76 E-07
cg09716613	13	33000534	*CG018*	572356	0.27	0.04	-0.00052	1.02 E-04	3.23 E-07
cg04387347	16	88537187	*ZFPM1*	171	0.22	0.05	-0.00075	1.48 E-04	3.94 E-07
cg02290168	1	151255971	*ZNF687*	25647	0.12	0.02	0.00030	5.98 E-05	5.23 E-07

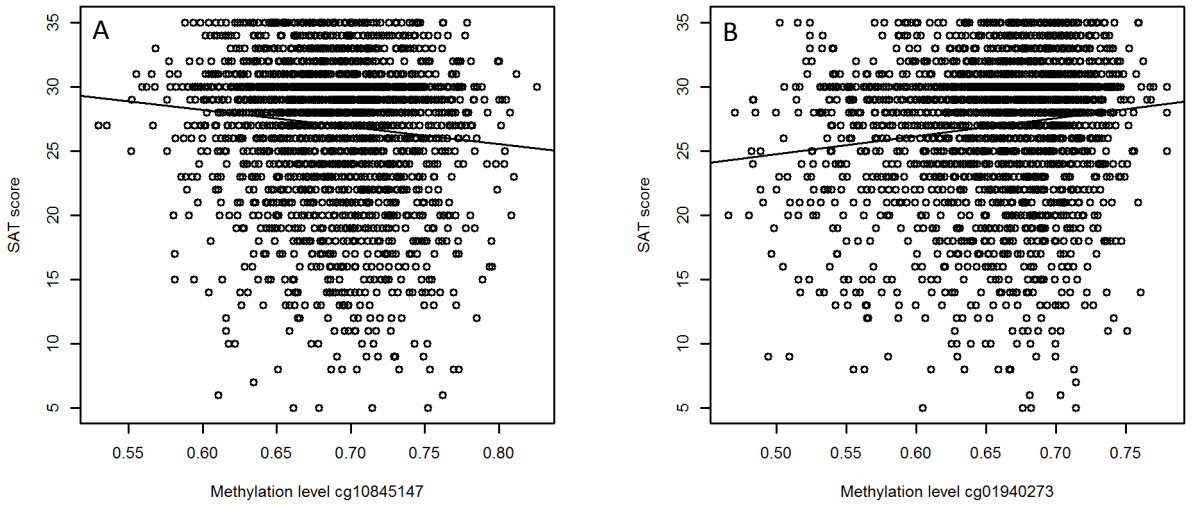
Top hits from the EWAS for the association between methylation and well-being

[A] Mean and standard deviation of the methylation proportion (β-value) in the entire 450K cohort

[B]Estimate from the regression of methylation proportion on well-being score.

[C]Robust standard error of the estimate (accounting for the clustering of observations within families)

Figure 3. Scatterplots for the two top CpGs based on the entire NTR study sample. Well-being scores are plotted against methylation level. **[**A] shows the relationship between WB and methylation level of CpG site cg10845147. [B] shows the relationship between WB and methylation level of CpG site cg01940273.



Next, we looked at the association with WB for all CpGs in relatively close proximity (within 10,000 bp = 10kb) of each significant CpG site (for an overview of all CpG sites located within this window and their p-value and regression coefficient, see Supplementary table S1). For the highest ranked CpG site (cg10845147, chromosome 5), CpG site cg07853407 was located closest at 2563 bps. This side showed no association with WB ($p = 8.17 * 10^{-1}$, ß $-1.22 * 10^{-5}$). The two genome-wide significant CpG site on chromosome 2 were located within 1606 bps from each other. Within this window, five additional probes were measured. Al of these probes showed a change in methylation in the same direction of association with WB (see Supplementary table S1). On chromosome 13, CpG site (cg12054869) was located closest to the significant CpG site (cg09716613) at 716 bps, while for chromosome 16, the CpG site located most closely to the leading CpG site (cg04387347) was located 73 bps away. Finally, the CpG site located closest to the leading CpG site at chromosome 1 was cg01062937 at 668 bps. For each of these probes, the regression coefficient for WB indicated a similar direction of effect as the significant probe in that specific region.

Enrichment of genomic locations

Table 4 shows the results of the regression of the EWAS test statistics on annotation categories across all genome-wide sites. Enrichment of signal was seen in the gene body (p =

$1.34 * 10^{-5}$), proximal promoters ($p = 6.01 * 10^{-18}$), CGI shores ($p = 9.26 * 10^{-10}$) and DHS ($p = 3.68 * 10^{-14}$). CpG sites with a lower mean methylation level, showed, on average, a stronger association with WB ($p = 9.13 * 10^{-15}$). To account for the fact that the errors in this regression are not normally distributed, jackknife standard errors were computed, but this analysis led to the same conclusions as the normal linear regression standard errors (see supplementary table S2). These findings indicate that methylation sites associated with well-being are enriched in gene bodies promoter areas and other regions of regulatory active DNA.

Table 4: Results from the regression of EWAS test statistics on genomic annotation categories.

Regression parameter	Estimate	Std. Error	t value	P value
Intercept	1.110	0.012	85.59	0
Gene Body	0.035	0.008	4.35	$1.34 * 10^{-5}$
Proximal Promotor	0.077	0.009	8.63	$6.01 * 10^{18}$
Distal Promotor	0.009	0.014	0.60	0.54
Downstream Region	0.022	0.021	1.05	0.29
DNase I hypersensitive site (DHS)	0.048	0.006	7.57	3.68 * 10-^{14}
CGI Shore	0.048	0.008	6.12	$9.26 * 10^{-10}$
CGI Shelf	0.002	0.010	0.23	0.82
CpG Island	0.001	0.008	0.06	0.94
Mean methylation level[A]	-0.086	0.011	-7.75	$9.13 *10^{-15}$
SD methylation level[B]	3.57	0.182	19.64	$6.63 * 10^{-86}$

[A]Mean methylation level of the site.

[B]Standard deviation of the methylation level.

Results based on jackknive are presented in Supplementary Table 2.

Gene ontology analysis

Gene ontology enrichment analysis based on EWAS p-value rank identified a large number of GO terms that were significantly enriched among higher ranked methylation sites. The strongest enriched GO term were positive regulation of biological processes (GO:0048518, $p = 5.38 * 10^{-21}$), positive regulation of cellular processes (GO:0048522, $p = 1.34 * 10^{-16}$) and developmental processes (GO:0032502, $p = 2.38 * 10^{-16}$). Also, many brain and central nervous system processes, such as regulation of neurogenesis (GO:0050767, $p= 3.72 * 10^{-12}$), neuron projector guidance (GO:0097485, $p = 5.77 * 10^{-10}$), neurotrophic signaling pathway (GO:0038179, $p = 2.53 * 10^{-8}$) as well as regulation of neuron differentiation (GO:0045664, $p = 1.81 * 10^{-8}$) were significant enriched among higher ranking methylations sites (see supplementary table S3 for a complete list of significant GO terms).

Discussion

By performing an epigenome-wide methylation analysis, the aim of the present study was to identify genomic locations at which differences in DNA methylation level are associated with differences in well-being in a population-based sample of adults. Six genome-wide significant hits were identified after correction for multiple testing (FDR q-value < 0.05), while two hits remained significant after applying the stricter Bonferonni correction. Annotation analysis showed that enrichment of signal was seen in the gene body, proximal promoters, CpG shores and DNase I hypersensitive sites. Gene ontology analysis, which tests categories of genes instead of single methylation sites, revealed that genes involved in regulation of cellular processes and central nervous system processes were enriched among higher-ranking genes from our EWAS. Here we describe the six CpGs that were genome-wide significant using an FDR q-value < 0.05. The gene located closest (at ~500kb distance) to our top-ranked CpG site cg10845147 (genomic location: chr5:172149624) is *DKFZP761M1511* (synonym is *NEURL1B*). *NEURL1B (*Neuralized E3 Ubiquitin Protein Ligase 1B) is a ligase that is involved in the regulation of the Notch pathway by influencing the stability and activity of several Notch ligands. Notch pathways acts as a regulator of cell survival and cell proliferation[37,38] and are suggested to play a role in human mammary development[39]. ENCODE data on Transcription Factor Binding site (TFBS) and DNase hypersensitivity sites (DHS) were accessed through the UCSC genome browser and showed that our top CpG, cg10845147 does not overlap with a TFBS but is located within a DHS peak in several cell types, suggesting that it is located within a regulatory region (ENCODE TFBS ChIP-seq data Mar 2012 Freeze).

The second and third ranked CpG site (cg01940273, location: chr2:233,284,934 and cg03329539, location: chr:233,283,329) are approximately 875kb located from the nearest gene called alkaline phosphatase, placental-like 2 (*ALLPL2*). Alkaline phpsphatases (ALPs) are responsible for the dephosphorylation of various molecules including proteins, nucleotides or alkaloids. Circulating ALP concentration is associated with premature birth[40], and low birth weight[41]. ALLP2 enzyme levels are increased up to 10-fold in 80% of cigarette smokers[42] and elevated in patients with a number of cancers [43]. Both CpGs found in our study have been associated with smoking in several studies[44–46]. In those studies, it was shown that methylation at multiple CpGs, including our two CpGs was decreased among heavy smokers, but slowly increased among former smokers. Because of their association with smoking, we tested whether adding smoking as covariate would alter the significance level of the two

CpGs. For both sites, the p-values did not reach the genome-wide significance threshold when adjusting for smoking (cg01940273, $p = 1.21 * 10^{-5}$ and cg03329539, $p = 7.89 * 10^{-5}$). Although not significant anymore, the association with WB is reduced rather than fully attenuated when correcting for smoking. The regression coefficient remained in the same direction as before, suggesting a positive relationship between WB and an increase in methylation. A growing field of research has been focusing on the effects of smoking cessation on well-being. The general findings of these studies are all pointing in the same direction: smoking cessation improves well-being. For instance, a study by Wilson et al. (1999) found that light, moderate, and heavy smokers scored significantly lower than never-smokers as well as ex-smokers on the health-related quality-of-life scale (HR-QoL), with the strongest difference between heavy smokers and never-smokers. A similar finding was found by (Piper et al. (2012) and Shahab & West (2012), who found that successful quitters reported improved subjective well-being, in contrast to continuing smokers, after one to three years. Finally, the genes, located nearby the other significant CpG sites, were *CGO18/ N4BP2L1* (chromosome 13), *ZFPM1* (chromosome 16) and *ZNF687* (chromosome 1) and have not been previously linked to well-being or related phenotypes.

Additionally, we looked up the top 3 probes associated with depression as reported by Dempster et al. (2014), and the genes located most closely to these probes (*DPYSL4, STK32C, KIF13B, DUSP4, PQLC3* and *KCNF1)* to investigate whether these genes are also associated with WB. The six genes were located in close proximity to 341 probes in our dataset, but none of these probes reach genome wide significance with p-values ranging from $p = 0.001$ to $p = 0.99$. Also, alterations in DNA methylation of the *BDNF* pathway have been associated with depression[16]. However, the 80 probes lying in close proximity in our dataset did not show an association with WB with p-values ranging from 0.003 to 0.99 (for a complete overview see Supplementary Table S4).

A limitation of this study is that we did not have access to a validation data set. Therefore, future studies are warranted, especially for follow-up of the CpG sites that reached genome-wide significance. Additionally, we limited this study to WB and did not consider other aspects of behaviour such as different personality traits or psychiatric symptoms. Since WB is strongly associated with a wide-range of mental health diseases like depression or neuroticism, DNA methylation levels associated with WB as measured in this study may be informative for associated phenotypes. The ideal EWAS approach would therefore encompass WB and related phenotypes (e.g. different aspects of personality and depression).

Such an approach would give insight into which methylation sites are common for WB and its related phenotypes and which sites are specific for WB.

In conclusion, this study provides the first genome-wide methylation association study of well-being. We found six genome-wide significant DNA methylation sites of which two remained significant after the more stringent Bonferonni correction. Once genetic variants have been identified for well-being, future studies that integrate both genetic and – epigenetic information are warranted to investigate the intriguing interplay between genetic and environmental mechanisms in a complex trait like well-being.

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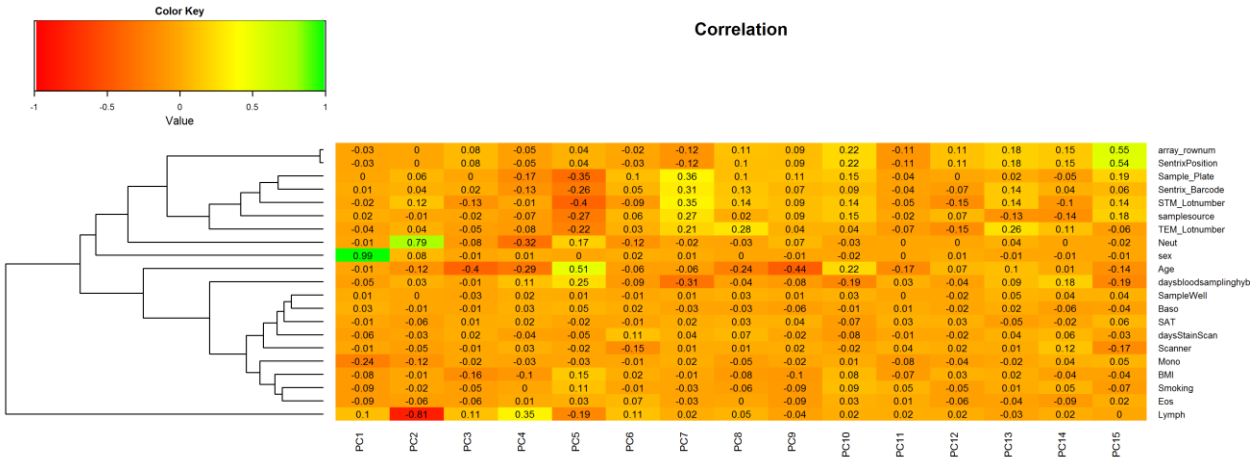
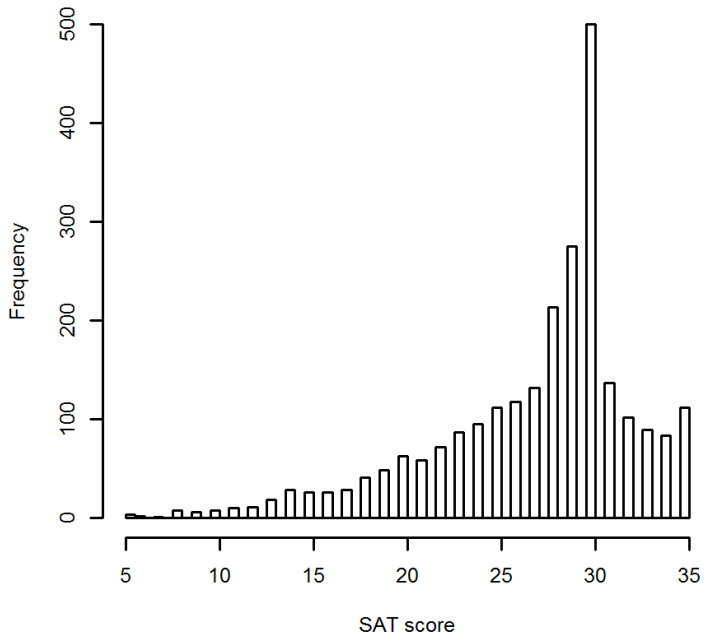
Supplementary Information

Supplementary Figure 1: Heatmap correlations between the PC's and the covariates



Supplementary Figure 2: Distribution of wellbeing scores used in this EWAS



06

Epigenome-wide analyses of well-being through direct epigenetic measurement and Mendelian Randomization

Abstract

We performed an epigenome wide association studies (EWAS) meta-analysis of well-being, where individual differences in CpG site methylation in whole blood are associated with individual differences in well-being. We control for two well-known confounders of epigenetic associations, smoking and BMI. However, we are aware of the effect that potential unmeasured confounders could have on our results as well as uncertain of the direction of causation of the association between well-being and CpG methylation. To guard against unmeasured confounding and to infer a direction of effect we perform Mendelian Randomization, specifically we perform summary-based Mendelian Randomization (SMR). We perform SMR in which the (*cis*) QTL effect of SNPs on methylation (*cis*-mQTL), and a large GWAS of wellbeing are combined to infer the (causal) effect of CpG methylation on well-being. We perform SMR leveraging *cis*-mQTLs discovered in both blood and brain tissues and compared results between tissues, and between SMR and EWAS. We found a high consistency of direction of effect ($r > .9$) between SMR results where the QTL is discovered in different whole blood datasets as well as high consistency between whole blood and fetal brain datasets ($r = .72$). However, when comparing the direction of effect between our EWAS and SMR results, no notable correlations were observed. Our results indicate that if the aim is to increase our understanding of the functional consequences of epigenetic changes on wellbeing, SMR may be preferred over EWAS in whole blood. If, however, the aim is to identify ways in which well-being Is itself a driver of environmental influences on differences in DNA methylation, possibly effecting gene-expression, a sufficient powered EWAS study will provide valuable information. The concurrent use of Mendelian Randomization and epigenome-wide association analysis proved to be a potent combination to further increase our understanding of the relation between well-being and CpG methylation.

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Introduction

Well-being is linked to numerous determinants and behaviors across the life course, such as income and employment, health, neighborhood environment (e.g. green space), air pollution, smoking, stress, alcohol use and several social factors such as friendship patterns (see review Diener and colleagues[22]). These exposures and behavioral characteristics are potential candidate drivers of differential epigenetic patterns between individuals having higher or lower levels of well-being. To our knowledge, our previous work is the only study that investigated the association between methylation differences and phenotypic variation in well-being (N = 2,519), reporting two CpG sites (cg10845147, $p = 1.51 * 10^{-8}$ and cg01940273, $p = 2.34 * 10^{-8}$) reached genome-wide significance after Bonferroni correction[23]. Gene ontology (GO) analysis highlighted enrichment of several central nervous system categories among higher-ranking methylation sites. However, replication of these results is warranted in larger samples.

Despite these positive outcomes from the epigenome-wide association studies (EWAS), there are interpretational problems which may complicate distilling etiology and biology from epigenetic studies[24–29]. The foremost interpretational difficulty is the uncertainty about cause and effect, e.g. does methylation causally influences complex trait outcomes, is the causal effect reverse, or does a third trait influences both methylation levels and traits? For instance, a recent study found using a stepwise Mendelian Randomization analysis, that differential methylation is the consequence of inter-individual variation in blood lipid levels and not vice versa[30]. Considering the tissue-specific nature of epigenetic processes, a second important consideration for EWAS is the assessment of methylation of trait relevant tissue. Empirical results suggest that easily accessible tissues, such as whole blood, cannot be used to address questions about inter-individual epigenomic variation in inaccessible tissues, such as the brain. Hannon et al. explored covariation between tissues and found that, for the majority of the genome, a blood-based EWAS for traits where brain is presumed to be the primary tissue of interest will provide limited information relating to underlying processes[31]. This finding is enforced by another study, which found that only 7.9% of CpG probes, obtained in a sample of epilepsy patients, showed a substantial and statistically significant correlation between blood and brain tissue[32].

However, there is reason for optimism, as recent studies utilizing GTEx data showed that the genetic correlation of gene expression between tissues in local regions (i.e < 1MB of the

transcription start site) is much higher than in distal regions[33,34]. This optimism is further supported by a recent study that found there is no evidence for tissue relevant eQTLs enrichment for associations with complex traits[35]. In this context, the question arises whether this holds for methylation QTLs (mQTLs) and to what extent the cis-genetic effects on DNA methylation in blood differ from those in brain. Capitalizing on this strong, cross tissue local genetics effects on methylation levels, Zhu *et al*. developed summary-based Mendelian Randomization (SMR), to infer the effect of eQTLS and mQTLs on complex traits[36]. *Mendelian Randomization* relies on the presence of genetic variants which confer a risk for an exposure of interest (CpG methylation in this case), as people do not self-select into a particular genotype group at birth, the genotype which indexes variation in CpG methylation can be considered random with respect to the outcome (well-being). Thus Mendelian Randomization offers a pseudo controlled experiment of the effect of variation in CpG methylation on well-being. SMR integrates summary-level data from GWAS together with data from eQTL *or* mQTL studies to identify genes whose expression levels, or CpG sites who's methylation level, are associated with a complex trait due to pleiotropy. Pleiotropy in this case refers to a single causal variant underlying differences in gene expression/DNA methylation *and* phenotypic variation, which is of more biological interest than linkage, where, in the case of two distinct causal variants, one affects gene-expression or CpG methylation and the other trait variation. The observed Pleiotropy between a CpG site and a trait will likely be caused by an effect of CpG methylation on the outcome. A previous study[37] leveraged SMR to infer the relationship between CpG methylation (either in blood or brain) in over 40 different complex traits. Their results were highly consistent between both tissues, specifically, because local *cis* genetic regulation of methylation does not differ strongly across blood and brain tissues. The cross tissue stability in *cis*-regulation was supported by a recent study[38]. that reported a high *cis*-genetic correlation (r = .78) between CpG methylation in brain and blood samples. Thus, several empirical results seem to support that while the relation between complex trait and methylation is tissue dependent, and individual differences in methylation (directly measured) do not correlate strongly between blood and brain tissues, local genetic regulation of methylation level is correlated across tissues.

The present study assessed methylation differences associated with differences in well-being using two study-designs.

1) Epigenome-wide Association meta-Analysis

We performed large association meta-analyses of well-being and genome-wide DNA methylation in whole blood (Illumina 450K array) samples of adult participants from twelve population-based cohorts (**Supplementary Table 1-3**). We performed two EWAS meta-analyses ; (1) A basic model not corrected for smoking behavior and body mass index (BMI; *N* = 9,496) and (2) An adjusted model corrected for smoking behavior and BMI (*N* = 8,463).

2) *Summary-based Mendelian Randomization (SMR) with genome-wide mQTL data*

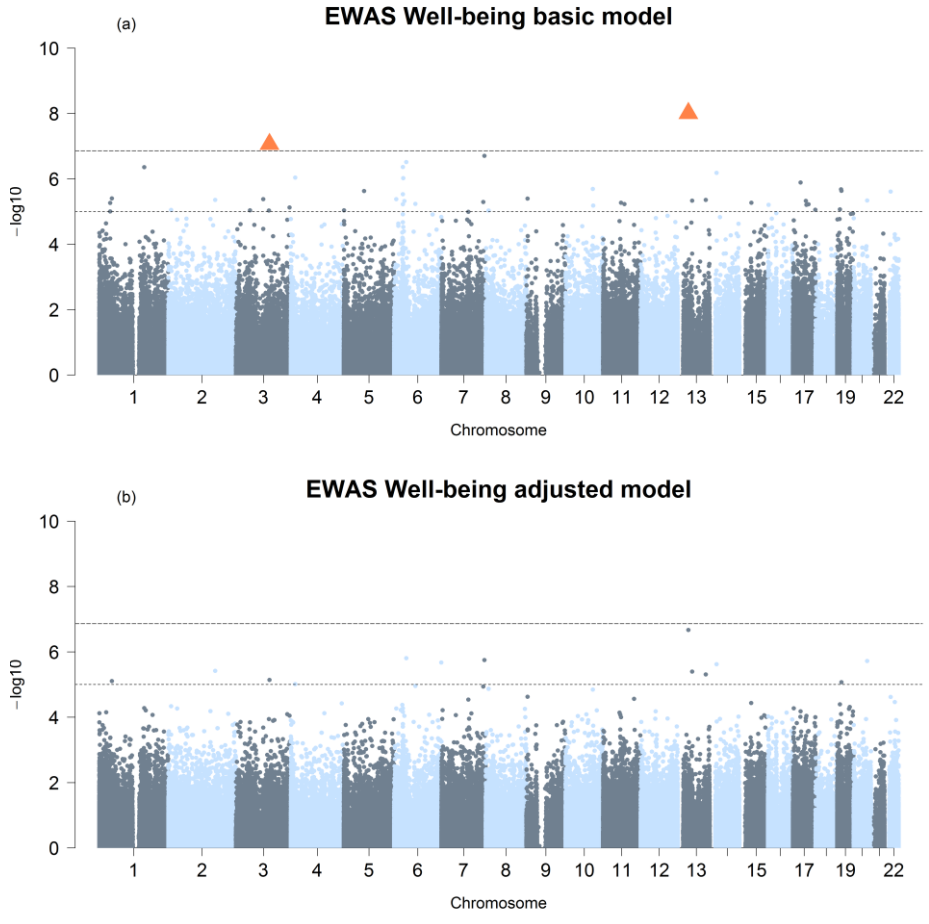
We performed a genome-wide meta-analysis (GWAMA) of well-being (**Supplementary Table 4**) and integrated the results with four publically available mQTL datasets (three whole-blood and one fetal brain dataset) using SMR[39,40,41]. CpG sites identified using this approach might provide important leads to design further functional studies to understand the mechanisms by which DNA variation leads to complex trait variation. Besides identifying CpG probes associated with well-being, an important aim of the current study was to assess the concordance between an EWAS, where a direct association between well-being and CpG methylation is tested, and SMR where the local genetic effects on methylation are used to infer which CpG sites effect well-being.

Results

Epigenome-Wide Meta-Analyses.

Genome-wide DNA methylation analyses were performed for our basic model (N = 9,496) and adjusted model (N = 8,463). Cohort specific EWAS summary statistics were combined in a fixed effects meta-analysis adjusted for test-statistic bias and inflation[42] (Bayesian estimates of bias and inflation from all analyses are provided in **Supplementary Table 3**). Our basic model (not adjusted for smoking and BMI) identified two probes (cg19275632; $P < 9.84 \text{ X } 10^{-9}$ and cg14535274: $P < 8.73 \text{ X } 10^{-8}$) significantly ($P < 1.38 \text{ X } 10^{-7}$) associated with well-being (**Figure 1A).** However, when adjusting for smoking and BMI, no genome-wide significant SNPs were observed (**Figure 1B).** As expected, a significant correlation between the Z-statistics of the basic –and adjusted model was observed (r =.98, $P < 2.2 \text{ X } 10^{-16}$; **Supplementary Fig 1**).

Fig. 1. Manhattan plots of the EWAS analyses. (**a**) EWAS from the basic model, and **(b)** EWAS from the adjusted model (corrected for smoking and BMI). The *x*-axis represents the chromosomal position of the CPG sites, and the *y*-axis represents the significance on a $-\log_{10}$ scale. Each approximately independent genome-wide significant association ("lead CPG site") is marked by Δ.



SMR analyses using methylation QTLs

We applied the SMR approach to test the association between DNA methylation probes and well-being, using mQTLs identified in a dataset of methylomic variation in whole blood and imputed SNP genotypes from the Lothian Birth Cohort (N = 1366)[44] in conjunction with a multivariate GWAMA[43] of well-being (N_{obs} = 491,455; **Supplementary Fig 2 and Supplementary Table 5**). The first stage of the SMR analysis identifies the most significantly associated SNP for a DNA methylation site (that is also present in the GWAMA dataset) as an instrumental variable for testing for association with well-being. This approach yielded 3 significant associations ($P < 5.65 \times 10^{-07}$ corrected for 88,531 tests; **Supplementary Table 6** and **Fig 2A**) between well-being and DNA methylation probes.

Because the associations can be driven by highly correlated yet different causal variants for well-being and DNA methylation, also known as linkage, the second stage in SMR tests for heterogeneity in the association analysis by performing a heterogeneity in dependent instruments (HEIDI) test. The 3 significant associations survived the HEIDI test ($P > 0.05$) and can be described as pleiotropic.

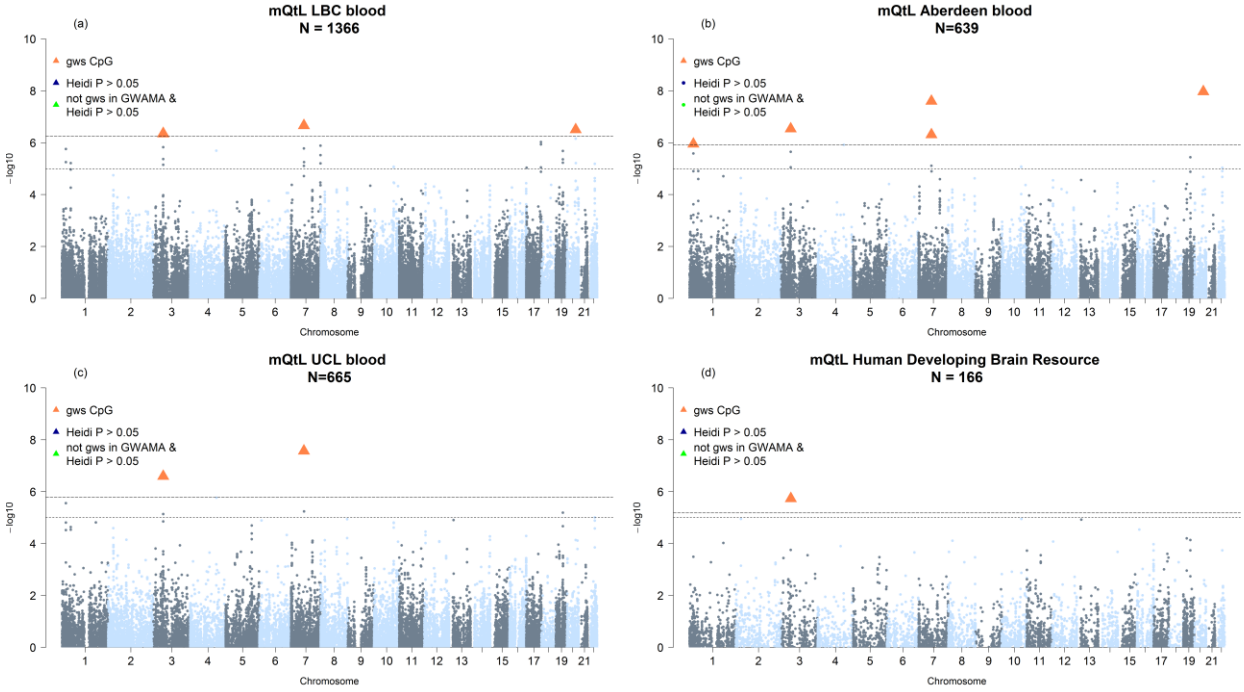
Replication in two independent whole-blood mQTL datasets

We were able to test for replication of the SMR results with mQTLs generated from two independent datasets (Aberdeen N = 639 and University College London N = 665)[40]. Using the same strategy as above, we identified five associations between well-being and DNA-methylation (**Fig 2B** and **Supplementary Table 6)** in the Abderdeen dataset ($P_{bonf} < 1.2 \times 10^{-06}$ corrected for 41,803 tests). Moreover, one out of three significant associations from the discovery dataset were genome-wide significant in the Abderdeen dataset cg07879825), whereas one association at chromosome 3 lies within 10 kb (cg11645453) form the discovery dataset. A significant correlation of the Z-statistics (r = 0.93; $P < 2.2 \times 10^{-16}$) indicates a large agreement in the direction of effect between both datasets (**Supplementary Fig 3A**).

Using UCL as the second replication dataset, we identified two associations between well-being and DNA-methylation ($P_{bonf} < 1.66 \times 10^{-06}$ corrected for 30159 tests; **Figure 2C** and **Supplementary Table 6)**). None of the significant associations from the discovery datasets were significant in the UCL dataset, although the association at chromosome 3 lies in close

proximity to the association found in the discovery dataset (< 10 kb). However, the high significant correlation (r = 0.91, P < 2.2 X 10^{-16}) between the Z-statistics is indicative of a large concordance between the LBC and UCL summary statistics (**Supplementary Fig 3B**).

Fig. 2. Manhattan plots of the SMR results. (**a**) LBC (whole blood) (**b**) Abderdeen (whole-blood), (**c**) University College Londen (whole blood), and **(d)** Human Developing Brain Resource. All plots in all panels are based on the same set of SNPs. The *x*-axis represents the chromosomal position, and the *y*-axis represents the significance on a $-\log_{10}$ scale. Each approximately independent genome-wide significant association is marked by Δ.



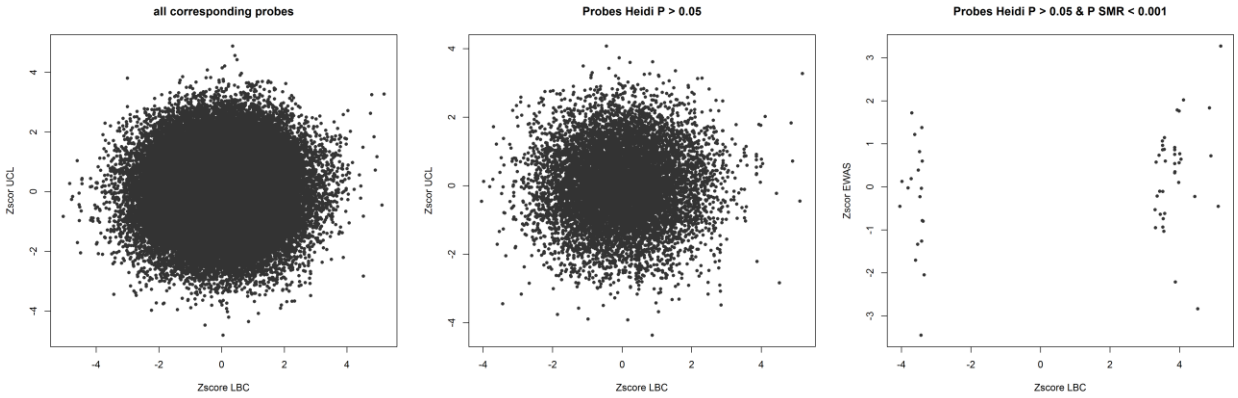
mQTL in whole blood versus brain

Given the tissue-specific and developmentally dynamic nature of gene regulation, we were interested in examining the consistency of the SMR findings in a different tissue. To this end, we repeated the SMR analysis on mQTLs in a dataset of human fetal brain derived from the Human Developing Brain Resource (HDBR; N = 166)[31]. We identified one association at chromosome 3 between well-being and DNA-methylation lying within 10 kb from the association present in our discovery dataset (P_{bonf} *<6.58 X* 10^{-6}*;* **Supplementary Table 6)***)* and a large correlation between *Z-statistics* of HDBR and LBC (r = 0.72, P < 2.2 X 10^{-16}), indicating consistency between both summary statistics (**Supplementary Fig 3C**).

Direct epigenetic measurement versus Mendelian Randomization

To assess the concordance between an EWAS, where well-being and CpG methylation are directly correlated, and SMR where the local genetic effects on methylation are used to infer which CpG sites effect well-being, we correlated the *Z*-statistics that were present in both datasets. When including all corresponding CpG probes (N = 70,564), the *Z*-scores hardly correlated (r = 0.02, P = 1.83 X 10^{-10}), which is indicative of little correspondence in direction of effects (**Fig 3A**). Next, we included only probes that survived the SMR HEIDI test (P > 0.05) and were present in both datasets (N = 10,072). The obtained correlation was still small but significant (r = 0.02, P = 0.02; **Fig 3B**). Finally, we tested the correlation between *Z*-statistics including probes with a SMR P < 0.001, N = 57) and found a small but non-significant correlation of .27 (P = .051; **Fig 3C**).

Fig. 3. Correlation of the Z-statistics between LBC and EWAS. (a) correlation between *Z*-statistics LBC and EWAS (adjusted model for all corresponding CpG probed (r = 0.02, P = 1.83 X 10^{-10}) **(b)** CpG probes surviving the HEIDI test (r = 0.02, P = 0.02), and **(c)** CpG probes surviving the Heidi test and P_{SMR} < 0.001 (r =.27, P = .051), In all plots, LBC is plotted at the x-axis.



Discussion

This study is one of the first large-scale investigations into epigenome-wide analyses of well-being through direct epigenetic measurement (EWAS) and summary based Mendelian Randomization (SMR). In the EWAS meta-analysis (N = 8,463), no genome-wide significant methylation hits were identified after correcting for multiple testing, smoking, and BMI.

In the Summary-based Mendelian Randomization analyses, we identified three genome-wide significant associations by integrating summary data from a GWAMA of well-being and the publically available mQTL dataset including participants from LBC (whole-blood). We were able to replicate one out of three associations using Abderdeen as an independent dataset, while another association lies in close proximity to the discovery dataset. In the third dataset (UCL), none of the three associations replicated although one lies in close proximity to the discovery dataset. Nevertheless we found high correlations of Z-statistics between the analyses with different datasets suggesting a large concordance in direction of effect. This replication confirms that the instruments used to index CpG methylation were consistent across multiple datasets, though we were not able to replicate the effects on well-being as a second sufficient powered independent GWAS of well-being is not available.

Next, we were interested in examining the consistency of our SMR findings in different tissue. Therefore, we repeated the SMR analyses in a dataset of human fetal brain derived from the Human Developing Brain Resource. The majority of SNP-DNA methylation relationships identified for SMR analysis in whole blood using the LBC dataset are characterized by a consistent direction of effect when tested in fetal brain.

Finally, we assessed the concordance between the EWAS, where well-being and CpG methylation are directly measured and SMR analyses, where the local genetic effects on methylation are used to infer which CpG sites effect well-being. Interestingly, no notable correlations were observed, even when different P-value threshold were used.

Our SMR results are in concordance with Hannon et al.[37] who found evidence for pleiotropic effects between SNPs that cause variation in CpG methylation and trait-associated genetic variation in over 40 complex traits with robust GWAS data. Moreover, similar to what we report in the present study, they found that a significant proportion of the associations (99.2%) with mQTLs using an independent whole-blood dataset had the same direction of association. The large consistency that we observe between associations with mQTL measured in whole-blood as well as in fetal brain (r = 0.72) is in concordance with previous studies[34,37,38] and

suggests that the correlation between mQTLs in local regions (i.e. ~1MB of the transcription start site) is fairly high. In addition, the lack of correlation between direct epigenetic measurement and summary-based Mendelian Randomization is in agreement with two previous studies[31,32] that found that most CpG probes are uncorrelated between whole-blood and brain tissues.

There are several explanations to explain the lack of correlation between EWAS and SMR. First of all, epigenome-wide analysis of well-being through direct measurement provides the association of all epigenetic markers (genome-wide) with a trait of interest *without* providing information about the possible mechanisms driving the association. There are five scenarios that theoretically can drive the association between DNA methylation and a trait; (1) There is an underlying single causal genetic variant that influence both DNA-methylation and trait, also known as pleiotropy, (2) There are 2 genetic variants (in high LD with each other) where on variant has an effect on DNA-methylation and one variant has an effect on trait variation, also known as linkage, (3) There is a causal relation where variation in CpG methylation will cause variation in well-being, inducing pleiotropy between the SNPs which influence CpG methylation, and well-being, (4) There were unmeasured confounding factors that have an effect on DNA methylation and trait variation, (5) There is reverse causation, where trait variation has an effect on differences in DNA-methylation instead of the other way around, and T (see **Fig. 4** for graphical representation of the five scenarios). Summary-based MR in combination with the HEIDI test can distinguish between pleiotropic and linkage effects of trait associated genetic variation on DNA-methylation. Doing so, SMR provides useful information on the mechanism underlying the association between DNA methylation and trait variation (e.g. well-being). The absence of correlation between the EWAS and SMR results suggests that the associations we observed in our epigenome-wide analyses through direct epigenetic measurement are mainly driven by processes other than pleiotropy or a direct causal effect of CpG methylation on well-being. This finding is consistent with the presence of a direct relation between CpG methylation and well-being that varies over tissues (i.e is not present in white blood cells, but may still be present in brain cells).

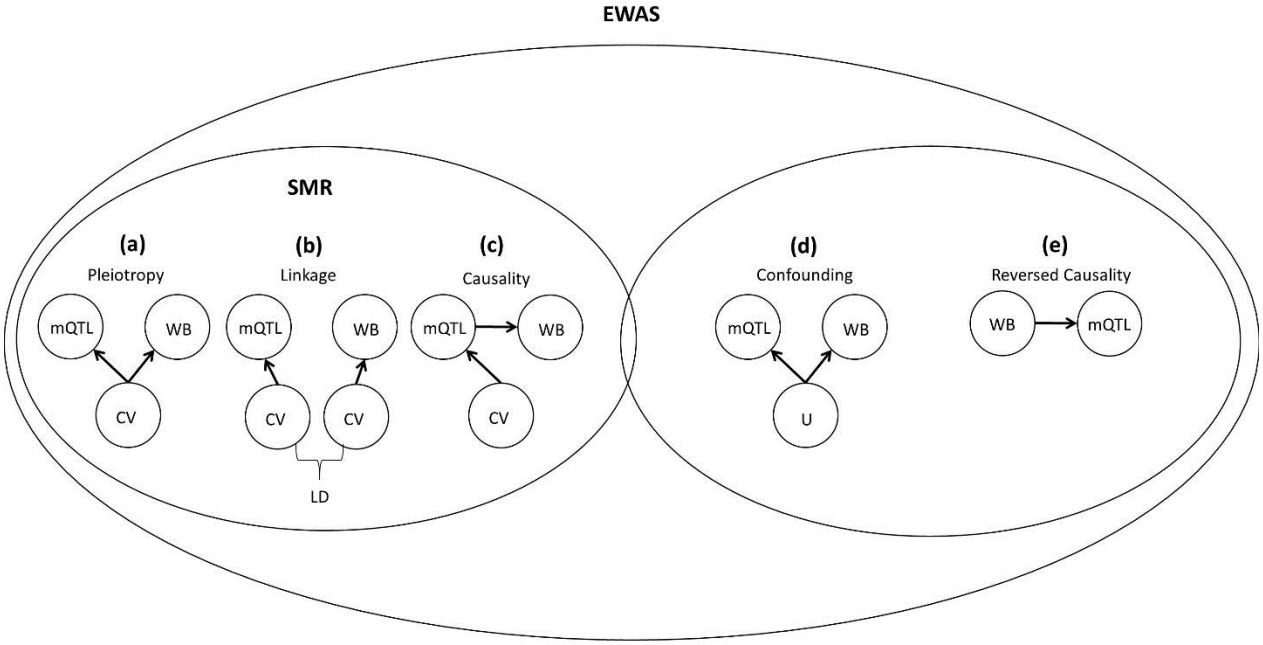
The current EWAS of well-being, is one of the largest conducted today with a sample size of ~8600, but was not capable to identify probes significantly associated with well-being after adjusting for smoking and BMI. Future EWAS with larger samples that have sufficient statistical power should be able to identify CpG probes associated with well-being. However, if a tissue of convenience (i.e whole blood, buccal) is used, it should be expected that a

substantial portion of the findings may reflect confounding (other than those confounders corrected for in the model) or reverse causation where variation in well-being influences CpG methylation. Reverse causation in itself may be a very interesting mechanism which could hold clues to health outcomes which are a consequence of modified epigenetic states as a causal consequence of systematically lowered well-being. As biobanks increase their dense phenotyping, further EWAS studies may be able to interrogate multiple tissues in relation to well-being, and for individual loci identify the tissue of action. These studies should where possible leverage SMR which we have shown to be a valuable addition to EWAS in the context of psychological traits.

Conclusion:

We performed the largest EWAS of well-being to date and no genome-wide significant methylation hits were identified after correcting for multiple testing, smoking and BMI. Using summary Mendelian Randomization, we identified three associations (discovery dataset) where well-being and variable DNA methylation are pleiotropically associated with genetic-variation. Moreover, a high concordance in direction of effect was observed using three independent mQTL dataset measured in blood (2x) and brain (1x). Our results indicate that if the aim is to increase our understanding of the functional consequences of genetic risk variants for a complex trait and to facilitate the localization of specific genes within genomic regions identified by GWAS, SMR seems to be a promising way to go forward. If however, the aim is to identify environmental influences on the epigenome, a sufficient powered EWAS study might provide valuable information if brain tissue is not the only predominant tissue of interest. Combined use of the two designs may prove a potent cocktail able to identify correlation between CpG methylation and well-being while testing the exact nature of the observed correlation.

Fig. 4. five scenarios that can drive the association between DNA methylation and a trait; **(a)** There is an underlying single causal genetic variant that influence both DNA-methylation and trait, also known as pleiotropy, **(b)** There are 2 genetic variants (in high LD with each other) where on variant has an effect on DNA-methylation and one variant has an effect on trait variation, also known as linkage; **(c)** There is a causal path from a genetic variant that influence DNA-methylation level an through DNA-methylation, trait variation will occur, **(d)**Tthere were unmeasured confounding factors have an effect on DNA methylation and trait variation, and **(e)** there is reverse causation, where trait variation has an effect on differences in DNA-methylation instead of the other way around.



Methods

Epigenome-wide association study

Data on well-being, body mass index (BMI), smoking, white blood cell counts, and methylation level were available for 13 cohorts: ALPSAC (*N* = 829), QIMR(*N* = 233), DTR (*N = 1012)*, FTC (*N* = 593), GENR (*N* = 643), KORAF4 (*N* = 660), LBC1921 (*N* = 376), LBC1936 (*N* = 697), LLD (*N* = 730), NFBC1966 (*N* = 803), NFBC1986 (*N* = 593), NAS (*N* = 1195), and NTR (*N* = 2519) (**Supplementary Table 1)**. All participants provided written informed consent, and all contributing cohorts confirmed compliance with their local research ethics committees or institutional review boards.

Well-being (WB) Measurements

All questionnaires, except LifeLines Deep (LLD), were measures of happiness or satisfaction with life (**Supplementary Table 2)**. LLD derived their questions from the positive-affect negative-affect (PANAS) questionnaire[44] with questions focusing on 'interested', 'enthusiastic', 'proud' or 'inspired'.

Participants inclusion criteria

We performed two EWAS meta-analyses. (1) The basic model without correcting for smoking and BMI (*N* = 9,496), and (2) the adjusted model corrected for smoking and BMI (*N* = 8,463). To be included in the two analyses, participants had to satisfy several criteria: (1) all relevant covariate data were available for each participant; (2) Participants passed the cohort-level methylation quality control and (3) Well-being was measured with either satisfaction with life measurements or happiness measurements. To be included in the adjusted model, covariate data on smoking and BMI should be present.

Epigenome-Wide Association study

To investigate associations between well-being and individual methylation markers the participating cohorts first performed cohort-level EWAS with a pre-specified analysis plan. As is standard, the EWAS was performed as a set of linear regressions in each cohort, one methylation marker at a time, with the methylation beta value (0– 1) as the dependent variable. The key independent variable was WB. We estimated two regression models that differ in the set of covariates included. In the basic model, the covariates were age, sex,

imputed or measured white blood cell counts, technical covariates from the methylation array, and four genetic principal components to account for population stratification. In the adjusted model, we additionally controlled for BMI (kg/m^2), smoker status (three categories: current, previous or never smoker), and As BMI and smoking are correlated with WB[45,46] and known to be associated with methylation[8,47] , the basic model may identify associations with WB that are actually due to BMI or smoking. Although the adjusted model reduces that risk, it may also reduce power to identify true associations with WB (by controlling for factors that are correlated with WB). We present the results for both models, but focus on the adjusted, more conservative, model.

EWAS QC and meta-analysis

Each participating cohort uploaded EWAS summary statistics to a central secure server for QC and meta-analysis. We removed probes with missing *P*-value, standard error, or coefficient estimate (*Beta*). Quantile-quantile (Q-Q) plots were made for the two models (basic versus adjusted). Additionally, we asked the participating cohorts to perform an EWAS on smoking and provide the corresponding *P*-values. From these *P*-values, also Q-Q plots were made for visual inspection. To account for test statistic bias and inflation we used the method described by Iterson *et al* [42] as implemented in the R-package *Bacon* (**Supplementary Table 3**). We performed a sample-size-weighted meta-analysis of the cleaned results using METAL[48]. The two EWAS meta-analyses were performed on 395,764 methylation sites (CpG sites present in all datasets). For all samples included in the EWAS, white blood cell counts were measured with the standard white blood cell differential as part of the complete blood count (CBC). A CpG site was considered to be genome-wide significant at the stringent Bonferroni level (alpha = 0.05/ 395,764 = 1.26 X 10^{-7}).

Summary-based Mendelian Randomization

mQTL summary statistic were from four publically available data sets. Peripheral blood from McRae *et al.*[39] (Lothian Birth Cohort; *N* = 1,366), Hannon *et al.*[40] (Aberdeen; *N* = 639, University College London; *N* = 665). Fetal brain summary statics were available from Hannon *et al.*[31] (Human Developmental Biology Resource; *N = 166)*.

We included in the SMR-analyses a multivariate GWAMA[43] of well-being measures. In the multivariate analyses, datasets from Okbay *et al.*[49](imputed to 1000G Phase 1 using the software tool DIST)[50] and UK Biobank (UK Biobank ID 20456 and 4526) were combined to

maximize power to identify genetic variants associated with well-being. Quality Control of UK Biobank data is described elsewhere [51]. In total 491,455 observations and 7,123,275 SNPs (MAF > 0.01) were included in the analyses.

Summary Based Mendelian Randomization Analyses

The method behind SMR is extensively described by Zhu *et al.*[36]. In short, the SMR test was developed to test the association between an exposure (e.g. DNA methylation) with an outcome (e.g. Well-being) using a genetic variant as the instrumental variable to remove non-genetic confounding. Let *x* be an exposure variable, *y* be an outcome variable, and *z* be an instrumental variable. The Mendelian Randomization (MR) estimate of the effect of exposure on outcome ($\hat{b}_{xy}$) is the ratio of the estimated effect of instrument on exposure ($\hat{b}_{zx}$) and that on outcome ($\hat{b}_{zy}$).

$$\hat{b}_{xy} = \frac{\hat{b}_{zy}}{\hat{b}_{zx}}$$

where $\hat{b}_{zx}$ and $\hat{b}_{zy}$ are available from mQTL and GWAMA summary data. One of the core assumptions for MR is that the instrument should be strongly associated with exposure. Therefore in the SMR analyses, only top mQTLs (at least $P < 1 \text{ X } 10^{-5}$) are included as instrument for an SMR analysis. A significant association detected by the SMR test above can result from either a pleiotropic model (i.e. the exposure and the outcome are associated by a *single* shared genetic variant) or a linkage model (two or more variants in LD affecting the exposure and outcome independently). To distinguish pleiotropy from linkage a heterogeneteity in dependent instruments (HEIDI) test was developed to test against the null hypothesis that there is a single causal variant underlying the association (pleiotropy model). For the HEIDI test we used multiple SNPs (e.g. the top 20 associated mQTLSs after pruning SNPs for either too strong or too weak LD in a *cis* region) to detect whether the association patterns across the region are homogeneous or not (a homogeneous pattern indicates a single shared causal variant). Thus, we assess the difference between $\hat{b}_{xy}$ estimated at the top significant associated instrument $\hat{b}_{xy(o))}$ and $\hat{b}_{xy}$ estimated at a less significant instrument $\hat{b}_{xy(i)}$:

$$\hat{d}_i = \frac{\hat{b}_{xy(i)}}{\hat{b}_{xy(o)}}$$

Under the null hypothesis (pleiotropic model), **d = 0.** If **d** significantly deviates from **0**, we reject the SMR association due to heterogeneity.

SMR correlations between the different datasets

As the number of methylation probes were different between the EWAS analyses and the SMR analyses, only corresponding probes were included for each analysis. SMR analyses output do not report *Z*-statistics. Therefore, we calculated for each SMR output:

$$Z_{SMR} = \frac{\beta_{SMR}}{\sigma_{SMR}}$$

The resulting Z_{SMR} test statistics were correlated (two sided) with each other as an indication for consistency of direction of effects between whole-blood mQTL dataset as well as consistency of effect between whole-blood and fetal brain mQTL datasets.

Direct epigenetic measurement versus Mendelian Randomization

To test the consistency of effect between the EWAS analyses and whole-blood mQTL SMR analyses we correlated the *Z* statistics of the LBC whole-blood mQTL dataset and *Z* statistics of the EWAS meta-analysis corrected for smoking and BMI. Correlation analyses were performed in R[52].

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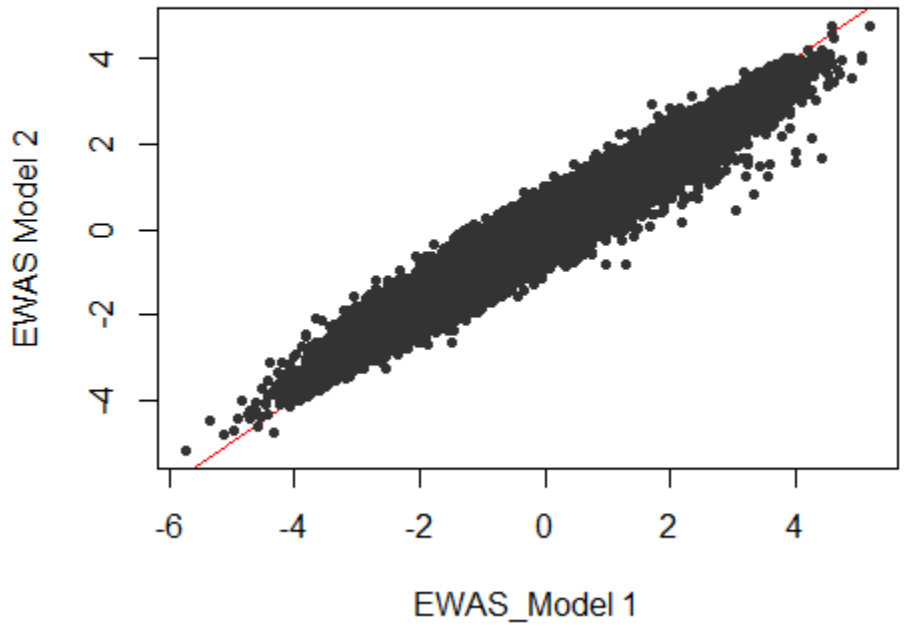
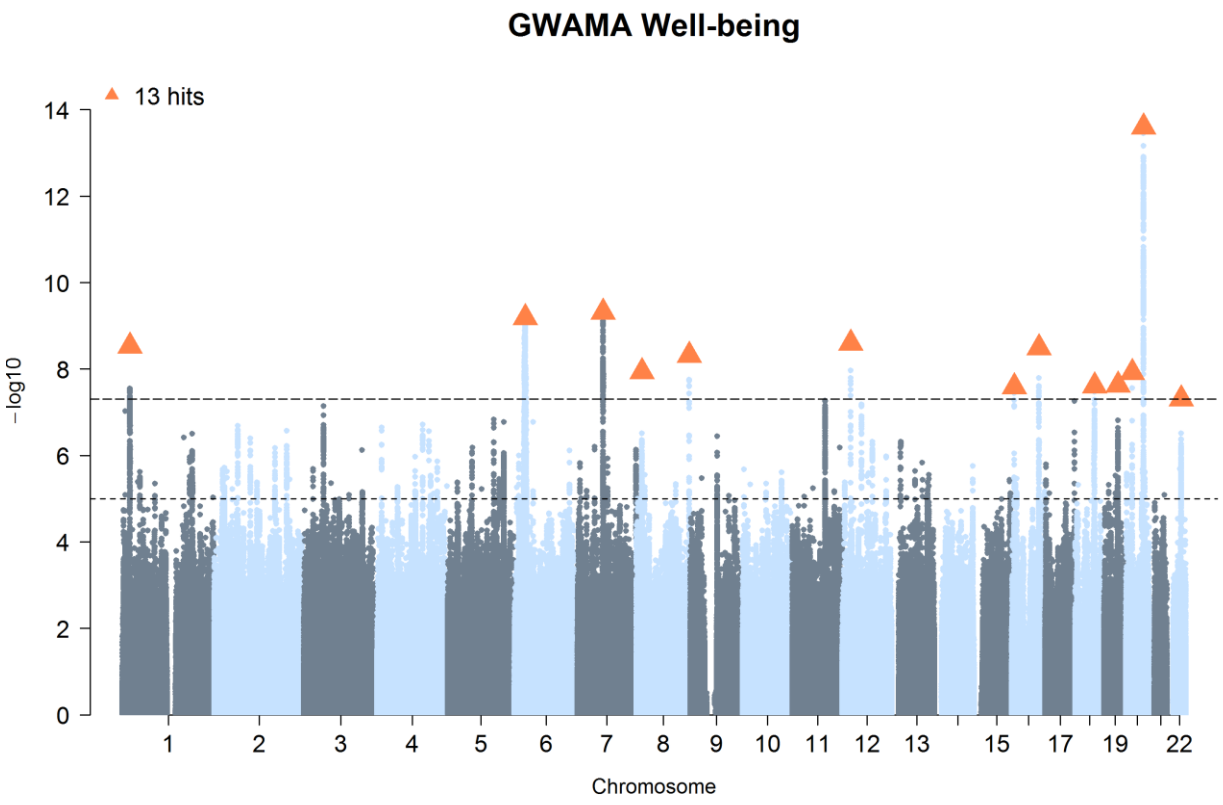
Supplementary Information

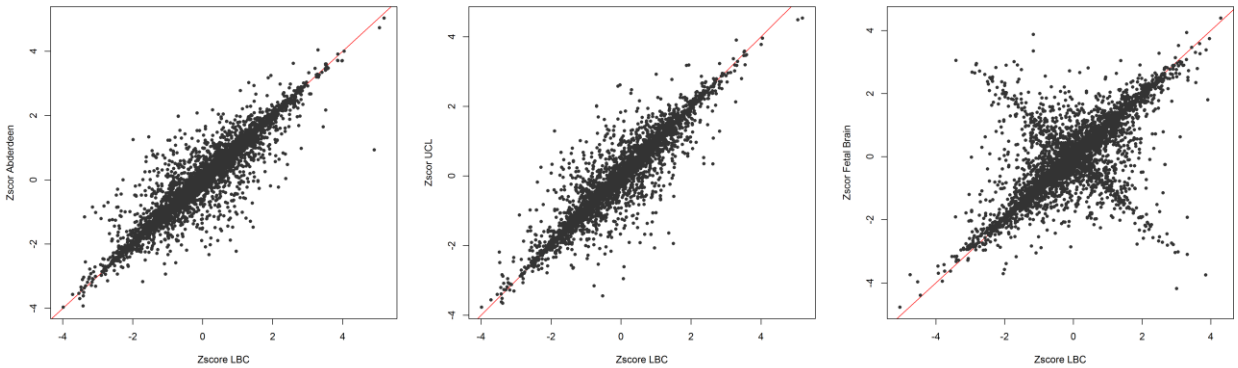
Supplementary Fig. 1. Correlation of the Z-statistics between EWAS basic model and adjusted model. X-axis representing the CpG probes of the basic model (model 1) and the Y-axis representing the CpG probes of the adjusted model (corrected for smoking and BMI). Correlation is r =.98, $P < 2.2 X 10^{-16}$.



Supplementary Fig. 2. Manhattan plots of N-weighted GWAMA of well-being. The *x*-axis represents the chromosomal position, and the *y*-axis represents the significance on a $-\log_{10}$ scale. Each approximately independent genome-wide significant association ("lead SNP") is marked by Δ.



Supplementary Fig. 3. Correlation of the Z-statistics between basic model and adjusted model. **(a)** correlation between *Z*-statistics LBC and Abderdeen (r = 0.93; $P < 2.2 \times 10^{-16}$), **(b)** correlation between LBC and UCL (r = 0.91, $P < 2.2 \times 10^{-16}$), and **(c)** correlation between LBC and fetal brain (r = 0.72, $P < 2.2 \times 10^{-16}$), In all plots, LBC is plotted at the x-axis.



07

A growing sense of well-being: a literature review on the complex framework well-being

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Abstract

This review examines the origin and structure of the complex well-being (WB) concept as it is currently applied in behavioral and social sciences. Current research on WB is often divided into two perspectives: subjective well-being (SWB) and psychological well-being (PWB), shaped by the philosophical concepts of hedonism and eudaimonism, respectively. How these different views relate to each other and to WB as a whole has not yet been clearly defined, leading to difficulties in interpretation. In this review, we aim to get more insight into the relation between SWB and PWB. We first present an overview of the philosophical history of SWB and PWB, followed by a systematic literature review. The goal of this review, based on 29 studies, was to investigate how much evidence there is for a conceptual overlap between SWB and PWB. A majority of the studies found appreciable shared variance between the constructs, suggesting that they might be more closely related than previously assumed. On the other hand, evidence from biological studies provides mixed results: a distinction between SWB and PWB based on unique biomarkers is reported, while recent molecular genetic studies show strong genomic overlap between SWB and PWB, but different gene-expression regulation. We end with a discussion on how these findings fit into a well-being framework, and describe some of the issues in the well-being field as we encountered them in our review followed by potential solutions to these problems.

Introduction

Over the past 20 years, the positive psychology movement has gained a lot of attention and popularity. This line of research examines the underpinnings of happiness and well-being[1]. From a theoretical perspective, two types of well-being can predominantly be distinguished in the field of positive psychology: subjective well-being (SWB) and psychological well-being (PWB). This distinction, though, has been discussed and criticized based on findings that indicate a substantial conceptual and, in some cases, biological overlap[2,3]. Moreover, with increasing numbers of concepts, constructs, and measurement instruments available[4], it has become ambiguous what we mean exactly when we claim to be measuring well-being. Furthermore, one of the largest complications in present-day well-being research is conceptual uncertainty: the validity of well-being constructs depends on which theory on the nature of well-being is correct[5]. Many theories have been proposed on the nature of well-being, yet, there is no unifying system that brings the different theories together[6,7]. To continue development in the area of well-being research, we must solve these issues. In this review, we aim to examine the complex framework well-being in three steps. First, we provide a brief overview of the philosophical roots of well-being and how this has shaped the modern SWB and PWB dimensions. Secondly, we perform a systematic literature review aimed at analyzing the current view on the relation between SWB and PWB. Lastly, we discuss the implications of the results from part 2 and offer suggestions for future well-being research.

PART 1 - THE PHILOSOPHICAL HISTORY OF WELL-BEING

For centuries, people have asked themselves questions about the nature well-being. This can be traced back to ancient Greek philosophers, such as Aristotle and Socrates, who already wondered about the prerequisites for living a good life[8]. In this part, we examine the ancient history of hedonic and eudaimonic well-being, followed by a discussion on how this history shaped present-day well-being theories.

Hedonia

"Pleasure is our first and kindred good. It is the starting-point of every choice and of every aversion, and to it we come back, inasmuch as we make feeling the rule by which to judge of every good thing"(Letter to Menoeceus, Epicurus).

Ancient hedonism is centered around pleasure, or how good a person feels about his or her life[9]. From this perspective, well-being consists in the balance of pleasure over pain, that is: how to maximize pleasure and minimize pain. Aristippus (c. 435 – c. 356 BCE), one of Socrates' students, was the founder of the Cyrenaic school of Philosophy, a school that taught that pleasure was the ultimate goal of human life and that the pursuit of pleasure was the purpose of human existence and is therefore considered one of the first that taught the hedonistic line of thought[10]. Epicurus popularized the same view on the good life but, different from the Cyrenaics, believed that pleasure was to be found in the absence of desire, and limiting what one wants, rather than striving to satisfy all one's desires as they are.

More recent examples of hedonists are Jeremy Bentham (1748-1832) and John Stuart Mill (1806-1873). Their emphasis on well-being as pleasure resulted from their utilitarian view on ethics, according to which one should maximize total well-being, rather than as a means to make one's own life good[11]. According to Bentham's narrow hedonism, different pains and pleasures possess different values, and their sum determines a person's hedonic level, his or her level of well-being. The two most fundamental aspects in this theory are duration and intensity: these factors determine the value of an individuals' pleasures and pains[12]. That is to say, the higher the intensity, and the longer the duration, the higher the value of a pain or pleasure. However, according to Mill, this form of hedonism lacks a dimension: quality. His objection to Bentham is that "*It is better to be a human being dissatisfied than a pig satisfied; better to be Socrates dissatisfied than a fool satisfied*"[13]. This implies that well-being is not a mere summation of quantities of pleasure, but that qualitatively better pleasures contribute more to well-being.

From hedonism to subjective well-being

Comparing these 19th century philosophers with their ancient counterparts, we see a more careful and detailed analysis of the concept of hedonic happiness. In modern-day behavioral and social sciences, the term hedonic well-being is becoming less and less frequent. However, this does not mean that the hedonistic line of thought is unpopular among contemporary social scientists. Rather, we observe a shift in terminology: contemporary social scientists prefer to use the terms subjective well-being (SWB) or happiness rather than pleasure and hedonism. A likely reason for this shift is that hedonism is a philosophical concept that has no clear method of measurement. Therefore, researchers have tried to redefine hedonism into an operational definition. While many methods have been proposed

for measuring and conceptualizing SWB[14], a widely adopted definition is that of Diener (1984). According to this conceptualization, SWB consists of three hallmarks: 1) it is subjective (objective influences are not necessarily part of the construct); 2) it includes positive measures (it is not just the absence of negative factors), and; 3) it includes a global assessment of all aspects of a person's life, not just of one or a few domains. Three separate components are used to measure this construct: positive affect, negative affect, and life satisfaction [16]. While the ancient concept of happiness is not exactly the same as modern SWB, evidence is pointing towards SWB existing as a result of the hedonic concept of well-being. Conceptually, positive and negative affect, collectively referred to as the affective/emotional aspect of SWB, are similar to the ancient ideas of pains and pleasures contributing to hedonic levels. Life satisfaction (also referred to as the cognitive component of SWB), defined as a global judgment of one's life, could intuitively be comparable to the overall hedonic level of an individual over his/her life as a whole, but life satisfaction could also be considered a newer addition to this type of well-being and not strictly a hedonic concept. A person's hedonic state is the overall balance of pleasure and pain experienced at a particular point in time. In contrast, life satisfaction is an evaluation a person makes about his or her life by his or her own standards. These two concepts do not necessarily coincide, as a person may be satisfied with states that might not feel good, like in the context of childbirth[17]. Moreover, in his *Conditions of Happiness*, Veenhoven (1984) mentions that ancient hedonists equate the evaluation of happiness with a focus on sensory pleasures, while the modern-day conception of happiness more strongly focuses on affective and cognitive pleasures. Taken together, while hedonic levels of well-being are an important aspect of SWB, it does not capture the complete SWB construct.

Eudaimonia

"Again, our definition accords with the description of the happy man as one who 'lives well' or 'does well'; for it has virtually identified happiness with a form of good life or doing well"(Aristotle, *Nicomachean Ethics*, trans. H. Rackham (Harvard University Press: London, 1943), 1098b).

Eudaimonia is a Greek word commonly translated as well-being or flourishing. Synonyms for eudaimonia are living well or doing well. Ancient eudaimonic philosophers based their ethical theories on the concept of eudaimonia[19], and ancient eudaimonism takes well-being to

be constituted by virtue and the fulfillment of human capacities. Whereas the hedonic tradition limited the concept of well-being to the balance of pleasure and pain, the eudaimonic tradition takes virtuous activity to be necessary for well-being as well. While hedonistic philosophers, such as Epicurus and Mill, may make space for virtue as a prerequisite or contributor to well-being, they do not take it to be necessary or essential for well-being[19]. Perhaps a more characterizing feature of the eudaimonic tradition of well-being is the principle of self-fulfillment. The most important contributor to, as well as founder of, the eudaimonic line of thought as discussed in this paper, is Aristotle (c. 384 – c. 322 BCE). Aristotle rejected the hedonistic definition of well-being, describing it as "*vulgar*"[20]. According to Aristotle, well-being can be interpreted as well-living: it is about the actualization of human potential. Virtue, defined as knowledge (practiced over time) about how to live well, is an important aspect of this theory[21]. Therefore, the Aristotelian conception of well-being has more to do with the fulfillment of a person's nature: it aims at reaching one's fullest potential in line with one's deeper principles[22]. Whereas hedonists have a purely individualistic notion of well-being, eudaimonia requires living well in one's social environment. A more modern, famous account that is strongly inspired by this conception is Maslow's hierarchy of needs, as proposed by Abraham Maslow (1943). This theory describes five different stages of human growth, starting at the most basic level of physiological needs. Every time the need belonging to a particular level is fulfilled, one moves up a stage in the hierarchy. The highest level a person can reach is self-actualization, which is (according to Maslow) only reached by one in a hundred people. In his theory of human motivation Maslow refers to self-actualization in the sense of the Aristotelian tradition: "*It refers to the desire for self-fulfillment, namely, to the tendency for him to become actualized in what he is potentially. This tendency might be phrased as the desire to become more and more what one is, to become everything that one is capable of becoming.*"

From eudaimonia to psychological well-being

Where the hedonic line of thought has largely been replaced by SWB in the empirical literature, the eudaimonic tradition has gradually shifted towards psychological well-being (PWB). Whilst creating valid measurement methods for SWB was starting to gain popularity amongst the social sciences around the 1970/1980s, valid measurements for PWB seemed to be lacking at that time. Especially the absence of self-actualization within PWB conceptualization was troubling and gave rise to a new formulation for capturing this construct[24]. This new formulation for PWB consists of six core dimensions: Self-Acceptance,

Positive Relations with Others, Autonomy, Environmental Mastery, Purpose in Life, and Personal Growth. While many other measurement instruments for PWB are available nowadays, these six core dimensions are still widely used to assess PWB. Notably, there are other modern perspectives on eudaimonia, such as Self-Determination theory[25]. However, here, we only present the PWB formulation as proposed by Ryff due to its frequent application in behavioral and social sciences. PWB, as proposed by Ryff, is without doubt a result of eudaimonic thinking: it was in her intention to create a measure that captures the eudaimonic line of thought: "*Indeed, the deeper philosophical roots of the new model of well-being resided in Aristotle's formulation of the highest human good, which in his Nichomachean Ethics he termed eudaimonia*"[26]. Therefore both PWB and eudaimonia are predominantly concerned with the development and self-realization of an individual[24]. However, a difference that can be pointed out between ancient eudaimonism and PWB is that in the Aristotelian tradition, eudaimonia did not just concern subjective experience but intersubjective experience: a way of being in the world[27]. Ryff's PWB scales, though, still have more focus on the subjective, individualistic values. This is not surprising since Western countries (in which Ryff's scales are often applied) mostly have individualistic values instead of collectivistic ones. The most intersubjective scale is the positive relations with others scale. While this scale does measure the concern someone has for others, it does not place as large emphasis on intersubjective values as the ancient eudaimonic tradition.

PART 2 – SYSTEMATIC LITERATURE REVIEW

In this review, we chose to adopt the following definitions (see Figure 1):

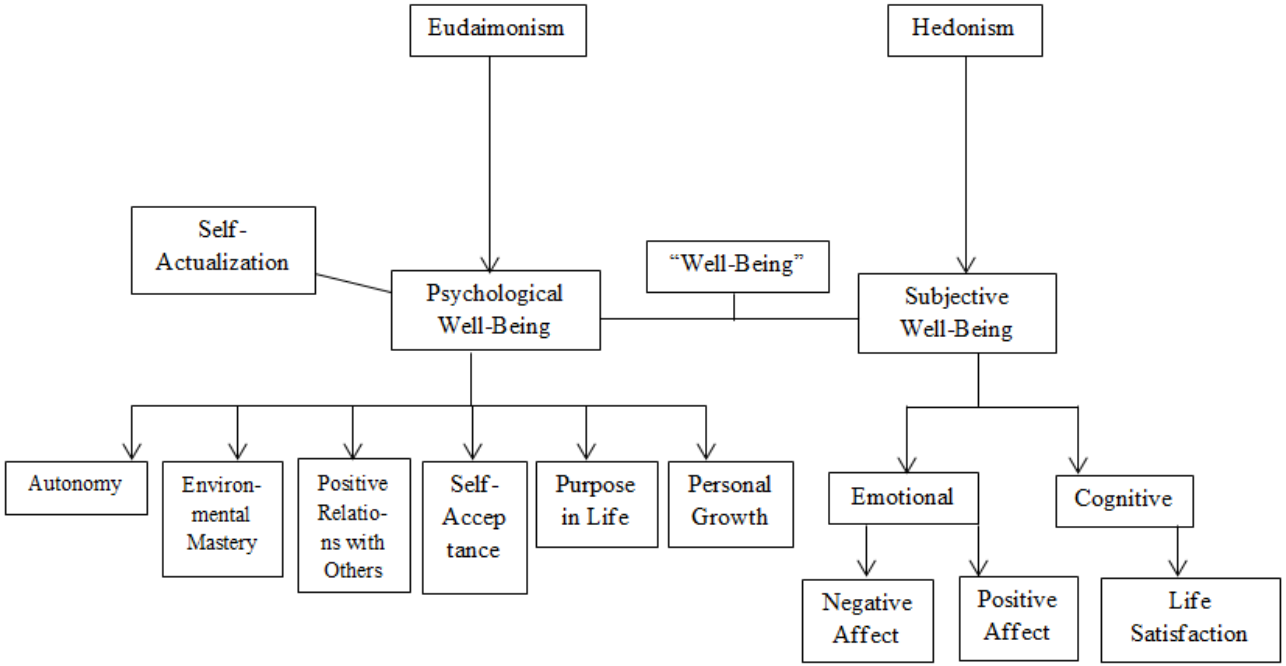
Subjective Well-Being. The term "subjective well-being" is used to refer to the domain of well-being inspired by the hedonistic line of thought. Unfortunately, the literature on well-being does not often distinguish between two notions of subjective: 1) subjectivity as pertaining to things internal to the individual, and 2) subjective as dependence on a person's attitudes[28]. We adopt the definition as proposed by Diener (1994), that splits subjective well-being into two domains: a cognitive domain and an affective domain. The cognitive domain is comprised of a person's general evaluation of his or her life. This domain can also be referred to as an individual's satisfaction with life. The affective domain refers to a person's long-term levels of negative and positive affect, in line with option (1) pertaining to subjectivity mentioned above.

Psychological Well-Being. The term "psychological well-being" is used to refer to the domain of well-being inspired by the eudaimonic line of thought. For the purpose of this paper, we refer to the formulation as proposed by Ryff (1995) where psychological well-being consists of the six core elements: 1) self-acceptance; knowing and accepting oneself, 2) positive relations with others, reflecting one's ability to empathize and to show affection, 3) autonomy; a sense of independence, 4) environmental mastery; the capacity to manage one's life and the surrounding world, 5) purpose in life, the belief that someone's life is purposeful, and 6) personal growth; the development of personal potential. Moreover, we consider "self-actualization", referring to the realization/fulfillment of one's potential, as a term employed to refer to psychological well-being (even though in a more restricted sense than Ryff's definition).

GOALS OF THE REVIEW

We conducted a systematic literature review with the goal of analyzing how the structure of well-being is currently viewed and measured. The goal of the review was to answer the following question: "How much evidence is there for a conceptual overlap between SWB and PWB?" For this purpose, we reviewed studies examining the relationship between SWB and PWB or the general structure of well-being.

Figure 1. A roadmap of the different well-being constructs employed in this review.



Method

Search Strategy

The search terms were entered into the electronic databases Web of Science and PubMed at 08-01-2018. Since many terms are used interchangeably in the well-being field, we selected different search terms for PWB and SWB in order to include as many relevant studies as possible. Table 1 shows the search terms used for the search. Every search included an entry of two search terms simultaneously. The terms in column 1 were entered in combination with the terms in column 2. Moreover, the terms in column 1 were entered in combination with each other, as well as the terms in column 2, which were also entered together using different combinations. The first author performed the literature search. A second assessor double-checked a random sample of 200 articles. Cohen's K was calculated to formally assess the degree of agree of inter-rater agreement.

Table 1

Search Terms for Literature search 1

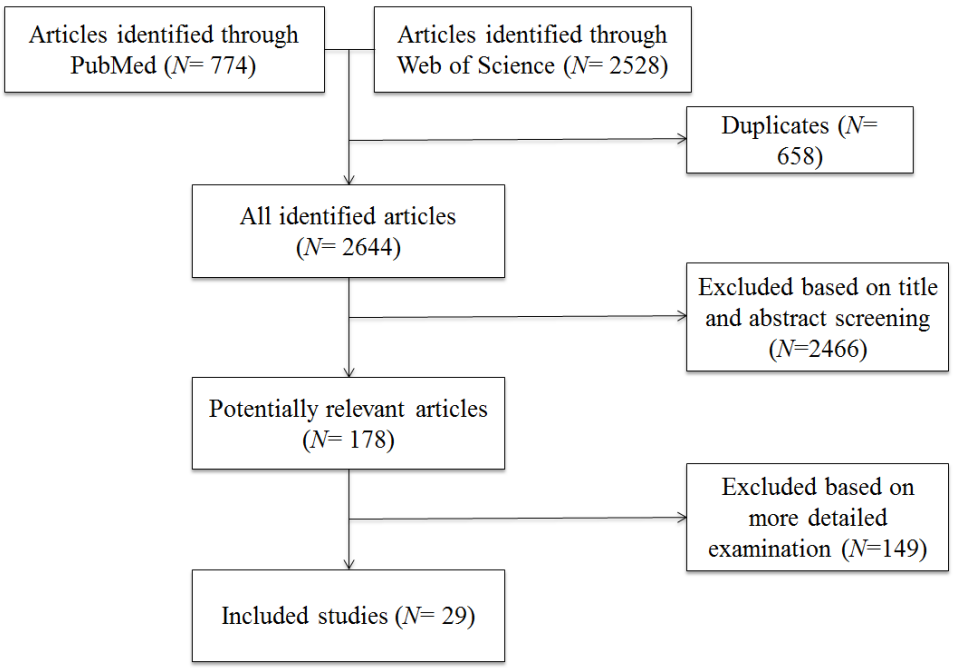
Term 1	Term 2
Psychological Well-Being	Happiness
Subjective Well-Being	Flourishing
	hedon*
	eudaimon*

Inclusion Criteria

Studies were included if they made some type of empirical comparison between psychological and subjective well-being. Meta-analyses, literature surveys, and theoretical papers were excluded from the review. Since well-being is under relatively different influences and genetically less stable in childhood and adolescence compared to adulthood[31], we only included studies in which the mean age of the participants was 18 or higher. Furthermore, since individualistic, Western countries value different things with respect to well-being and apply different standards than countries with a collectivistic/eastern culture,

we decided to only include countries that can be categorized as Western[32]. This included: Europe, the USA, Canada, and Australia. The paper has to be written in English and, in order to ensure the quality of the paper, be published in a peer-reviewed journal. In order to get the broadest scope of information, we did not apply any constraints regarding the time frame in which the studies were published. When age or origin of the participants could not be derived from a paper, it was excluded to prevent bias from unknown factors.

Figure 2. Flowchart for literature search.



Results

Figure 2 displays a flowchart depicting the search process. The inter-rater agreement was 91%. There was moderate agreement between the two assessors (κ= .452, 95% C.I., 0.210 to 0.694, p=.002). The initial electronic database search resulted in 774 hits in PubMed and 2528 hits in Web of Science. After removing the duplicates from the two searches together, we ended with a list of 2644 articles. After scanning the abstracts and titles of these 2644 articles based on the selection criteria mentioned above, we were left with 178 articles. These articles were examined in greater detail by reading them fully. Eventually, 29 articles met our selection criteria and were thus included in the study. Broadly, the resulting articles could be split up in three domains: 1) correlational studies, 2) studies looking at the factor structure of well-being, and 3) studies looking at biological/genetic factors influencing well-being. Therefore, we present our results for these domains separately. Importantly, we focused on the measurement instruments used in a study and the descriptions of the constructs as provided by the authors rather than the names used for the constructs. The reason for this approach is that there seems to be an inconsistent use of terminology in the field of well-being. For example, sometimes, hedonic measures are referred to as psychological well-being. Categorizing the constructs under the names provided by different authors in this review would therefore lead to false comparisons. An overview of all the different measurement instruments used to measure well-being in these studies can be found in Table 2.

1. Correlational Studies

Table 3 shows the three studies that fell under the category "correlational studies". Important in this table (and the following tables) is that the column named "research question" denotes the (sub)question in this study relevant for the purpose of this literature review. This means that the questions in this column do not necessarily reflect the main question in a study. From the studies in Table 3, one (nr 1) study suggests that the two constructs share considerable overlap, but are also distinguishable. In this study, personal expressiveness (their measure of eudaimonia) was a sufficient, but not a necessary condition for hedonic happiness. Furthermore, one (nr 2) study found that the relationship between life satisfaction and 4 of the 6 PWB scales was mediated by affect balance. The remaining two PWB scales were nevertheless also associated with life satisfaction. Lastly, one (nr 3) study suggests that PWB and SWB might not be as separated as previously claimed. In this study, this was suggested

because of the highly similar patterns of social reputations, clinician judgments, and behaviors associated with self-reports of subjective happiness and psychological well-being. These mixed results show that correlational analyses do not provide us with enough information to draw conclusions about the relationship between eudaimonic and hedonic well-being.

2. Studies examining the factor structure of well-being

Table 4 shows the 17 studies that fell under the category "studies examining the factor structure of well-being". These studies made use of several analyses, including: exploratory and confirmatory factor analysis (EFA and CFA), structural equation modeling, and Bayesian structural equation models. In this domain, a variety of models was tested. Five studies (nr 5, 13, 17, 18, and 19) approached well-being from Keyes' perspective, in which a three-factor model was tested with emotional, social, and psychological well-being as sub-domains of well-being[33]. Since the emotional domain in Keyes' model is an indicator of hedonic well-being, we decided these studies were relevant for the purpose of this study as well. One of these studies (nr 19) even labels the three-factor model as such, with the sub-domains subjective, psychological, and social well-being. These studies all conclude that the three-factor model is an appropriate fit to their data. According to these studies, well-being can most accurately be measured by considering these three dimensions simultaneously. An interesting finding was that in study 5 and 19, exploratory structural equation modeling was used and compared to CFA. The authors of this study conclude that CFA results in inflated inter-factor correlations, thereby overestimating the correlations between SWB and PWB domains, suggesting potential inflation in the inter-factor correlations for the studies in this review that made use of CFA. Eleven (nr 4, 6, 7, 8, 10, 11, 12, 14, 15, 16, and 19) studies approached well-being from a two-domain perspective, with SWB/ hedonic well-being as one domain, and PWB/eudaimonic well-being as the other. The conclusions from these studies were in agreement: PWB and SWB are overlapping, yet distinguishable constructs. Interestingly, one of these studies (nr 4) found that the cognitive aspect of SWB was more closely related to eudaimonic well-being than to the affective aspect of SWB. This further demonstrates the interrelatedness of the constructs. The remaining study (nr 9) examined well-being as consisting of the domains: SWB, personal growth, religiosity. In this study, evidence was found for this tripartite structure.

3. Studies examining biological/genetic factors of well-being

Table 5 shows the 10 studies that fell under the category "studies examining biological/genetic factors of well-being". Note that the two studies by Fredrickson et al. (2013 and 2015) have been the subject of discussion and that two critiques on this paper are also included in the review. Again, we observe differences between the conclusions from these studies. From the seven biological studies (the two critiques not included), six (nr 20, 22, 24, 26, 28 and 29) found evidence for distinct biological factors influencing SWB and PWB and one (nr 25) found evidence for a shared biological factor. These studies included a wide range of biological factors, such as CTRA gene expression profile (nr 20, 22, 29), neural activation patterns (nr 25) and inflammatory factors (nr 24, 26). The only twin-family design included (nr 27) found evidence for a single genetic factor and some trait-specific genetic factors. Together, these studies suggest overlap as well as distinctiveness between the SWB and PWB.

How much evidence is there for a conceptual overlap between SWB and PWB?

This literature review consisted of 29 studies investigating the conceptual overlap between SWB and PWB that could be split up into the following domains: correlational studies, studies examining the factor structure of well-being, and studies examining biological/genetic factors of well-being. The results from this review show that:

- Studies employing correlational analyses provide us with mixed results and cannot be used to infer clear conclusions about the relationship between SWB and PWB.
- Studies examining the factor structure of well-being find that there is a large overlap between PWB and SWB, but that the constructs can also be distinguished based on considerable unshared variances.
- Studies examining biological/genetic factors of well-being found evidence for distinct biological factors associated with the traits. However, there was also evidence for a single genetic factor influencing PWB and SWB.

PART 3 – DISCUSSION, ISSUES AND RECOMMENDATIONS

In this review, we examined the complex framework of well-being. The first part of the review provides a brief review of the philosophical history of hedonism and eudaimonism, the two philosophical disciplines that inspired researchers to start operationalizing and

measuring SWB and PWB. In the second part of the review, we sought to investigate what empirical research can tell us about the distinction between the two constructs by conducting a systematic literature review. The review consisted only of studies directly examining the relationship between the two constructs. These studies employed correlational, factor analytical, and biological methods to assess to what extent the two constructs can be separated or united. The high correlations between the constructs showed that there is indeed overlap between them. However, considering the distinct biological correlates and the fact that the two constructs both explain unique as well as overlapping parts of the variance in "general" well-being, these studies also showed that PWB and SWB can be distinguished. In this part of the review, we will discuss the implications of the review and formulate some recommendations based on these implications. Lastly, we provide some general recommendations for follow-up research that could extend the findings from the current search.

Issue 1: The relationship between philosophical constructs and psychological measures

When ancient philosophers started defining hedonism and eudaimonism, their definitions and ideas were not designed to ensure they could be translated into measurable constructs. It is therefore not surprising that measuring well-being (subjective or psychological) has turned out to be a complex task. This is evident when looking at Table 2. Especially for SWB, the amount of measurement instruments applied is extensive. It is clear that there is a lack of consensus amongst researchers concerning the measurement of well-being. A question one could raise is whether the current distinction between SWB and PWB might be a result of the ancient distinction between hedonism and eudaimonism. Since measurement instruments are only as reliable as the theories underlying them, this would mean that most of these instruments might not be measuring the constructs they ought to measure.

Recommendation 1: Re-define the framework

The literature searches in this review revealed that SWB and PWB are largely overlapping, but also partly distinct. This suggests that the distinction between SWB and PWB is not as large as proposed by the theories underlying them. The ancient separation between hedonism and eudaimonism can be explained from the (data-free) theoretical philosophical perspective, but cannot accurately be captured by questionnaires employed in modern-day social/behavioral research. While the traditional view of hedonism and eudaimonism has been very important in terms of its contribution to the theoretical framework, it fails to

provide sufficient guidance for the formulation of empirical constructs. We propose that an empirical well-being framework should be developed considering the actual empirical data rather than the ideas that inspired the research[6]. In the context of the social and behavioral sciences, the well-being framework can best be described as one hierarchical construct including both SWB and PWB constructs. This means that hedonism and eudaimonism are not to be defined as two clearly separated streams, but as related underlying domains of the same construct.

Issue 2: *Semantic Ambiguity and Inconsistency – do we mean the same things?*

It is often taken for granted that when we are using the same words, we mean the same things. As it turns out, at least in the field of well-being, we should be more cautious about this assumption. An obvious issue we encountered in our search was the inconsistent use of terminology. The problem as we encountered it was twofold; First, there was a diverse range of terms used to denote similar concepts. Second, the terms were used in an inconsistent manner. The first problem, that of diversity, is the least problematic. We dealt with this problem in the early stages of the review, where we used a variety of terms to expand our search. For example, SWB can be referred to as "happiness", "hedonism", "subjective happiness", "emotional well-being" and "affective well-being". The largest consequence of this lack of a common language is that it potentially blocks advancement in science since it makes it more difficult for researchers to learn from each other's work. The second issue, that of inconsistency, has more severe consequences. With inconsistency, we refer to the inappropriate application of terminology within and beyond the field as a whole, and sometimes within one study. Often, "subjective well-being" is used to refer to both the psychological as well as the subjective domain of well-being. Presumably, this issue arises due to the fact that both SWB and PWB are measured using questionnaires, meaning that participants will give a subjective evaluation in both cases. The same holds for the term "psychological well-being". General well-being was often referred to as psychological well-being, which was thereafter split up in multiple domains that could be identified as the PWB and SWB domain. Terms that are used most inconsistently were those that are used less frequent. An example is the term "flourishing", which was sometimes used to denote SWB, sometimes to denote PWB, and sometimes to describe a combination of both. This issue causes confusion in a more severe sense: when not described properly, it could cause readers to draw the wrong conclusions.

Recommendation 2: Be detailed

Both problems, though one more severe than the other, have the same consequence: confusion. While preventing this inconsistency would be the most effective solution, this is not easily manageable since it would require a consensus concerning the use of terminology in this field. Therefore, the most feasible solution would be for researchers to be detailed about the constructs they aim to be measuring and about the scope of their study. This means that researchers should: 1) be consistent in their use of terminology; 2) give detailed descriptions of their most basic terms and constructs, and; 3) keep in mind that the results of their study might not cover well-being in its entirety. Especially (2) is important, since it can help prevent confusion for the careful reader even if (1) and (3) are not fulfilled. In this way, even if there is no consensus concerning terminology, different studies can more easily be compared and interpreted.

Follow-up research

A last issue that has not yet been discussed is that if one were to draw conclusions about "well-being" in general, but only uses measurement instruments related to one domain of well-being, the conclusion would depend on which domain is included (given the partial distinction between the constructs). To evaluate to what extent this might influence research outcomes, it is important for future studies to examine whether SWB and PWB relate to external correlates in the same way and to a similar degree. For example, in a study by Aghababaei & Arji (2014), the personality domain honesty-humility (H-H) was unrelated to SWB, but related to PWB. This means that if one were to draw conclusions about H-H and "well-being" in general, but only used measurement instruments related to one domain of well-being, the conclusion would depend on which domain is included. At the moment, it is not clear whether these types of discrepancies often occur in the literature. Without this knowledge, the safest approach is to take a multifaceted approach and use multiple measurement instruments aimed at SWB *and* PWB. In this way, researchers can overcome the risk of drawing firm conclusions based on incomplete assessment of well-being. In case such an approach is too time- or money-consuming, another solution is to make explicit which domain of well-being the results of a study refer to.

Conclusion

In this review, we examined the origin and structure of the complex framework well-being as applied in behavioral and social sciences. A systematic literature review was performed that examined how much evidence there is for a conceptual overlap between SWB and PWB. We find that SWB and PWB are related constructs that are likely domains of a general factor well-being. However, while the constructs are related, they are not interchangeable and can be distinguished both conceptually and biologically. In order to continue development in this field of research, we advise to view SWB and PWB as related domains of the same overarching well-being construct. Moreover, to avoid inconsistency and confusion, it is important for researchers to be very detailed about the constructs they aim to be measuring and to keep in mind that to measure well-being most accurately, both SWB and PWB should be included.

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Table 2

Measurement Instruments used in studies from the Literature Search

Subjective Well-Being Measures			Psychological Well-Being		
Instrument	Studies	Total	Instrument	Studies	Total
The Satisfaction with Life Scale	Sanjuan, 2011; Vanhoutte & Nazroo, 2014; McMahan & Estes, 2011; Compton, 2001; Chen, Jing, Hayes, & Lee, 2013; Huta & Ryan, 2010; Kafka & Kozma, 2002; Urry et al., 2004; Compton, Smith, Cornish, & Qualls, 1996.	18	Ryff's Scales of Psychological Well-being (several versions with varying amount of items)	Ryff, Singer, & Love, 2004; Sanjuan, 2011; Nave, Sherman, & Funder, 2008; Joshanloo, 2016; Compton, 2001; Keyes, Shmotkin, & Ryff, 2002; Chen, Jing, Hayes, & Lee, 2013; Gallagher, Lopez, & Preacher, 2009; Kafka & Kozma, 2002; Kokko, Korkalainen, Lyyra, & Feldt, 2013; Robitschek & Keyes, 2009; Fredrickson et al., 2015; Friedman, Hayney, Love, Singer, & Ryff, 2007; Urry et al., 2004; Keyes, Myers, & Kendler, 2010; Walker, 2016, Compton, Smith, Cornish, & Qualls, 1996; Joshanloo, 2017.	28
The Positive and Negative Affect Scale	Ryff, Singer, & Love, 2004; Sanjuan, 2011; Burns, Anstey, & Windsor, 2011; Chen, Jing, Hayes, & Lee, 2013; Gallagher, Lopez, & Preacher, 2009; Urry et al., 2004.	12	The Personally Expressive Activities Questionnaire	Waterman, 1993.	1
The short-form MASQ	Ryff, Singer, & Love, 2004.	1	The CASP scale	Vanhoutte & Nazroo, 2014.	1
The Personally Expressive Activities Questionnaire	Waterman, 1993.	1	Beliefs about Well-Being Scale	McMahan & Estes, 2011.	1
The Subjective Happiness Scale	Nave, Sherman, & Funder, 2008; Gallagher, Lopez, & Preacher, 2009.	3	The Meaning in Life Questionnaire-Presence Subscale	McMahan & Estes, 2011.	1
the General Health Questionnaire	Vanhoutte & Nazroo, 2014.	1	The Subjective Vitality Scale	McMahan & Estes, 2011; Huta & Ryan, 2010.	2
the CES-D	Vanhoutte & Nazroo, 2014.	1	The hedonic and eudaimonic motives for activities scale	Bujacz, Vitterso, Huta, & Kaczmarek, 2014.	1

Mroczek and Kolarz's positive and negative affect scales	Joshanloo, 2016; Joshanloo, 2017.	1	Connor-Davidson Resilience Scale & Personal Mastery Scale	Burns, Anstey, & Windsor, 2011.	1
Satisfaction with Life: unspecified Instrument	Joshanloo, 2016; Joshanloo, 2017.	2	Meaning & elevating experience as assessed with a self-created scale.	Huta & Ryan, 2010.	1
Beliefs about Well-Being Scale	McMahan & Estes, 2011.	1	"11 Well-Being items measuring the frequency of negative and postive affect & evaluation of people's lifes"	Kim, Lehning, & Sacco, 2016.	1
the Intensity and Time Affect Scale	McMahan & Estes, 2011.	1	The Short Flourishing Scale	Fredrickson et al., 2013; Walker, 2016.	2
The hedonic and eudaimonic motives for activities scale	Bujacz, Vitterso, Huta, & Kaczmarek, 2014.	1	Mental Health Continuum—Short Form	Fredrickson et al., 2015; Walker, 2016.	2
The Happiness Measure	Compton, 2001; Compton, Smith, Cornish, & Qualls, 1996.	3	the CASP-19	Steptoe, Demakakos, de Oliveira, & Wardle, 2012.	1
The Affect Balance Scale	Compton, 2001.	1	The Short Index of Self-Actualization	Compton, Smith, Cornish, & Qualls, 1996.	2
Cantril's Self-Anchoring Scale	Keyes, Shmotkin, & Ryff, 2002.	1	the Perceived Self Questionnaire	Compton, Smith, Cornish, & Qualls, 1996.	2
The Midlife Development Inventory affect scales	Keyes, Shmotkin, & Ryff, 2002; Gallagher, Lopez, & Preacher, 2009.	2			
The HEMA	Huta & Ryan**,** 2010.	1			
the brief NA and PA scales developed by Diener and Emmons	Huta & Ryan, 2010.	1			
The Memorial University of Newfoundland Scale of Happiness	Kafka & Kozma, 2002.	1			
"11 Well-Being items measuring the frequency of negative and postive affect & evaluation of people's lifes"	Kim, Lehning, & Sacco, 2016.	1			
the Brief Mood Introspection Scale	Kokko, Korkalainen, Lyyra, & Feldt, 2013.	1			
the Life Situation Questionnaire	Kokko, Korkalainen, Lyyra, & Feldt, 2013.	1			

A Personal Interview	Kokko, Korkalainen, Lyyra, & Feldt, 2013.	1			
An adaption of Cantril's Self-Anchoring Scale & Bradburn's affect balance Scale	Robitschek & Keyes, 2009.	1			
The Short Flourishing Scale	Fredrickson et al., 2013; Walker, 2016.	2			
Mental Health Continuum—Short Form	Fredrickson et al., 2015; Walker, 2016.	2			
The short-form Mood and Anxiety Symptom Questionnaire	Friedman, Hayney, Love, Singer, & Ryff, 2007; Ryff, Singer, & Love, 2004.	2			
Unspecified: positive affect & Life Satisfaction	Keyes, Myers, & Kendler, 2010.	1			
the CASP-19	Steptoe, Demakakos, de Oliveira, & Wardle, 2012.	1			
The quality of life scale	Compton, Smith, Cornish, & Qualls, 1996.	1			

Table 3

Correlational Studies

Nr	Article	Research Question	Participants	Analysis	Outcome	The structure of Well-Being
1	Waterman, 1993	Is it possible to distinguish between personal expressiveness (eudaimonia) and hedonic enjoyment?	Study 1: *N*=140 undergraduates (18-23 years) & 69 graduates (22-65 years). Study 2: *N*=193 undergraduates (18-46 years) & 56 graduates (22-52 years) (Trenton State College)	Correlational analysis	"Eudaimonia is a sufficient, but not a necessary condition for hedonic happiness."	Empirically, hedonic and eudaimonic enjoyment are related but can also be distinguished
2	Sanjuan, 2011	How are psychological and subjective well-being related?	*N* =250 Spanish participants, age(*M*= 36.46, *SD*= 10.83)	Regression analyses	"The results obtained here show that experiencing positive relations with others, autonomy, purpose in life, and personal growth is associated with positive feelings, which, in turn, leads to judgments of satisfaction with life."	Psychological and subjective well-being are independent, but there is a strong relationship which is (for some scales) mediated by affect balance.
3	Nave, Sherman, & Funder, 2008	What are the patterns of social reputations, clinician judgments and behaviors associated with self-reports of subjective happiness (SH) and psychological well-being?	*N*= 196 undergraduate students from the university of California-Riverside (age not reported, mean age assumed above 18 due to college sample).	Correlational analyses	"The results of this study suggest that the pattern of social reputations, clinician judgments, and behaviors associated with self-reports of SH and PWB are remarkably similar."	“One might question whether SH and PWB are truly distinct psychological constructs"

Table 4

Studies examining the factor structure of well-being

Nr	Artikel	Research Question	Participants	Analysis	Outcome	The structure of Well-Being
4	Vanhoutte & Nazroo, 2014	To what extent do empirical measures confirm to the theoretical divide between hedonic and eudaimonic aspects of subjective well-being?	*N* =3703 participants of the English population, age > 50	Confirmatory Factor Analysis (CFA)	"The difference between hedonic and eudaimonic well-being has been exaggerated in the literature"	A three-fold structure, distinguishing affective, emotional & eudaimonic well-being.
5	Joshanloo, 2016	What is the relationship between hedonic and eudaimonic aspects of well-being when investigated with ESEM (as compared to CFA)?	*N* =3986 adults from the United States, age(*M*=56.12, *SD*= 12.33)	CFA & Exploratory Structural Equation Modeling (ESEM)	"The inter-correlations between well-being factors may have been overestimated in the previous research due to the inherent limitations of traditional CFA."	"The hedonic and eudaimonic factors are correlated yet distinct factors, with considerable unshared variance."
6	McMahan & Estes, 2011	To what degree do eudaimonic and hedonic dimensions of individual conceptions of well-being differentially associate with self-reported well-being?	Study 1: *N* =115 American undergraduate students, age(*M*=21, *SD*=3.71). Study 2: *N* = 240 non-student American participants, age(*M*=31.9, *SD*=14.19)	Regression & CFA	"Eudaimonic dimensions of conceptions of well-being are more robustly associated with experienced well-being than hedonic dimensions."	"Conceptualizing well-being in eudaimonic terms may be relatively more important for positive psychological functioning."

7	Bujacz, Vitterso, Huta, & Kaczmarek, 2014	What is the relationship between factors of stable hedonic and eudaimonic orientations to well-being across 2 different nations?	*N* =386 Polish adults, age(*M*=21.26, *SD*= 1.75), 429 North American Anglophone participants, age(*M*=19.19, *SD*=1.92)	CFA, Maximum Likelihood estimation & Bayesian Structural Equation Models	"Stepwise analyses were conducted to establish a factor structure of the scale, revealing three correlated factors: two hedonic and one eudaimonic."	A three factor model with hedonic pleasure, hedonic comfort and eudaimonic factors was confirmed for motives for activities.
8	Burns, Anstey, & Windsor, 2011	Are the related PWB and SWB constructs independent?	*N* =3989 randomly selected individuals from the electoral rolls of Canberra/ Queanbeyan, Australia. Age= 20-24 & 40-44 at baseline	Exploratory Factor Analysis with principal axis factoring with direct oblimin oblique rotation	"Principal axis factoring of the resilience, mastery, and PANAS items revealed a four-factor structure whereby items loaded onto factors that corresponded with the original measures."	The measures reflect different cognitive and affective components of well-being, whilst moderate correlations between these constructs at a first-order factor level, indicate PWB and SWB as related.
9	Compton, 2001	What is the factor structure of well-being?	*N* =242 participants from the USA, age(*M*=25.9, *SD*=7.5)	Principal Components Analyses	"A large first factor that appeared to be a subjective well-being factor, a second factor that seemed to be a religiosity factor, and two other factors that were related to measures of personal growth, autonomy, and positive relationships.”	A tripartite structure with SWB, personal growth & religiosity that is characterized by other-centeredness.
10	Keyes, Shmotkin, & Ryff, 2002	Do indicators of PWB and SWB constitute taxonomically distinct reflections of well-being?	*N* =3032 participants from the USA. Age(weighted)(*M*=45.3, *SD*=13.5)	Exploratory & Confirmatory Factor Analyses	"The best fitting model is one that posits two correlated latent constructs, namely SWB and PWB, rather than two orthogonal factors (or one general factor)."	SWB and PWB represent related but distinct conceptions of well-being.

11	Chen, Jing, Hayes, & Lee, 2013	Is there a meaningful differentiation between psychological well-being and subjective well-being?	Study 1: *N* =795 undergraduate psychology students from the university of Delaware, age(*M*=19.27, *SD*=1.78). Study 2: *N*= 4032 Americans, age(*M*=56.25, *SD*=12.39)	A bifactor model	"PWB and SWB form a general factor of global well-being, which captures the common ground shared by the two types of well-being.[..] the components of PWB and of SWB form specific factors, which capture their unique variances."	"Both perspectives on well-being have merit, depending on the level of analysis (i.e., general or specific)."
12	Gallagher, Lopez, & Preacher, 2009	What is the latent structure of well-being?	*N* =591 undergraduates of a Mid-western university, age(*M*=18.94, *SD*=1.65) & 4032 American Adults, age(*M*=56.25, *SD*=12.39).	CFA techniques	"The model containing three second-order factors of hedonic, eudaimonic, and social well-being provided the best representation of the hierarchical structure of well-being."	A tripartite model with hedonic, eudaimonic & social domains.
13	Huta & Ryan, 2010	How do hedonia and eudaimonia (as motives for activities)& their combination relate to well-being? (only study 1 included)	*N* =300 undergraduates at a private northeastern US university, age(*M*=19.7, *SD*=1.3).	Principal Components Analysis, MANOVA	"The HEMA scales not only confirmed the distinction between eudaimonia and hedonia, but also had good reliabilities for our research."	Hedonia and eudaimonia occupy overlapping and distinct niches.
14	Kafka & Kozma, 2002	What is the relationship between Ryff's scale of PWB and measures of SWB?	*N* =277 university students at the Memorial University of Newfoundland and the University of Winnipeg, age(*M*=21.31, *SD*= 3.76)	PCA with varimax rotation	"While the MUNSH and the SWLS loaded on the same factor identifiable as a higher order SWB factor, subscales of the SPWB produced two additional factors."	If the SPWB reflects psychological functioning, then it is clear that such functioning is not the same as such SWB constructs as "happiness" or "life satisfaction.

15	Kim, Lehning, & Sacco, 2016	Should hedonic and eudaimonic components of the NHATS be measured separately or on a single scale?	*N* =6602 older adults (USA), ages 65 and over	CFA	"The single factor structure indicates that among community-dwelling older adults in the NHATS sample, the hedonic and eudaimonic aspects of well-being may be intertwined."	It appears that while there is a conceptual distinction between these two views of well-being, the hedonic and the eudaimonic perspectives can be measured as a single scale.
16	Compton, Smith, Cornish, & Qualls, 1996	What is the factor structure of well-being?	*N*= 338 US students, age (*M*=25.8, *SD*=10.6)	Principal Components Analysis with Oblique rotation	"Mental Health appears to be defined by two factors: SWB and Personal Growth, with SWB accounting for the larger portion of variance in the measures used for this study"	"Theories of personal growth and subjective well-being describe related, but not identical, constructs"
17	Kokko, Korkalainen, Lyyra, & Feldt, 2013	What is the structure of well-being?	*N* = 219 Finnish adults at age 36 & 42	Structural Equation Modeling	"Our findings showed that well-being in mid-adulthood can be described in terms of a higher-order core factor comprising the three dimensions from Keyes' tripartite model of well-being, that is, emotional, psychological and social well-being, as well as by low depression."	These results imply that instead of following separate lines of theory and research, it is more relevant to consider the different dimensions of well-being simultaneously.
18	Robitschek & Keyes, 2009	Is there support for the three-factor model of mental health (as proposed by Keyes) in a sample of college students?	*N*=467 students from a large Southwestern university, age(*M*=19.67, *SD*=1.71)	CFA	"Results of confirmatory factor analyses supported this 3-factor model of psychological, social, and emotional well-being, consisting of 14 subdimensions."	A three-factor model is supported.

19	Joshanloo, 2017*	What is the structural and discriminant validity of the tripartite model of mental well-being?	*N*=2732 US participants, age (*M*= 63.64, *SD* = 11.35)	CFA & ESEM	"Both in CFA and ESEM, a three-dimensional model of mental well-being was supported over the one- and two-factor models."	"Subjective, psychological and social dimensions of mental well-being constitute distinct factors, with a substantial amount of unshared variance."

*This author published three studies concerning the same research question in 2017 (with different populations), only one was included due to similar conclusions

Table 5

Studies Focusing on Biological Measures/Genetics and SWB & PWB

Nr	Artikel	Research Question	Participants	Analysis	Outcome	The structure of Well-Being
20	Fredrickson et al., 2013	What are the biological implications of hedonic & eudaimonic well-being?	*N* =80 healthy adults from Chapel Hill, NC, ages 35-64.	Generalized Linear Model Analyses	"Hedonic and eudaimonic well-being, although correlated, have markedly divergent gene transcriptional correlates in human immune cells."	The different streams of well-being have a different molecular physiology.
21	under debate!	see Brown, MacDonald, Samanta, Friedman, & Coyne, 2014				
22	Fredrickson et al., 2015	Is CTRA gene expression associated with eudaimonic (and hedonic) well-being?	Confirmation study (CS): *N* =122 adults from the Durham and Orange County regions of NC, age(*M*=48.4, *SD*= 8.8). Generalization study(GS): *N* = 107 participants from the Vancouver BC metropolitan area, age(*M*= 45.3, *SD*=5.6)	Mixed effect linear model analyses to predict reduced CRTA gene expression	"Sub-dimensions of eudaimonic well-being as promising targets for CTRA gene expression, and provide no support for any independent favorable contribution from hedonic well-being."	Distinct molecular basis for hedonic and eudaimonic well-being.
23	under debate!	see Brown, MacDonald, Samanta, Friedman, & Coyne, 2016				
24	Friedman, Hayney, Love, Singer, & Ryff, 2007	Can different measures of well-being predict plasma levels of inflammatory factors in aging women?	*N* =135 aging women (USA), age(*M*=74.02, *SD*=7.08)	Regression analyses between plasma IL-6 & sIL-6R levels and well-being	"The only measures that were significantly related to IL-6 and sIL-6R were measures of eudaimonic well-being."	Distinct biomarkers for hedonic and eudaimonic well-being.
25	Urry et al., 2004	What are the frontal neural activation patterns of eudaimonic and hedonic well-being?	*N* =84 adults who were Wisconsin high school seniors in 1957, age(*M*=58.49, *SD*=0.81)	Simultaneous and Hierarchical Regression Analysis	Greater left than right superior frontal activation was positively associated with both forms of well-being, but only with PWB when controlling for dispositional positive	Both PWB and SWB are associated with greater left frontal activation.

					affect.	
26	Ryff, Singer, & Love, 2004	What are the biological correlates of eudaimonic- and hedonic well-being?	*N* =135 aging women (USA), age=61-91	Correlational analysis	"Eudaimonic and hedonic well-being may not have equivalent neurobiological correlates"	"There is a pressing need to have measures of both eudaimonic and hedonic well-being incorporated in national-level health statistics across multiple countries."
27	Keyes, Myers, & Kendler, 2010	What is the structure of the genetic and environmental influences on mental well-being?	*N* = 670 same-sex twins pairs (USA) + 46 individual twins, age(*M*=44.6 years)	Twin Analyses	"A common pathway model fit our data best, suggesting the existence of a latent propensity to mental well-being."	"The tripartite structure of well-being observed at the phenotypic level is caused by the latent, higher-order variable of mental well-being that has its own genetic and environmental influences."
28	Steptoe, Demakakos, de Oliveira, & Wardle, 2012	What is the relationship between a range of biological measures and eudaimonic & hedonic well-being?	*N*= 3540 English men (age: *M*=65.6, *SD*=9.3) & 4255 women (age: *M*=65.6, *SD*=9.7).	Multivariate linear regression	There were few differences in the associations between biological function and affective and eudaimonic well-being.	This study suggests that both types of well-being are similarly related to a range of biological measures.
29	Walker, 2016	Can the results from Fredrickson (2013 & 2015) regarding CTRA expression levels and well-being measures be replicated using OLS & GEE (with Monte Carlo simulation as a check for performance)?	*N*=108 participants from Fredrickson, 2013 & 2015 (see above)	Multivariate (OLS) linear models and generalized estimating equation (GEE) models	"The OLS estimates combined with the permutation F-tests provide some evidence of a very small negative association between Eudaimonia and mean CTRA expression, although the Monte Carlo results of these F tests raise some concern about the sign of this effect."	CTRA gene expression might only be related to eudaimonia, but the effect is very minor.

08

A genetic perspective on the relationship between eudaimonic –and hedonic well-being

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Abstract

Whether hedonism or eudaimonia are two distinguishable forms of well-being is a topic of ongoing debate. To shed light on the relation between the two, large-scale available molecular genetic data were leveraged to gain more insight into the genetic architecture of the overlap between hedonic and eudaimonic well-being. Hence, we conducted the first genome-wide association studies (GWAS) of eudaimonic well-being (*N* = ~108K) and linked it to a GWAS of hedonic well-being (*N* = ~ 222K). We identified the first two genome-wide significant independent loci for eudaimonic well-being and six independent loci for hedonic well-being. Joint analyses revealed a moderate phenotypic correlation (*r* = 0.53) and a high genetic correlation (r_g = 0.78) between eudaimonic and hedonic well-being. This indicates a substantial shared genetic etiology with genetic factors having effects on both phenotypes while there are also divergent (environmental) factors having an effect on hedonic and eudaimonic well-being. Loci regulating expression showed significant enrichment in the brain cortex, brain cerebellum, frontal cortex, as well as the cerebellar hemisphere for eudaimonic well-being. No significant enrichment for hedonic well-being is observed, although brain tissues were top ranked. Genetic correlations patterns with a range of positive and negative related phenotypes were largely similar for hedonic –and eudaimonic well-being. Our results reveal a large genetic overlap between hedonism and eudaimonia.

Introduction

For centuries, people have asked themselves questions about well-being with hedonic well-being and eudaimonic well-being as its major philosophical schools of thoughts. Hedonic well-being concerns the balance of pleasure over pain, with Aristippus (c. 435 –c. 356 BCE), as one of its founders[1]. Whereas the hedonic tradition focused on what is good for a person, the eudaimonic tradition took well-being to centre around virtuous activity, defined as knowledge (practiced over time) and the fulfilment of human capacities[2]. One of the important founders of eudaimonic well-being is Aristoteles (c. 384 – c. 322 BCE), who was a true opponent of the hedonistic school of thought describing it as "*vulgar*"[3]. According to Aristotle, eudaimonic well-being is more than being happy and is it about the actualization of the human potential [4].

In contemporary behavioural and social sciences, the term hedonic well-being is used less frequently. A reason for this is that hedonism as a theoretical (data-free) concept is difficult to quantify. To redefine the hedonic line of thought in an operational construct, the subjective well-being (SWB) definition, as proposed by Diener[5], is widely adopted. Herein, SWB consists of three hallmarks: 1) it is subjective; 2) it includes positive measures (not just the absence of negative measures), and 3) it includes a global assessment of all aspects of a person's life. SWB has been repeatedly found to be associated with health and mortality e.g.[6–9]. Analogous to hedonism, the term eudaimonic well-being has gradually shifted towards psychological well-being (PWB) in contemporary science. To assess PWB, six core dimensions are widely used: self-acceptance, positive relations with others, autonomy, environmental mastery, purpose in life, and personal growth[10]. Several studies have found that people who believe their lives have meaning or purpose appear better off, with better mental and physical health and engagement in healthier life styles[11–16].

Although, it is recognized that modern-day hedonism and eudaimonia are central concepts of well-being, the overlap and distinction between these two forms of well-being is a topic of an ongoing debate[1,17–23]. Factor analytic studies show that hedonic and eudaimonic aspects of well-being load on separate yet highly correlated factors, with correlations in the range of 0.81 to 0.92[24–26]. Application of less restrictive exploratory structural equation modelling, results in a correlation of 0.60 between hedonic and eudaimonic well-being[22]. A more in-depth overview of the reported correlation between hedonic and eudaimonic uncovers a wide spread in correlations resulting from differences in degree of centrality (if the hedonic measures are the core aspect of the analyses or if the correlation is based on correlates of the concepts),

application of different categories of analyses (if hedonia and eudaimonia is considered an orientation, behavior, experience, or function) and level of measurement (state versus trait)[20].

A way to provide more clarity on the overlap and distinction of hedonic and eudaimonic well-being is by exploring the underlying sources of overlap. Differences in both hedonic and eudaimonic well-being have been found to be partly genetic. Twin-family studies, which contrast the resemblance of monozygotic (MZ), dizygotic (DZ) twins and their non-twin siblings or other family members, report heritability estimates in the range of 30 – 64% for both hedonic and eudaimonic well-being[27,28]. Most molecular genetic work, so far, focused on hedonic measures of well-being. Initially a handful of studies attempted to associate specific candidate genes (e.g. *5-HTTLPR*, *MAOA*, *FAAH*) to hedonic well-being[29–32]. However, these studies were most likely underpowered and results have not been replicated. More recent molecular genetic approaches revealed that 5-10% of the variation in responses to single-item survey hedonic measures (happiness) is accounted for by genetic variants measured on presently used genotyping platforms[33]. Additionally, a recent large genome-wide association study (GWAS; N = 298,420) identified the first three genetic variants (two at chromosome 5 (rs3756290 and rs4958581) and one at chromosome 20 (rs2075677)) associated with SWB, defined as a combination of hedonic measurements like happiness and satisfaction with life[34].

There have only been two attempts to use molecular genetic data to reveal the overlap and distinction between hedonic and eudaimonic well-being[35,36]. The first study showed divergent transcriptome profiles between both measurements[35]. Hedonic well-being was associated with up-regulated gene expression of a conserved transcriptional response to adversity (CTRA), while eudaimonic well-being was associated with CTRA down-regulation. After substantial critiques and replies[37–40], the authors of the initial finding replicated part of the results by showing a significant inverse relation between down-regulated CTRA expression and eudaimonic well-being[36]. Based on these results, the authors conclude that eudaimonic well-being might play a more significant role in the link between well-being and health, than hedonic well-being.

The availability of large-scale molecular data make it possible to gain more insight into the genetic factors underpinning overlap and distinction between hedonic and eudaimonic well-being. In the current paper, we therefore leverage data from the UK Biobank and estimate the molecular genetic based heritability and bivariate genetic correlation. To this end, we conduct the first genome-wide association study (GWAS) to identify genetic variants associated with

eudaimonic well-being as well as a GWAS for hedonic well-being. For eudaimonic well-being we used the question: “To what extent do you feel your life to be meaningful” as a proxy phenotype. For hedonic well-being, we used ”In general how happy are you” as a proxy phenotype. As the genetic architecture can be a reflection of common biology, we annotate the genome-wide association results using gene-mapping and tissue specific enrichment analyses. Finally, we estimate whether hedonic and eudaimonic well-being show different genetic correlations patterns with positively and negatively related traits.

Results

Descriptive statistics and phenotypic correlation

For eudaimonic well-being, females and males mean scores were similar (mean =3.69, sd = 0.82 and 0.83, t = -0.79, $P = 0.43$). For hedonic well-being, males were significantly, but only slightly, happier (mean 4.52, sd = 0.74) than females (mean 4.51, sd =0.72) (t = 4.00, $P < 0.001$). Eudaimonic and hedonic well-being were moderately correlated ($r = 0.53$, $P < 0.001$).

Genome-wide association analyses

For eudaimonic well-being, 2 genetic variants reached genome-wide significance (**Table 1 and Figure 1A).** The two univariate GWAS for hedonic well-being (UKB ID 4526 and UKB ID 20458) identified, respectively 1 and 2 genome-wide significant hits (**Supplementary Table 2 and Supplementary Figure 1-2**). The genomic inflation factor (lamda Genomic Control) of eudaimonic well-being (λ_{GC} = 1.14) and hedonic well-being (λ_{GC}_UKB ID 4526 = 1.13 and λ_{GC}_UKB ID 20458 = 1.13) were inflated. The estimated intercept from LD Score regression, though, did not exceed 1.02, indicating that nearly all the inflation is the GWAS analyses is due to polygenic signal rather than bias[41] (**Supplementary Table 3**). Based on the high genetic correlation between the two hedonic well-being measures ($r_g = 0.99$, $P < 0.001$), we performed a multivariate N-weighted GWAMA to increase the effective sample size. The multivariate N-weighted GWAMA for the two hedonic GWAS analyses yielded 6 genetic variants for hedonic well-being that reached genome-wide significance (λ_{GC} = 1.21, LD intercept = 1.00; **Figure 1B, Table 1, and Supplementary Table 3**). The significant SNPs associated with eudaimonic well-being had low *P*-values (7.6×10^{-4} for rs79520962 and 3.4×10^{-5} for rs7618327) in the hedonic analyses. Three out of 6 significant SNPs associated with hedonic well-being had low *P*-values ($P < 3.6 \times 10^{-5}$) in the eudaimonic GWAS.

Validation genome-wide significant results

To validate our analyses, we cross-checked our GWAS results against a published GWAS of multiple positive affect measurements ($N \sim 133K$)[42] omitting UK Biobank samples. For hedonic well-being we identified 5 genome-wide significant SNPs present in both our current results and the previous published GWAS. All betas showed a similar direction of effect in both studies (**Supplementary Table 4)**. For eudaimonic well-being, the genome wide significant SNP (rs7618327) is also present in the previous published GWAs with similar direction of effect in both studies. From the 20 SNPs with a *P*-value < 1 X 10^{-5}, eighty-five percent had

similar direction of effects showing a significant relation ($\chi^2(1) = 7.54$, $P = 0.006$; **Supplementary Table 5)**.

Figure 1: Manhattan Plot for GWAS results. Result is shown for **(a)** Univariate GWAS of eudaimonic well-being and, **(b)** N-weighed GWAMA of hedonic well-being. The *x* axis shows chromosomal position, and the *y* axis shows association significance on a −log10 scale. The upper dashed line marks the threshold for genome-wide significance ($P = 5\times10^{-8}$), and the lower dashed line marks the threshold for nominal significance ($P = 1\times10^{-5}$). Each approximately independent genome-wide significant association (lead SNP) is marked by an orange **Δ**. Each lead SNP is the SNP with the lowest *P* value within the locus, as defined by our clumping algorithm

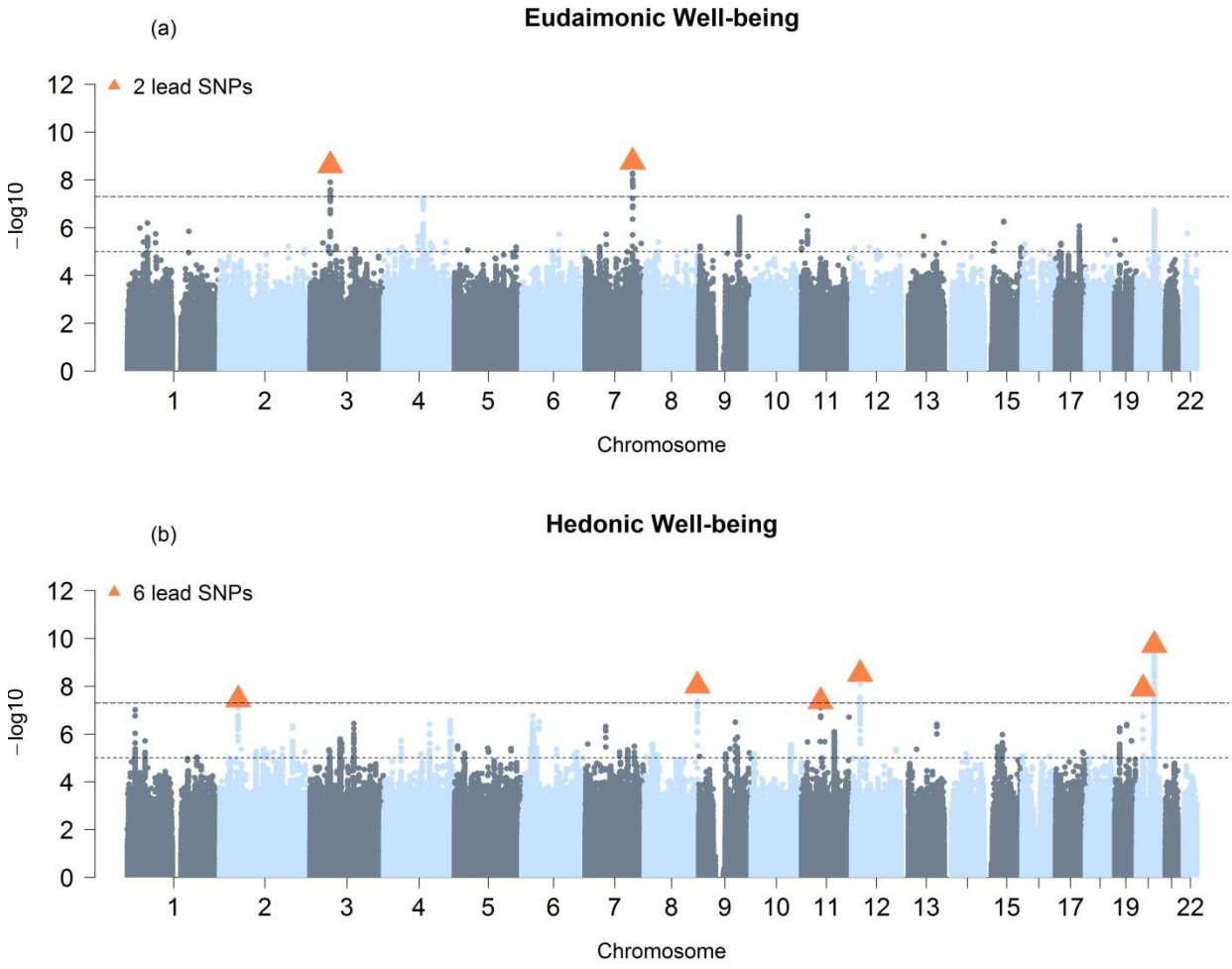


Table 1: Genome-wide significant hits for eudaimonic and hedonic well-being.

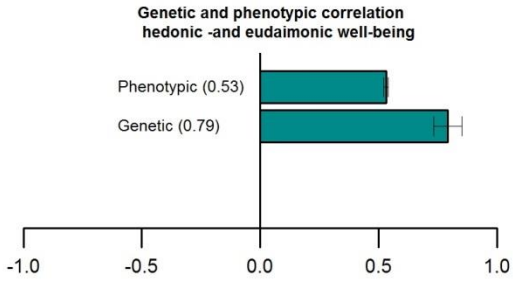
Eudaimonic well-being											
SNP	**RS**	**CHR**	**BP**	**A1**	**A2**	**Z**	***P***	**N**	**EAF**	**BETA**	**SE**
7:127671511	rs79520962	7	127671511	A	G	-6.015	1.80E-09	108154	0.05	-0.051	0.009
3:54376990	rs7618327	3	54376990	G	A	-5.961	2.52E-09	108154	0.12	-0.033	0.006
Hedonic well-being Multivariate											
20:47746974	rs34841991	20	47746974	C	T	6.367	1.92E-10	221575	0.24	0.022	0.004
12:22874365	rs261909	12	22874365	C	G	5.925	3.12E-09	221575	0.44	0.018	0.003
8:142617261	rs746839	8	142617261	G	C	-5.739	9.53E-09	221575	0.38	-0.018	0.003
20:17445078	rs4239724	20	17445078	G	A	-5.689	1.28E-08	221575	0.22	-0.021	0.004
2:49222872	rs6732220	2	49222872	C	G	5.506	3.68E-08	221575	0.77	0.020	0.004
11:51477511	rs146213057	11	51477511	A	G	5.476	4.36E-08	221575	0.01	0.084	0.015

CHR = chromosome, BP= Base Pair, A1 = Effect allele, A2 = Other allele, Z = Zscore, *P* = P-value, N = sample size, EAF = Estimated Allele Frequency, SE = Standard Error

SNP heritability and Genetic Correlation

For eudaimonic well-being, SNP h^2 was 6.2% (se = 0.005), while for hedonic well-being the SNP h^2 was 6.2% (se = 0.005) (UKB ID 4526) and 6.4% (se =0.005) (UKB ID 20458; **Supplementary Table 3**). The genetic correlation between the two measurements of hedonic wellbeing was –as expected- extremely high (0.99, $P < 0.001$). Additionally, the genetic correlation between eudaimonic and hedonic well-being was $r_g = 0.79$, ($P < 0.001$, **Figure 2 and Supplementary Table 6)**.

Figure 2: Phenotypic and genetic correlations between eudaimonic and hedonic well-being with their corresponding 95% confidence intervals.



Polygenic prediction

Polygenic scores were calculated for 10 *P*-value thresholds, using Caucasian UK Biobank participants with non-British ancestry as an independent sample. PRS based on the hedonic well-being GWAMA explained 0.83% ($P = 2.81x10^{-18}$) of the variance in eudaimonic well-being whereas PRS based on the eudaimonic well-being GWAS explained 0.43% ($P = 2.60x10^{-10}$) of the variance in hedonic well-being. A complete overview of the polygenic scores including all thresholds can be found in **Supplementary Table 7 and Supplementary Figure 3.**

Functional annotation

Eudaimonic well-being

We searched the NHGI GWAS catalog to determine which of the lead SNPs ($P < 5x10^{-8}$, independent from each other at $r^2 < 0.1$) associated with eudaimonic well-being have been previously reported. This search initially revealed that none of the variants are previously reported. However, if we look at the results of the gene-based test as computed by MAGMA

including all SNPs with a P value below 0.05, genes associated with Educational attainment[43] (*ARFGEF2*), Subjective Well-being[34] (*ARFGEF2, CSE1L*) and height[44] (*STAU1, ZFAS1*) were found.

Based on the eudaimonic well-being GWAS, 3 genes were found through positional mapping, 1 through eQTL mapping, and 13 through chromatine interaction-mapping (**Supplementary Tables 8-10**). Looking at the results of the gene-based test as computed by MAGMA including all SNPs with a *P* value below 0.05, 10 genes were associated with eudaimonic well-being (**Supplementary Table 11**). Of these 27 genes in total, one gene (*SND1*) was implicated in all four methods. The *SND1* gene encodes a transcriptional co-activator that interacts with the acidic domain Epstein-Barr virus nuclear antigen (EBNA 2), a transcriptional activator that is required for B-lymphocyte transformation. Proteins encode by this gene are thought to be essential for normal cell growth (https://www.ncbi.nlm.nih.gov/gene/27044).

Hedonic well-being

We first searched the NHGI GWAS catalog to determine which of the lead SNP associated with hedonic well-being have been previously reported. Here we found that the variants have been reported in Educational attainment[43] (*ARFGEF2*), Obesity-related traits[45] (*PCSK2, ARFGEF2*), Subjective Well-being [34] (*ARFGEF2, CSE1L*) and height[44] (*STAU1, ZFAS1*) (**Supplementary Table 12**).

Based on the multivariate N-weighted GWAMA, 7 genes were implicated through positional mapping, 9 through eQTL mapping, and 50 through chromatine interaction-mapping (**Supplementary Tables 13-15**). Using the results of the gene-based test as computed by MAGMA including all SNPs with a P value below 0.05, 35 genes were associated with hedonic well-being (**Supplementary Table 16**). Of these 101 genes in total, 16 were found in more than one strategy. Of these, two genes (*CSE1L, STAU1*) were implicated by all four methods. Proteins encode by *CSE1L*, may play a role in apoptosis and in cell proliferation (https://www.ncbi.nlm.nih.gov/gene/1434?otool=inlvulib). The *STAU1* gene is a member of the family of double stranded RNA (dsRNA)-binding proteins involved in the transport and/or localization of mRNAs to different subcellular compartments. *STAU1* contains a microtubule-binding domain similar to that of microtubule-associated protein 1B (*MAP1B*) and bind tubulin (https://www.ncbi.nlm.nih.gov/gene/6780).

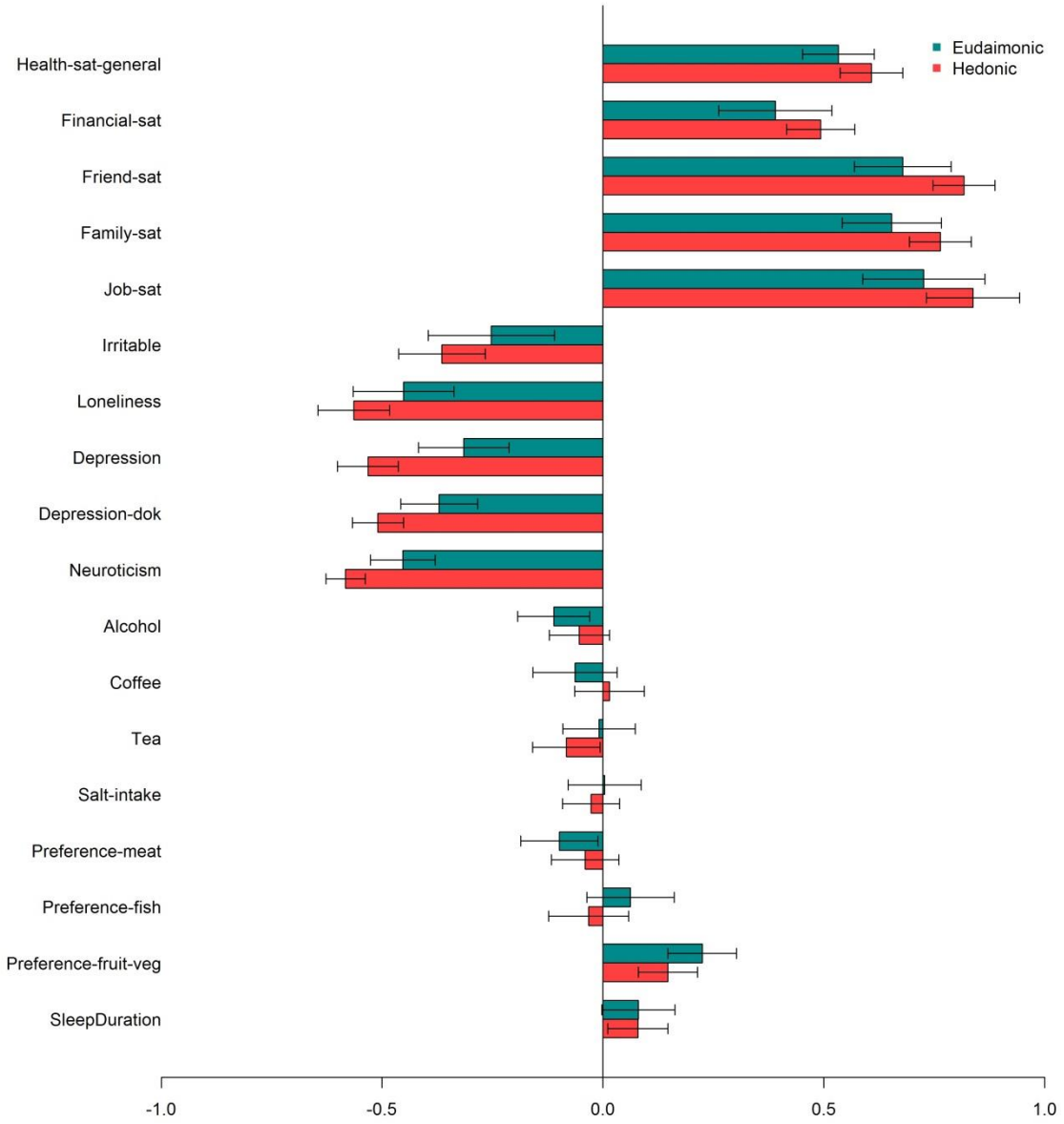
Tissue Specific expression

Tissue expression analysis, performed on GTEx RNA-sq data, showed significant enrichment in the brain cortex, brain cerebellum, frontal cortex, as well as the cerebellar hemisphere for eudaimonic well-being. In contrast, no significant results were found for hedonic well-being, although brain tissues were top ranked in their enrichment (**Supplementary Table 17, 18, Supplementary Figure 4**).

Genetic Correlations

Another way to study the relationship between eudaimonic and hedonic well-being is by comparing their genetic correlation patterns with positive and negative related traits. Overall, we found a similar pattern for both eudaimoninc and hedonic well-being. Both were positively correlated with satisfaction with health (*rgEUD* = 0.53, *rgHED* = 0.61), financial satisfaction (*rgEUD* = 0.39, *rgHED* = 0.49), friendship satisfaction (*rgEUD* = 0.68, *rgHED* = 0.81), family Satisfaction (*rgEUD* = 0.65, *rgHED* = 0.76) and job satisfaction (*rgEUD* = 0.73, *rgHED* = 0.84). Negative correlations were found for irritable (*rgEUD* = -0.25 , *rgHED* = -0.36), loneliness (*rgEUD* = -0.45 , *rgHED* = -0.56), depressive symptoms (*rgEUD* = -0.32 , *rgHED* = -0.53), depression diagnosed by doctor (*rgEUD* = -0.37 , *rgHED* = -0.51), and neuroticism (*rgEUD* = -0.45 , *rgHED* = -0.58; **Figure 3 and Supplementary Table 19)**. These similar patterns support the finding of a large genetic overlap between eudaimonic and hedonic well-being.

Figure 3: Genetic correlations between eudaimonic (blue) –and hedonic well-being (red) with (from top to bottom): satisfaction with health, financial satisfaction, friendship satisfaction, familial satisfaction, job satisfaction, irritable, loneliness, depression, depression diagnosed by a doctor, neuroticism, alcohol use, coffee use, tea use, salt intake, meat preference, fish preference, fruit preference and sleep duration. 95% confidence intervals are provided.



Discussions

In this article, we provide evidence for a strong genetic overlap between hedonic and eudaimonic well-being. Our analyses revealed a moderate phenotypic correlation (r = 0.53), but a high genetic correlation (r_g = 0.78), suggesting a large shared genetic etiology. Our results include the first two genome-wide significant independent loci for eudaimonic well-being and six independent loci for hedonic well-being. Biological annotation points to a central role for the central nervous system in both forms of well-being. Loci regulating expression showed significant enrichment in the brain cortex, brain cerebellum, frontal cortex, as well as the cerebellar hemisphere for eudaimonic well-being. No significant enrichment for hedonic well-being is observed, although brain tissues were top ranked.

To validate our genome-wide analyses, we performed a direction of effect test with a previous GWAS study including multiple positive affect measurements (N = ~ 133K). Significant SNPs for both hedonic -and eudaimonic well-being have similar directions in the previous published GWAS of positive affect, whereas 17 out of 20 SNPs (eighty-five percent) of the suggestive eudaimonic SNPs had similar direction of effects. Moreover, we obtained significant polygenic score predictions for both eudaimonic and eudaimonic well-being. Although the explained variance is small (< 1 %), due to the small effect sizes of the genetic variants, our results are in line with previous studies[46,47]. Given these results, together with the multiple robustness checks (e.g. LD Score intercept of one, large genetic correlation with each other and similar patterns of genetic correlation with related traits), we are, beyond reasonable doubt, convinced that our genome-wide associations findings are credible findings.

The high genetic correlation between the two forms of well-being can be a product of a causal relationship between the two traits. The direction of effect between hedonic –and eudaimonic well-being can be assessed using a two-sample Mendelian Randomization (MR) design. However, given the relatively small sample size and limited genetic variants reaching genome-wide significance, we are not able to construct strong instrumental variables that are needed for trustworthy interpretations of the direction of effect between hedonic –and eudaimonic well-being. However, recent-non-genetic studies investigating the relationship between subjective well-being (SWB) and psychological well-being (PWB) found stronger evidence for a causal relation from PWB to SWB than vice versa[48–50]. It would be very interesting for future studies to investigate the causal relationship between hedonic-and eudaimonic well-being in a genetically informed dataset to be able to investigate causality and (genetic) pleiotropy.

Further evidence for a shared genetic architecture between hedonic and eudamonic well-being is provided by the similar patterns of genetic correlations with other traits. Largest correlations were found for job satisfaction followed by friendship –and family satisfaction and general health satisfaction. Remarkably, in contrast to job satisfaction, financial satisfaction showed the lowest correlation with both eudaimonic –and hedonic well-being. Genetic correlations with negative related phenotypes were for both measures largest for neuroticism followed by loneliness, depression (2X) and irritable. Thus, genetic correlations showed similar patterns for both measures of well-being, with largely overlapping confident intervals (CIs). However, point estimates for hedonic well-being were systematically larger compared to eudaimonic well-being, which is unlikely due to chance. Therefore, it would be interesting for future studies with larger samples to test whether hedonic well-being indeed a shows stronger associations with related phenotypes. Moreover, the lower phenotypic correlation suggests that there are divergent (environmental) factors having an effect on hedonic –and eudaimonic well-being. It would be very interesting to identify these factors in future studies. In this light our results are supportive of a two-factor model with highly correlated constructs.

Besides adding to the ongoing debate on the overlap and distinction between hedonic and eudaimonic well-being the current study provides novel insight into the genetics of well-being by identifying genome-wide significant genetic variants that explain differences in eudaimonic well-being. These variants have not been associated with a complex trait before, and thus warrant replication. Robustness of the current findings, though, is reflected by our validation analyses. Moreover, the genome-wide significant genetic variant at chromosome 20 identified in the hedonic well-being GWAMA lies in close proximity (< 50 kb) to a genetic variants previously associated with subjective well-being[34].

The findings of this study should be interpreted in light of the following limitations. One is that eudaimonic and hedonic well-being are based on single item measurements. Ideally, measurements with multi-item measurements would be included. For eudaimonic well-being, principal factor analysis of the 8-item Flourishing scale [51] showed that all items of this scale, which included our included question: “To what extent do you feel your life to be meaningful”, load all on one single factor. Moreover, our question showed the highest correlation with all other items as well as with the total score. For Hedonic well-being, Bartels and Boomsma[27] have shown that both multi-item and single-item questionnaires load on a single well-being factor. We, however, have explicitly chosen not to include all other available hedonic results of our previous work[34,52], to leverage the power of homogeneity of the UK Biobank dataset and to

ease the interpretation of our findings. Research studying higher-quality measures of the various facets of well-being is a critical next step. Our results can help facilitate such work because, if the variants we identify are used as candidates, studies conducted in the smaller samples in which more fine-grained phenotype measures are available can be well powered. Additionally, it is known that participants of the UK Biobank have a specific age range (40-70 years). In previous work we, however, showed that the variance explained by genetic factors for well-being over time is stable[53] and that genetic innovation is not likely to take place in adulthood [54]. Therefore, we are confident that this characteristic of the UKbiobank sample will not have a large effect on the results.

In conclusion, we found a moderate phenotypic correlation between eudaimonic and hedonic well-being and report a strong genetic correlation, indicating that from a genetic perspective there is a large shared etiology. Future studies should acknowledge the strong genetic correlation between eudaimonic and hedonic well-being and include both to increase our understanding of the (genetic) etiology of well-being.

Methods

Participants

We analyzed data from the UK Biobank project[58]. The UK Biobank is a prospective study designed to be a resource for research into the causes of disease in middle and old age. The study protocol and information about data access are available online (http://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf) and more details on the recruitment and study design have been published elsewhere[58]. The UK Biobank study was approved by the North West Multi-Centre Research Ethics Committee (reference number 06/ MRE08/65), and at recruitment all participants gave informed consent to participate in UK Biobank and be followed-up, using a signature capture device. All experiments were performed in accordance with guidelines and regulations from these committees. In brief, all participants were registered with the UK National Health Service (NHS) and lived within 25 miles (40 km) of one of the assessment centres. The UK Biobank invited 9.2 million people to participate through postal invitation with a telephone follow-up, with a response rate of 5.7%. A total of 503,317 men and women aged 40–70 years were recruited in assessment centres across England, Wales and Scotland, between 2006 and 2010. In total, 608 participants have subsequently withdrawn from the study and their data were not available for analysis. Participants attended 1 of 22 assessment centers across the UK, at which they completed a touch-key questionnaire, had a face-to-face interview with a trained nurse, and underwent physical assessments. Participants completed sociodemographic questionnaires, which included questions on financial satisfaction and income as well as questionnaires about their physical and mental health.

Data access permission was granted under UKB application 25472 (PI Bartels). For the discovery genome-wide association analyses we used data of ≈ 110K UK-habitant Caucasian individuals only. A full overview of the included participants with valid phenotypic measurements as well as genetic data is presented in **Supplemental Table 1**.

Phenotypic data

Eudaimonic well-being was assessed in the online follow-up with its core element meaning in life ("To what extent do you feel your life to be meaningful?"; UKB Data-Field 20460). Answers were provided on a 5-item likert scale that ranged from "Not at all" (score 1) to "An extreme amount" (score 6). Information on eudaimonic well-being and genotypic data were available for 108,154 UK Biobank participants (56% female).

Hedonic Well-being was assessed with its core element general happiness ("In general how happy are you?"; UKB Data-Field 4526 & UKB Data-Field 20458). Answers were provided on a 6-item likert scale that ranged from "Extremely happy" (score 1) to "Extremely unhappy" (score 6). Scores were reversed so that a higher score was associated with higher levels of happiness. Hedonic well-being, as part of the touchscreen questionnaire on psychological factors and mental health (data-field 4526), was available for 111,470 individuals. Hedonic well-being was also assessed in the online follow-up (data-field 20458) and this measure is available for 110,105 individuals. Almost forty thousand individuals (n=39,999) participated in both assessments. In total, information on hedonic well-being and genotypic data were was available for 181,578 unique UK Biobank participants (49% female; **Supplementary Table 1**).

Because the online follow-up questionnaire (ID 20458) of hedonic well-being took place at a later stage (~4 years later), there is a possible discrepancy between the genetic and psychological assessment. To study whether this has an effect on the genetic analyses we will calculate the genetic correlation between both measurements of hedonic well-being. Doing so allows us to investigate whether the same genes have an effect on both measurements.

Genotypic data

Participants were genotyped using one of two platforms: The affymetrix UK BiLEVE Axiom array or the Affymetrix UK Biobank Axiom array. The genetic data underwent rigorous quality control and was phased and imputed against a reference panel of Haplotype Reference Consortium (HRC), UK10K and 1000 Genomes Phase 3 haplotypes[59]. Due to an issue with the imputation of UK10K and 1000 Genomes variants, analyses were restricted to HRC variants only. Samples were excluded based on the following genotype-based criteria; non-European ancestry, relatedness, mismatch between genetic sex and self-reported gender, outlying heterozygosity, and excessive missingness[59]. For more details on the UK Biobank genotyping, imputation, and quality control procedures see[60].

Descriptive statistics and phenotypic correlation

Descriptive statistics and spearman's rank correlation between eudaimonic and hedonic well-being were calculated in R. We, furthermore, tested for sex and age effects on mean levels.

Univariate Genome-wide association analyses

Univariate genome-wide association analyses for eudaimonic well-being and for hedonic well-being (touchscreen measure and online follow-up separately) were performed in PLINK[61,62] using a linear regression model of additive allelic effects. Standard pre-GWAS- quality control filters were applied, which included removing SNPs with minor allele frequency < 0.005 and/or with an INFO-score < 0.8 for imputed SNPs, and removing individuals with ambiguous sex and/or non-British ancestry. We, furthermore, randomly selected 1 individual from each closely related pair (i.e. parent offspring pairs, sibling pairs). The GWAS included 40 principal components, age, sex, and a chip dummy as covariates. Additionally, following a pre-specified analysis plan, we conducted a stringent post-GWA quality control (QC) protocol based on the paper of Winkler and colleagues[63].

Multivariate Genome-wide association analyses

To increase the effective sample size, we conducted multivariate N-Weighted genome-wide association meta-analyses (GWAMA) by leveraging the association between the two hedonic well-being univariate GWAS analyses (UKB Data-field 4526 and 20458, n_{obs} total = 221,575). The dependence between effect sizes (error correlation) induced by sample overlap in both these GWAMAs was estimated from the genome-wide summary statistics of the univariate GWAS analyses using LD score regression[64]. Knowledge of the error correlation between the univariate GWAS analyses allowed us to meta-analyze them together, providing a gain in power while guarding against inflated type I error rates. For a detailed description on performing N-weighted GWAMA, please see Baselmans and colleagues[52].

Validation genome-wide significant results

To validate our analyses, we cross-checked our GWAS results against a published GWAS of multiple positive affect measurements (N ~ 133K)[42] omitting UK Biobank samples. The positive affect GWAS used the HapMap2 CEU as reference sample (~2.2 million SNPs), which contains considerable less SNPs compared to the roughly 8.6 million SNPs (1000G, phase 3) present in the UK Biobank analyses. We used the following strategy to identify proxy genome-wide significant SNPs present in both datasets. First, we extracted the genome-wide significant ($P < 5 \times 10^{-8}$ from the GWAS of hedonic well-being) and suggestive SNPs ($P < 1 \times 10^{-5}$, GWAS of eudaimonic well-being) and matched these to the corresponding positive affect SNPs of the published GWAs. Next, using a clumping procedure (250kb window and $R2 < 0.1$), we identified the independent SNPs present in both datasets, which will be used for testing the direction of effect. When there is a discrepancy in direction of effect between the two datasets, a Chi-square test of independence was calculated to test the significance of the relation.

SNP heritability and Genetic Correlation

SNP heritability for eudaimonic and hedonic well-being separately was estimated using bivariate LD Score Regression[64,65]. The same methodology was used to estimate the genetic correlation between the two measures of hedonic well-being and between eudaimonic and hedonic well-being. LD scores regression produces unbiased estimates even in the presence of sample overlap and only requires summary statistics and a reference panel from which to estimate each SNP's "LD score" (the amount of genetic variation tagged by a SNP). We used the file of LD scores computed by Finucane et al.[66] using genotypic data from a European-ancestry population (see https://github.com/bulik/ldsc/wiki/Genetic-Correlation, accessed September 8, 2017).

Polygenic prediction

We performed polygenic risk score prediction (PRS) using Caucasian UK Biobank participants with non-British ancestry as independent prediction sample (n_{obs} = 28,582). For eudaimonic well-being, polygenic prediction was performed in 9,088 individuals. For hedonic well-being, we used phenotypic measurements closest to genotype-collection (UKB Data-Field 20458) for polygenic scores and scores were available for 9,276 individuals. The weights used for the polygenic scores are based on the univariate GWAS (eudaimonic) and multivariate GWAMA

(hedonic well-being). Polygenic scores were based on the genotyped SNPs (n_{obs} = 619,049). To calculate the incremental R^2, the phenotypes (eudaimonic and hedonic well-being) were standardized and regressed on sex and age as well as principal components, which were included to correct for ancestry. Next, the same analysis was repeated with inclusion of the polygenic scores. The differences in R^2 between both regression is referred to as incremental R^2. To obtain 95% confidence intervals (CI) around the incremental R^2 's, bootstrapping was performed with 2000 repetitions.

Functional annotation

Functional annotation was performed in FUMA[67] (http://fumactglab.nl) for the eudaimonic well-being GWAS and the hedonic well-being GWAMAs. Lead SNPs were defined as having a genome-wide significant P values ($5x10^{-8}$) and being independent from each other ($r^2 < 0.1$). Functional annotation was performed on these lead SNPs and SNPs with P < 0.05, MAF < 0.01, and in high LD ($r^2 > 0.6$) with those lead SNPs.

Gene-mapping

This set of SNPs was mapped to genes in FUMA using three strategies. The SNPs were mapped to genes based on 1) their physical distance (i.e. within 10kb window), 2) significant eQTL association (i.e. the expression of that gene is associated with allelic variation at the SNP). eQTL mapping in FUMA uses information from the GTEx, Blood eQTL browser, and BIOS QTL browser, and is based on cis-eQTLs that can map SNPs to genes up to 1MB apart. A false discovery rate (FDR) of 0.05 was applied to define significant eQTL associations. 3) a significant chromatin interaction between a genomic region and promoter regions of genes (250bp up and 500bp downstream of transcription start site (TSS)). Chromatine interaction mapping can involve long-range interaction as it does not have a distance boundary as in eQTL mapping. We used a FDR p-value of $1x10^{-5}$ to define significant interactions.

Finally, given our modest sample size and expected polygenicity of our phenotypes, we added an extra strategy in which all SNPs (P < 0.05) were included and mapped to genes based on physical distance (i.e. within 10kb window) from known protein coding genes (GRCh37/hg19). Genome-wide significance for this test was defined at P = 0.05/ 18187 = $2.74x10^{-6}$.

Tissue Expression Analysis (MAGMA)

To test the relationship between highly expressed genes in a specific tissue and genetic associations, gene-property analysis is performed using average expression of genes per tissue type as a gene covariate. Gene expression values are $\log^2$ transformed average RPKM (Reads Per Kilobase Million) per tissue type after winsorized at 50 based on GTEx RNA-seq data. Tissue expression analysis is performed for 53 specific tissue types separately. The result of the gene analysis (gene-based P value) were used in MAGMA to test for one-side increased expression conditioned on average expression across all tissue types.

Genetic Correlation

To test whether hedonic or eudaimonic well-being are genetically differently correlated with a set of related phenotypes, bivariate LD Score regression was applied with both measures of well-being and the following UK Biobank summary statistics: satisfaction with health (UKB ID 20459), financial satisfaction (UKB ID 4581), friendship satisfaction (UKB ID 4570), family satisfaction (UKB ID 4559), job satisfaction (UKB ID 4537), irritable (UKB ID 4653), loneliness (UKB ID 2020), depressive symptoms (UKB ID 2100), depression diagnosed by doctor (UKB ID 2090), neuroticism (UKB ID 20127). To test the relationship between hedonic/eudaimonic well-being with less established phenotypes we included the following phenotypes: alcohol (UKB ID 1558), coffee (UKB ID 1498), tea (UKB ID 1488), salt (UKB ID 1478), food preference meat (UKB ID 1349), food preference fish (UKB ID 1329), food preference fruit/vegetarian (UKB ID 1289), sleep duration (UKB ID 1160). For every genetic correlation 95% confident intervals were calculated.

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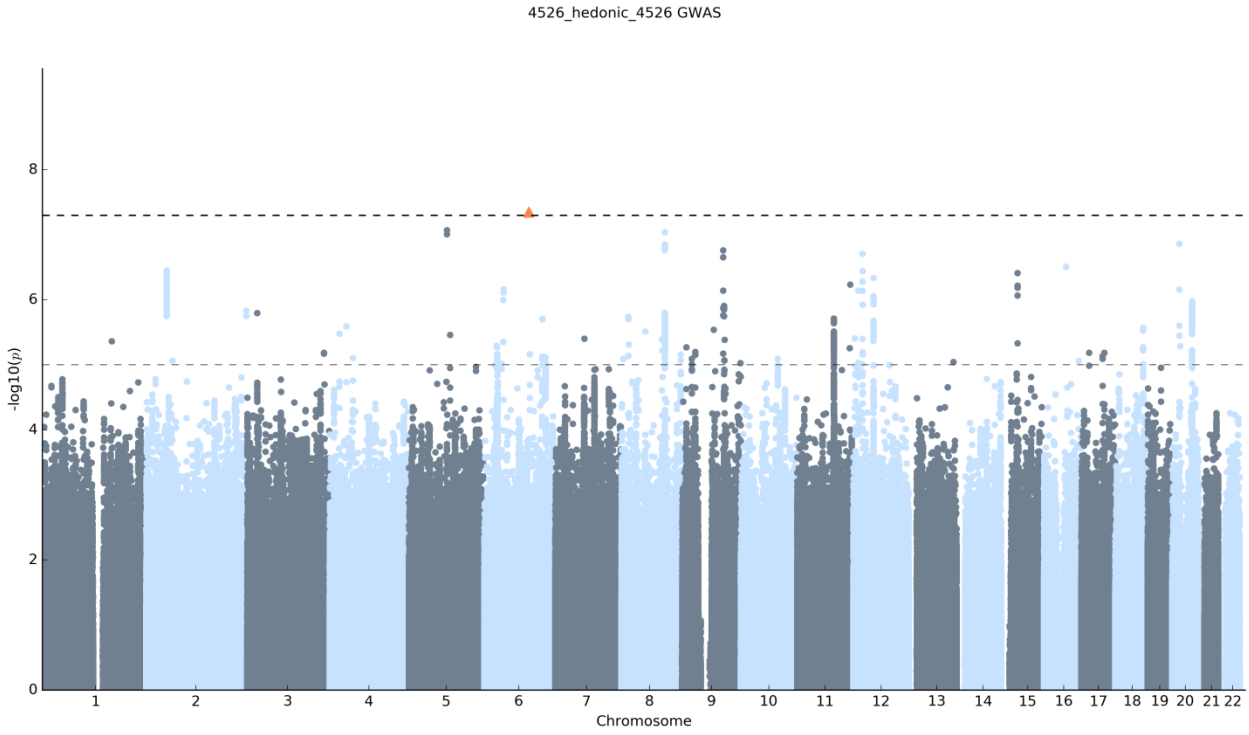
Supplementary Information

Supplementary Figure 1: Manhattan Plot for GWAS results. Result is shown for Hedonic well-being (UKB ID 4526). The *x* axis shows chromosomal position, and the *y* axis shows association significance on a −log10 scale. The upper dashed line marks the threshold for genome-wide significance ($P = 5 \times 10{-}8$), and the lower dashed line marks the threshold for nominal significance ($P = 1 \times 10{-}5$). Each approximately independent genome-wide significant association (lead SNP) is marked by an orange **Λ**. Each lead SNP is the SNP with the lowest *P* value within the locus, as defined by our clumping algorithm.



Supplementary Figure 2: Manhattan Plot for GWAS results. Result is shown for Hedonic well-being (UKB ID 20458). The *x* axis shows chromosomal position, and the *y* axis shows association significance on a −log10 scale. The upper dashed line marks the threshold for genome-wide significance ($P = 5 \times 10{-}8$), and the lower dashed line marks the threshold for nominal significance ($P = 1 \times 10{-}5$). Each approximately independent genome-wide significant association (lead SNP) is marked by an orange **Δ**. Each lead SNP is the SNP with the lowest *P* value within the locus, as defined by our clumping algorithm.

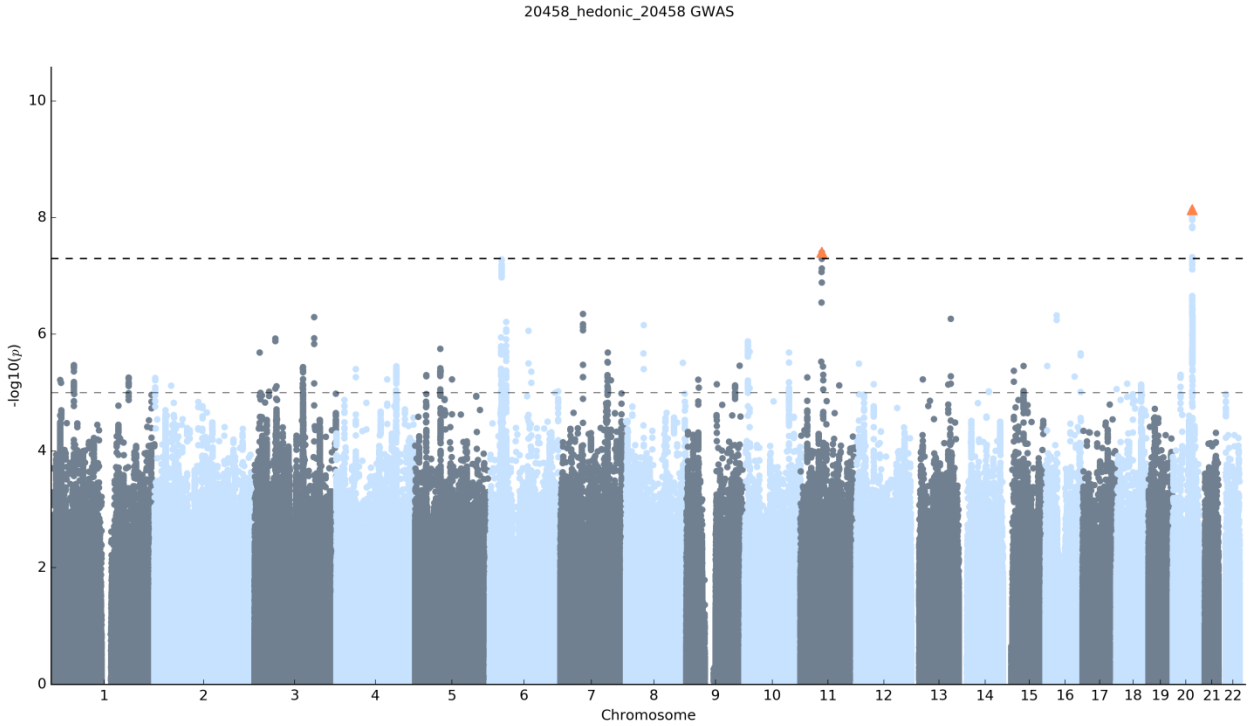


Figure 3: Polygenic scores thresholds for **(a)** Eudaimonic well-being, and **(b)** Hedonic well-being. The y axis shows the explained variance in percentage.

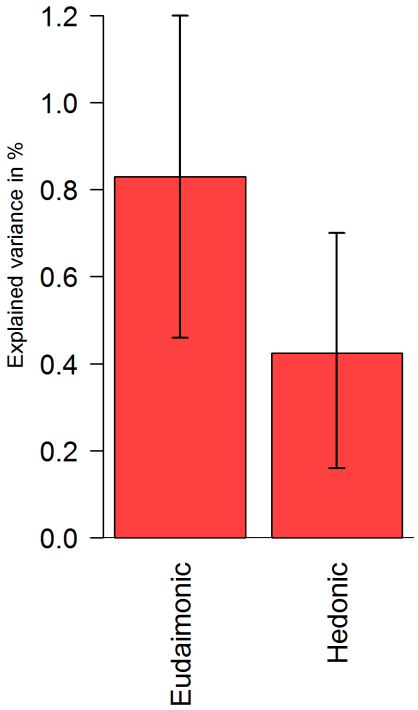
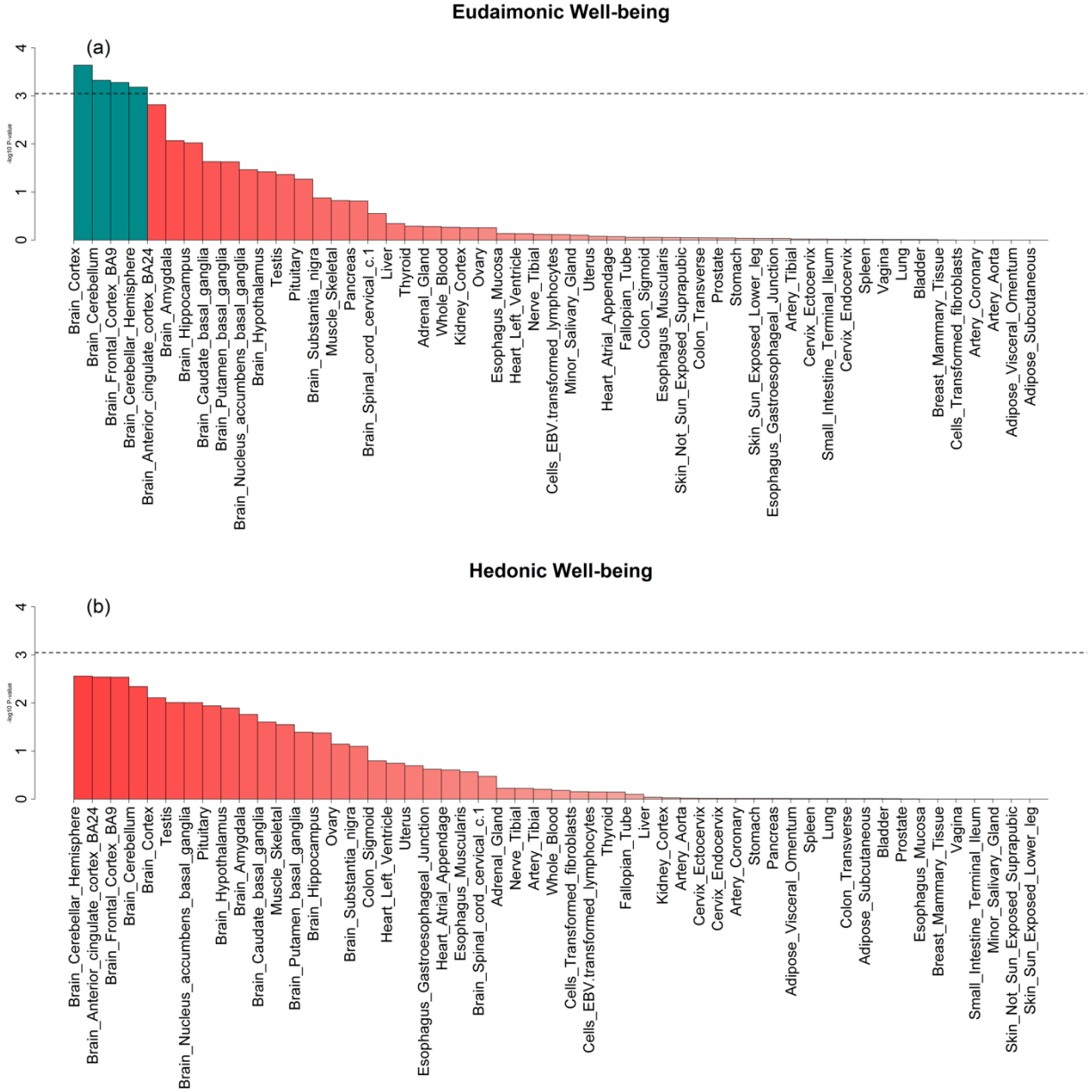


Figure 4: Tissue specific enrichment using 53 specific tissue types for **(a)** Eudaimonic, **(b)** Hedonic. Bar-graphs above the dashed line are significantly enriched. The x axis shows the 53 different categories whereas the y axis shows the $-\log^{10}$ P value. Bars in blue are significant enriched.



09

A Genetic Investigation of the Well-Being Spectrum

Revise and resubmit as:

Baselmans, B.M.L., van de Weijer, M.P., Abdellaoui, A., Vink, J.M., Hottenga, J.J., Willemsen, G., Nivard, M.G., de Geus, E.J.C., Boomsma, D.I., Bartels, M (2018). A Genetic Investigation of the Well-Being Spectrum

Abstract

The interrelations among well-being, neuroticism and depression can be captured in a so-called well-being spectrum (3-phenotype well-being spectrum, 3-WBS). Several other human traits are likely linked to the 3-WBS. In the present study, we investigate how the 3-WBS can be expanded. First, we constructed polygenic risk scores for the 3-WBS and used this score to predict a series of traits that have been associated with well-being in the literature. We included information on loneliness, big five personality traits, self-rated health, and flourishing. The 3-WBS polygenic score predicted all the original 3-WBS traits and additionally loneliness, self-rated health, and extraversion (R^2 between 1.52-0.69%). Next, using LD score regression, we calculated genetic correlations between the 3-WBS and the traits of interest. From all candidate traits, loneliness and self-rated health were found to have the strongest genetic correlations (r_g= .78, and r_g= .65, respectively) with the 3-WBS. We propose to include these traits in the well-being spectrum and use a 5-phenotype well-being spectrum in future studies to gain more insight into the determinants of human well-being.

Introduction

Many mental disorders share a common genetic liability[1–3]. This common genetic liability offers an explanation as to why many disorders are comorbid or represent highly similar behaviours. While there have been detailed investigations of the genetic similarity and comorbidity of mental disorders, there is much less information about the genetic similarity of mental health traits such as happiness, satisfaction with life, personality, loneliness, self-rated health, and flourishing. Studies on traits that could be considered to be part of a well-being spectrum are important given the large collection of studies pointing to the emotional, cognitive, and interpersonal benefits of high levels of well-being above and beyond the absence of mental disorders[4–6]. Therefore, the aim of this study was to investigate the genetic similarity between several traits associated with well-being, collectively referred to as the "well-being spectrum".

Well-being is a broad and complex construct used to describe optimal psychological functioning[7] and it has been recently proposed to use a spectrum approach[8]. This well-being spectrum (the 3-phenotype well-being spectrum; 3-WBS) captures the phenotypic and genetic overlap between subjective well-being, neuroticism, and depressive symptoms, as has been found in a large genome-wide association study[9]. There are nonetheless other traits that could be considered candidates for inclusion in a broader well-being spectrum. From a phenotypic perspective, it is important to identify such traits in order to get more insight into the aspects influencing human well-being. From a genetic perspective, it is important to identify these traits since their inclusion into the spectrum will help identify more genetic variants that influence human well-being.

One of the associations that has been studied thoroughly is the relationship between well-being and personality. Especially extraversion and conscientiousness have been established as strong positive phenotypic correlates of well-being[10], while neuroticism has been identified as an important negative correlate of well-being[11]. Furhermore, existing literature has established that loneliness, characterized by a sense of emptiness, worthlessness, and a lack of control[12], is negatively associated with well-being[13]. Moreover, self-rated health, a subjective evaluation of one's current health status, has also been pointed out as an important predictor of well-being, due to its high proportion of shared variance with well-being[14,15]. Lastly, while the 3-WBS has included subjective/ hedonic well-being measures (such as satisfaction with life and subjective happiness), it did not yet include psychological or eudaimonic well-being

measures, a well-being domain that involves the fulfilment of human potential [7]. An example of such a measure is flourishing: a person's self-perceived success in several life areas. Previous research on the relationship between psychological/ eudaimonic- and subjective/hedonic well-being have revealed that these two lines of research reflect highly correlated, yet distinguishable constructs[16,17]. Therefore, including both types of well-being could theoretically yield a more integrated conception of the well-being spectrum.

Contrary to the phenotypic associations, few studies have investigated the genetic associations for well-being and associated traits. A genetic investigation of loneliness[18] revealed a strong negative association between a polygenic score for loneliness and subjective well-being, and a positive association with neuroticism and depression, indicating genetic links between the 3-WBS and loneliness. With regard to self-rated health, twin studies have demonstrated that both genes and the environment contribute to the association with well-being[19], but to our knowledge no molecular genetic study is conducted yet. A twin study on the relationship between subjective/hedonic- and psychological/eudaimonic well-being (PWB) indicates a single, genetic factor that accounts for the high heritability in both these constructs[20]. Likewise, a genome-wide association study on hedonic and eudaimonic well-being showed that there is a large overlap in the sets of genes influencing these two traits[17] Lastly, extraversion, and conscientiousness show not only strong phenotypic, but also genetic associations with well-being[21,22].

In this study, we aim to further investigate the well-being spectrum from a genetic perspective. We perform two different types of analyses to compute the genetic associations between the 3-WBS and the likely candidates. We use summary statistics from a large multivariate GWAMA of the 3-WBS[8] to calculate polygenic risk scores to predict satisfaction with life, happiness, neuroticism, depressive symptoms, loneliness, openness to experience, conscientiousness, extraversion, agreeableness, self-rated health, and flourishing.Since the amount of variance explained by polygenic scores can be small even though two traits are highly genetically correlated, we also calculate the standardized proportion of the variance shared by the traits that can be attributed to genetic factors, known as the genetic correlation, using LD score regression.

Materials and Methods

Participants

Participants are voluntary participants in the studies of the Adults Netherlands Twin Register[23,24]. Participants were included if they had filled out questionnaires on one or more of the relevant traits and provided a blood or buccal cell sample for DNA isolation and genotyping. Based on the availability of the data, sample size per analyses varied. An overview of the sample characteristics can be found in Table I and details are provided below.

Subjective Well-Being - Satisfaction with Life

Satisfaction with life was assessed using the satisfaction with life scale[25]. The satisfaction with life scale contains 5 items measuring global cognitive judgments of satisfaction with one's life on a scale from 1 (strongly disagree) to 7 (strongly agree). Items were summed to calculate an individual's final score ranging from 0 to 35. A mean was calculated when satisfaction with life was assessed on more than one occasion. In total, data on satisfaction with life were available for 5344 individuals.

Subjective Well-Being - Happiness

Happiness was assessed using an adaptation of the subjective happiness scale[26]. The adapted subjective happiness scale contains 4 items measuring global subjective happiness on a scale from 1 (strongly disagree) to 7 (strongly agree). Items were summed to calculate an individual's final score, ranging from 0 to 28. A mean was calculated when subjective happiness was assessed on more than one occasion. In total, data on subjective happiness were available for 5350 individuals.

Depressive Symptoms

Depressive symptoms were assessed using the DSM-oriented depressive problem scale of the Adult Self Report[27]. This scale contains 14 items measuring depression symptoms on a scale from 0 to 2 (0= not true, 1= somewhat true, 2= very true or often true). The items were summed to create a sum score ranging from 0 to 28, a higher score representing higher levels of depressive symptoms. A mean was calculated when the depression problems were assessed on more than one occasion. In total, data on depressive symptoms were available for 8667 participants.

Loneliness

Loneliness was assessed using the short scale for assessing loneliness in large epidemiological studies[28,29]. This scale contains 3 items from the R-UCLA loneliness scale and asks participants to score how often they identify with the items on a scale from 1 to 3 (1= hardly ever, 2= some of the time, 3=often). The items were summed to obtain a sumscore with possible scores between 3 and 9, a higher score representing higher levels of loneliness. In total, data on loneliness were available for 8817 participants. A mean was calculated when loneliness was assessed on more than one occasion. We log-transformed the loneliness scores since they were highly positively skewed.

Personality

The Big Five personality traits were measured using the NEO-FFI[30,31]. This scale measures the Big Five personality traits (openness to experience, conscientiousness, extraversion, agreeableness, and neuroticism) with 60 items in total. Participants were asked to respond on a 5-point scale, ranging from 1 (strongly disagree) to 5 (strongly agree). The 12 items per trait were summed to obtain one sumscore for each personality trait with possible scores between 12 and 60, a higher score representing higher levels of that particular personality trait. When personality data were available for more than one occasion, we calculated an individual's mean personality score per scale. In total, data on each personality scale were available for 8622 individuals.

Self-Rated Health

Self-rated health was assessed using a single item: "How, in general, is your health?"[32]. The item was rated on a 5-point scale, on which participants could respond with: "Bad", "Poor", "Fair", "Good" or "Excellent". A mean was calculated when Self-rated health was assessed on more than one occasion. In total, 8667 participants had data available for self-rated health.

Flourishing

Flourishing was assessed using the Flourishing Scale[33]. This scale contains 8 items measuring a person's self-perceived success in multiple life domains on a scale from 1 to 7, ranging from strong disagreement to strong agreement. The items were summed to create a sumscore ranging from 8 to 56, a higher score representing higher levels of positive flourishing. In total, data on flourishing were available for 2200 participants.

Genotyping, Quality Control, Imputation, and PCA

Genotyping was done on several genome-wide SNP micro-arrays[24]. Genotyped data were cross-platform imputed using the Genome of the Netherlands (GoNL)[34,35] as a reference set to infer the SNPs missing per platform in the combined data[36]. Alleles with reference set allele frequency differences of >10%, SNPs with MAF <.005, deviation from Hardy-Weinberg Equilibrium with $p<10^{-12}$, and a genotyping call rate <.95 were excluded for pre-imputation quality control. Samples that had a genotyping call rate <.90, inbreeding coefficient from PLINK (F) < -.075 or >.075[37], Affymetrix Contrast Quality Control metric <.40, Mendelian error rate >5 standard deviations from the mean, or gender or Identity-by-State status that did not agree with known relationship status and genotypic assessment were excluded. MaCH-Admix software[38] was used for phasing and imputation. SNPs that were significantly associated with genotyping platform ($p<10^{-5}$), that had an allele frequency difference of >10% with GoNL reference set, HWE $p<10^{-5}$, Mendelian error rate >5 SD from the mean over all markers, or an imputation quality R^2<.90 after imputation were excluded. In order to exclude individuals with a non-Dutch ancestry and to control for Dutch population stratification, we performed Principal Components Analysis (PCA) following procedures described by Abdellaoui et al. (2013). The remaining SNPs (N=1,224,793) were used to construct polygenic scores.

Phenotypic Correlations

Phenotypic correlations were calculated between all the traits using the gee package to correct for familial relatedness using in R statistical software[40]. The results were visualized using the corrplot package. The significance threshold for the phenotypic correlations was set at a Bonferonni corrected value of α =.005/55 = 0.00009, where 55 represents the number of correlations that were calculated in total.

Table I : Sample characteristics

Trait	Age *M*(*SD*)	*N* participants (% males)	Score *M*(*SD*)
Satisfaction with Life	40.94(15.83)	5344 (37.18%)	26.96(4.70)
Happiness	39.36(15.59)	5350 (37.14%)	22.48(4.17)
Neuroticism	42.09(15.94)	8622 (36.29%)	22.21(8.10)
Depressive Symptoms	38.68 (16.00)	8667 (36.45%)	3.58(3.19)
Loneliness	43.38(16.37)	8817 (36.43%)	3.82(1.03)
Openness to Experience	42.09(15.94)	8622 (36.29%)	29.65(6.58)
Conscientiousness	42.09(15.94)	8622 (36.29%)	37.96(6.28)
Extraversion	42.09(15.94)	8622 (36.29%)	34.27(6.73)
Agreeableness	42.09(15.94)	8622 (36.29%)	37.42(5.98)
Self-Rated Health	38.44(15.68)	8667 (39.26%)	4.07(.59)
Flourishing	40.16(14.96)	2200 (35.95%)	46.84(6.47)

Power Analysis

We used an online power-calculator based on code provided by Dudbridge (2013) to investigate whether the 3-WBS summary statistics[8] had sufficient power to predict the phenotypes that are considered to become part of the well-being spectrum. The power was computed as a function of the following discovery trait parameters: 1) the discovery sample size set based on the maximum sample size from the multivariate analyses (2,370,390) and 2) the discovery trait SNP heritability (h_{snp}) set at 0.02. Concerning the target trait parameters, we adjusted the parameters according to the different phenotypes mentioned above and set the significance threshold at a Bonferroni corrected value of $\alpha = .005/11 = 0.0005$, where 11 represents the number of phenotypes to be predicted with the polygenic scores. Table II shows an overview of the different input parameters and the results of the power analyses. The estimated SNP heritability for personality, self-rated health, loneliness, and depressive symptoms was based on results from previous studies[9,42–44]. The SNP heritability for the 3-WBS was estimated using LD score regression[45]. Since there has been no genome-wide association study for flourishing, we estimated the SNP heritability to be approximately as high as the SNP heritability for subjective well-being and meaning in life, which are estimated at ~.04[9] and ~.06[17], respectively. The power for all traits was very high, assuming a medium

to high genetic correlation, with the exception of flourishing, where (due to smaller sample and low SNP heritability) the power to detect effects was somewhat lower, around .60 (assuming a genetic correlation of ~.8).

Table II. *Power Calculation Parameters for the Polygenic Prediction*

Discovery Trait Parameters						
	N_{obs}	SNP heritability				
Well-Being Spectrum	2370390	0.021				
Target Trait Parameters						
	Input Sample Size	SNP heritability	Power if r_g=.2	Power if r_g=.4	Power if r_g=.6	Power if r_g=.8
Subjective Well-Being	5300	0.04	.07	.46	.90	.99
Neuroticism	8600	0.12	.56	.99	1	1
Depressive Symptoms	8600	0.05	.18	.70	.99	1
Loneliness	8800	0.16	.74	1	1	1
Openness to Experience	8600	0.11	.51	.99	1	1
Conscientiousness	8600	0.10	.45	.99	1	1
Extraversion	8600	0.18	.79	1	1	1
Agreeableness	8600	0.09	.4	.99	1	1
Self-Rated Health	8600	0.13	.61	.99	1	1
Flourishing	2200	0.04	.02	.15	.44	.77

Polygenic Prediction

The polygenic scores were created using LDpred[46]. LDpred takes into account linkage disequilibrium (LD) among SNPs in creating the polygenic risk scores. We calculated the mean causal effect size of each marker using the SNP effect sizes from the recent multivariate 3-WBS GWAMA, where SNP effects were reversed for depressive symptoms and neuroticism, ensuring that a higher score reflects higher levels of well-being[8]. The LD structure from the European populations in the 1000 Genomes reference set[47] was used to calculate polygenic scores in the target sample. In order to avoid an over-estimation of the association between the polygenic scores and phenotypes, summary statistics in the discovery set were re-computed, excluding NTR subjects. The polygenic scores were calculated with the expected fraction of causal genetic variants (the fraction of markers with non-zero effects) set at .10. Generalized Estimating Equation (GEE) modelling was used to test whether the 3-WBS polygenic scores significantly predict satisfaction with life, happiness, neuroticism, depressive symptoms, loneliness, openness to experience, conscientiousness, extraversion, agreeableness, self-rated health, and flourishing. An exchangeable conditional covariance

matrix was used to account for family relatedness and tests were based on robust (sandwich-corrected) standard errors[48]. Age, age^2, sex, and the first ten genomic principal components (PCs) (three ancestry-informative PCs and seven PCs accounting for genotyping batch effects) were included as covariates. To obtain 95% confidence intervals (CI) around the R^2's, we performed bootstrapping with 2000 repetitions. All analyses were performed in R[40].

Genetic Correlations

We used LD score regression[45] to compute the genetic correlations between the 3-WBS and the candidate traits for which GWAS summary statistics were available. This method distinguishes bias and inflation from a true polygenic signal by quantifying the contribution of each through examining the relationship between linkage disequilibrium and test statistics. For neuroticism, depressive symptoms, positive affect, and life satisfaction, we used the univariate summary statistics from the multivariate 3-WBS GWAMA[8]. For all personality measures except neuroticism, we used summary statistics from a subset of 23andme participants[42].

We obtained summary statistics for self-rated health and loneliness by running GWASs on data from UK Biobank (UK Biobank ID 20459 and 2020, respectively). Genome-wide association analyses were performed in PLINK[37] in a linear regression model of additive allelic effects. Standard pre-GWAS- quality control filters were applied, which included removing SNPs with minor allele frequency < 0.005 and/or with an INFO-score < 0.8 for imputed SNPs, and removing individuals with ambiguous sex and/or non-British ancestry. Furthermore, we randomly selected 1 individual from each closely related pair of relatives (i.e. parent offspring pairs, sibling pairs). The GWAS included 40 principal components, age, sex, and a chip dummy as covariates. The summary statistics from these GWASs were used as input for LD score regression analyses. The significance threshold for the genetic correlations was set at a Bonferonni corrected value of $\alpha = .005/55 = 0.00009$).

Results

Figure 1 (and Online Resource 1) shows the phenotypic correlation structure between the traits as measured in the NTR. The well-being phenotypes satisfaction with life and happiness were significantly associated with all traits except openness to experience. Neuroticism was associated with all traits except conscientiousness. All traits were significantly correlated with depressive symptoms. The 3-WBS traits were most significantly associated with each other, followed by the correlations between the 3-WBS phenotypes and loneliness (weakest *r*=-.38 and strongest *r*=.54), self-rated health (weakest *r*=-.24 and strongest *r*= .34), and flourishing (weakest *r*=-.29 and strongest *r*=.40).

Figure. 1 Phenotypic Correlations Between the Different Traits. SWL= Satisfaction with Life, HAP = Happiness, NEU= Neuroticism, DEP= Depressive Symptoms, LON= Loneliness, OPEN= Openness to Experience, CON= Conscientiousness, EXTR = Extraversion, AGREE= Agreeableness, SRH= Self-Rated Health, FLOUR= Flourishing. Upper triangle, phenotypic correlation displayed in numbers, where red coloured numbers are negative phenotypic correlations and blue coloured numbers are positive phenotypic correlations. Lower triangle is a visualisation of the strength of the phenotypic correlations.

Figure 2 (and Online Resource 2) show the results from the GEE analyses where the polygenic scores for the 3-WBS were used to predict the eleven outcome variables. As a proof of principle, we found that the traits used to create the polygenic scores (satisfaction with life, happiness, neuroticism, and depressive symptoms) were significantly associated (standardized *b*= between -.123 and .084) with the polygenic score. From the candidate traits to be added to

a well-being spectrum, four were significantly associated with the 3-WBS polygenic score. The strongest association was found for loneliness (standardized b=-.091, p=3.14 x 10^{-16}, R^2= 0.84%), followed by self-rated health (standardized b=.083, p=3.71 x 10^{-14}, R^2= 0.69%) and extraversion (standardized b=.069, p=6.63 x 10^{-9}, R^2= 0.47%). Conscientiousness, agreeableness and flourishing were not significantly associated with the 3-WBS polygenic score.

Fig. 2 The amount of variance explained by the polygenic risk score for each of the traits. SWL= Satisfaction with Life, HAP = Happiness, NEU= Neuroticism, DEP= Depressive Symptoms, LON= Loneliness, OPEN= Openness to Experience, CON= Conscientiousness, EXTR = Extraversion, AGREE= Agreeableness, SRH= Self-Rated Health, FLOUR= Flourishing

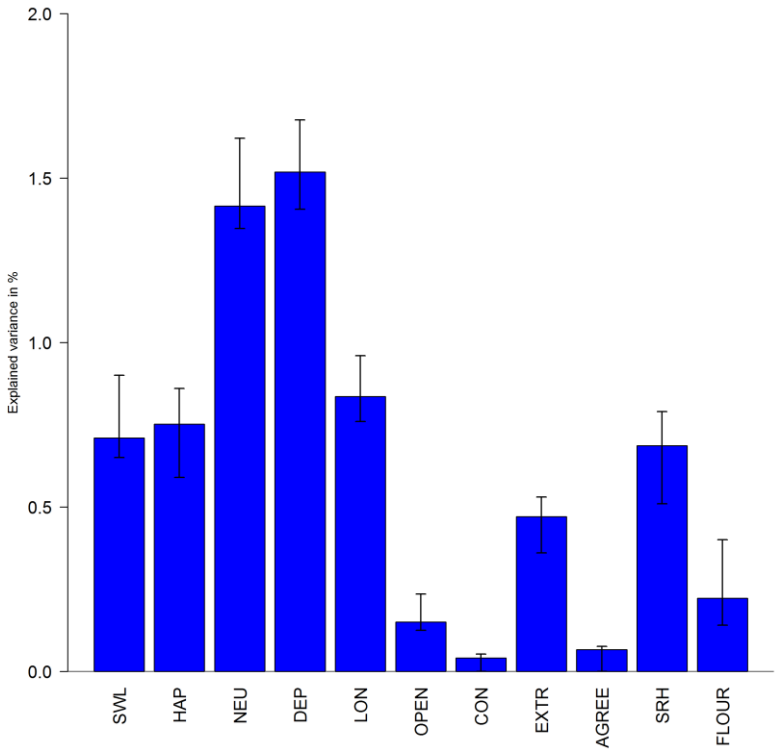
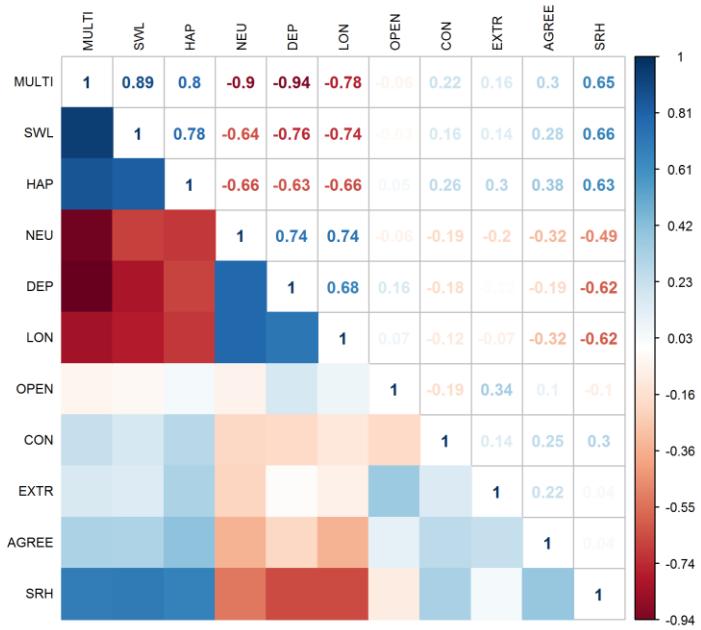


Figure 3 (and Online Resource 3) depict the genetic correlations obtained using LD score regression. As expected, the genetic correlations were strongest between the 3-WBS and the traits originally included in the spectrum, life satisfaction (r_g = .89), positive affect (r_g = .80), neuroticism (r_g = -.90), and depressive symptoms (r_g = -.94). Next, loneliness had the strongest genetic correlation with 3-WBS (r_g = .78), followed by self-rated health (r_g = .65), agreeableness (r_g = .30), conscientiousness (r_g = .22), and extraversion (r_g = .16). The only

trait that did not have a significant genetic association with 3-WBS was openness to experience (r_g = .06).

Fig. 3 Genetic Correlations Between the Different Traits. WB= Well-Being Spectrum, SWL= Satisfaction with Life, PA = Positive Affect, NEU= Neuroticism, DEP= Depressive Symptoms, LON= Loneliness, OPEN= Openness to Experience, CON= Conscientiousness, EXTR = Extraversion, AGREE= Agreeableness, SRH= Self-Rated Health. Upper triangle, genetic correlation displayed in numbers, where red coloured numbers are negative genetic correlations and blue coloured numbers are positive genetic correlations. Lower triangle is a visualisation of the strength of the genetic correlations.



Discussion

Well-being is a broad construct, with many traits contributing to its variation. In this study, we applied two types of genetic analyses to examine the genetic boundaries of a well-being spectrum. The traits we examined included the 3-WBS, as well as loneliness, openness to experience, conscientiousness, extraversion, agreeableness, self-rated health, and flourishing, conscientiousness, agreeableness, and openness to experience. First, we used publicly available GWAMA summary statistics to construct polygenic scores that reflect a genetic propensity for higher levels of well-being to predict several traits that have previously been associated with well-being. Second, we calculated genetic correlations between the 3-WBS and these traits.

The strongest associations with the polygenic score for the 3-WBS were found for the traits originally included in this spectrum. This shows that the scores are a good reflection of the proposed well-being spectrum when it is split up into its subdomains and supports the earlier findings of shared risk genes for these domains[9]. Moreover, these traits show high genetic correlations with the 3-WBS, as well as with each other, confirming previous findings that indicated high genetic correlations between life satisfaction, positive affect, neuroticism, and depressive symptoms[9].

Out of all candidate traits, loneliness showed the strongest phenotypic and genetic correlation with the well-being spectrum. As expected, lower well-being was found in people reporting higher levels of loneliness. The 3-WBS polygenic score predicted loneliness to a similar extent as it predicted subjective well-being measures. Even though the polygenic score predicted only a small amount of the variation in loneliness (0.84%), the genetic correlation between loneliness and well-being was in the same range as the that of the traits in the 3-WBS amongst themselves. Given these high phenotypic and genetic correlations, loneliness is a first good candidate to be added to the well-being spectrum.

Self-rated health constitutes a second good candidate. Self-rated health is a subjective measure of how individuals rate their current health status and has been established a good predictor of important objective health measures, such as mortality and the use of health services[49]. The 3-WBS polygenic score was found to predict self-rated health to a similar extent as subjective well-being. The genetic correlation between self-rated health and the 3-WBS was also relatively high (r_g= .65) confirming that people with a genetic predisposition for higher levels of well-being are more likely to rate themselves positively concerning their

health. For personality, we report a genetic association between the 3-WBS and extraversion and conscientiousness, but not for openness to experience or agreeableness. These results suggest that individuals with a genetic predisposition for higher levels of extraversion and conscientiousness also have a genetic predisposition to experience higher levels of well-being. These findings are in line with previous studies identifying extraversion and conscientiousness (in addition to neuroticism) as the strongest personality correlates of well-being[50,51]. Moreover, these results further support the findings by Weiss, Bates & Luciano (2008), where the genetic variance underlying subjective well-being was also responsible for individual differences in neuroticism, extraversion, and conscientiousness. The finding that openness to experience was not associated with well-being at a phenotypic and genetic level was not surprising and also found in previous research.

The genetic correlations revealed that only a small part of the genes that are important for extraversion and conscientiousness are also associated with well-being. Whereas the genetic correlation between agreeableness and well-being suggested they also share genetic factors, the polygenic score did not predict agreeableness. As shown in our power analyses, the power to predict agreeableness, given a genetic correlation of ~.30, is between .4 and .99. Therefore, it is likely that this seemingly contradictory finding is a result of the polygenic prediction for agreeableness having too little power to detect a polygenic association. Taken together, the evidence for a genetic correlation between well-being and multiple personality domains do not strongly support the inclusion of personality traits other than neuroticism in the well-being spectrum. A surprising finding was that the polygenic score did not significantly predict flourishing. Since the flourishing scale is a measure of PWB, and PWB is phenotypically highly associated with subjective well-being[52,53], we expected that, in line with the recent work of Baselmans and Bartels[17], part of this association could be explained by genetic factors. Two explanations are possible for our observations. The first explanation is that the relationship between 3-WBS and PWB as defined in this study is mainly a result of environmental factors. The second explanation is that, since our study had relatively low power to detect associations for flourishing, there is genetic overlap, but that these genetic effects remained unnoticed in this study. Unfortunately, we could not calculate the genetic correlation between the 3-WBS and flourishing due the constraint of the absence of a genome-wide association summary statistics for flourishing. However, future studies with larger sample sizes for PWB measures could elucidate which of these explanations is correct.

We note that the genetic correlations suggest large genetic overlap between the several traits, whereas the polygenic risk scores only explain a small part of the variance in each trait even with our large discovery sample. This discrepancy can be expected since the genotyped SNPs do not necessarily tag all causal variants, and not all SNPs were genotyped. Moreover, since measurement error accumulates across all the markers, sampling variation has a large influence on the predictive accuracy of the polygenic score[41]. We are therefore optimistic that, with increasing sample sizes and increased accuracy in the estimation of SNP effects, well-being polygenic scores will turn into clinically relevant tools for the prediction of outcomes such as loneliness or depression.

While these results provide us with important information on the genetic architecture of the well-being spectrum, the results should be interpreted with caution. As shown in Table II, the power of the polygenic prediction is dependent on sample size, especially when the genetic correlation between traits is low. Thus, better predictive accuracy and power could be achieved with larger sample sizes. Moreover, while including more traits in the well-being spectrum can lead to greater power for detecting genetic variants, the number of genetic variants influencing all traits will decline.

The results from the present study provide us with useful information on the determinants of individual differences in human well-being. Even though not all traits examined here can be included in the well-being spectrum from a genetic point of view, most of them are phenotypically and genetically related to well-being. It is important to know these determinants, since it could help us improve policy making and clinical interventions aimed at improving human well-being.

To conclude, in this study we found evidence for a shared genetic aetiology between several traits associated with well-being. The strongest relationships were found for loneliness and self-rated health. Our findings suggest that these two traits should be further investigated for potential inclusion in the well-being spectrum to increase our understanding of the causes and links between well-being and several mental/behavioural traits.

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Supplementary Information

Supplementary Table 1

Phenotypic Correlations Between the Traits (NTR Data).

	SWL	HAP	NEU	DEP	LON	OPEN	CON	EXTR	AGREE	SRH	FLOUR
SWL	1	-	-	-	-	-	-	-	-	-	-
HAP	.65*	1	-	-	-	-	-	-	-	-	-
NEU	-.48*	-.47*	1	-	-	-	-	-	-	-	-
DEP	-.48*	-.45*	.61*	1	-	-	-	-	-	-	-
LON	-.45*	-.38*	.54*	.53*	1	-	-	-	-	-	-
OPEN	-.01	<.01	.32*	.08*	.12*	1	-	-	-	-	-
CON	.23*	.20*	.01	-.29*	-.15*	.34*	1	-	-	-	-
EXTR	.28*	.33*	-.05*	-.33*	-.21*	.38*	.58*	1	-	-	-
AGREE	.11*	.11*	.20*	-.09*	-.06*	.44*	.54*	.49*	1	-	-
SRH	.34*	.24*	-.31*	-.34*	-.21*	.02	.12*	.20*	.04	1	-
FLOUR	.40*	.32*	-.29*	-.30*	-.26*	.06	.26*	.30*	.17*	.23*	1

Note. NEU= Neuroticism, DEP= Depressive Symptoms, SWL= Satisfaction with Life, HAP = Happiness, LON= Loneliness, SRH= Self-Rated Health, EXTR = Extraversion, FLOUR= Flourishing, CON= Conscientiousness, AGREE= Agreeableness, OPEN= Openness to Experience.

*p-value significant at α=.00009 (0.005/55).

Supplementary Table 2

Outcomes GEE analyses

Outcome Variable	**Standardized *b*(*se*)**	***P*-value**	R^2
Satisfaction with Life	.084(.015)	2.15×10^{-8}*	0.71
Happiness	.087(.015)	7.81×10^{-9}*	0.75
Neuroticism	-.119(.012)	$5.3x\ 10^{-23}$*	1.42
Depressive Symptoms	-.123(.012)	$7.57x\ 10^{-25}$*	1.52
Loneliness	-.091(.011)	3.14×10^{-16}*	0.84
Openness to Experience	-.039(.012)	0.002	0.15
Conscientiousness	.020(.012)	0.092	0.04
Extraversion	.069(.012)	6.63×10^{-9}*	0.47
Agreeableness	.026(.012)	0.027	0.07
Self-Rated Health	.083(.011)	3.71×10^{-14}*	0.69
Flourishing	.047(.047)	0.027	0.22

*p-value significant at α=.0005.

Supplementary Table 3

Genetic Correlations (SE) Between the Different Traits (Data From Several GWAS).

	MULTI	SWL	PA	NEU	DEP	LON	OPEN	CON	EXTR	AGREE	SRH
MULTI	1	-	-	-	-	-	-	-	-	-	-
SWL	.889(.084)*	1	-	-	-	-	-	-	-	-	-
PA	.798(.014)*	.777(.051)*	1	-	-	-	-	-	-	-	-
NEU	-.902(.006)*	-.645(.057)*	-.660(.018)*	1	-	-	-	-	-	-	-
DEP	-.937(.004)*	-.759(.085)*	-.631(.023)*	.738(.015)*	1	-	-	-	-	-	-
LON	-.781(.019)*	-.735(.068)*	-.658(.026)*	.740(.017)*	.678(.022)*	1	-	-	-	-	-
OPEN	-.056(.046)	-.029(.065)	.046(.043)	-.062(.048)	.162(.042)	.069(.051)	1	-	-	-	-
CON	.218(.042)*	.164(.068)	.258(.045)	-.194(.038)*	-.183(.043)*	-.117(.048)	-.187(.064)	1	-	-	-
EXTR	.155(.037)*	.137(.055)	.296(.034)*	-.204(.037)*	-.018(.036)	-.074(.038)	.341(.045)*	.145(.053)	1	-	-
AGREE	.299(.045)*	.284(.072)*	.375(.046)*	-.322(.047)*	-.192(.042)*	-.320(.055)*	.095(.073)	.252(.065)	.221(.052)	1	-
SRH	.652(.037)*	.663(.076)*	.629(.034)*	-.492(.046)*	-.617(.038)*	-.616(.044)*	-.098(.056)	.305(.054)*	.039(.047)	.036(.063)	1

Note. WB=Well-Being Spectrum, NEU= Neuroticism, DEP= Depressive Symptoms, SWL= Satisfaction with Life, PA= Positive Affect, LON= Loneliness, SRH= Self-Rated Health, EXTR= Extraversion, CON= Conscientiousness, AGREE= Agreeableness, OPEN= Openness to Experience.
*p-value significant at α= .00009 (0.005/55)

10

Summary, General Discussion & Future Perspectives

Genetics of Well-Being; an update

Where are we now and where are we heading

Introduction

Considering the beginning of my PhD trajectory in 2014, and as described in **chapter 1**, there has been major progress in the field of (molecular) genetics and complex traits like well-being. Up until 2014, most studies investigating the molecular genetics of well-being used either linkage or candidate gene analyses. As pointed out in **chapter 1**, linkage analysis is a powerful approach to detect genetic variants with large effect but has more difficulties detecting genetic variants with small effects. The candidate gene approach, on the other hand, theoretically has enough power to detect genetic variants with small effect. It requires, however, a sound theoretical mechanism with functional candidate genes a priori, knowledge that is still limited despite our increasing understanding of biological processes of complex traits. For these reasons, results based on these methods have shown to be extremely difficult to replicate[1] and the valid question arose whether it would be possible at all to identify genetic variants explaining phenotypic variance in well-being.

Since then, though, game-changing progress in the field of molecular/statistical genetics and bioinformatics has been made, resulting in the GWAS Era. One of the first promising signs indicating that it might become possible to detect genetic variants associated with well-being arose from results of a Genome-wide Complex Trait Analysis (GCTA)[2]. Rather than testing the association of a particular SNP with well-being, GCTA estimates how much of the variance in a trait can be accounted for by the genetic variance based on common SNPs, resulting in a heritability estimate based on molecular genetic data. Using GCTA in a sample of ~11,500 unrelated individuals, it was estimated that about 5-10% the variance in well-being could be explained by common SNPs[3]. Therefore, in **chapter 1**, we hypothesized that future genome-wide large-scale efforts to search for SNPs associated with well-being might have the potential to become successful.

However, in order to make it an success, it became obvious that sample size was the key issue. For instance, the first genetic variant robustly associated with schizophrenia was identified in 2009 using a sample of ~3,300 cases and ~3,500 controls[4]. In 2014, using a sample of ~35,000 cases and 110,000 controls, 108 genetic variants were associated with schizophrenia[5]. Like Schizophrenia, well-being is a polygenic trait, i.e. each individual will carry multiple alleles that increase his or her level of well-being, and multiple alleles that will decrease his or her level of well-being. Therefore, each individual variant will typically explain only a very small proportion of the variance in well-being. In addition, because of

many potentially different combinations of these risk alleles, it is likely that each individual carries a unique set of alleles. To detect these genetic variants with small effects, large sample sizes are required. Furthermore, it has been shown that the distribution of a phenotype in the population has an effect on the power to identify SNPs associated with it[6]. Higher levels of well-being are more prevalent in the population than psychiatric disorders. As a consequence, the sample size to detect SNPs robustly associated with well-being should be even larger than the sample size to detect genetic variants for psychiatric disorders.

Genome-Wide Association Studies

Using this information as *a priori* knowledge, we, together with the Social Science Genetic Association consortium (SSGAC; https://www.thessgac.org/) collected genetic and phenotypic data from 59 cohorts with a combined sample size of 298,420 individuals. **Chapter 3** describes this large-scale GWAS meta-analysis of well-being that led to the identification of the first three independent genetic variants associated with trait variation in well-being. Supplementing this analysis, we performed a GWAS meta-analysis of depressive symptoms (N = 180,866) and neuroticism (N = 170,910) and identified the first two genome-wide significant variants for depressive symptoms and eleven genome-wide significant variants for neuroticism. Additionally, the concordance of the allelic effect between the three traits was assessed, using a recently developed software tool called *Linkage Disequilibrium Score Regression* (LDSC)[7,8]. Within this approach, an "LD score" is computed for each SNP, taking the sum of correlation between that SNP and all neighboring SNPs. Under a polygenic model, these LD scores are expected to show a linear relationship with the GWAS test statistics of the corresponding SNPs, where the slope is proportional to the SNP heritability. Using LDSC, in **chapter 3,** we report a high genetic correlation between well-being, depressive symptoms, and neuroticism ($| r_g | > .75$), which corresponded to the genetic correlations derived in **chapter 2** using a large twin design. These high genetic correlations indicate common underlying biology between the three traits.

Multivariate Genome-wide association meta-analysis

Recognizing this large overlap, together with the knowledge that increasing samples sizes are required to detect genetic variants with small effects, we introduced two multivariate genome-wide association meta-analysis methods in **chapter 4**. Both methods enable analyzing clusters of correlated traits while handling bias resulting from inevitable sample overlap. Method 1, N-

weighted multivariate genome-wide association meta-analysis (N-GWAMA), assumes a single underlying construct with a *unitary* effect of the SNP on all included traits. Method 2, model averaging GWAMA (MA-GWAMA), relaxes this assumption and allows different effects on the various traits. We applied both methods on measures of life satisfaction, positive affect, neuroticism, and depressive symptoms, which we referred to as the *well-being spectrum* (N_{obs} = 2,370,390). Collectively, we found 319 genome-wide significant genetic variants associated with this spectrum.

Thus, in just over 2 years, the field of genetics and well-being progressed from the first three genetic associated with well-being to 319 genome-wide significant genetic variants. This spectacular increase in significant associations is representative of the enormous progress in the field of complex traits genetics in the last couple of years, reflected in findings for phenotypes such as educational attainment[9,10] and neuroticsm[11,12]. A significant player in the field of this progress is the UK Biobank (http://www.ukbiobank.ac.uk/), with the release of genome-wide genetic data on ~500,000 individuals in the summer of 2017[13]. The UK Biobank is a prospective study designed to be a resource for research into the causes of disease in middle and old age. Participants were recruited between 2006 and 2010 and completed a broad range of questionnaires. By meta-analyzing (smaller) cohort-data together with data derived from the UK Biobank, many studies, including ours described in **chapter 3, 4, 6, 8, and 9,** were able to increase the statistical power to find genetic variants associated with a specific trait of interest.

Phenotypic Heterogeneity

Although combining smaller cohort-data together with UK biobank has been proven successful, there is a downside to combining multiple measures of a trait (e.g. well-being). When combining multiple measures, there is always a dilemma; on the one hand including a cohort in the meta-analysis will increase the sample-size and consequently the power to find genetic variants of interest; on the other hand, including that specific cohort may bias the GWAS results, since combining different measures introduces phenotypic heterogeneity. In the studies described in **chapter 3** and **4**, we included multiple measures of positive affect as well as life satisfaction, neuroticism, and depressive symptoms leading to more noise in the GWAs analysis. To quantify the effects of this kind of phenotypic heterogeneity, **chapter 3** describes a "quantity-quality tradeoff" analysis that shows that in a *realistic* GWAS meta-analysis scenario with high genetic correlations (r_g > 0.6) between two measures of well-

being, the inclusion of a second cohort will reduce the measurement error in most cases. Therefore, based on the analysis of the costs and benefits of pooling heterogeneous measures, it can be concluded that pooling genetically associated traits increases the statistical power to detect genetic variants.

Mixing different measures, though, will result in a drop of the SNP heritability (h^2_{SNP}), as the included measures are partly influenced by different genetic factors. This is indeed what we observed in chapter **3**, **4**, and **8.** In **chapter 8**, we performed a GWAS of a homogenous measure of hedonic well-being resulting in a h^2_{SNP} of ~6.2%. This percentage dropped to four percent in the GWAS comprised of multiple well-being measures as described in **chapter 3**. Moreover, in **chapter 4,** where we performed a multivariate GWAMA including measures of well-being, neuroticism, and depressive symptoms, SNP heritability dropped to 2.1%. On the other hand, if we consider the GCTA h^2_{SNP} estimates of 5-10% for single-item well-being measures as an upper bound[3], then we approached it pretty closely with our GWAS using a homogenous measure of well-being (h^2_{SNP} of ~6.2%). Additionally, Rietveld et al.[3] state that 12-18% of the h^2_{SNP} could be captured after correcting for measurement error. Therefore, a promising but challenging way to go forward is to re-measure well-being using similar questionnaires and perform a GWAS on the unified measures.

Biological Analyses

To shed some light on the possible biological mechanisms underlying our findings we performed several bioinformatics analyses. Previous work has demonstrated that some functional categories of the genome contribute disproportionally to the heritability of complex behavior[2,14,15]. Build on this observation Finucane et al.[16] developed stratified LD Score Regression (SLDSC), which requires only GWAS summary data together with LD information from an external reference panel matching the population structure of the GWAS. Doing so, SLDSC can distinguish between h^2_{SNP} explained by different functional categories of the genome, for instance in the central nervous system (CNS), while accounting for influence of the remaining functional categories (e.g. blood, bone, and muscle tissues). Using SLDSC, in **chapter 3** we report significant enrichment in the CNS for well-being, depression, and neuroticism, which we confirm in **chapter 4** for the well-being spectrum. **In chapter 4,** we expanded these analyses by leveraging the genome-wide results, LDSC, and an atlas of brain gene expression. Doing so, we were able to pinpoint brain regions where genes that are significantly associated with well-being are significantly enriched in their effects. We report

evidence for enrichment of genes differentially expressed in the Ventral Tegmental Area (VTA), as well as in the subiculum (part of the hippocampal formation). Furthermore, we report significant enrichment of glutamatergic neurons in the CA1 and CA3 of the hippocampus and in the prefrontal cortex as well as enrichment of GABAergic interneurons. However, as we only had specific cell types for specific regions (hippocampus and prefrontal cortex), there are some interpretational limitations. Gene expression is known to vary systematically between cell-types within the brain[17] (e.g neurons, microglia, astrocytes) and developmental phases[18] (prenatally, childhood, adulthood and old age). Although we find specific cell type enrichment for well-being, it stands to reason that the same cell type specific enrichment in other regions might exist, which we now missed. This limitation needs to be addressed in future well-being research. However, capitalizing on ongoing efforts to categorize gene expression across the human brain at increased (single cell) resolution, this will be a promising future approach to understand biological processes underlying phenotypic variation of well-being.

Epigenome-Wide Association Studies

Besides genetic influences, environmental factors play an important role in explaining variance in well-being, as evidenced by multiple twin-family studies and described in **chapter 2**. Additionally, epigenetic regulation of gene expression by mechanisms such as DNA methylation may mediate the interplay between the genetic make-up of individuals and their exposure to the environment[19,20]. In humans and animals, various early life exposures can induce stable long-term changes in DNA methylation[21–23]. Examples include early postnatal maternal behavior[23], childhood abuse [22], and prenatal maternal nutrition[21]. Later life exposures also induce changes to the methylome, for example exposure to cigarette smoke[24,25].

Recently, using epigenome-wide association studies (EWAS), changes in DNA methylation have been implicated in various complex traits such as obesity[26], type 2 diabetes[27,28,29], and educational attainment[30,31]. In **chapter 5,** we performed the first EWAS on well-being in a population-based sample (N = 2519) of adults from the Netherlands Twin Register (NTR)[32]. We identified two genome-wide significant methylation probes after correction for multiple testing (Bonferroni correction). Moreover, gene ontology (GO) analyses highlighted enrichment of several CNS categories among higher-ranking methylation probes. However, replication of these results is warranted in larger samples as (1) we are aware that potential

unmeasured confounders could have an effect on our results, and (2) we are uncertain of the direction of causation of the association between well-being and CpG methylation.

The foremost interpretational difficulty in EWAS is the uncertainty about cause and effect, e.g. does methylation causally influence complex trait outcomes, is the causal effect reverse, or does a third trait influence both methylation levels and traits? For instance, a recent study found that differential methylation is the consequence of inter-individual variation in blood lipid levels and not vice versa[33]. A second important consideration for EWAS is the assessment of methylation in trait relevant tissue. Empirical results suggest that easily accessible tissues, such as whole blood, cannot be used to address questions about inter-individual epigenomic variation in inaccessible tissues, such as the brain[34,35].

To examine these interpretational issues, we performed an EWAS meta-analysis of well-being controlled for two well-known confounders of epigenetic associations, smoking and BMI, in **chapter 6** (*N* = ~8,600). To guard against unmeasured confounding and to infer a direction of effect we performed summary-based Mendelian Randomization (SMR). In SMR, SNP effects on *cis*-methylation (*cis*-mQTLs), and a large GWAS of well-being were combined to infer the (causal) effect of CpG methylation on well-being. To assess concordance between blood and brain tissue, we performed SMR leveraging *cis*-mQTLs present in both blood and brain tissues and compared results between tissues, and between SMR and EWAS. Doing so, we found a high consistency of direction of effect ($r > .9$) between SMR results, where the mQTL was discovered in two whole blood datasets, as well as high consistency between whole blood and fetal brain datasets ($r = .72$). However, when comparing the direction of effect between our EWAS and SMR results, no notable correlations were observed. These results indicate that, if the aim is to increase our understanding of the functional consequences of epigenetic changes on wellbeing, SMR may be preferred over EWAS in whole blood. If, however, the aim is to identify ways in which well-being is itself a driver of environmental influences on differences in DNA methylation, possibly effecting gene-expression, a sufficiently powered EWAS study will provide valuable information. The concurrent use of Mendelian Randomization and epigenome-wide association analysis proved to be a potent combination to further our understanding of the relation between well-being and CpG methylation.

Well-being framework

It is well known that several mental health issues, such as anxiety, depression, neuroticism, and loneliness share a common genetic liability[36–38]. This common genetic liability offers an

explanation as to why many disorders are comorbid or present highly similar behaviors. While there have been detailed investigations of the genetic similarity and comorbidity of mental disorders, there is much less information about the genetic similarity of mental health traits such as happiness, satisfaction with life, personality, self-rated health, and flourishing. The multivariate approach in **chapter 4**, focused on the overlap within a mental health spectrum, and leveraged the genetic overlap between well-being, neuroticism, and depressive symptoms to identify genetic variant for this 3-phenotype well-being spectrum (3-WBS). Studies on traits that could additionally be considered as part of a well-being spectrum are important given the large collection of studies pointing towards the emotional, cognitive, and interpretational benefits of high levels of well-being beyond the absence of mental disorders[39–41]. In the literature, several other traits, such as loneliness, self-rated health, and personality have been found to be strongly associated with well-being. Therefore, the aim of **chapter 9** was to investigate the genetic overlap between well-being and these proposed traits. Using polygenic scoring and genetic correlations, we report that the 3-WBS is strongly genetically associated with loneliness and self-rated health. These findings suggest that these traits are interesting candidates to be included in the well-being spectrum and may increase our understanding of the causes and links between well-being and several mental and behavioral traits.

Conceptualization the well-being framework

So far, we have identified multiple genetic variants associated with well-being (**chapter 3** and **4**) and showed that well-being is related to a broad range of mental –and behavioral traits (**chapter 2**, **3**, **4**, and **9**). These studies have in common that they all use measures of life satisfaction and positive affect, which are often referred to as subjective well-being (SWB) measures. However, from a theoretical perspective, two types of well-being can predominantly be distinguished: subjective well-being (SWB) and psychological well-being (PWB), shaped by the philosophical constructs hedonism and eudaimonism, respectively. Ancient hedonism is centered around pleasure, or how good a person feels about his or her life[42]. From this perspective, well-being consists in the balance of pleasure over pain, that is: how to maximize pleasure and minimize pain (Aristippus (c. 435 – c. 356 BCE)). In contrast, eudaimonism, is more about virtue (defined as knowledge about how to live well) and human capacities. Although in contemporary sciences the terms hedonism and eudaimonism have gradually shifted to SWB and PWB, there is still an ongoing debate how these concepts relate to each other[42,44–48]. Therefore, to examine the complex framework of well-being, we

performed a literature study aiming at analyzing the current view on the relation between SWB and PWB (**chapter 7**). We found that the main consensus is that SWB and PWB are related constructs that are likely domains of a general factor well-being. However, while the constructs are related, they are not interchangeable and can be distinguished both conceptually and biologically. Based on these findings we provide some general recommendations for follow-up research.

(1) *Re-define the well-being framework.* We propose that an empirical well-being framework should be developed considering the actual empirical data rather than the ideas that inspired the research[50]. In the context of the social and behavioral sciences, the well-being framework might be best described as one hierarchical construct including both SWB and PWB constructs. This means that hedonism and eudaimonism are not to be defined as two clearly separated streams, but as related underlying domains of the same construct

(2) *Be detailed.* It is often taken for granted that when we are using the same words, we mean the same things. As it turns out, at least in the field of well-being, we should be more cautious about this assumption. For example, SWB can be referred to as "happiness", "hedonism", "subjective happiness", "emotional well-being" and "affective well-being". This inconsistency might lead to interpretational issues of study results. To overcome this, the most feasible solution would be for researchers to be detailed about the constructs they aim to be measuring and about the scope of their study. This means that researchers should: 1) be consistent in their use of terminology; 2) give detailed descriptions of their most basic terms and constructs, and; 3) keep in mind that the results of their study might not cover well-being in its entirety.

To add weight into the discussion to what extent hedonic –and eudaimonic well-being relate to each other, we had to wait for the availability of a sufficient powered molecular-genetic dataset with measures of eudaimonic well-being (**Chapter 8**). With the release of the UK Biobank data, we were able to conduct a GWAS, where the question: "*To what extent do you feel your life to be meaningful*" served a proxy-phenotype for eudaimonic well-being in ~110,000 participants. Paired to this analysis, we conducted a GWAS where the question: "*In general how happy are you*" served as a proxy phenotype for hedonic well-being. We identified the first two genetic variants associated with eudaimonic well-being as well as six genetic variants for hedonic well-being. Moreover, the genetic correlation between both measures was, as expected, large (r_g = 0.78), suggesting a large shared genetic etiology.

Further evidence for a shared genetic architecture between both measures is provided by the similar patterns of genetic correlations with other traits (e.g. depressive symptoms, personality, and loneliness). These results complement our results found in the literature review (**chapter 7**) and indicate that both constructs can be seen as two related underlying domains of the same construct.

Future perspectives

Enormous progress has been made in the field of human genetics the last four years, with a tsunami of genetic associations with numerous traits identified as a consequence. In line with this progress, we reported the first 3 genetic variants associated with well-being in 2016, while two years later, this number increased to 319 genetic variants (**chapter 3** and **4).** Similar progress has been made for other phenotypes, like depression[51], education attainment[10], neuroticism[12] and human intelligence[52]. These studies are staggering proof that the field of complex traits genetics has become increasingly successful in the last couple of years. With this progress, new questions arise. Valid questions, like how we should interpret these results and what the next steps are to take. Of course, there are no conclusive answers to these questions yet, but for (genetic)-research involving well-being, the following opportunities are worth exploring.

From association to causation

The high genetic correlation between different measures of well-being, as well as between well-being and other complex traits, such as neuroticism, depressive symptoms, and self-rated health, can be a product of a causal relationship between the traits, a third factor that influences the traits or a combination of both mechanisms. Although progress is being made in detecting causal relationships between correlated traits using Mendelian Randomization (MR), presence of horizontal pleiotropy can bias results. Horizontal pleiotropy occurs when the variant has an effect on the outcome outside of its effect on the exposure in MR. The presence of horizontal pleiotropy has been demonstrated by a recent study that developed a software tool called MR-PRESSO, showing that horizontal pleiotropy was detectable in over 48% of significant causal relationships reported in MR-analyses[53]. A solution to overcome biased results in MR analyses is to include very strong instrumental variables. Given that the genome-wide significant SNPs associated with well-being explain typically little of the phenotypic variance (~ 0.01%), it will be difficult, to construct strong instrumental variables for well-being. There is, however, reason for optimism. Many methods that are better able to

cope with pleiotropy have been proposed recently, such as the genetic instrumental variable (GIV) regression[54] and two-sample MR (2S-MR)[55–57]. In addition to these MR methods for inferring causal relationships between two traits, one could ask how much of the relationship is mediated by a third factor. Given the high correlations between well-being and numerous traits (see **chapter 8** and **9**), this would be a reasonable scenario. To test this, the recently developed Genomic structural equation modelling SEM approach[58] might be an informative way to go forward and lay the groundwork for a novel multi-faceted approach in investigating the well-being spectrum, and progress from showing association, to understanding direction of causation.

From genetic variants to biological functioning

The number of identified genetic loci for well-being has increased spectacularly in recent years as described in multiple chapters in this thesis. These findings are largely driven by the release of large-scale genetic-data sets such as the UK biobank. The next challenge is to improve our understanding of the biological effects of these genetic risk loci, especially since the actual genes mediating phenotypic variation are not necessary proximal to the lead SNPs identified in genome-wide association studies (GWASs). Supported by the observation that GWAS variants are preferentially located in enhancers and open chromatin regions[59,60], the majority of common genetic risk factors are predicted to influence gene regulation, either directly or through modifiable epi-genetic processes, rather than directly affect the coding sequence of transcribed proteins[61]. Therefore, a promising way to go forward is to first identify the causal variants (eQTL) influencing gene-expression, using for instance SMR. Next, software tools like FUMA (Functional Mapping and Annotation of Genome-Wide Association Studies[62]), which utilize information from different databases and methods can be used. Using FUMA, functional consequences on gene functions, deleteriousness, regulatory functions, and biological pathways can be revealed from the causal SNPs identified in the first step. In chapter **4** and **5** we made a first step in identifying causal variants influencing gene-expression or methylation –expression, and it is expected that this strategy will result in new insights in the biological underpinnings of the well-being spectrum.

The effect of parental genotypes

Another promising way to go forward is to include, the often ignored, genetic variants in the parental genomes that are *not* transmitted to a child in the studies of well-being. A recent paper Kong et al.[63] showed that non-transmitted alleles canstill affect a child through the

impact of the alless on the parents themselves or on other relatives (such as siblings), a phenomenon they called "genetic nurturing". Kong et al. showed, using education attainment as an example, that polygenic scores computed from the non-transmitted alleles have an estimated effect on the educational attainment of that child that is roughly 30% of the magnitude of the polygenic scores based on the transmitted alleles. It would add a novel layer to "*the genetics of well-being"* if it could be demonstrated that genetic nurturing exists and has an impact on the variance of well-being in the off-spring.

Phenotypic innovations

Beside the progress in the field of human genetics, there have been major methodological advances in measuring complex behaviors .

Social Media

For example, recent work in language use has shown its innovative power to assess complex behavior. Self-report surveys provide a snapshot in time. Online social media data, on the other hand, may 'fill in the gaps' with ongoing 'in the moment measures' of a broad range of people's thoughts and feelings and provide real-time assessment of well-being. For instance, it has been shown that patterns in a community's Twitter language predict several health outcomes, including community-level disease mortality[64], depression and mental illness[65], and ADHD[66]. Moreover, it has been shown that social media language derived personality assessments match the psychometric quality of observer-report through surveys[67]. It would be very interesting to examine whether language use expressed through social media predict levels of well-being and to assess the genetic component of it. As pointed out in in **chapter 3, 4, 8,** and **9,** well-being is related to a broad range of positive *and* negative traits. The widespread use of social media may therefore provide additional opportunities to the detection of otherwise undiagnosed cases.

Sensor data

Besides social media use, sensors in everyday devices, such as our phones, wearables, and computers, leave a stream of digital traces. These traces can be captured, analyzed ,and related to human behavior (for review see Mohr et al.)[68]. For example, by leveraging built-in sensors, a number of smartphone-based sensing systems have been developed to passively monitor sleep periods. Several groups have shown that sleep duration can be estimated with approximately 90% accuracy, without asking the user to do anything special with the

phone[69,70]. In turn, these sleep period markers have been correlated to the severity of depressive symptoms[71] and a strong genetic correlation has been onserved between well-being and insomnia (Hammerslag et al., 2017). Although numerous challenges must be overcome before these types of measures become viable for large scale epidemiological deployment, recent technological progresses in machine learning methods give rise to a certain level of optimism. It would be very interesting for future studies to focus on sensor dating in relation to well-being and related traits.

Societal Impact

Well-being and the prevention of Mental Illness

Happy people are healthy people: they live longer, function better, and are less susceptible to mental illness[41]. Given the power and potential of happiness, the previous lack of insight into the causes of individual differences in happiness and the persistence of isolated approaches from different disciplines was surprising. With the work in my thesis I have added some pieces to the complex puzzle of well-being. As a future perspective, I anticipate that a focus on well-being could be very beneficial for the society at large. In the field of epidemiology, for example, it has been proposed that larger benefits to overall public health are to be expected when the bell curve of mental health in the human population is shifted a little to the healthy side, the so-called population strategy[75,76]. So, a relatively slight increase in the level of well-being of the majority of the population may have a larger preventive effect than targeting the much smaller group of people at high risk or in the early stages of mental illness. To this end, knowledge on the causes of individual differences in well-being and modifiable risk and protective factors is crucial.

Support for the potential preventive role of well-being to prevent mental illness is provided in **chapter 2**, where I showed that the phenotypic relationship between well-being and depressive symptoms is largest in adolescence and young adults, with genetic effects explaining most of this correlation. In other words, a genetic predisposition for increased levels of well-being will probably have a protective effect in developing these depressive symptoms. In combination with the strong genetic correlations of well-being with depressive symptoms, neuroticism, loneliness, and self-rated health as reported in **chapter 3**,**4,** and **9**, it might be worth to investigate the effects of positive psychology interventions for prevention of (mental) illness.

To date, two meta-analyses that examined the overall effects of positive psychology interventions (PPI) have been published. The first meta-analysis[77] included 51 controlled studies and found that PPI significantly enhance well-being (mean r = 0.29) *and* decrease depressive symptoms (r = 0.31). The second meta-analysis included 39 randomized controlled trial studies (N ~ 6,100)[78], including PPIs such as self-help interventions, group training and individual therapy. They reported a standardized mean difference of 0.34 for subjective well-being, 0.20 for psychological well-being and 0.23 for depressive symptoms. These effect sizes attenuated at follow up (3 to 6 months) but were still significant, indicating that effects are fairly sustainable. Together, these studies indicate that engaging in simple positive activities can reliably increase an individual's level of well-being as well as decrease someone's depressive symptoms. Given that there is some evidence that Positive Psychology interventions might be effective, it is essential to understand the causes of difference in intervention response. As a first step, Haworth and colleagues[79] revealed minimal changes in the overall magnitude of genetic and environmental influence on individual differences during the intervention, despite significant improvements in overall well-being. They furthermore showed that the genetic factors important for intervention response were the same as those influencing baseline well-being scores. This indicates that the genetic findings in my thesis could be informative in the development of personalized positive prevention interventions.

To conclude, during my PhD trajectory, I have witnessed the enormous progress the field of human genetics has made from the frontline. On this wave of progress, my work has contributed to a better understanding of the factors influencing phenotypic variation in well-being, a phenotype that is affecting us all.

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Nederlandse Samenvatting

Dit proefschrift bestaat uit een aantal studies waarin onderzocht is waar individuele verschillen vandaan komen in welbevinden (WB) en gerelateerde menselijke eigenschappen, zoals depressieve symptomen (DS) en neuroticisme (NEU). Uit eerder onderzoek is gebleken dat WB erfelijk is, wat wil zeggen dat verschillen in WB voor een deel komen door verschillen in genetische aanleg. Echter, er is nog maar weinig bekend over de specifieke genetische factoren en biologische mechanismen die hier aan ten grondslag liggen. Daarom was het belangrijkste doel van mijn proefschrift om de genetische varianten, genen en biologische mechanismen te identificeren die geassocieerd zijn aan individuele verschillen in WB. Hoewel WB een verzamelterm is die ik in mijn proefschrift gebruik zijn er verschillende subtypen. Zodoende richt een deel van mijn onderzoek zich op tevredenheid van leven en kwaliteit van leven en je gelukkig voelen, terwijl een ander deel meer de nadruk legt op een betekenisvol leven.

Tweelingstudies en de samenhang tussen welbevinden en depressieve symptomen

In **hoofdstuk 2** van mijn proefschrift laat ik aan de hand van een tweeling design zien dat genetische invloeden op WB (gemeten als kwaliteit van leven) even groot zijn op verschillende leeftijden. Zowel in kindertijd (7, 10 en 12 jaar), adolescentie (14, 16 jaar) en volwassenheid (18-27 en >27 jaar) verklaarden genetische factoren ongeveer 40 procent van de fenotypische variantie. Dit wil zeggen dat ongeveer 40% van de verschillen in WB tussen mensen verklaard worden door genetische verschillen. In dezelfde studie zagen we dat het genetisch effect op DS ook een stabiel karakter heeft. Ongeveer 55% van de fenotypische variantie in DS kan verklaard worden door genetische effecten. Als we kijken naar de relatie tussen WB en DS vonden we dat deze sterker werd in de adolescentie en jong volwassenheid ten opzichte van de kindertijd. Dit betekent dat individuen met een hoger WB lager scoorden op DS en vice versa. Daarnaast vonden we dat de relatie tussen WB en DS bij adolescenten en volwassenen voornamelijk verklaard wordt door genetische effecten. Tot slot onderzochten we of de set genen die een effect op WB hebben ook een effect op DS hebben, ook wel genetische correlatie genoemd. Hier vonden we wederom dat wanneer de leeftijd toenam, dezelfde genen een rol spelen bij zowel WB als DS.

Genetische varianten voor welbevinden

Van de resultaten beschreven in **hoofdstuk 2** weten we dat genetische effecten een substantieel gedeelte van de fenotypische variantie in WB verklaren. In **hoofdstuk 3** ga ik op zoek naar waar op het genoom de genetische varianten liggen die hier verantwoordelijk voor zijn. Dit heb ik, in een grootschalige internationale samenwerking, gedaan met een zogenaamde genoom-brede-associatie studie ook wel GWAS (Genome-wide Assocation Study) genoemd. Voor deze analyse hebben we gebruik gemaakt van een groep van ongeveer 300,000 individuen waarbij DNA is afgenomen en hun WB is gemeten met verschillende vragenlijsten. Door het analyseren van miljoenen genetische varianten in deze grote groep, ook wel meta-analyse genoemd, konden we de eerste drie genetische varianten identificeren die robuust met WB geassocieerd zijn. Daarnaast vonden we in dezelfde studie de eerste twee genetische varianten geassocieerd met DS alsmede 11 genetische varianten geassocieerd met NEU. In vervolganalyses hebben we gekeken waar in het lichaam deze genetische varianten de meeste erfelijkheid verklaarden voor deze drie fenotypen. Voor zowel WB, DS als NEU vonden we dat de genetische varianten die tot expressie kwamen in het centrale zenuwstelsel meer van de erfelijkheid verklaarden dan genetische varianten die bijvoorbeeld tot expressie kwamen in andere delen van het lichaam zoals botten of spieren. Vervolgens hebben we gekeken naar de genetische overlap tussen de drie fenotypen. Een groot deel van de genetische varianten die een invloed hebben op WB hebben ook een effect op DS en NEU terwijl de genetische varianten die een effect hebben op DS ook een effect hebben op NEU. Dit betekent dat er vanuit een genetisch perspectief een sterke aanwijzing is dat er een gedeelde etiologie is voor de drie fenotypen.

De laatste bevinding uit **hoofdstuk 3** vormde het uitgangspunt van **hoofdstuk 4** waar we twee nieuwe methoden ontwikkeld hebben die het mogelijk maken om fenotypen samen te analyseren. Dit levert een toename in statistische power op om genetische varianten te identificeren die geassocieerd zijn met hun gedeelde etiologie. Als we WB, DS en NEU afzonderlijk analyseerden, vonden we een totaal van 241 genetische varianten. Door het samen analyseren van deze drie fenotypen, het WB spectrum genoemd, vonden we 319 genetische varianten, een toename van 32%.

Daarnaast vonden we dat de genetische predictie, de voorspellende waarde van de gemeten genetische varianten om verschillen in bijvoorbeeld het WB spectrum te verklaren, met 38% toenam. In vervolg analyses hebben we bevestigd dat het centrale zenuwstelsel een rol speelt in de etiologie van het WB spectrum. Bovendien zijn we nu een stap verder gegaan door op zoek te gaan naar de locaties in de hersenen die daarbij betrokken zijn. Hier vonden we dat genen die tot uiting kwamen in de subiculum (behorend bij de hippocampale formatie) geassocieerd waren met het WB spectrum. De subiculum speelt een rol in onze reactie op stress. Als laatste hebben we gekeken welke cel typen in het brein betroken waren en vonden dat GABAergic interneuronen, betrokken bij informatieoverdracht, een mogelijke rol spelen. Samengenomen zijn zowel **hoofdstuk 3** en **4** een goed voorbeeld van de enorme progressie die gemaakt is op het gebied van moleculaire genetica en de identificatie van genetische varianten geassocieerd met WB.

Epi-genetica

Naast genetische effecten spelen omgevingsfactoren ook een belangrijke rol bij het verklaren van verschillen in WB, zoals ook beschreven in **hoofdstuk 2.** Epigenetische regulatie van gen expressie door middel van DNA methylatie speelt mogelijkerwijs een mediërende rol tussen iemands genetische aanleg en de blootstelling aan omgevingsinvloeden. Met andere woorden, doordat iemand zich in een bepaalde omgeving bevindt (bijvoorbeeld chronische stress), kan er een methyl deeltje zich op het DNA bevestigen waardoor genen moeilijker worden afgelezen, wat kan leiden tot veranderingen in gevoelens en gedrag. In **hoofdstuk 5** heb ik daarom de eerste epi-?genetische studie uitgevoerd in een groep van ongeveer 2,500 tweelingen van het Nederlands Tweelingen Register. Bij deze mensen is bloed afgenomen waardoor we in staat waren om het epi-genetisch profiel in kaart te brengen op ongeveer 450,000 locaties. Daarnaast is WB gemeten aan de hand van verschillende vragenlijsten. Deze studie, ook wel een epigenoom-brede associatie studie genoemd, werkt volgens hetzelfde principe als de studie beschreven in **hoofdstuk 3.** Echter, in plaats van dat we genetische varianten meten, kijken we naar methylatie-deeltjes op het genoom en proberen we die te linken aan verschillen in WB. Met deze methode konden we de eerste twee methylatie-deeltjes identificeren die geassocieerd waren met WB. Hierbij moet wel in acht worden genomen dat deze studie in een relatief kleine groep (ongeveer 2,500 personen) is uitgevoerd, waardoor replicatie noodzakelijk is. Daarnaast is het belangrijk om te onderzoeken of methylatie, gemeten in bloed, relevant is voor fenotypen waar biologische processen

voornamelijk in de hersenen afspelen, zoals we voor WB hebben aangetoond in **hoofdstuk 3** en **hoofdstuk 4**.

In **hoofdstuk 6** hebben we geprobeerd om op deze vragen antwoord te geven. Als eerste hebben we de onderzoeksgroep uit **hoofdstuk 5** vergroot, door data van 12 verschillende onderzoeksgroepen samen te voegen. Hierdoor konden we het sample vergroten naar ongeveer 9,000 individuen waarvan het epi-genetisch profiel en WB is gemeten. Van al deze personen is het epigenetische profiel gemeten met behulp van een bloedsample. Vervolgens hebben we onderzocht of we de gemeten methyl-deeltjes konden linken aan WB. Echter, als we corrigeerden voor de gebruikelijke confounders zoals roken en BMI, konden we geen significante relatie vinden tussen methyl-deeltjes en WB. Op dit moment zijn we dus nog niet in staat om de resultaten van **hoofdstuk 5** te repliceren. In het tweede deel van **hoofdstuk 6** hebben we onderzocht of methylatie gemeten in bloed ook informatief is voor fenotypen waarvan biologische processen voornamelijk in de hersenen afspelen. Hiervoor hebben we gebruik gemaakt van een dataset waarin methylatie gemeten is in het brein afkomstig van donoren. Door deze dataset te combineren met de resultaten van onze genoom-brede associatie studie van WB, vonden we op 1 locatie in de hersenen dat methylatie gelinkt kon worden aan WB. Vervolgens hebben we onderzocht of methylatie gemeten in bloed gelinkt kan worden aan methylatie gemeten in de hersenen. Als dit zo zou zijn zou je een positieve relatie verwachten. Met andere woorden, de effecten die gevonden zijn in bloed (uit het onderzoek met de 9000 mensen zoals hierboven beschreven) en uit de hersenen (uit het onderzoek met de breindonoren en de WB GWAS resultaten) komen overeen. Echter, deze relatie was niet significant. Dit geeft aan dat we op dit moment nog geen duidelijke conclusies kunnen trekken over mogelijke epigenetische processen voor WB. Het geeft tevens aan dat epigenetische processen in het bloed niet direct een afspiegeling zijn van epigenetische processen in de hersenen. Vervolgonderzoek is dan ook noodzakelijk om meer inzicht te krijgen in dit complexe samenspel tussen genetische aanleg, het tot uitkomen van genen, en invloeden uit de omgeving.

Het raamwerk van welbevinden

Tot zover hebben we meerdere genetische varianten kunnen linken aan WB (**hoofdstuk 3** en **4**) en hebben we aangetoond dat WB is gerelateerd aan een scala van gedragseigenschappen zoals DS en NEU (**hoofdstuk 2, 3, 4)**. Wat deze studies allemaal met elkaar gemeen hebben is dat ze WB meten met vragenlijsten over tevredenheid met leven en hoe gelukkig je bent, vaak

ook wel subjectief welbevinden of subjectief well-being (SWB) genoemd. Echter, vanuit een historisch perspectief kunnen we twee verschillende typen WB van elkaar onderscheiden: SWB en psychologisch WB (PWB). Deze zijn gebaseerd op de filosofische stromingen hedonisch WB en eudaimonisch WB. Hedonisch WB draait vooral om het hebben van zoveel mogelijk plezier, of hoe goed een persoon zich over zijn of haar leven voelt. Eudaimonisch WB gaat meer over deugdzaamheid of een goed leven leiden. Hoewel de termen hedonisch en eudaimonisch WB langzaam zijn overgegaan in respectievelijk SWB en PWB, is er nog steeds een discussie over hoe deze twee concepten zich tot elkaar verhouden. Om een beter beeld te krijgen van het WB raamwerk hebben we in **hoofdstuk 7** een literatuurstudie uitgevoerd waarin we de huidige standpunten ten opzichte van de relatie tussen SWB en PWB hebben onderzocht. Hier vonden we ondanks dat beide constructen erg aan elkaar gerelateerd zijn, ze niet compleet inwisselbaar zijn en dus van elkaar onderscheiden kunnen worden.

Om de conclusie uit hoofdstuk 7 empirisch te testen hebben we in **hoofdstuk 8** met behulp van data van meer dan 220 duizend individuen GWAS studies voor (1) eudaimonisch WB en (2) hedonisch WB uitgevoerd. Hierdoor waren we in staat om de eerste twee genetische varianten voor eudaimonisch WB te identificeren en zes genetische varianten voor hedonisch WB. Daarnaast hebben we gekeken naar de genetische samenhang tussen beide vormen van WB door de genetische correlatie te berekenen. Oftewel, hebben de genetische varianten die een effect hebben op hedonisch WB ook een effect op eudaimonisch WB. Hier vonden we inderdaad een grote positieve genetische correlatie, wat er op duidt dat er een grote overlap is tussen beide vormen van WB. Deze resultaten ondersteunen de bevindingen van de literatuurstudie zoals beschreven in **hoofdstuk 7.**

Wellbevinden Spectrum

Naast het WB spectrum zoals beschreven in **hoofdstuk 4** kunnen er waarschijnlijk nog meer fenotypen aan WB gelinkt worden. Om dit te onderzoeken hebben we in **hoofdstuk 9** gekeken of we het WB spectrum konden uitbreiden. Als eerste hebben we een poly-genetische score berekend door het effect van alle genetische varianten geassocieerd met het WB spectrum bij elkaar op te tellen. Deze score hebben we vervolgens gebruikt om fenotypen te voorspellen die eerder gelinkt zijn aan WB. Hiervoor hebben we onder andere data gebruikt van eenzaamheid, verschillende vormen van persoonlijkheid en gezondheid (zelf beoordeeld). Daarnaast hebben we de genetische correlatie tussen het WB spectrum en deze fenotypen berekent. Van alle fenotypen vonden we dat vooral eenzaamheid en gezondheid (zelf

beoordeeld) een sterke relatie met het WB spectrum hebben en mogelijk extra inzicht geven in de factoren die van invloed zijn op verschillen in WB tussen mensen.

Conclusie

Gelukkige mensen zijn gezonde mensen: ze leven langer, functioneren beter en zijn minder vatbaar voor mentale aandoeningen. Gegeven deze voordelen, is het enigszins verbazingwekkend dat er zo weinig onderzoek is gedaan naar de oorzaken van individuele verschillen in WB. Het werk in mijn proefschrift heeft bijgedragen aan een beter begrip van de verschillende factoren die van invloed zijn op WB. Voor de toekomst verwacht ik dat een focus op WB van groot nut kan zijn voor de samenleving. Verschillende studies hebben al aangetoond dat een kleine toename in WB bij de algemene populatie een groot preventief effect heeft op de ontwikkeling van mentale aandoeningen. Om dit te bewerkstellingen is kennis over de oorzaken van individuele verschillen in WB en het in kaart brengen van risicofactoren en beschermende factoren cruciaal.