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A genetically informative study of addictive behaviour with a focus on smoking

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door

Jorien Lisanne Treur

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promotoren: prof.dr. D.I. Boomsma
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Chapter 1.

Introduction

Nicotine is one of the most frequently used addictive substances and, through cigarette smoking, a major contributor to morbidity and mortality. Worldwide, smoking causes 6 million deaths each year (1). While smoking rates have gone down in the Netherlands over the last few decades, still 28% of men and 22% of women were smokers in the year 2014 (2). A better understanding of the causes and consequences of smoking could help to further decrease smoking and thereby improve public health. The aim of this thesis is to explore environmental and genetic influences on addictive behavior with a focus on smoking. It is well known that smoking co-occurs with the use of substances such as alcohol and cannabis (3, 4). Much less clarity exists regarding the relationship between smoking and caffeine use and the relationship between substance use and the consumption of sugar (considered by some as potentially addictive). These two relationships are thus a focus of this thesis. Another key point of this thesis concerns the effect of smoking on mental health. Smoking has been robustly associated with the two most common mental disorders; depressive and anxiety disorders (5, 6). Another disorder that affects more smokers than nonsmokers is ADHD (attention-deficit/hyperactivity disorder) (7). Some evidence suggests that smoking causally increases ADHD symptoms, but this is limited to animal research (8). To further explore this and the other issues described here, data from a unique and large sample of twin families registered at the Netherlands Twin Register (NTR) are utilized.

Smoking behaviour and risk factors

In the Netherlands, 28% of men were *current smokers* in 2014 while an additional 32% were *former smokers*. For women, these percentages were slightly lower at 22% and 28%, respectively (2). Male smokers also smoked more cigarettes per day (n = 11.4) than female smokers (n = 10.0). When combining the statistics on current and former smoking, the majority of men (60%) and half of women were considered *ever smokers*, meaning that they smoked regularly at some point in their life-time. The remaining 40% of men and 50% of women were consequently *never smokers*; those who had never regularly smoked. Smoking rates differed across age groups. Only 5% of the 12-16 year olds smoked in 2014 while this was 23% in the age category 16-20 years. Smoking was most prevalent in 20-30 year olds (37%) and the prevalence was as low as 9% in those aged 75 years or older. When categorizing the Dutch population into four levels of educational attainment going from low to high, smoking prevalence was 30%, 27%, 20% and 16%, respectively (2).

All aspects of smoking behaviour show individual variation and a large body of research has identified risk factors that are associated with smoking initiation, smoking quantity/nicotine dependence, and smoking cessation. Smoking is usually initiated during adolescence, at which age peers are very important and peer pressure to smoke can affect adolescent behaviour (9). In an adolescent sample from the NTR, smoking status of friends was much more predictive of adolescent's smoking than the smoking status of parents (10). This is probably because adolescents model themselves more to their peers, who are of the same age, than to their parents, who differ from them in age. Low correlations between smoking behaviour of parents

and offspring were also found in an earlier NTR-study. These correlations were not dependent on the sex of the parent or offspring and the resemblance between parents and offspring was explained entirely by genetic relatedness (11). In another, longitudinal sample of Dutch adolescents, a decrease in refusal self-efficacy (the confidence an adolescent has in his/her ability to stay a nonsmoker and refuse a cigarette) predicted smoking initiation (12). Besides the influence of peers, there are individual characteristics associated with an increased risk of taking up smoking. Young males were more likely to initiate smoking than females (13) and a lower education in adolescence was strongly associated with a higher chance of smoking in adulthood (14). Smoking was also more often initiated by Dutch (young) adults living in deprived areas compared to those living in affluent areas, even after correcting for education and income (15). Personality traits are important, with individuals who are more impulsive or prone to experiment being more likely to light up a first cigarette (16) and to do so at a younger age (17). Finally, being a regular smokers was associated with higher levels of extraversion and neuroticism and with lower conscientiousness (self-control and allegiance to social norms) (18).

Once (regular) smoking has been initiated, several factors are associated with individual differences in the number of cigarettes smoked per day and the degree of dependence to smoking. An often used measure of nicotine, or smoking, dependence is the Fagerström Test for Nicotine Dependence (FTND) (19). In a sample of approximately 2,500 current and former smokers registered at the NTR, FTND scores were not associated with age or gender but did show a low, negative correlation with age at first cigarette and a positive correlation with the total number of years a person had smoked (20). In addition, fewer years of education, a lower income and a lower occupation were all associated with higher smoking heaviness and/or FTND scores (21). When comparing light (nondependent) smokers to heavy smokers, the latter reported higher perceived stress than the former while there was no difference between the two groups in level of impulsivity (22).

Most smokers want to quit smoking and have attempted to do so at least once (23). In Dutch current smokers in 2014, 30% of men and 38% of women said to have had a (unsuccessful) quit attempt in the past 12 months (2). Multiple factors are related to the chance that a smoker quits (*smoking cessation*) or continues to be a smoker (*smoking persistence*). For instance, individuals who successfully quit smoking were less likely to report symptoms of emotional distress, had a higher self-reported health, drank less alcohol and reported less medical conditions (24). Other predictors of successful smoking cessation were a higher age and a higher educational level while higher FTND scores were associated with smoking persistence (25). Finally, higher levels of neuroticism predicted smoking relapse in former smokers (18).

Genetic underpinnings of smoking

Genes play an important role in smoking behaviour and twin studies have been crucial in

estimating how much of the variation in the different aspects of smoking is due to genetic factors. The main premise of the twin model is that the resemblance between two types of twins is compared: monozygotic twins (MZ; share ~100% of their segregating genes and shared environment) and dizygotic twins (DZ; share ~50% of their segregating genes and shared environment). When MZ twins are more similar than DZ twins for a particular trait, genetic influences are implied. When the correlation between DZ twins is larger than half of the correlation between MZ twins, this suggest that there is an influence of the common environment that the twins share. In the NTR it was demonstrated that individual differences in smoking initiation were explained for 44% by genetic factors (26). Most of the remaining variation was explained by common environmental factors shared by the twins (51%), while a very small part was due to unique environmental factors (5%). This moderate influence of genes on whether or not someone starts to smoke is most likely mediated through personality traits such as impulsivity and extraversion which increase the chance of smoking initiation and are moderately to highly heritable (27, 28). Individual differences in smoking heaviness are for the most part genetic in nature. In Dutch twins, 75% of the variation in nicotine dependence was explained by genetic factors with the remaining 25% being due to unique environmental factors (26). There was no influence of the common environment that the twins share. Lastly, approximately half of the individual differences in the ability to quit smoking (smoking cessation) was due to genetic factors in a Finnish twin study, while the other half was due to unique environmental factors (29).

The above described twin studies demonstrate that the *phenotype* smoking is moderately to largely influenced by a person's *genotype*. With the introduction of genome-wide association studies (GWAS), a hypothesis free method to search for genetic variants associated with a complex trait such as smoking became available (30). In GWAS, hundreds of thousands of single nucleotide polymorphisms (SNPs) are measured across the genome. A SNP is a single nucleotide in the genome that is polymorphic, meaning that more than one form is common in the population. For example, at a particular location, or locus, most people have the letter G while a minority of the population has the letter A. In GWAS, the frequency of all included SNPs is compared between individuals with a certain trait or condition (cases) and those without it (controls). In the year 2010, three large GWA meta-analyses were published, investigating the genetics of smoking behaviour (31-33). In a set of pooled analyses of these three studies, several genome-wide significant 'hits' were found for smoking behaviour. First, four loci were associated with the number of cigarettes smoked per day. The strongest of these associations was found for rs1051730 which is located in the nicotinic receptor gene *CHRNA3*. The A allele of this SNP was associated with increased smoking heaviness. Rs1051730 is in very high linkage disequilibrium (LD) with rs16969968 (meaning that these SNPs are usually transmitted together). The *CHRNA3* gene codes for the expression of nicotine receptors in the brain, thus providing a plausible explanation for its association with smoking heaviness. For smoking initiation, eight SNPs reached genome-wide significance. The strongest effect was found for rs6265, with carriers of the C allele being at increased risk of smoking.

This SNP is located in the *BDNF* gene, which codes for a neurotrophin that regulates synaptic plasticity and the survival of cholinergic and dopaminergic neurons. It is highly expressed in the prefrontal cortex and hippocampus. These brain regions had previously been found to affect cognitive-enhancing effects of nicotine. Lastly, one SNP was genome-wide significantly associated with smoking cessation (being a former vs. a current smoker). The G allele of SNP rs3025343, located near the *DBH* gene, was associated with an increased odds of successful smoking cessation. The *DBH* gene codes for a protein that converts dopamine into norepinephrine.

The introduction of GWAS also made it possible to estimate how much of the variation in smoking is explained by all of the measured SNPs. Lubke *et al.* (2012) utilized two methods to estimate such SNP-based heritability for smoking, one developed by Yang *et al.* (34) and one by So *et al.* (35). When applying both of these methods Lubke *et al.* (2012) found a heritability of 19% and 28%, respectively for smoking initiation and of 24% and 44%, respectively for current smoking. Corresponding heritability estimates from twin studies were 44% for smoking initiation and 79% for current smoking (36). These findings show that with currently available genotype data, it is possible to explain a considerable part of the heritability of smoking behaviour. However, much of the heritability as found by twin studies remains unexplained. Possible explanations for this so-called 'missing heritability' are that twin/family studies have overestimated heritability, that there are many causal variants which each explain a tiny amount of the variation and they therefore do not reach genome-wide significance and/or that causal variants are not in sufficient LD with the SNPs that are genotyped and therefore their effects are not fully captured. More research is needed to uncover the exact explanation (37).

Smoking and other addictive behaviours

Several traits co-occur with smoking, meaning that they are present more often in current smokers compared with never smokers (with former smokers often showing intermediate levels). Generally, there are two mechanisms that can explain such an association. 1: A causal effect of smoking on the co-occurring trait or of the co-occurring trait on smoking. 2: The two traits have common genetic or environmental influences. The most prominent association is the one between smoking and the use of other addictive substances, such as alcohol and cannabis. In a large review including 56 studies from around the world the majority reported a strong correlation between alcohol and smoking (4) and in an American study 90% of cannabis users reported that they smoked at some point during their life, compared with 47% of non-cannabis users (3). Less is known about the association between smoking and caffeine use. The strongest contributor to human caffeine consumption is coffee, which showed a heritability of 39% in Dutch twins (38). The influence of genetics on coffee use is thus moderate compared to genetic influences on smoking. This difference in heritability may be due to the fact that caffeine is much less addictive compared with nicotine (39, 40), and heaviness of caffeine use is therefore determined more by environmental factors. Strong

observational associations have been found between smoking and coffee use (41-43). For instance, in an American sample 4.8% of men and 8.1% of women who never drank coffee were smokers, compared with 34.7% and 48.1%, respectively in men and women who drank 6 or more cups of coffee per day (41). Investigations on smoking and caffeinated drinks other than coffee, such as tea, cola and energy drinks, are scarce. Since caffeine is the most used psycho-active substance worldwide (39), a better understanding of the association with smoking is needed.

Besides co-occurring with the use of 'conventional' addictive substances, smoking is positively associated with the consumption of sugar (44), a nutrient that is considered by some as potentially addictive (45). Alcohol or drug dependent individuals also have a higher sweet preference than individuals who are not substance dependent (46-48). The consumption of sugar contributes greatly to the rising prevalence of (morbid) obesity worldwide (49). This was for instance shown in a randomized controlled trial where the consumption of sugar through drinks caused weight gain and fat accumulation (50). To date, there is very little research on the association between substance use on the one hand and sugar consumption/liking on the other hand. Interestingly, the consumption of sugar promotes the release of dopamine in the brain and thus has rewarding properties similar to substances such as nicotine or alcohol (51). Given these overlapping effects on the brain's reward system, sugar consumption and substance use may have common genetic foundations. Twin data are perfectly fit to test mechanisms underlying the association between these two traits.

Consequences of smoking

Smoking is a major cause of morbidity and mortality, with some of the most severe consequences being lung cancer (52) and cardiovascular disease (53). Furthermore, smoking has been shown to be correlated with mental health such that smokers are diagnosed with depressive and anxiety disorders more often than nonsmokers (5, 6). Smoking also co-occurs with less prevalent mental disorders such as ADHD. Significantly higher smoking rates have been found in individuals diagnosed with ADHD compared to those without the disorder (7), with one study finding that 40% of adults with ADHD smoke against 26% of the general population (54). It is often assumed that the explanation for this association is that individuals with ADHD or attention problems are more likely to initiate smoking. There is supporting evidence for this explanation from longitudinal studies showing that ADHD leads to smoking, also referred to as the 'self-medication' hypothesis (55, 56). Recently, animal research provided compelling evidence for an additional explanation, namely that cigarette smoking causally increases attention problems. In rats, exposure to nicotine during adolescence lead to a decrease in attentional performance, which lasted into adulthood (8). Evidence for such a causal mechanism is not yet available from human studies but could be tested with data of twins.

Content of this thesis

Given the current state of knowledge, there are some unresolved questions. These questions can be addressed by utilizing data of twin-families. To this end I analyzed previously collected data from the NTR and I collected and analyzed new data on addictive behaviour, including smoking, caffeine use and sugar consumption. A brief description of the content of each of the chapters in this thesis is provided below.

Chapter 2 describes the large-scale data collection that has taken place within the Netherlands Twin Register (NTR) as part of this PhD project. Data collection comprised of a survey containing questions on health, personality and behaviour, sent in 2013 and 2014. Approximately 20,000 NTR participants participated in the study by completing the survey. This chapter gives an elaborate description of the methods of data collection and an accurate account of the response rate. In the following chapters of this thesis, previously collected data from the NTR as well as these newly collected data are utilized.

Chapter 3 explores 'smoking expectancy', a measure that is obtained by asking people whether they think they will smoke in a year's time, with answer categories ranging from 'certainly not' to 'absolutely yes' on a 5-point scale. The meaning of the answer to this question differs depending on whether the person in question is a never smoker (expectancy to initiate smoking), current smoker (expectancy to continue smoking) or former smoker (expectancy to take up smoking again). In a longitudinal design, it is tested whether such a relatively simple question can predict future smoking behaviour. These analyses are corrected for age, gender, educational attainment, self-reported health and smoking quantity and frequency. By employing data of twins, it is also estimated whether a person's ability to correctly predict future smoking behaviour is influenced by genetic and/or environmental factors.

Chapter 4 describes a study on spousal resemblance for smoking. Spouses resemble each other more than would be expected by chance with the strongest spousal correlations being found for smoking behaviour (57). As of yet, the nature of this association is largely unclear. There are three possible mechanisms that are most often referred to as underlying spousal resemblance. First, spouses may resemble each other due to *phenotypic assortment* in which case someone's choice of spouse is directly based on phenotype. Second, *social homogamy* could pose an explanation, meaning that spouses resemble each other because they are from similar (social) surroundings and were therefore more likely to meet and pair up. Third, spouses may resemble each other because they influence each other while being in a relationship together, in which case there is *marital interaction*. In this chapter the exact mechanism behind spousal resemblance is elucidated by utilizing data from a large sample of twins, spouses of twins and parents of twins. The effects of research cohort (time of data collection) and age of the participants on spousal resemblance are also explored.

Chapter 5 gives an extensive account of the observational association between smoking behaviour and caffeine consumption. While a correlation between smoking and coffee use has often been reported, associations between smoking and other caffeinated drinks (tea, cola, energy drinks) are less clear. In addition it is unknown if such associations are consistent across (European) countries with contrasting patterns of caffeine consumption, such as the Netherlands (a 'coffee drinking' country) and the United Kingdom (a 'tea drinking' country). This chapter provides an answers to these two research questions in two large, population-based samples, one Dutch and one British.

Chapter 6 continues with the topic of smoking and caffeine consumption. There is contradictory evidence on the nature of this association. High observational correlations between smoking and caffeine could be due to causal effects of smoking on caffeine or vice versa, or due to an overlap in genetic and/or environmental factors. This chapter describes a study where three different methods are utilized to test these hypotheses, namely bivariate twin modeling, LD Score regression and Mendelian randomization. With bivariate twin modeling and LD Score regression it can be tested whether genetic and/or environmental risk factors for smoking overlap with genetic and/or environmental risk factors for caffeine use. Mendelian randomization analysis tests whether there are causal effects.

Chapter 7 turns the focus to the association between substance use and the consumption of sugar, a nutrient which some claim has addictive potential (45). In this study, bivariate twin modeling is employed to explore the association between substance use (smoking, alcohol, cannabis, caffeine and illicit drugs) and sugar consumption through drinks. By using data of twins, it can be tested whether genetic and/or environmental risk factors for substance use overlap with genetic and/or environmental risk factors for sugar consumption. This endeavor will further our understanding of the etiology of different types of addictive behaviours, among which the excessive consumption of sugar.

Chapter 8 aims to confirm an important finding from animal research that suggests smoking during adolescence causally increases attention problems (8). It was previously known that smoking and ADHD symptoms, or attention problems, show a high correlation. A commonly posed explanation for this association was that ADHD symptoms causally increase smoking (self-medication hypothesis). Animal research has thus pointed to another option; smoking affects the developing brain and thereby increases attention problems. As of yet there is no such evidence from human studies. This chapter describes the first human, longitudinal study in twins investigating the causal effect of smoking on attention problems, utilizing MZ twins who are discordant for smoking (one twin smokes while the other doesn't). Twins from these discordant twin pairs are compared on attention problems. When the smoking twin has more attention problems than the non-smoking co-twin, a causal effect of smoking is suggested. Because MZ twins share ~100% of their genetic make-up and a large part of their environment, the design corrects for genetic and most environmental factors.

Chapter 9 concludes this thesis with an overall summary and a general discussion.

Chapter 2.

Data collection

Introduction

The Netherlands Twin Register (NTR) was established around 1987. Since 1991, an invitation to complete a survey is sent to adolescent and adult (ANTR) participants once every two to three years (58). Surveys have been sent to ANTR families in *1991* (survey 1), *1993* (survey 2), *1995* (survey 3), *1997* (survey 4), *2000* (survey 5), *2002* (survey 6), *2004-2008* (survey 7), *2009-2012* (survey 8) and *2011-2012* (survey 9). A considerable part of the results described in this thesis were based on these existing data, but new data were also collected. This new data collection took place in *2013* and *2014* (survey 10). The present chapter describes the content of survey 10, the methods of data collection and the final response rates.

One of the main focusses of survey 10, and the main focus of this thesis, was substance use. In addition to questions on physical and mental health, personality and behaviour, survey 10 contained many questions on the use of different substances. Some of these questions were recurrent in the NTR data collection, while others were included for the first time. For instance, recurring questions on the frequency and quantity of alcohol use and cigarette smoking were complemented with new questions on exposure to second-hand cigarette smoke prenatally, during childhood and later in life. Cannabis use was investigated more thoroughly compared with earlier surveys with questions on past and current frequency of use, age at first use and age at the time the substance was most regularly used. Another new addition to survey 10 was a comprehensive set of questions on the consumption of different kinds of drinks. These included both caffeinated drinks (such as coffee, black tea, and energy drinks) and non-caffeinated drinks (decaffeinated coffee and herbal tea), as well as drinks with sugar (such as soft drinks and fruit juices) and those that are sugar free (diet soft drinks and diet fruit juices). With the answers to these questions an accurate assessment of caffeine and sugar consumption through drinks was obtained. Finally, questions on the use of novel and upcoming 'substances' including e-cigarettes and water pipe (also referred to as 'hookah' or 'shisha') were included in survey 10.

Two versions of the survey were developed, one which was tailored specifically to an older group of participants (survey 10-O) and one standard version, which was sent to all other (adult) participants (survey 10-S). Both versions of survey 10 and the exact methods of data collection are described below, followed by some specific points of interest related to the data collection. The complete content of survey 10-O and survey 10-S can be found in appendix I. Data collection started with Survey 10-O.

Survey 10-O

Brief study description

Survey 10-O was developed specifically for an older population. It was approximately twice as short in length and contained a version of the ASR for older subjects (the OASR (59)). Some other questions (such as questions on living arrangements) were also amended in order to tailor the survey to an older age group. The survey was sent in paper form only, i.e. it was not

available online. Survey 10-O was sent to participants registered at the NTR who were aged 65 years or older, or those who were 60-65 years old and whose spouse or older sibling was aged 65+. The latter was done to prevent misunderstandings among the participants as a result of one person in a family receiving the survey. This selection resulted in a total of *4,788* participants who were eligible for survey 10-O. Participants for whom there were no spouses registered at the NTR received an extra survey for their potential spouse, with the aim of encouraging more spouses of twins to participate in the NTR research.

Protocol

Survey 10-O was sent according to a set protocol. A global time-line of this protocol is depicted in figure 1. In the *beginning of May 2013*, participants were invited to take part in the newest study of the NTR. Through mail, they received a package containing an invitation letter, a brochure with extra information on the study, the paper survey and a reply envelope. Participants for whom there was no spouse registered at the NTR also received a paper survey and reply envelope for their spouse. An example of the invitation letter and the brochure can be found in appendix II and appendix III. The letter introduced the study and invited people to participate, while the brochure provided additional information. When a participant had completed the survey and sent it back to the NTR, he or she received a 'thank you' card to express our gratitude for their participation (see appendix IV). By the *end of June 2013* (approximately 6 weeks after the first invitation was sent), a reminder card was sent to the participants who had been invited to complete survey 10-O but hadn't done so yet (see appendix V). This reminder card reminded them of the study and encouraged them to contact the NTR when they had lost their survey and required a new one. In weeks 8 through 11 after the first invitation, follow-up telephone calls were made in an additional effort to increase the response rate. For these follow-up calls a group of *300* people who had regularly participated in NTR surveys in the past, and were thus considered as 'loyal' participants, were selected.

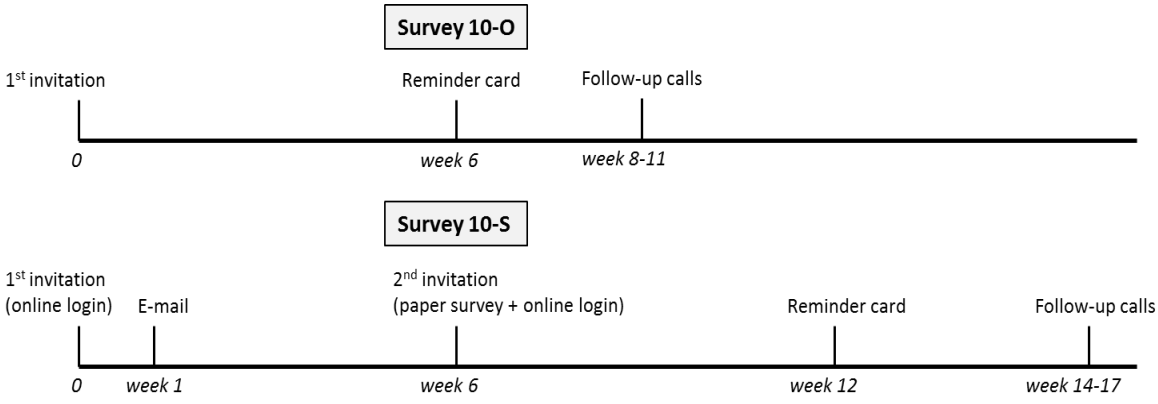

Figure 1. Global time-line of the sending of survey 10-O and survey 10-S

Survey 10-S

Brief study description

Survey 10-S (the 'standard', non-shortened version of survey 10) was sent to all adult participants (aged 18+), who were not previously approached for survey 10-O. In contrast to survey 10-O, survey 10-S was available online as well as in paper form. The online version of survey 10-S was tailored based on participation in previous surveys of the NTR. It was programmed in such a way that for participants who had completed one of the more recent NTR surveys (survey 8 and / or survey 9), certain questions were automatically skipped. This benefitted loyal participants by making the survey considerably shorter. The questions which were skipped based on previous participation are indicated in the overview of items and scales of survey 10-S in appendix I. Due to the large number of participants eligible for survey 10-S, it was sent in two batches which are described below.

Protocol

Batch 1

Mid October 2013, the first batch of participants was invited for survey 10-S. See figure 1 for a global time-line of the whole protocol. Participants who were previously approached for survey 10-O (older population) were excluded. For the selection of batch 1, only participants who enrolled into the NTR as an adolescent or adult were included, the so-called ANTR participants (as opposed to participants who were enrolled as a child, the so-called YNTR participants). This selection resulted in a total of *19,973* participants and included twins, siblings, parents, spouses of twins and offspring of twins aged 18 years or older. Through mail, these participants received a package containing an invitation letter and a brochure with extra information. In the letter, personalized login details for the online survey were provided as well as the link to the webpage for the online survey. An example of this invitation letter can be found in appendix VI. The letter invited people to complete the survey online, but also stated that if they preferred to complete the survey in paper form they would receive a paper survey in approximately 6 weeks. To further facilitate participation in the study, we sent participants with a valid e-mail address an e-mail with the link to the online survey and their login details approximately one week after the initial invitation per mail (see appendix VII). The paper survey was sent 6 weeks after the first invitation in the *beginning of December 2013*. It also constituted as a reminder, aiming to increase the number of people who would complete the survey (see appendix VIII). When a survey was completed and received at the NTR, participants were sent a 'thank you' card to express our gratitude for their participation. By the *end of January 2014* (approximately 3 months after the first invitation was sent), a reminder card was sent to the participants who were invited to complete survey 10-S but hadn't done so yet. In weeks 14 through 17 follow-up calls were made to a group of 450 'loyal' participants. Finally, a group of 400 participants who completed survey 10 and for whom there was no spouse registered at the NTR received an extra survey for their potential spouse in *November 2014*.

Batch 2

Mid February 2014, the second batch of participants was invited to participate in survey 10-S. Participants who were previously approached for survey 10-O (older population) or the first batch of survey 10-S were excluded. For the selection of batch 2, twins who were enrolled into the NTR as a child and their parents and siblings were included (YNTR participants). This selection resulted in a total of *40,696* adult participants. These participants first received a package containing an invitation letter with personalized login details for the online survey and a brochure with extra information. Again, it was stated in the letter that if they preferred to complete the survey in paper form they would receive a paper survey in approximately 6 weeks. All participants with a valid e-mail address also received an e-mail with the link to the online survey and their login details one week after the initial invitation per mail. The paper survey was sent in the *beginning of April 2014* and it constituted as a reminder for people to participate in the study when they hadn't done so yet. Thank you cards were sent to participants who had completed the survey and in *mid May 2014* (approximately 3 months after the first invitation was sent), a reminder card was sent to those who hadn't completed survey 10-S yet. Follow-up calls were made in weeks 14 through 17, to a group of 450 participants who were considered to be 'loyal' participants.

Updating of address information

During the mail-out of survey 10, a new method of updating address information in the NTR system became available. Permission was obtained for the NTR to utilize the national administration of municipalities, or in Dutch the 'Gemeentelijke Basis Administratie' (GBA), in order to retrieve the current residential addresses of NTR participants. Before this option became available, information on the residential addresses of participants who had moved was retrieved by contacting the concerning municipalities. This was often a difficult and time-consuming task. Because of these difficulties in finding out where people had moved to, the current residential address was unavailable for a considerably large group of NTR participants. This meant that in some cases, participants who might be willing to participate in research could not do so because we were not able to reach them and invite them for a new study.

With the newly gained access to the GBA it was possible to retrieve the last known address of NTR participants. We did not check the address for every survey we sent so we relied on undeliverable letters being returned to us in order to trace participants who had moved. In the initial phases of survey 10, all mail which was returned to the NTR as being undeliverable (meaning the addressee was not residing on the address it was sent to) was registered. In a next step, a list of moved participants was created and entered into the GBA database. The resulting output gave us the most recent residential addresses of these NTR participants, which was then updated in the NTR database. Finally, survey 10 was resent to all participants for whom the new residential address was obtained.

Personal feedback

Another new feature that was implemented during the collection of the 10th survey of the NTR was the use of an online portal called 'Mijn NTR'. Participants have to activate the portal themselves and can then access this portal by going to a webpage and logging in with their own personal login details. After logging in, participants are able to access the results and outcomes of previously completed NTR studies. Not only can they obtain outcomes in the form of the scientific papers which have been published using these data, they can also receive their own personal scores for some surveys. Personal scores are for instance available for certain personality scales and exercise behaviour. Based on survey 10, personal scores on the consumption of caffeine and sugar through drinks were available. In the report, participants can see how much caffeine and sugar they consume on average per day and can compare this with the average of all NTR participants who completed survey 10. An example of this report can be found in appendix IX.

Response rate

Survey 10 was sent to *65,442* registered NTR participants and completed by *19,371* of those participants. In total, this makes for a response rate of 29.6%. An additional *265* newly recruited spouses of twins also completed the survey. With the help of the GBA database we were able to update address information and resent the invitation for survey 10 to participants for whom the invitation letter was returned to us as being undeliverable (4.2%). However, it is likely that there were more participants who moved but for whom we did not receive back the undeliverable letter. To get an impression of the percentage of participants for whom the address information in our database was incorrect, we entered a list of 1000 participants into the GBA database. Of this group, 30% had moved to a different address. Even though these 1000 participants were all residing in the city of Amsterdam, and may therefore not be representative for all NTR participants, this shows that we probably did not reach all 65,442 participants and the actual response rate could be higher than what is presented here.

When there was an option to choose between the paper or the online version of the survey (i.e. for survey 10-S batch 1 and batch 2), the majority (69.3%) completed the survey online, while the remaining 30.7% completed the survey in paper form (see Table 1). Response rates differed across survey version/batch, with the highest rate for survey 10-O (50.2%), a considerably lower rate for survey 10-S batch 1 (33.4%) and the lowest rate for survey 10-S batch 2 (25.3%).

Table 1. Total response rate of survey 10

	N sent	N received	Response	Response adjusted	Ratio paper/online
Survey 10-O	4,773	2,397 (excl. 165 new spouses)	50.2%	**51.3%**	-
Survey 10-S batch 1	19,973	6,676 (excl. 100 new spouses)	33.4%	**33.7%**	1,654 (25.2%) / 5,022 (75.0%)
Survey 10-S batch 2	40,696	10,298	25.3%	**25.4%**	3,559 (34.6%) / 6,739 (65.4%)
Total	65,442	19,371(excl. 265 new spouses)	29.6%	**29.8%**	5,213 (30.7%) / 11,761 (69.3%)

For survey 10-O the ratio paper/online is not provided because only paper surveys were sent (it was not available online). 'Response adjusted' represents the response rate excluding the participants who we were not able to reach via mail (mail was sent back undeliverable) and excluding participants of whom we were notified that they were deceased or unable to complete the survey due to illness.

One possibility is that this difference in response rates is due to the fact that survey 10-O was sent to an older age group (60 years or older) compared to survey 10-S and that these older participants were more willing and/or able to participate and had not moved. However, when stratifying response rates on age group within the different survey versions, no consistent effect of age was seen (Table 2). Within survey 10-O, there was neither an increase nor a decrease in response rates going from <=65 years (53.6%) to >80 years (49.1%). The two batches of survey 10-S differed from each other in response rate, such that batch 1 showed a higher response rate than batch 2. When stratifying on age within each batch it seems that response rates more or less follow a 'U-shaped' curve, with the lowest response rates in the 35-45 years group in both batches (29.1% for batch 1 and 17.3% for batch 2). Another feature that stands out, is that identical age categories showed different response rates across the two batches of survey 10-S. For instance in the 25-35 years group, the response rate was 34.1% in batch 1 compared with 25.1% in batch 2 (the same is true for age groups 35-45 and 45-55 years). It thus seems the response rate in batch 2 was overall lower, independent of age distribution.

Table 2. Response rate of survey 10 stratified on age groups

Survey 10-O				Survey 10-S batch 1				Survey 10-S batch 2			
Age groups	**N sent**	**N received**	**Response**	**Age groups**	**N sent**	**N received**	**Response**	**Age groups**	**N sent**	**N received**	**Response**
<=65 years	571	306	53.6%	*<=25 years*	176	99	56.3%	*<=20 years*	6,283	1,478	23.5%
65-70 years	2,285	1,164	50.9%	*25-35 years*	2,926	999	34.1%	*20-25 years*	12,600	2,978	23.6%
70-75 years	1,121	557	49.7%	*35-45 years*	9,104	2,650	29.1%	*25-35 years*	4,879	1,224	25.1%
75-80 years	447	202	45.2%	*45-55 years*	3,455	1,395	40.4%	*35-45 years*	640	111	17.3%
>80 years	328	161	49.1%	*55-65 years*	3,021	1,404	46.5%	*45-55 years*	10,275	2,681	26.1%
				>65 years	261	99	37.9%	*>55 years*	5,920	1,814	30.6%
Total	4,752	2,390	50.3%	*Total*	18,943	6,646	35.1%	*Total*	40,597	10,286	25.3%

Participants for whom age was missing were excluded from this table. Appropriate age groups were created, based on the age distribution within each survey version.

Another characteristic that might be associated with differences in response rate is gender. Table 3 shows response rates stratified on gender for the different surveys of version 10. The table clearly shows that response rates were consistently higher in women when compared

with men. The difference was largest in survey 10-S where in both batches, the response rate was 10.6% lower in men than in women (compared to a difference of 4.2% in survey 10-O).

Table 3. Response rate of survey 10 stratified on gender

	Survey 10-O			Survey 10-S batch 1			Survey 10-S batch 2		
	N sent	**N received**	**Response**	**N sent**	**N received**	**Response**	**N sent**	**N received**	**Response**
Men	2,219	1,065	48.0%	8,478	2,341	27.6%	19,482	3,865	19.8%
Women	2,551	1,331	52.2%	11,319	4,329	38.2%	21,164	6,431	30.4%
Total	4,770	2,396	50.2%	19,797	6,670	33.7%	40,646	10,296	25.3%

Participants for whom gender was missing were excluded from this table.

Table 4 depicts response rates across family role (being a multiple, sibling, parent or spouse of a multiple). Multiples and spouses of multiples seemed to be the most willing to participate in survey 10-O and survey 10-S batch 1, while in survey10-S batch 2 the siblings were the most cooperative (spouses are not included in the table for batch 2 because the number of spouses was very low as many young participants do not yet have a stable relationship). When breaking down the group of multiples on zygosity (monozygotic [MZ] or dizygotic [DZ]), MZ were more cooperative compared with the DZ multiples.

Table 4. Response rate of survey 10 stratified on the most common family roles

	Survey 10-O			Survey 10-S batch 1			Survey 10-S batch 2		
	N sent	**N received**	**Response**	**N sent**	**N received**	**Response**	**N sent**	**N received**	**Response**
Multiple	738	575	77.9%	10,927	3,931	36.0%	21,023	4,826	23.0%
MZ	448	363	81.0%	4,464	2,113	47.3%	6,658	1,891	28.4%
DZ	271	208	76.8%	4,439	1,405	31.7%	12,642	2,636	20.9%
Sibling	321	134	41.7%	2,850	851	29.9%	2,839	882	31.1%
Parent	3,529	1,559	44.2%	4,008	1,069	26.7%	16,761	4,581	27.3%
Spouse	159	111	69.8%	1,595	553	34.7%	-	-	-
Total	4,747	2,379	50.1%	19,380	6,404	33.0%	40,623	10,289	25.3%

Family roles for which there were less than 20 received surveys (for example children of twins or spouses in survey 10-S batch 2) were excluded from this table. MZ = monozygotic, DZ = dizygotic.

Finally, Table 5 shows the response rate for survey 10-S batch 1 and batch 2, conditional on the color code that was used for tailoring the online survey. There were three color codes based on participation in the most recent ANTR surveys. The color coding thus reflects how 'loyal' participants were in completing surveys in the last couple of years. The more color codes a person was assigned to (blue and/or green and/or red), the more questions from the online survey were skipped. There was a major difference in response rate between participants who were assigned none of the color codes and all others, with the former showing the lowest response rates (10.3% for batch 1 and 19.1% for batch 2). This means that individuals who participated less often in recent surveys, were also less likely to participate in

survey 10. Among participants assigned one or more color codes, those with all three color codes showed the highest response rates by far (79.5% for batch 1 and 78.7% for batch 2).

Table 5. Response rate of survey 10-S stratified on color coding used to tailor the online survey

			Survey 10-S batch 1			Survey 10-S batch 2		
Blue	*Green*	*Red*	**N sent**	**N received**	**Response**	**N sent**	**N received**	**Response**
0	*0*	*0*	10,907	1,125	10.3%	34,530	6,583	19.1%
0	*0*	*1*	895	448	45.6%	845	446	52.8%
1	*0*	*0*	390	183	46.9%	-	-	-
1	*0*	*1*	45	29	64.4%	-	-	-
1	*1*	*0*	2,847	1,005	35.3%	2,199	811	36.9%
1	*1*	*1*	4,889	3,886	79.5%	3,119	2,456	78.7%
Total			19,973	6,676	33.4%	40,696	10,298	25.3%

Blue = survey 8 and/or the introductory (basislijst) survey was completed, green = survey 8 was completed, red = survey 9 was completed. 0 = not completed the survey 1 = completed the survey. Combinations 0 1 0 and 0 1 1 are not possible since a person who completed survey 8 will be assigned color code green and blue. Combinations for which there were less than 20 received surveys were excluded from this table.

Figure 2 provides a completer picture of how often survey 10 participants have been taking part in surveys over the years. In this figure, the number of previously completed surveys (ANTR survey 1 till 9) is given for all participants who completed survey 10. A minimum of 0 and a maximum of 9 previous surveys could be completed. Of the 2,397 individuals who completed survey 10-O, 1,081 (45.1%) also completed five or more previous ANTR surveys. For the 6,676 participants who completed survey 10-S batch 1, this proportion was slightly lower at 2,499 (37.4%). Of the 10,298 participants who completed survey 10-S batch 2, almost none completed five or more surveys (n=7). This is because batch 2 consisted of YNTR participants who were not invited to take part in ANTR research until survey 8, and only if they were aged 18 years or older at that time. This is reflected in Figure 2 with most survey 10-S batch 2 participants completing none or 1-2 previous surveys (10,081 [97.9%]).

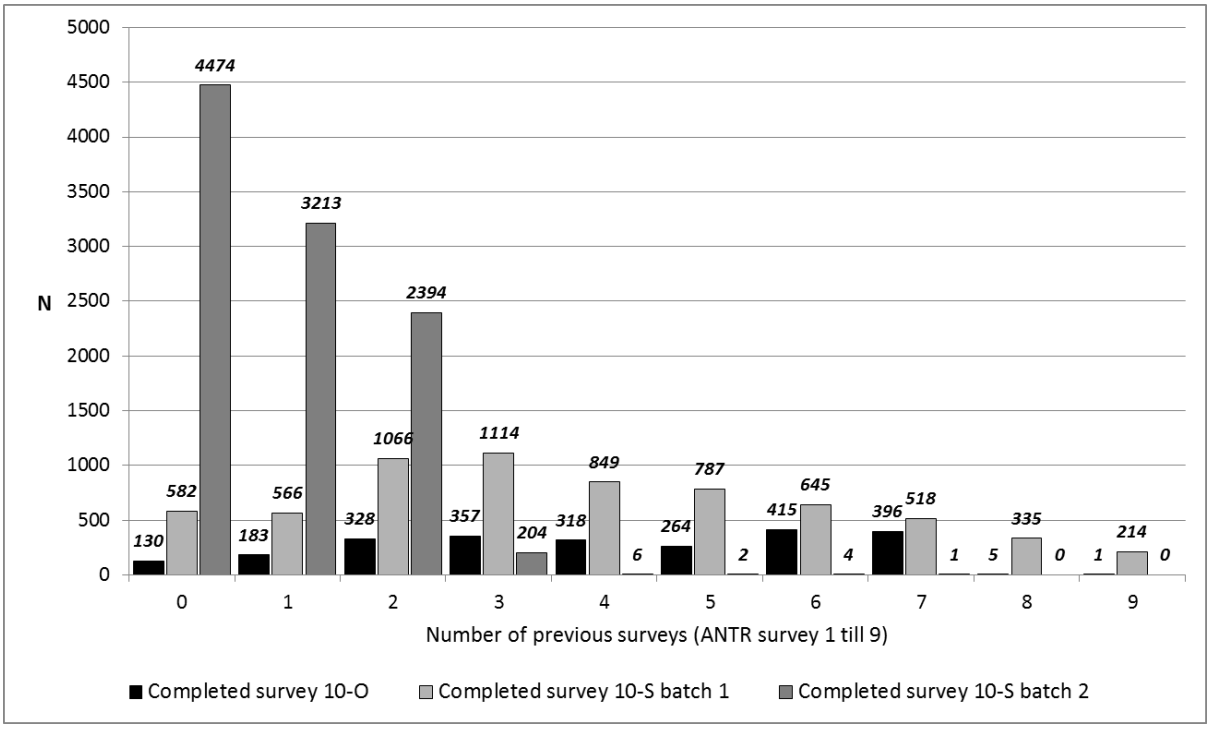

Figure 2. Number of previous surveys (ANTR survey 1 till 9) completed by participants who completed survey 10-O, survey 10-S batch 1 or survey 10-S batch 2.

Chapter 3.

The predictive value of smoking expectancy and the heritability of its accuracy.

This chapter is based on:

Treur JL, Boomsma DI, Lubke GH, Bartels M and Vink JM (2014). The predictive value of smoking expectancy and the heritability of its accuracy. Nicotine and Tobacco Research, 16(3):359-368

Abstract

Introduction: In smokers, former smokers, and never-smokers, this study aimed to (a) determine the predictive value of smoking expectancy on future smoking status and (b) test the relative contribution of genes and environment to a person's ability to accurately predict future smoking status. For smokers, smoking expectancy reflects the intention to continue smoking, for former smokers to take up smoking again, and for never-smokers to initiate smoking. **Methods:** A longitudinal design was employed in which participants of the Netherlands Twin Register completed 2 consecutive surveys 2 years apart between 1993 and 2011 (3,591 adolescents, aged 14–18 years), or between 1993 and 2004 (11,568 adults, aged 18+ years). Smoking expectancy was measured by asking 'Do you think you'll smoke in a year's time?', with answer categories ranging from 'Certainly not' to 'Absolutely yes' on a 5-point scale. To determine the predictive value of smoking expectancy, analyses were performed in smokers, former smokers, and never-smokers separately. Data of 2,987 adolescents and 4,911 adult twins were analyzed to estimate heritability. A dichotomous variable reflected the ability to predict future smoking status ('correct'/'incorrect'). **Results:** Smoking expectancy significantly predicted future smoking status in former smokers and never-smokers. The ability to accurately predict future smoking status was explained by additive genetic factors for 59% in adolescents and 27% in adults, with the remainder being explained by unique environmental factors. **Conclusions:** A single question on smoking expectancy helps predict future smoking status. Variation in how well subjects predict their future smoking behaviour is influenced by genetic factors, especially during adolescence.

Introduction

Smoking remains a major public health problem worldwide and can cause severe morbidity (60). The World Health Organization has estimated that up to half of all tobacco users will eventually die from a tobacco-related disease (61). Despite these facts, 28% of adult men and 26% of adult women in the Netherlands were smokers in 2010 (62), while 16% of adolescents smoked occasionally, and 10% vs. 9% of male and female adolescents smoked daily (63). Smoking cessation leads to a significant decrease in the risk of serious health problems (64), and a complete understanding of smoking behaviour and its predictors might aid in developing successful intervention programs. This study explores the expectancy people have about their own future smoking behaviour. Such 'smoking expectancy' may predict not only whether smokers continue smoking but also whether former smokers will relapse or never-smokers will initiate smoking.

In general, adolescent never-smokers are more susceptible to the initiation of smoking than (young) adults. In Dutch youngsters, the mean age at first cigarette is 15 years (65), with 89% of ever-smokers having started smoking before the age of 18 years (66). Young males were more likely to initiate smoking than females (13, 14), and a lower educational level in adolescence was associated with a higher chance of smoking in young adulthood (67).

In current smokers, the intention to quit predicted a future quitting attempt and was higher in smokers who smoked for a shorter period of time and/or smoked fewer cigarettes per day (68). Whether or not smokers expected to be successful in quitting was also predictive of a future quit attempt (69). Together, attitude toward quitting smoking, opinions of friends and family about smoking, and the extent to which one believes to be able to quit accounted for approximately 30% of the variance in quitting intentions (70). Past quit attempts and having concerns about the health effects of smoking were predictive of making a quit attempt in the future while succeeding in quitting was influenced by cigarette dependency (71). Having had health problems in the past increased the intention to quit and the chance to make a quit attempt (69).

In former smokers, risk factors for relapse include a lower abstinence self-efficacy and a higher frequency of urges to smoke (72, 73). A higher former cigarette dependence increased the chance of relapse following a quit attempt (74), while a higher educational level and a higher self-reported health were associated with a lower risk or relapse (24, 75, 76).

Smoking expectancy might be able to predict future smoking behaviour in never-smokers, current smokers, and former smokers, making it a useful tool for identifying risk groups. Up until now, publications on smoking expectancy (measured by asking if a person thinks he/she will smoke next year) are scarce. In adolescents from New Zealand, a higher age was associated with a higher chance of being a smoker and thus with a higher expectancy to (still) smoke in the future (77). Smoking adolescents tended to underestimate the chances of

continuing, while nonsmoking adolescents underestimated the chances of initiating smoking (78). In addition, susceptibility to smoking (defined as not being able to rule out the possibility of smoking in a year) was a strong predictor of starting smoking in nonsmoking adolescents (79).

Multiple aspects of smoking behaviour are influenced by genetic factors, including smoking initiation, nicotine dependence (26, 80, 81), and smoking cessation (29). Genetic factors may also influence people's ability to accurately predict their own future smoking status and understanding how individual differences can be explained may assist in tailoring of prevention strategies. As for every human complex trait, we expect people to differ in how well they assess their own future smoking behaviour. Some might be overly optimistic about their ability to quit, while others may be more capable of predicting their future smoking behaviour. Such individual differences are likely to have a heritable component, possibly related to genetically influenced personality traits like optimism. Self-knowledge about ability to quit may also depend on experience and age. The extent to which this knowledge depends on genotype may be age dependent as well, decreasing when people gain more experience about their own behaviour. We, therefore, investigate the heritability of predicting future smoking status in both adolescents and adults.

Longitudinal data on smoking expectancy were collected in two large groups of participants from the Netherlands Twin Register (NTR; 3,591 adolescents and 11,568 adults). Within each age group, smoking expectancy for current smokers reflects the intention to continue smoking, while for former smokers, it reflects the intention to take up smoking again, and for never-smokers, the intention to initiate smoking. We aimed to (a) determine the predictive value of smoking expectancy on future smoking status through longitudinal analyses and (b) estimate the relative contribution of genetic and environmental factors to the ability to accurately predict future smoking status through genetic analyses of twin data.

Methods

Subjects

All participants are enrolled in longitudinal survey studies of the NTR (58, 82). The young NTR consists of participants who were recruited as newborn twins from 1987 onwards and their siblings who were included later on. The adult NTR comprises adolescents and adult twins and their family members who were recruited since 1990.

Data were analyzed separately for adolescents (aged 14–18 years) and adults (aged 18+ years). We first selected 3,591 adolescents and 11,568 adult participants who completed at least two successive surveys approximately two years apart. After discarding participants with an unknown smoking status, the adolescent group consisted of 3,114 twins and their siblings (40% male; 4% nontwin; mean age 15.7 years, *SD* = 1.1). Between 1993 and 2011, the adolescents completed two surveys either around 14 and 16 or 16 and 18 years. The adult

group contained 10,468 participants (41% male; 53% nontwin; mean age 37.0 years, *SD* = 14.3). Adults completed two or more consecutive surveys in 1993, 1995, 1997, 2000, 2002, and/or 2004. Data on smoking expectancy were collected in all surveys, except in 2004.

For the genetic analyses, data from 2,987 adolescent twins (1,106 complete pairs and 775 twins from incomplete pairs) were available. This group included 422 monozygotic male (MZM), 348 dizygotic male (DZM), 800 monozygotic female (MZF), 499 dizygotic female (DZF), and 918 dizygotic opposite sex (DOS) twins. In the adult group, a total of 4,911 twins (1,911 complete pairs and 1,089 twins from incomplete pairs) were available including 727 MZM, 486 DZM, 1,641 MZF, 903 DZF, and 1,154 DOS twins. Zygosity was based on DNA typing for 27% of the adolescents and 54% of the adult twin pairs. For the remaining pairs, survey questions about similarity between the twins were used. Agreement between zygosity based on survey data and DNA data was 96.1%.

Measures

Smoking expectancy was assessed at baseline (time point 1 [T1]) by asking 'Do you think you'll smoke in a year time?', with the answers being measured on a 5-point scale ranging from 'Certainly not' to 'Absolutely yes'. Smoking status (smoker, former smoker, or never-smoker) was established at baseline and at follow up (time point 2 [T2]) by asking 'Have you ever smoked?' (answer categories 'No', 'A few times just to try' and 'Yes') and 'How often do you smoke now?' (answer categories 'I don't smoke regularly', 'I've quit smoking', 'Once a week or less', 'A few times a week', and 'Once a day or more'). In adolescents, only participants who stated that they had smoked more than 50 cigarettes when asked 'How many cigarettes have you smoked till now?' could be classified as former smokers. For participants who answered 'Yes' when asked 'Have you ever smoked?', but gave no further information on current smoking status or frequency, smoking status was coded as unknown. When participants stated that they (regularly) smoked before but subsequently answered 'I have never been a regular smoker' when asked 'How often do you smoke now?', smoking status was also coded as unknown. Participants classified as smokers or former smokers at T1 and as never-smokers at T2 were excluded from analysis (see Supplementary Figure S1). All additional covariates are depicted in Table 1.

A new variable was created reflecting whether someone was able to predict his or her future smoking status. The 5-point scale for smoking expectancy was dichotomized into 'No' (answer categories 'Certainly not' or 'Probably not') and 'Yes' (answer categories 'I don't know', 'Probably', and 'Absolutely yes'), with the latter reflecting the inability to exclude the possibility of smoking, as was previously done by Forrester *et al*. (2007) (79). This dichotomized variable at baseline was compared with smoking status at follow up, and a dichotomous variable was defined reflecting a correct (0) or an incorrect (1) prediction of future smoking status (see Supplementary Table S1).

Statistical Analysis

Data management and Pearson's chi-square tests, to test differences in sample characteristics across smoking statuses, were performed using SPSS (version 17.0). To account for family relatedness, chi-square tests were repeated in a subsample of unrelated individuals. Regression analyses were carried out in Stata Statistical Software (version 9.0) and corrected for family clustering by employing the robust cluster option, which uses information on family relatedness to correct for the correlation within the families (i.e., clusters). Logistic regression analysis determined the predictive value of smoking expectancy at T1 (independent variable) on smoking status at T2 (dependent variable). We used a two-step approach to quantify the predictive effect of smoking expectancy over and above commonly used predictors. Smoking expectancy was first regressed on the predictors of smoking behaviour at T1 by means of linear regression analysis (see Table 1 for these predictors), after which the resulting residuals were used as a predictor of smoking status at T2 in a second step (residual model). This approach completely eliminates the effects of the predictors of smoking behaviour at T1, providing a conservative estimate of the impact of smoking expectancy. Analyses were carried out for smokers, former smokers, and never-smokers, both in adolescents and adults.

The classical twin model was used to estimate the heritability of the ability to predict future smoking status by comparing the correlations of monozygotic (MZ) and dizygotic (DZ) twin pairs. MZ twins share (nearly) 100% of their DNA, while DZ twins share on average 50% of their segregating genes. If the ability to predict future smoking status is influenced by additive genetic factors (A), the correlation between MZ twins is expected to be twice as large as the correlation between DZ twins. When the correlation of DZ twins is larger than half the correlation of MZ twins, the environment that is shared by both twins is also of influence. When the correlation of DZ twins is smaller than half that of MZ twins, genetic non-additive effects (D) are likely. Structural equation modeling was performed in OpenMx (83). Ability to predict future smoking status was analyzed in a threshold model with the underlying liability being a function of genetic and environmental factors (84). A single threshold divides individuals into those who correctly predicted their future smoking status and those who did not. Since smoking initiation at the time of measuring smoking expectancy might affect the chance of making a correct prediction, it was added to the model as a covariate (0: never smoked and 1: ever smoked). The threshold was modeled as follows: $T = X + \beta_{covariate}$, where T is the estimated value of the threshold, X is the value of the threshold when the covariate is 0 (never smoked), and β represents the deviation on the threshold in subjects who initiated smoking.

First, five twin correlations (MZM, DZM, MZF, DZF, and DOS) were estimated in a saturated model. The threshold and the effect (β) of ever smoking were estimated separately for males and females (nine free parameters). Constraints were then imposed on the model in a stepwise manner (models 2, 3, and 4). Next, the influences of additive genetic factors (A), non-additive or dominance deviations (D), and unique environmental factors (E) were estimated in

a univariate ADE model (models 5, 6, and 7). With likelihood ratio tests, the fit of the different nested models was tested by subtracting the negative log-likelihood (−2LL) of a nested model from the −2LL of the more extensive model. The difference in −2LL follows a χ^2 distribution with *df* equal to the difference in *df* of the two models. In order to achieve the most parsimonious and best-fitting model, constraints were retained whenever they did not significantly deteriorate the fit (p >.05).

Table 1. Sample characteristics at baseline (time point 1 [T1]) for adolescent and adult smokers, former smokers and never-smokers.

Adolescent group (n=3,114)		*Smokers (n=196)*	*Former smokers (n=50)*	*Never-smokers (n=2,868)*	**Adult group** (n=10,468)		*Smokers (n=2,512)*	*Former smokers (n=2,324)*	*Never-smokers (n=5,632)*
Smoking expectancy	Certainly not	7 (3.6)	9 (18.0)	2268 (79.1)	Smoking expectancy	Certainly not	73 (2.9)	1814 (78.1)	5188 (92.1)
(n (%))	Probably not	32 (16.3)	15 (30.0)	419 (14.6)	(n (%))	Probably not	269 (10.7)	405 (17.4)	328 (5.8)
	I don't know	63 (32.1)	15 (30.0)	163 (5.7)		I don't know	681 (27.1)	96 (4.1)	94 (1.7)
	Probably	60 (30.6)	9 (18.0)	11 (0.4)		Probably	1135 (45.2)	7 (0.3)	19 (0.3)
	Absolutely yes	34 (17.3)	2 (4.0)	7 (0.2)		Absolutely yes	354 (14.1)	2 (0.1)	3 (0.1)
Age *(M (SD))*		16.3 (0.9)	16.0 (1.0)	15.7 (1.1)	Age *(Mean (SD))*		36.7 (13.7)	46.2 (12.5)	33.3 (13.6)
Sex	Male	68 (34.7)	23 (46.0)	1164 (40.6)	Sex	Male	1153 (45.9)	1042 (44.8)	2088 (37.1)
(n (%))	Female	128 (65.3)	27 (54.0)	1704 (59.4)	*(n (%))*	Female	1359 (54.1)	1282 (55.2)	3541 (62.9)
Educational achievement	Low	85 (51.5)	24 (70.6)	800 (31.2)	Educational achievement	Low	812 (34.0)	836 (36.9)	977 (18.5)
(n (%))	Intermediate	47 (28.5)	5 (14.7)	728 (28.4)	*(n (%))*	Intermediate	854 (35.7)	676 (29.8)	1824 (34.5)
	High	33 (20.0)	5 (14.7)	1037 (40.4)		High	724 (30.3)	753 (33.2)	2490 (47.1)
Health	Poor	1 (0.5)	0 (0)	2 (0.1)	Health	Poor	10 (0.4)	12 (0.5)	16 (0.3)
(n (%))	Fair	2 (1.0)	2 (4.2)	27 (0.9)	*(n (%))*	Fair	60 (2.4)	63 (2.8)	88 (1.6)
	Reasonable	34 (17.5)	4 (8.3)	187 (6.6)		Reasonable	342 (13.8)	318 (14.0)	457 (8.2)
	Good	112 (57.7)	30 (62.5)	1505 (52.9)		Good	1583 (63.8)	1374 (60.3)	3331 (59.7)
	Excellent	45 (23.2)	12 (25.0)	1122 (39.5)		Excellent	485 (19.6)	511 (22.4)	1691 (30.3)
Number of cigarettes per day *(n (%))*	Less than one a day	25 (13.8)	5 (12.2)	-	Number of cigarettes per day	Less than one a day	158 (6.3)	15 (0.7)	-
	1-5 a day	57 (31.5)	21 (51.2)	-	*(n (%))*	1-5 a day	424 (16.9)	476 (21.1)	-
	6-10 a day	59 (32.6)	7 (17.1)	-		6-10 a day	618 (24.7)	556 (24.6)	-
	11-20 a day	36 (19.9)	8 (19.5)	-		11-20 a day	982 (39.2)	801 (35.5)	-
	21-30 a day	4 (2.2)	0 (0)	-		21-30 a day	282 (11.3)	320 (14.2)	-
	More than 30 a day	0 (0)	0 (0)	-		More than 30 a day	39 (1.6)	90 (4.0)	-
Age at first cigarette	Younger than 12 years	13 (7.1)	6 (12.5)	-	Age at first cigarette	Younger than 12 years	87 (5.4)	65 (5.5)	-
(n (%))	12-13 years	76 (41.8)	29 (60.4)	-	*(n (%))*	12-13 years	330 (20.5)	271 (22.8)	-
	14-15 years	79 (43.4)	12 (25.0)	-		14-15 years	569 (35.3)	384 (32.2)	-
	16-17 years	13 (7.1)	1 (2.1)	-		16-17 years	387 (24.0)	280 (23.5)	-
	Older than 17 years	1 (0.5)	0 (0)	-		Older than 17 years	240 (14.9)	190 (16.0)	-
Smoking frequency	Once a week or less	22 (12.1)	-	-	FTND score *(M (SD))*		2.7 (2.3)	2.5 (2.4)	-
(n (%))	Few times a week	30 (16.5)	-	-	Number of years smoked *(M (SD))*		17.8 (12.7)	15.0 (10.2)	-
	Once a day or more	130 (71.4)	-	-	Number of times tried quitting *(M [SD])*		1.8 (1.9)	2.1 (2.5)	-

Age was a continuous variable measured in years; sex was measured as 1 (male) and 2 (female); educational achievement was a continuous variable with categories: primary school/lower vocational schooling (low), intermediate vocational/upper secondary school (intermediate) and upper vocational/university (high); health/age at first cigarette/number of times tried quitting/number of years smoked/smoking frequency and number of cigarettes per day were continuous variables; the Fagerström Test for Nicotine Dependence (FTND) was a continuous variable measuring severity of nicotine addiction on a scale of 0 to 10 (number of cigarettes per day and FTND in former smokers concerned the period they smoked the heaviest).

Results

Subject Characteristics

Table 1 summarizes the distribution of smoking expectancy across smoking status at baseline in adolescents (p <0.001) and adults (p <0.001). Most adolescent smokers expected to continue smoking or did not know, while former smokers scored lower on smoking expectancy (58% answered 'Certainly not' or 'Probably not'). Although the large majority of never-smokers expected that they will 'Certainly not' smoke a year from now, more than 6% of never-smoking adolescents say 'I don't know', 'Probably', or 'Absolutely yes'. In adults, smoking expectancy was similarly distributed with a more pronounced difference between former smokers and smokers. The majority of former smoking adults (78.1%) stated that they will certainly not smoke a year from now. Though percentages are lower compared with adolescents, there are some never-smoking adults (2.1%) who expected to start smoking or state they do not know.

In adolescents and adults, never-smokers more often attained a higher education than (former) smokers (p <0.001). Subjective health differed significantly across smoking status in adults and adolescents, with never-smokers reporting an 'excellent' health more often than former and current smokers (p <0.001). In adults, the ratio of males vs. females differed across smoking status (p <0.001), while in adolescents, there was no significant difference (p = 0.169).

Transitions in Smoking Behaviour Over Time

Adolescents and adults who were smokers at baseline mostly remained smokers at follow up (Figure 1). As might be expected, there was a difference between adult and adolescent never-smokers, with adolescent never-smokers starting smoking at T2 more often than adult never-smokers (8% vs. 3%, respectively). In both adolescents and adults, a small proportion of never-smokers became former smokers at T2, implying they started smoking and stopped in the approximately two years in between baseline and follow up. In former smoking adolescents at T1, a high percentage started smoking again at T2 (70%), while for adults, the percentage of people who relapsed was considerably lower (14%).

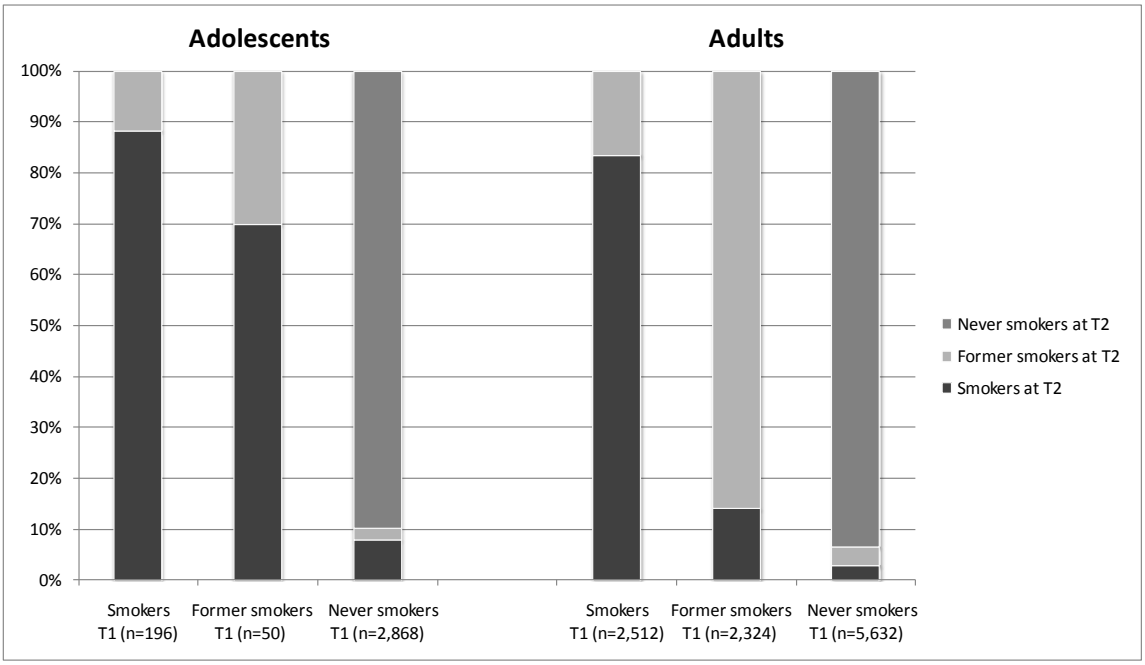

Figure 1. Transitions in smoking status from baseline (time point 1 [T1]) to follow up (time point 2 [T2]), depicted for adolescents and adults separately. There is approximately two years in between T1 and T2.

Predictive Value of Smoking Expectancy

Smoking expectancy was a strong predictor of future smoking status in univariate analyses of all groups (see Table 2). Odds ratios (*ORs*) represent the odds of smoking at T2 (as opposed to not smoking) for an individual who responds one answer category higher than another individual on the scale of smoking expectancy (at T1). Overall, associations were strongest for former smokers (*OR* 3.02 [confidence interval, *CI* = 1.37 to 6.68] in adolescents and 3.01 [*CI* = 2.51 to 3.62] in adults) and never-smokers (*OR* 3.39 [*CI* = 2.88 to 3.98] in adolescents and 4.93 [*CI* = 4.06 to 6.01] in adults). When correcting for the impact of age, sex, education, health, and smoking behaviour at T1, smoking expectancy remained a significant predictor of future smoking status in never-smokers and former smokers, but not in smokers (*OR* 1.46 [*CI* = 0.74 to 2.85] in adolescents and 1.04 [*CI* = 0.82 to 1.32] in adults). The group of adolescent former smokers was too small to analyze with a residual model. Other than that, results for adolescents and adults were similar.

Table 2. Results of logistic regression analysis with smoking expectancy at baseline (time point 1 [T1]) as the independent variable and smoking status approximately two years later (time point 2 [T2]) as the dependent variable, for adolescent and adult smokers, former smokers and never smokers

Adolescents (n=3,114)	**Smokers (n=196)**			**Former smokers (n=50)**			**Never smokers (n=2,868)**		
	N	OR *(95% CI's)*	*Pseudo R²*	*N*	OR *(95% CI's)*	*Pseudo R²*	*N*	OR *(95% CI's)*	*Pseudo R²*
Univariate model	196	**2.12** (1.18 to 3.79)	0.09	50	**3.02** (1.37 to 6.68)	0.18	2,868	**3.39** (2.88 to 3.98)	0.12
Residual model	141	1.46 (0.74 to 2.85)	0.02	-	Not applicable	-	2,383	**1.83** (1.56 to 2.15)	0.06
Adults (n=10,468)	**Smokers (n=2,512)**			**Former smokers (n=2,324)**			**Never smokers (n=5,632)**		
	N	OR *(95% CI's)*	*Pseudo R²*	*N*	OR *(95% CI's)*	*Pseudo R²*	*N*	OR *(95% CI's)*	*Pseudo R²*
Univariate model	2,512	**1.58** (1.41 to 1.76)	0.03	2,324	**3.01** (2.51 to 3.62)	0.08	5,632	**4.93** (4.06 to 6.01)	0.12
Residual model	591	1.04 (0.82 to 1.32)	0.00	459	**2.03** (1.29 to 3.19)	0.03	4,844	**3.44** (2.56 to 4.61)	0.05

CI = confidence interval; OR = Odds Ratio. OR represents the odds of smoking at T2 (as opposed to not smoking) for an individual who responds one answer category higher than another individual on the scale of smoking expectancy at T1 (categories 'certainly not', 'probably not', 'I don't know', 'probably', 'absolutely yes'); Pseudo R^2 = explained variance; not applicable: too little cases left for analysis; univariate model: smoking expectancy as the independent variable; residual model: smoking expectancy corrected for all predictors of smoking behaviour at T1 as the independent variable; significant OR's are depicted in bold.

Genetic Modeling

Prevalence

Prevalences for the ability to predict future smoking status were significantly different for ever-smokers than never-smokers in both adolescents and adults (see Table 3; model 2). The proportion of adolescents accurately estimating their future smoking status did not differ significantly between boys and girls (model 3). In adolescents, 89% of never-smokers accurately predicted their future smoking status in comparison with 78% of ever-smokers. In adults, 91% of never-smoking men and 94% of never-smoking women predict future smoking status accurately, in comparison with 79% of ever-smoking men and 77% of ever-smoking women.

Twin Correlations

There were no differences in twin resemblance between men and women (model 4), but twin correlations were higher for MZ (0.58 [*CI* = 0.40 to 0.73] for adolescents and 0.27 [*CI* = 0.09 to 0.42] for adults) than for DZ pairs (0.17 [*CI* = −0.03 to 0.35] for adolescents and 0.09 [*CI* = −0.07 to 0.24] for adults).

Heritability

The pattern of MZ and DZ correlations suggests non-additive genetic influences, but formal testing indicated that a model including only additive genetic effects was sufficient to explain familial resemblance (model 6). The heritability in adolescents was estimated at 0.59 (*CI* = 0.41 to 0.74) with the remaining variance explained by unique environmental influences (0.41 [*CI* = 0.26 to 0.59]). For adults, the heritability was lower with a point estimate of 0.27 (*CI* = 0.11 to

0.42) with the largest part of the variance (0.73 [*CI* = 0.58 to 0.89]) explained by unique environmental influences.

Table 3. Structural equation models to explore genetic and environmental influences on the ability to accurately predict future smoking status

Adolescents (n=1,881)	**Estimated parameters**	**-2LL**	**df**	**Compared to**	**X^2**	**P-value**
1. Saturated 5 group model	9	2088.83	2978	-	-	-
2. βs covariate set on 0	7	2110.59	2980	1	21.77	<0.001
3. Thresholds and β's Male=Female	7	2090.99	2980	1	2.17	0.34
4. Correlation MZM=MZF + Correlation DZM=DZF=DOS	**4**	**2098.35**	**2983**	**3**	**7.36**	**0.06**
5. ADE model	5	2098.35	2983	1	29.87	0.23
6. AE model	**4**	**2098.42**	**2984**	**5**	**0.08**	**0.78**
7. E model	3	2131.1	2985	6	32.68	<0.001
Adults (n=3,000)	**Estimated parameters**	**-2LL**	**df**	**Compared to**	**X^2**	**P-value**
1. Saturated 5 group model	9	3370.06	4902	-	-	-
2. βs covariate set on 0	7	3621.51	4904	1	251.45	<0.001
3. Thresholds and β's Male=Female	7	3383.23	4904	1	13.18	<0.001
4. Correlation MZM=MZF + Correlation DZM=DZF=DOS	**6**	**3377.43**	**4905**	**1**	**7.38**	**0.06**
5. ADE model	7	3377.43	4905	1	33.61	0.07
6. AE model	**6**	**3377.49**	**4906**	**5**	**0.05**	**0.82**
7. E model	5	3387.6	4907	6	10.11	<0.001

DOS = dizygotic opposite sex; DZF = dizygotic female; DZM = dizygotic male; MZF = monozygotic female; MZM = monozygotic male; LL = log-likelihood. Best fitting models are depicted in bold; threshold: the value which forms two distinct categories in the underlying liability which stand for the proportions of individuals who accurately predicted their future smoking status and the individuals who did not; β: the effect of the covariate smoking initiation (0='never smoked', 1='ever smoked') on the threshold. ADE model: additive genetic (A), dominance (D) and unique environmental effects (E) are estimated, AE model: only A and E are estimated, E model: only E is estimated.

Discussion

Smoking expectancy significantly predicted future smoking status over and above commonly used predictors of smoking in former and never-smokers, but not in current smokers. The ability to accurately predict future smoking status was influenced by genetic factors, more so in adolescents than in adults. In never-smokers, a higher score on smoking expectancy was associated with a higher chance of initiating smoking, both in adolescents and adults. It is the first time that this association has been demonstrated in adults. Similar results have only been reported in never-smoking adolescents, where smoking susceptibility (not being able to rule out the possibility of smoking next year) was a predictor of future smoking status (79). Measuring smoking expectancy is particularly valuable in efforts to prevent smoking initiation in adolescents, as they are most vulnerable to starting smoking (65, 66). Not many adult never-smokers started smoking, but we still observed that smoking expectancy was an accurate predictor of future smoking behaviour.

In both adolescents and adult smokers, a higher smoking expectancy was associated with a higher chance of remaining a smoker two years later. When taking demographic variables and variables related to smoking into account, associations were still significant in never-smokers and former smokers but not in smokers. This could be due to a relatively small sample size,

caused by the fact that some covariate data were only available in a subsample. Previous studies in smokers showed that the intention to quit smoking predicts making a quit attempt (68-70). A 'Motivation To Stop Scale' consisting of one item with seven response categories was able to accurately predict future quitting attempts (85). Our measure of smoking expectancy may reflect not only a persons' willingness to quit smoking but also their estimation of whether or not they will succeed. The importance of self-efficacy in successfully quitting smoking has been shown in several papers (70, 86, 87).

In former smokers, a higher smoking expectancy resulted in a significantly higher chance of relapse two years later. This result corresponds to findings from the International Tobacco Control survey, which showed that a lower abstinence self-efficacy (measured by asking 'How sure are you that you can stay quit?') was associated with a higher risk of relapse (72). Smoking expectancy can be an additional, relatively easy tool to predict which former smokers will start smoking again. Knowledge on who is most vulnerable to relapse is crucial in developing intervention programs because many people attempt to quit smoking, but a lot of them will fail in remaining abstinent (88, 89).

When analyzing data from smokers, former smokers, and never-smokers simultaneously and correcting for current smoking status (data not shown), smoking expectancy remained a significant positive predictor for future smoking status in both adolescents (*OR* 2.83, $p < 0.001$, $n = 3{,}114$) and adults (*OR* 1.63, $p < 0.001$, $n = 10{,}468$). This emphasizes the unique predictive effect of smoking expectancy on future smoking status over and above the effect of current smoking status.

Being able to predict future smoking status is explained by genetic factors for 59% and 27% of the variance in adolescents and adults, respectively. Environmental factors explained the remaining portion of the variance. The heritability may be mediated by genetically influenced personality traits such as optimism or sensation seeking. Research in twins has shown that 36% of the variation in optimism can be explained by genetic effects (90) and that heritability estimates for sensation seeking range from 48% to 63% (91). Optimism may lead people to make a better prediction of their own ability to quit or refrain from smoking, while a high score on sensation seeking may make them more willing to seek out new experiences and change their behaviour. In older participants, previous (failed) quitting attempts may have given them more experience, explaining the larger influence of environmental factors. Failure to predict future smoking status in (former) smokers is probably also related to smoking dependence and the inability to quit, with the latter being explained by genetic factors for approximately 50% (29, 92). Low numbers for the responses 'Probably' and 'Absolutely yes' prevented us from to re-analyzing the twin data while assigning the response 'I don't know' to the 'No' category of the dichotomized version of smoking expectancy.

Heritability is a measure that estimates the contribution of genetic differences to observed differences within a group of individuals (93). As well as other behavioural traits, smoking behaviour is a complex trait that is influenced by numerous genes and environmental factors. Estimating the heritability of such traits can give new insights into the mechanisms behind the trait (94). This study shows that genetic factors play a considerable role in the ability to predict future smoking status, especially in younger people. Knowledge that individual differences in smoking trajectories have a heritable component justifies ongoing efforts into the tailoring of prevention strategies.

Heritability estimates were larger in adolescents than in adults ($p = 0.03$), while the heritability of substance use typically increases over age (95). Differences in heritability between age cohorts indicate gene by age (gene × age) interaction (96). The influence of environmental factors on the ability to predict future smoking status is larger in adults than in adolescents, lowering the relative contribution of A (heritability). Besides unique environmental factors, E comprises measurement error or 'noise'. Changes in social norms can affect the magnitude of genetic influences by maximizing noise (97-99). In adults, social norms on smoking might be more negative, thereby influencing them not to smoke. In adolescents on the other hand, peers stimulate the initiation of smoking (100). In both age cohorts, there was some suggestion that nonadditive genetic influences might play a role, but although statistical power was sufficient (101), these influences were not significant.

A limitation of this study is its reliance on self-reported smoking status. A recent review demonstrated that in 5%–9% of the cases, self-report did not detect someone as a smoker while biochemical validation did (102). To study the reliability of self-reported smoking status, we used a powerful alternative of a test–retest approach, by studying the similarity within MZ (genetically identical) twin pairs. For several traits, it has been shown that the difference between MZ twins was almost equal to that between two consecutive measurements of the same individual (103, 104), making the similarity within MZ twin pairs a suitable test of reliability. About 94% of adolescents and 78% of adult twin pairs were concordant for smoking status, implying self-reported smoking status is reliable. The reliability of self-report in adolescents was also shown in a study of 150 Finnish youngsters (mean age 15 years), where the sensitivity for detecting smokers was 81%–96% (comparing questionnaire data to biochemical measurements; (105)).

Another limitation is that covariate data were not available for the total sample, so the residual model was analyzed in a smaller subsample. Previous studies demonstrated that individuals with missing data (less cooperative subjects) tended to score slightly more unfavorable on lifestyle variables but differences were not significant (106, 107).

This is the first study examining longitudinal data on smoking expectancy in adolescents and adults across smoking status. A recently much debated topic is 'precision medicine', involving

tailoring of care/treatment to suit the different genetic backgrounds of patients (108). Smoking expectancy could provide another way of delivering a personalized approach, by effectively tailoring guidance, counseling, and possibly treatment. A big advantage of quizzing people on smoking expectancy is that it is based on a simple question, which can be employed in smokers, former smokers, and never-smokers.

Supplement

Methods

We identified participants with a baseline measurement (time point 1 [T1]) and a follow-up measurement (time point 2 [T2]) for smoking behaviour. Participants were included when they completed two consecutive surveys, approximately 2 years apart (see Figure S1). Some participants completed more than two consecutive surveys. In the adolescent group, when data were available at the ages 14, 16 and 18 years, the most recent data were used. This resulted in the information coming from participants who completed two surveys at 16 and 18 years in approximately 38% of the cases and from participants completing two surveys at 14 and 16 years for approximately 62%. When participants in the adult group completed two successive surveys more than once, data from the two most recent succeeding surveys were used. For approximately 63% of the adult group, information was obtained from the two most recent surveys, namely the 2002- and the 2004-survey. For the remaining 37%, information was obtained from two other successive surveys.

In the YNTR survey around age 18 and in the 2004 and 2009 ANTR surveys, no question on smoking expectancy was included. Therefore, these surveys were only used as follow-up to determine smoking status at T2.

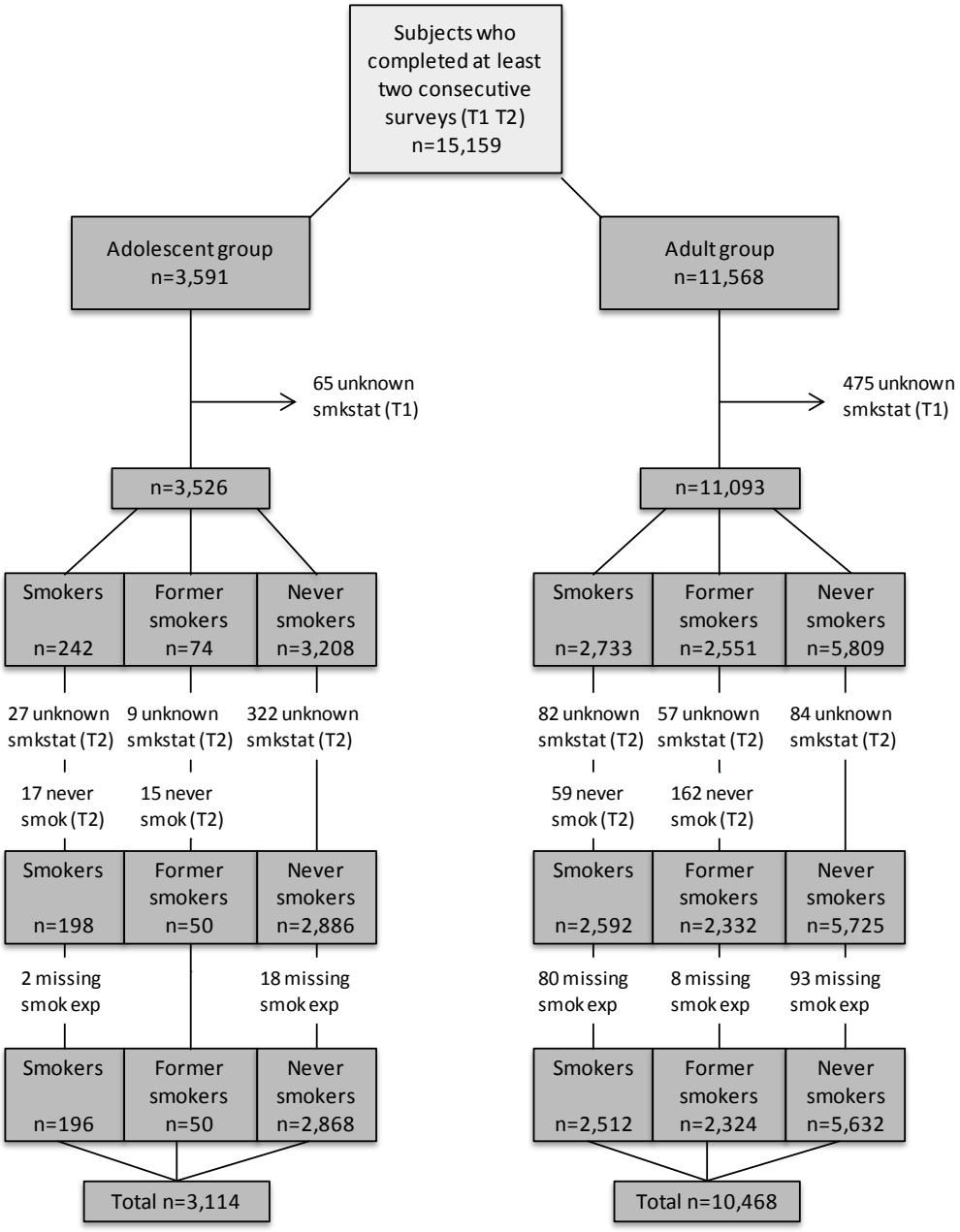

Figure S1. Flow chart of participants included in the final analyses per smoking status**.** T1: time point 1 (baseline), T2: time point 2 (follow-up), with approximately two years in between; smkstat = smoking status; never smok = never smokers, smok exp = smoking expectancy.

Table S1. Accuracy of predicting future smoking status, the light grey boxes show correct predictions and the darker gray boxes show incorrect predictions.

Adolescent group (n= 2,987)			Smoking status T2				
Smoking status T1	Smoking expectancy →	Dichotomized	Never smoker	Former smoker	Smoker	Total	% correct
Never smoker	Certainly not	*No*	2054	27	108	2189	92%
	Probably not		326	18	51	395	
	I don't know		91	15	52	158	
	Probably	*Yes*	2	1	8	11	47%
	Absolutely yes		1	0	6	7	
Former smoker	Certainly not	*No*	-	5	3	8	43%
	Probably not		-	4	9	13	
	I don't know		-	3	12	15	
	Probably	*Yes*	-	1	8	9	84%
	Absolutely yes		-	0	1	1	
Smoker	Certainly not	*No*	-	3	4	7	33%
	Probably not		-	9	20	29	
	I don't know		-	5	53	58	
	Probably	*Yes*	-	4	53	57	92%
	Absolutely yes		-	2	28	30	

Adult group (n= 4,911)			Smoking status T2				
Smoking status T1	Smoking expectancy →	Dichotomized	Never smoker	Former smoker	Smoker	Total	% correct
Never smoker	Certainly not	No	2733	102	0	2835	95%
	Probably not		161	64	0	225	
	I don't know		36	30	0	66	
	Probably	*Yes*	2	10	0	12	51%
	Absolutely yes		1	1	0	2	
Former smoker	Certainly not	*No*	-	365	55	420	82%
	Probably not		-	96	46	142	
	I don't know		-	21	25	46	
	Probably	*Yes*	-	2	2	4	54%
	Absolutely yes		-	0	0	0	
Smoker	Certainly not	*No*	-	12	22	34	29%
	Probably not		-	35	94	129	
	I don't know		-	61	257	318	
	Probably	*Yes*	-	81	428	509	84%
	Absolutely yes		-	15	154	169	

T1: time point 1 (baseline), T2: time point 2 (follow-up), % correct: the percentage of individuals who made a correct prediction depicted for the categories 'No' and 'Yes' separately (and for all the different smoking statuses

Chapter 4.

Spousal resemblance for smoking: Underlying mechanisms and effects of cohort and age.

This chapter is based on:

Treur JL, Vink JM, Boomsma DI and Middeldorp CM (2015). Spousal resemblance for smoking: Underlying mechanisms and effects of cohort and age. Drug and Alcohol Dependence, 153:221-22

Abstract

Background: In this study we ask why spouses resemble each other in smoking behaviour and assess if such resemblance depends on period of data collection or age. Spousal similarity may reflect different, not mutually exclusive, processes. These include phenotypic assortment (choice of spouse is based on phenotype) or social homogamy at the time spouses first meet, and marital interaction during the relationship. **Methods:** Ever and current smoking were assessed between 1991 and 2013 in surveys of the Netherlands Twin Register for 14,230 twins and 1,949 of their spouses (mean age 31.4 [*SD* = 14.0]), and 11,536 parents of twins (53.4 [*SD* = 8.6]). Phenotypic assortment and social homogamy were examined cross-sectionally by calculating the probability of agreement between twins and their spouses, twins and their co-twin's spouse and spouses of both twins as a function of zygosity. Marital interaction was tested by investigating the association between relationship duration and spousal resemblance. **Results:** Between 1991 and 2013 smoking declined in all age groups for both genders. Spousal resemblance for ever and current smoking was higher when data were more recent. For ever smoking, a higher age of men was associated with lower spousal resemblance. Phenotypic assortment was supported for both smoking measures, but social homogamy could not be excluded. No effect of marital interaction was found. **Conclusions:** Differences in smoking prevalence across time and age influence spousal similarity. Individuals more often choose a spouse with similar smoking behaviour (phenotypic assortment) causing higher genotypic similarity between them. Given the heritability of smoking this increases genetic risk of smoking in offspring.

Introduction

Many risk factors contribute to human smoking behaviour, including environmental (13) and genetic factors (80). From previous work in a Dutch sample, we know that spouses often show similar smoking behaviour (10). In fact, a systematic review of the literature on spousal resemblance for traits associated with coronary heart disease found that smoking was one of the most strongly correlated traits between spouses (57). High spousal correlations (0.19 to 0.55) were also reported by Kuo *et al.*. (2007) and by Clark and Etilé (2006), who showed that the chance of a smoker having a smoking partner is approximately 50%. Boomsma *et al.* (1994) found that the correlation between husband and wife for 'currently smoking' (r = 0.43) was larger than for 'ever smoked' (r = 0.18) (11, 109, 110)

There are different, not mutually exclusive, explanations as to how spousal resemblance arises. The three most frequently investigated mechanisms are phenotypic assortment, social homogamy and marital interaction. In case of primary, or *phenotypic assortment*, individuals tend to choose a spouse that is phenotypically similar (84). If a trait is heritable, phenotypic assortment is associated with a higher genotypic similarity between spouses, causing a greater phenotypic and genotypic similarity between them and their offspring (84, 111, 112). Alternatively, spouses may be more similar to each other due to *social homogamy* (113). Then, individuals are more likely to meet and pair up because they are from similar (social) surroundings. It can also be described as a 'passive' influence on mate selection, as opposed to the 'active' influence which occurs with phenotypic assortment (114). Under social homogamy, the genetic resemblance between parents and offspring or between siblings is not expected to increase (84). Lastly, spousal resemblance may be due to *marital interaction* reflecting that two individuals start to resemble each other because they influence each other while being in a relationship together. Here, a longer relationship is associated with more similar behaviour of spouses due to their interaction. Increasing similarity with marriage duration in cross-sectional data could also result from selection: those who are more similar to each other are more likely to remain together. Marital interaction does not have consequences regarding genetic similarity in the next generation.

As phenotypic assortment can be associated with a higher genotypic resemblance between spouses, spousal resemblance can influence the genetic resemblance between relatives. In addition, it shapes the environment to which the offspring of smoking parents is exposed. Data from twins and their spouses can inform on the underlying mechanisms of spousal resemblance (113, 115), but studies employing this design for smoking are scarce. In a Swedish sample of 507 twin pairs and 273 twin-spouse couples, Reynolds *et al*. (2006) found support for phenotypic assortment for quantity of tobacco, while social homogamy explained spousal resemblance for current tobacco use (yes/no) (116). Phenotypic assortment was demonstrated for ever regular smoking in 914 Australian twin-spouse couples (117), while evidence for both phenotypic assortment and social homogamy was found in a larger US-based study of 14,756 twins and 4390 spouses (118). After initial mate selection, a person's

smoking status was not influenced by their spouse (117), arguing against marital interaction for the initiation of smoking. These studies give an indication of the factors behind spousal resemblance for smoking, but only one addressed phenotypic assortment, social homogamy and marital interaction simultaneously (118).

Since smoking behaviour is often measured as a dichotomous variable (current smoking yes/no or ever smoking yes/no), resemblance between spouses will depend on smoking prevalence (84). In the Netherlands, smoking prevalence has dropped considerably in the past decades, partly due to nationwide (media) campaigns and tobacco control policies (119, 120). This decrease has been observed in countries worldwide (121, 122). Age is also associated with smoking behaviour, such that ever smoking initially increases with age (109) while older age groups show lower rates of current smoking (123). Age differences can be due to effects of age itself or differences in birth cohort. Trends across time and age and their effect on spousal similarity must be assessed when studying spousal resemblance for smoking.

The current study explores trends in ever and current smoking in a large sample of 27,715 Dutch twins, spouses of twins and parents of twins, and investigates spousal resemblance for ever and current smoking conditional on period of data collection (1991–1997, 2000–2004, 2009–2013) and age. Phenotypic assortment, social homogamy and marital interaction are investigated as causes of spousal resemblance.

Methods

Subjects

This study is part of ongoing longitudinal survey studies of the Netherlands Twin Register (NTR) (58). The NTR consists of adolescent and adult twins and their family members who have completed surveys since 1991. For the current study, cross-sectional data on smoking behaviour were available for 27,715 individuals (40.5% male, originating from 10,905 families), consisting of 14,230 twins and 1,949 spouses of twins (mean age 31.3 [*SD* = 13.9]) as well as 11,536 parents of twins (mean age of 53.4 [*SD* 8.6]). Figure 1 depicts a flowchart of all included subjects and corresponding analyses.

Data were retrieved from surveys completed in 1991, 1993, 1995, 1997, 2000, 2002, 2004, 2009, 2011 and 2013. Three research cohorts based on time of data collection were created: 1991–1997, 2000–2004 and 2009–2013. Surveys were sent at the family level to the 1991–1997 cohort, while participants were approached individually in the 2000–2004 and 2009–2013 cohorts. If smoking data were available from more than one survey, preference was given to the survey that was completed by most members of a family to increase the number of complete pairs of relatives available for analysis. Recently collected data were preferred over earlier data to ensure the inclusion of as many spouses of twins as possible. Spouses of twins were not invited to participate until the 2000-survey, with recruitment continuing till the year 2013. In total, 1,949 spouses were included for 14,553 twins (13.4%). This rather low

percentage of participating spouses is not due to twins being in a steady relationship less often. This was shown by a previous study comparing twins with siblings (124) and was confirmed by self-reported data on marital status in 9,247 twins indicating that the proportion of twins with a spouse was 61.7%. In the final data set, 16.1% of smoking data came from surveys sent in 1991 to 1997, 28.9% from 2000 to 2004 and 55.0% from 2009 to 2013.

Spousal pairs were excluded from analysis when the duration of relationship that spouses reported differed for more than 2 years between them, since this could indicate that the spouses are separated and report on the relationship duration with a new romantic partner (see Figure 1). Only parents of twins aged 17+ were invited to participate in NTR surveys. Parents who stated to be in a steady relationship for <17 years were excluded since these were presumably reporting on the relationship duration with a new romantic partner. The final number of pairs with complete data on smoking was 5,537 for twin pairs, 1,734 for twin-spouse pairs, 1,346 for pairs consisting of twins and their co-twin's spouse, 325 for pairs consisting of spouses of both twins and 3,725 for parents of twins (father–mother) pairs.

Measures

Participants were classified as current smokers, former smokers or never smokers, based on the questions 'Have you ever smoked?' ('No', 'A few times just to try', and 'Yes') and 'How often do you smoke now?' ('I don't smoke regularly', 'I've quit smoking', 'Once a week or less', 'A few times a week', and 'Once a day or more'). Those who said 'Yes' to the first question and subsequently stated to smoke once a week or more were classified as current smokers. Answering 'I've quit smoking' to the second question resulted in a classification as former smoker. In case of contradictory answers, or when answers to one of the two main questions were missing, additional questions were used to determine classification. Additional questions were, for example, 'How many cigarettes a day/a week do you smoke on average?' (for smokers; when a valid answer was given, current smoking was assumed) or 'At what age did you quit smoking' (when an age was given, former smoking was assumed). When participants said 'Yes' to the question 'Have you ever smoked?', but no additional information on their current smoking status was available, smoking status was coded as unknown. These individuals were not included in the analyses or the flowchart in Figure 1.

Two variables were created reflecting *ever* and *current* smoking. For ever smoking, all participants classified as never smokers were assigned the value '0', while all others (current and former smokers) were assigned the value '1'. For current smoking, both never and former smokers were assigned '0', and only current smokers were assigned '1'. Covariates were age (in years, continuous), gender (0 = male, 1 = female) and duration of relationship (in years, continuous). Duration of relationship was determined by asking participants 'For how long have you had a steady relationship with/have you been married to your spouse?'. Information on relationship duration was available for data collected between 2000 and 2013.

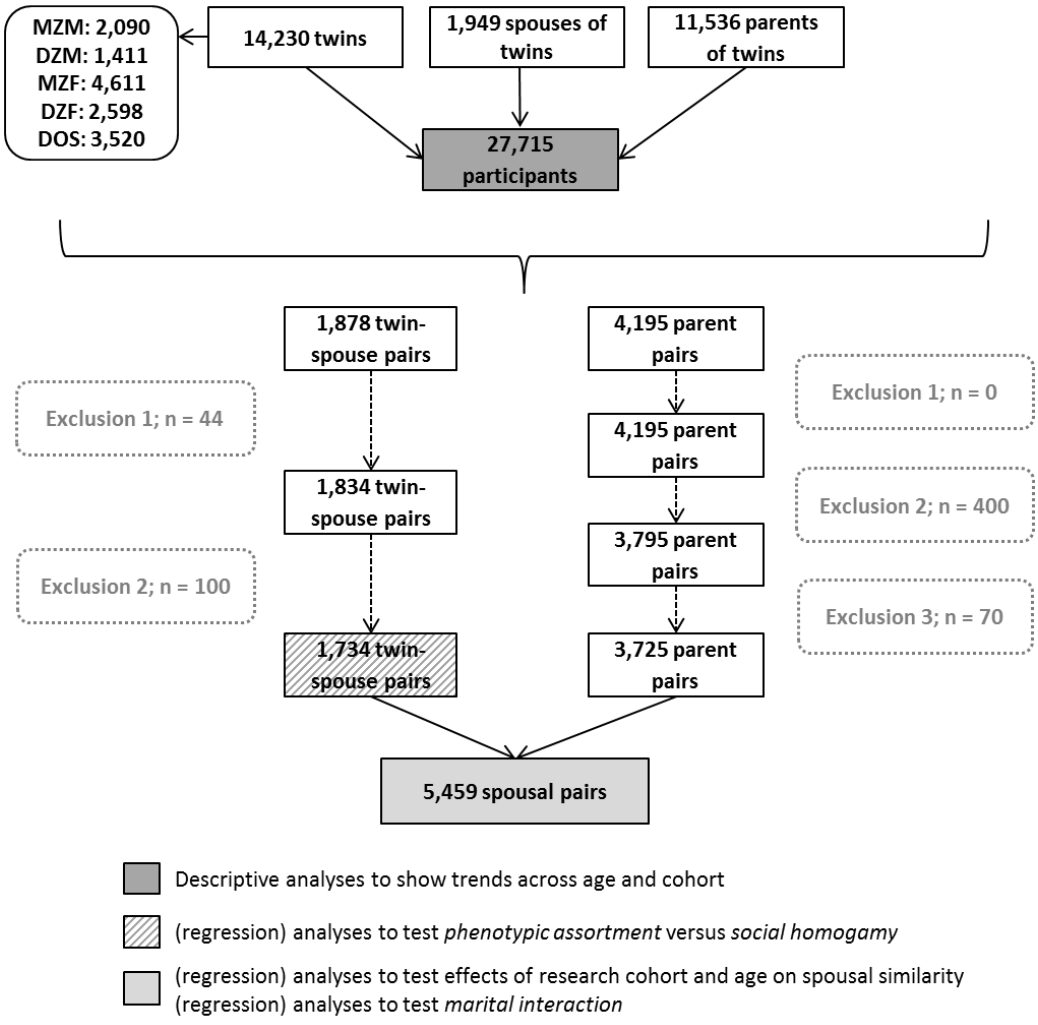

Figure 1. Flowchart of the groups of subjects and their corresponding analyses. MZM = monozygotic male twins; DZM = dizygotic male twins; MZF = monozygotic female twins; DZF = dizygotic female twins; DOS = dizygotic opposite sex twins; exclusion 1 = exclusion of same-sex spousal pairs; exclusion 2 = exclusion of pairs for which the answer regarding duration of relationship differed for more than 2 years between spouses; exclusion 3 = exclusion of parent pairs which said to have been in a steady relationship or marriage for less than 17 years (given that only parents of twins aged 17+ were invited to participate, these were presumably reporting on the relationship duration with a new romantic partner).

Statistical analyses

Data management and statistical analyses were conducted in SPSS (version 21). To describe age and cohort differences, ever and current smoking rates were stratified by research cohort (1991–1997, 2000–2004, 2009–2013), age in categories (18–24, 25–34, 35–44, 45–54, 55–64 and ≥65 years) and gender. The effects of research cohort and age on smoking rates were determined through logistic regression analysis with smoking as the outcome variable and both research cohort and age as the independent variables. For age, the resulting odds ratio (*OR*) reflected the odds of being a(*n*) ever/current smoker compared to a non-smoker at a particular age compared with being one year younger. For research cohort the *OR* reflected the odds of smoking for a particular research cohort compared with an older research cohort.

The probability of agreement (PA) between two individuals of a pair determined their resemblance and was obtained as follows: *n pairs in agreement/(n pairs in agreement + n pairs not in agreement)*. Pairs with both individuals having the same smoking status are in agreement while pairs who differ in their smoking status are not in agreement. Three sets of analyses were performed, which are described below.

- PA was calculated for all spousal pairs (twin-spouse pairs and parents of twins). To test whether research cohort or age affected spousal resemblance, a logistic regression analysis was then performed with the following regression formula:
  $Y = \beta_0 + \beta_1 x_{cohort} + \beta_2 x_{age\_person1} + \beta_3 x_{age\_person2} + \varepsilon$. *Y* represents the resemblance between spouses (either ever or current smoking; 0 = not in agreement 1 = in agreement), β_0 is the intercept (i.e. the value of *Y* when independent variables are 0), independent variable are x_{cohort} (regression coefficient for cohort; 1991–1997, 2000–2004 or 2009–2013, treated as a continuous variable), $x_{age\_person1}$(regression coefficient age person 1 in years [continuous]; person 1 is always the man), $x_{age\_person2}$ (regression coefficient age person 2 in years [continuous]; person 2 is always the woman), and ε is the error term.
- To explore phenotypic assortment and social homogamy as causes of spousal resemblance, PA was calculated between twins ($PA_{tw1–tw2}$), twins and their spouses ($PA_{tw–sp}$), twins and their co-twin's spouses ($PA_{cotw–sp}$) and spouses of both twins ($PA_{sp1–sp2}$). The pattern of resemblance between these different pairs of (extended) family members provides the information to determine the mechanism(s) of assortment (113, 125). If the following is found; $PA_{tw–sp} > PA_{cotw–sp} > PA_{sp1–sp2}$, phenotypic assortment (mate choice is based on phenotype), is likely to be the exclusive mechanism. If genetic influences on the phenotype are present, as in smoking, it is also expected that $PA_{cotw–sp}$ and $PA_{sp1–sp2}$ are higher in families of MZ (monozygotic) twins compared to families of DZ (dizygotic) twins. MZ twins are genetically (almost) 100% identical while DZ twins share only 50% of their segregating genes. Any predisposition for smoking and preference for a spouse with the same smoking status will thus be more similar in MZ vs. DZ twins. In contrast, when $PA_{tw–sp}$, $PA_{cotw–sp}$ and $PA_{sp1–sp2}$ are almost equal and there are no MZ-DZ differences, social

homogamy is more likely. It was tested in a logistic regression analysis whether zygosity had a significant effect on the PA, while correcting for differences due to research cohort and age. These analyses included participants of the 2000–2004 and 2009–2013 research cohorts (data on spouses of twins were not available for the 1991–1997 cohort). The formula was as follows:
$Y = \beta_0 + \beta_1 x_{zygosity} + \beta_2 x_{cohort} + \beta_3 x_{age\_person1} + \beta_4 x_{age\_person2} + \varepsilon$, with Y representing the resemblance between two individuals (either ever or current smoking; 0 = not in agreement 1 = in agreement) and independent variables being $x_{zygosity}$ (regression coefficient for twin zygosity; 0 = MZ 1 = DZ), x_{cohort} (regression coefficient for cohort; 0 = 2000–2004 1 = 2009–2013), $x_{age\_person1}$ (regression coefficient age person 1 in years [continuous]; person 1 is twin1 in twin1–twin2 pairs, twin in twin-spouse pairs, co-twin in co-twin-spouse pairs and spouse1 in spouse1–spouse2 pairs) and $x_{age\_person2}$ (regression coefficient age person 2 in years [continuous]; person 2 is twin2 in twin1–twin2 pairs, spouse in twin-spouse pairs, spouse in co-twin-spouse pairs and spouse2 in spouse1–spouse2 pairs).

- In the case of marital interaction, spouses who have been together for longer will be more similar. A logistic regression analysis similar to those described above was carried out to test whether duration of relationship has a significant effect on spousal resemblance: $Y = \beta_0 + \beta_1 x_{duration} + \beta_2 x_{cohort} + \beta_3 x_{age\_person1} + \beta_4 x_{age\_person2} + \varepsilon$. Independent variables were $x_{duration}$ (regression coefficient for duration of relationship in years [continuous]), x_{cohort} (regression coefficient for cohort; 0 = 2000–2004 1 = 2009–2013), $x_{age\_person1}$ and $x_{age\_person2}$.

The likelihood that spouses have the same smoking status (PA) depends heavily on how many individuals smoke. When smoking prevalence is for example very low, many spousal pairs will consist of two non-smokers, resulting in high spousal resemblance. To address this issue, Cohen's kappa (*k*) was reported alongside all probabilities of agreement. *k* is also a measure of similarity, but takes into account agreement occurring by chance (the proportion of spousal pairs in agreement when mating occurs at random). The first step in calculating *k* is to subtract all agreement arising by chance from the PA. After it is 'normed', *k* equals 1 if there is full agreement and 0 when there is no agreement at all above that expected by chance (126). For all statistical tests, a *p*-value of <0.05 was considered statistically significant.

Results

Trends in ever and current smoking

In the total sample of 27,715 subjects, the prevalence of ever smoking was 47.1% while for current smoking it was 21.3%. Table 1 gives ever and current smoking rates for different age and gender groups, stratified by research cohort. Additional smoking characteristics such as age at first cigarette and total number of years smoked are shown in Table S1. Men smoked more often than women, but these differences were not large in most groups. A decrease in both ever and current smoking was seen over time, with the lowest rates for the most recently

collected data (2009–2013). This was confirmed with regression analyses including both research cohort and age as independent variables. An increasing age was associated with a higher prevalence of ever smoking and a lower prevalence of current smoking, but effect sizes were small (see last two columns of Table 1).

Table 1. Rates of ever smoking and current smoking, stratified by research cohort (1991-1997, 2000-2004 or 2009-2013), age (18-24, 25-34, 35-44, 45-54, 55-64 or >=65 years when completing the survey) and gender.

	1991-1997		**2000-2004**		**2009-2013**		**Total**		**Effect research cohort**	**Effect age**
	% smk	*N*	*% smk*	*N*	*% smk*	*N*	*% smk*	*N*	*OR (95% CI)*	*OR (95% CI)*
Ever smoking										
Men										
18-24 years	44.8	764	38.1	488	23.4	1498	32.0	2750	0.59 (0.56-0.62)	1.045 (1.043-1.048)
25-34 years	50.7	67	42.6	1321	36.1	368	41.6	1756		
35-44 years	75.9	348	39.0	354	37.3	585	48.2	1287		
45-54 years	78.4	811	65.9	428	50.5	1446	61.4	2685		
55-64 years	81.1	169	68.9	499	69.6	1022	70.5	1690		
>=65 years	90.0	30	70.2	198	72.6	817	72.6	1045		
Total	65.8	2189	50.2	3288	47.7	5736	52.0	11213		
Women										
18-24 years	37.7	697	34.9	855	21.5	2814	26.7	4366	0.73 (0.70-0.77)	1.029 (1.027-1.031)
25-34 years	46.0	63	35.5	1748	30.7	841	34.2	2652		
35-44 years	72.0	600	44.6	619	42.9	1162	50.7	2381		
45-54 years	64.4	779	61.9	730	56.6	2634	59.0	4143		
55-64 years	48.5	134	50.6	545	62.6	1164	58.1	1843		
>=65 years	n.a.	1	27.4	223	43.9	891	40.5	1115		
Total	56.8	2274	42.0	4720	41.8	9506	43.9	16500		
Current smoking										
Men										
18-24 years	38.0	764	29.5	488	19.0	1498	26.1	2750	0.56 (0.53-0.60)	0.990 (0.987-0.993)
25-34 years	44.8	67	29.6	1321	19.6	368	28.1	1756		
35-44 years	40.8	348	26.6	354	15.4	585	25.3	1287		
45-54 years	37.4	811	27.6	428	16.0	1446	24.3	2675		
55-64 years	35.5	169	21.2	499	13.9	1022	18.2	1690		
>=65 years	20.0	30	18.2	198	9.3	817	11.3	1045		
Total	38.0	2189	27.0	3288	15.6	5736	23.3	11213		
Women										
18-24 years	31.0	697	27.0	855	15.8	2814	20.4	4366	0.61 (0.58-0.64)	0.994 (0.992-0.997)
25-34 years	28.6	63	23.1	1748	12.2	841	19.8	2652		
35-44 years	40.0	600	21.2	619	16.8	1162	23.8	2381		
45-54 years	31.3	779	27.1	730	17.8	2634	22.0	4143		
55-64 years	26.9	134	18.5	545	15.7	1164	17.4	1843		
>=65 years	n.a.	1	8.1	223	7.1	891	7.3	1115		
Total	33.2	2274	22.9	4720	15.3	9506	20.0	16500		

% smk = percentage of ever or current smokers; n.a=not applicable, numbers were too low; OR = odds ratio representing the odds of being a(n) ever/current smoker compared to a non-smoker at a particular age compared with being one year younger, or the odds of being a(n) ever/current smoker compared to a non-smoker in a particular research cohort compared with an older research cohort; 95% CI = 95% confidence interval. Two individuals were excluded from this table due to missing information on gender.

Effect of research cohort and age on spousal resemblance

In all 5,459 spousal pairs, resemblance between spouses, reflected by the probability of agreement and by Cohen's kappa (*k*), was higher for current (79.7%, $k = 0.39$ $p < 0.001$) than for ever smoking (65.3%, $k = 0.30$ $p < 0.001$). Spousal resemblance differed across research cohort (1991–1997, 2000–2004 and 2009–2013). The odds of a couple being in agreement vs. not being in agreement for ever smoking was 1.13 (95% *CI* = 1.05 to 1.22) for a more recent compared to a less recent cohort. For current smoking this was 1.85 (*CI* = 1.70 to 2.02). The age of men showed a significant, negative association with spousal resemblance for ever smoking (*OR* 0.97 [*CI* = 0.96 to 0.99]), but not for current smoking (*OR* 0.99 [*CI* = 0.97 to 1.01]). The age of women was not significantly associated with spousal resemblance for either ever or current smoking. Duration of relationship and age difference within spousal pairs, stratified by research cohort, are given in Table S2.

Phenotypic assortment vs. social homogamy

The probability of agreement was calculated for pairs of family members to test phenotypic assortment vs. social homogamy as mechanisms explaining spousal resemblance for smoking (see Table 2). The probability of agreement and Cohen's kappa were higher in MZ families compared with DZ families in twin1–twin2, co-twin-spouse and spouse1–spouse2 pairs for both ever and current smoking. This finding, showing that twins and their co-twin's spouse and spouses of both twins are more similar in MZ twins compared to DZ twins, is supportive of phenotypic assortment (113, 125). In most zygosity groups, twin-spouse probability of agreement was higher than co-twin-spouse and spouse1–spouse2 probability of agreement, again implying phenotypic assortment. However, similarity within spouse1–spouse2 pairs was relatively high (in some cases equal to twin-spouse similarity), which could indicate an influence of social homogamy. Under social homogamy, one would expect an equal resemblance within twin-spouse, co-twin-spouse and spouse1–spouse2 pairs.

Marital interaction

The probability of agreement and Cohen's kappa were calculated for differing durations of relationship (≤9 years, 10–19 years, 20–29 years and ≥30 years) in Figure 2. When marital interaction is the reason that spouses are similar on smoking behaviour, a higher resemblance is expected for couples with a longer duration of relationship. Though the probability of agreement and Cohen's kappa decreased somewhat when comparing categories ≤9 years and 10–19 years to 20–29 years and ≥30 years, logistic regression analysis (corrected for age and research cohort) showed no significant effect of duration of relationship on spousal resemblance (*OR* 1.00 [*CI* = 0.99 to 1.02]). The same was true for current smoking, where logistic regression analysis found no significant effect of relationship duration on spousal resemblance either (*OR* 1.02 [*CI* = 0.99 to 1.04]).

Table 2. Probability of agreement for pairs of (extended) family members, stratified by twin zygosity

	MZM		MZF		DZM		DZF		DOS		Total		Effect of zygosity on PA
	PA	***k***	**PA**	***k***	**PA**	***k***	**PA**	***k***	**PA**	***k***	**PA**	***k***	***OR* (95% CI)**
Ever smoking													
Twin1-twin2	83.1%	0.63***	85.3%	0.67***	69.2%	0.37***	72.6%	0.41***	68.0%	0.33***	77.3%	0.51***	0.41 (0.35 to 0.46)
Pairs (*N*)	842		1943		530		1018		1204		5537		
Twin-spouse	79.1%	0.58***	77.4%	0.53***	76.7%	0.54***	73.9%	0.48***	77.9%	0.55***	77.1%	0.53***	0.86 (0.69 to 1.08)
Pairs (*N*)	273		671		146		287		357		1734		
Co-twin-spouse	67.2%	0.34***	70.0%	0.38***	59.2%	0.19*	60.6%	0.21**	63.5%	0.26***	66.0%	0.31***	0.69 (0.55 to 0.87)
Pairs (*N*)	217		573		98		231		227		1346		
Spouse1-spouse2	75.0%	0.48***	86.2%	0.69***	62.5%	0.25	75.0%	0.50**	62.5%	0.25	76.9%	0.52***	0.40 (0.23 to 0.68)
Pairs (*N*)	56		145		24		44		56		325		
Current smoking													
Twin1-twin2	85.6%	0.58***	86.7%	0.50***	73.0%	0.33***	77.2%	0.30***	69.9%	0.21***	79.8%	0.39***	0.43 (0.38 to 0.50)
Pairs (*N*)	842		1943		530		1018		1204		5537		
Twin-spouse	86.1%	0.60***	87.0%	0.52***	81.5%	0.47***	84.7%	0.50***	84.9%	0.59***	85.5%	0.54***	0.87 (0.66 to 1.14)
Pairs (*N*)	273		671		146		287		357		1734		
Co-twin-spouse	78.8%	0.37***	78.3%	0.18***	68.3%	0.08	69.3%	0.06	66.1%	0.05	74.1%	0.16***	0.61 (0.48 to 0.79)
Pairs (*N*)	217		573		98		231		227		1346		
Spouse1-spouse2	85.7%	0.51***	86.2%	0.37***	75.0%	0.24	72.7%	0.17	59.0%	-0.06	78.8%	0.26***	0.35 (0.20 to 0.62)
Pairs (*N*)	56		145		24		44		56		325		

MZM = monozygotic male twins; DZM = dizygotic male twins; MZF = monozygotic female twins; DZF = dizygotic female twins; DOS = dizygotic opposite sex twins; PA = probability of agreement; k = Cohen's kappa; OR = odds ratio representing the odds of a pair being in agreement on smoking compared to not being in agreement for families of DZ twins, compared to families of MZ twins, corrected for research cohort (0=2000-2004 1=2009-2013), age of person 1 in years (continuous) and age of person 2 in years (continuous); 95% CI = 95% confidence interval. The analyses included only participants of the 2000-2004 and 2009-2013 cohorts, because data on spouses of twins were not available for the 1991-1997 cohort. *p<0.05 **p<0.01 ***p<0.001

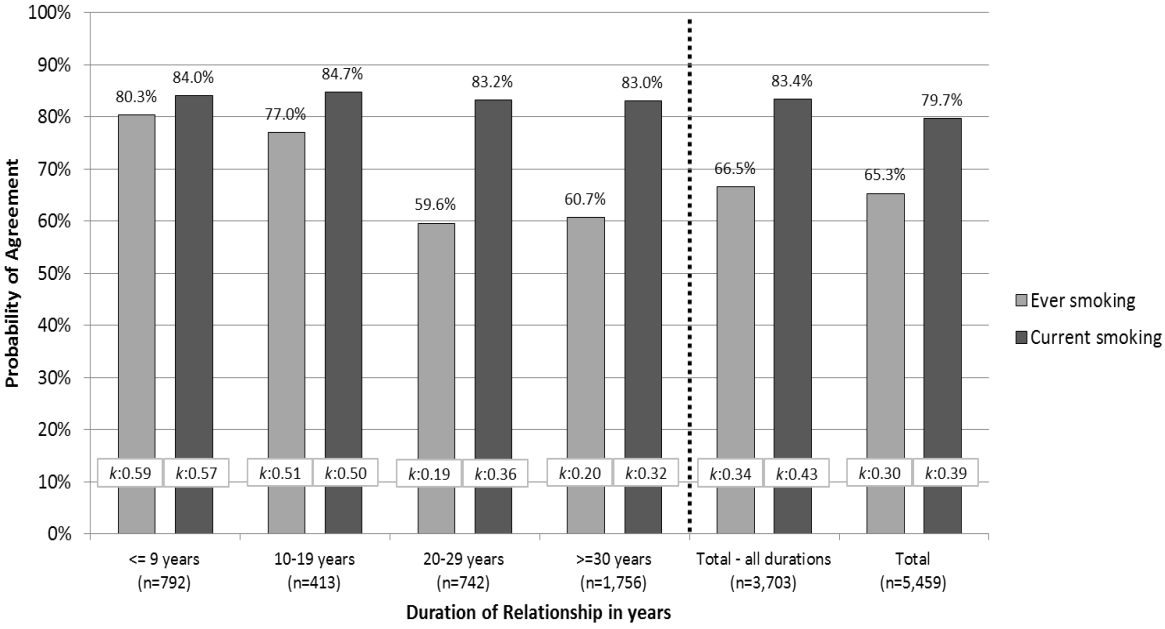

Figure 2. Similarity within spousal pairs, stratified by duration of relationship in years (<=9, 10-19, 20-29 or >=30 years). Probability of agreement = n pairs in agreement / (n pairs in agreement + n pairs not in agreement); *k* = Cohen's kappa; all *k* statistics were significant at p<0.001; in the group 'Total – all durations', only spousal pairs with information on duration of relationship were included while the group 'Total' contained all available spousal pairs; the spousal pairs comprised both twin-spouse and parent pairs; analyses with information on duration of relationship included only participants of the 2000-2004 and 2009-2013 cohorts, since this information was not available for the 1991-1997 cohort.

Discussion

In this large, population based, twin-family study, we studied spousal resemblance for smoking, investigated effects of research cohort and age on this resemblance, and explored the underlying mechanisms. For both measures of smoking (ever and current), spousal resemblance increased significantly in the more recent research cohorts. This increase in resemblance was mostly driven by an increase in the number of nonsmoking couples, while the number of couples in which both had ever smoked or currently smoked decreased across research cohort (data not shown). These findings are consistent with the decrease in both ever and current smoking rates in the more recent research cohorts. Cobb *et al.* (2014) reported similar results for current smoking, showing a sharp decrease in number of couples where both husband and wife were currently smoking over the course of the study (1986–1998) (127).

Spousal resemblance for ever smoking was lower when men were older, while the age of women did not matter. This difference between genders can be explained by the fact that the prevalence of ever smoking in men increased greatly over age, while in women this increase was more modest and followed by a decrease. Kuo *et al.* (2007) reported a similar finding, with higher spousal correlations for lifetime smoking in a younger group of twins and their spouses compared to their parents and grandparents, but it was not investigated if this effect was specific for the age of men only (109). A previous study by our own research group reported the risk of current smoking when having a smoking spouse to be higher compared with having a non-smoking spouse, and this risk decreased with age. There were gender differences too, with a somewhat stronger influence of men on their (female) spouse compared to the influence of women on their (male) spouse (10). Resemblance between spouses for smoking may be due to the fact that spouses are usually of a similar age, and age is strongly associated with smoking prevalence. The current study implies that not taking age and time period into account when measuring spousal resemblance for smoking or when making comparisons between different populations, could lead to incorrect conclusions.

Most of the evidence pointed to phenotypic assortment explaining spousal resemblance for smoking behaviour. The main indicator was the fact that co-twin-spouse pairs and spouse1–spouse2 pairs were significantly more similar in MZ families compared with DZ families. The underlying assumption being that the degree of social homogamy is similar in MZ compared with DZ twin families. For ever smoking, these findings comply to the conclusions of Agrawal *et al.* (2006) and those of an earlier study investigating the three main mechanisms of assortative mating simultaneously (117, 118). Our findings on current smoking do not corroborate with the only previous study employing twins and spouses to study spousal resemblance for current smoking (116), which reported that social homogamy was the most probable underlying mechanism. This discrepancy might have to do with the time of data collection. Reynolds *et al.* (2006) analyzed data from a Swedish sample collected in 1977 (116). Since then, major changes have taken place. Public opinion about smoking has changed, smoking rates have decreased and gender differences in smoking have all but disappeared (128). One could speculate that in the sample of Reynolds *et al.* (2006), individuals were not specifically rejected by (or attracted to) a person's smoking status because the social 'stigma' on smoking was not as large as it is today (128). Thus, people may have been less concerned about smoking behaviour when choosing a spouse. In such a situation, similarity in smoking status may well be a cause of social homogamy. It demonstrates how time of data collection can lead to different conclusions about the mechanism(s) through which spousal resemblance for smoking arises. Since the spouse1–spouse2 resemblance for ever and current smoking was relatively high, social homogamy could not be entirely excluded in the current study either. Etcheverry and Agnew (2009) conducted a prospective, multi-wave study in young adults and concluded that spousal similarity on smoking is due to the selection of a spouse more similar to oneself (129). As one of the very few studies employing twin-family data to

explore mechanisms of spousal resemblance for smoking, we have provided further support for phenotypic assortment.

Duration of relationship was not associated with spousal similarity for ever or current smoking, indicating that marital interaction is not of influence. This is in contrast to previous studies reporting that smoking behaviour of one's romantic partner significantly influences smoking initiation in adolescents aged 11–14 years (130) and young adult women (mean age of 26.8 [*SD* = 5.8]) (131). The specific samples of young participants might be the reason that we couldn't replicate these findings. In our sample, with a mean age of 48.1 [*SD* = 15.1] for women and 50.4 [15.1] for men, spousal influence on ever smoking is not very likely given that smoking is usually initiated in adolescence or early adulthood (13). Another study finding evidence for marital interaction (132), suggested that the influence between spouses is more prominent in the early phase/years of a relationship. The spousal couples in the current sample have a mean duration of relationship of 26.2 years (*SD* = 14.7) with only 21.3% reporting to be together ≤9 years and 5.1% ≤2 years, which could explain why we found no evidence for this process. Our findings are in agreement with other reports, showing that spouses do not become more similar for smoking across time (110, 117, 118, 133). In a smaller sample of NTR participants we previously found a decrease in spousal similarity for current smoking with relationship duration (134). This was not replicated in the present (larger) study, but both results suggest that marital interaction is not the main mechanism causing spousal resemblance for smoking.

There are some limitations to consider. Since mechanisms underlying spousal resemblance can differ across time, population and/or country, our findings may not be generalizable to all populations. The relatively small number of spouses of twins also made it difficult to disentangle effects of phenotypic assortment from those of social homogamy. Yet, we still found significant effects of zygosity on similarity in co-twin-spouse and spouse1–spouse2 pairs, pointing to phenotypic assortment. Sample sizes were too small to investigate the interaction between research cohort/age and the mechanisms underlying spousal resemblance (phenotypic assortment, social homogamy and marital interaction).

Phenotypic similarity caused by phenotypic assortment also reflects a higher genotypic similarity. It can therefore have important implications for smoking susceptibility in offspring. Children receive both their (family) environment and their genetic material from their parents. Under phenotypic assortment, offspring of two smoking parents are at an increased risk of smoking by receiving the 'risk' genes from both parents. Despite the genetic influences on smoking behaviour, measures taken by the government to discourage smoking have been highly effective in reducing smoking prevalence (119, 120). This makes sense considering that individual differences in both the initiation of smoking and nicotine dependence can be explained by genetic influences (respectively 44% and 75%) but also for a considerable part by environmental influences (remaining 56% and 25%) (26). Especially for smoking initiation,

there is a lot to be gained from preventive measures. These prevention programs have so far focused on the general (smoking) population. It might also be beneficial to develop prevention programs focusing on individuals who are the most susceptible. This has been shown to be more effective than applying the same programs to the general population (135). Given the importance of genetic factors, these prevention programs should select individuals that are at high genetic risk. Our findings suggest that high risk groups are best identified by selecting children from families where both parents smoke (or have smoked).

Spousal resemblance for both ever and current smoking was associated with research cohort (with a higher resemblance for more recent research cohorts), while only for ever smoking spousal resemblance was associated with the age of men (with a lower resemblance for a higher age). Spousal resemblance for smoking is most likely the result of phenotypic assortment, where spouses select each other directly on their phenotype, but a small influence of social homogamy could not be ruled out. Spousal resemblance was not associated with duration of relationship, arguing against marital interaction.

Supplement

Table S1. Smoking characteristics for ever smoking participants, stratified by research cohort (1991-1997, 2000-2004 or 2009-2013) and gender

		1991-1997		2000-2004		2009-2013	
Ever smokers		Male	Female	Male	Female	Male	Female
Age at first cigarette n (%)	<= 11 years	86 (7.9)	35 (3.8)	12 (4.8)	17 (5.6)	69 (4.4)	72 (2.9)
	12-13 years	273 (25.0)	154 (16.7)	54 (21.8)	61 (20.1)	290 (18.5)	460 (18.5)
	14-15 years	364 (33.4)	273 (29.6)	73 (29.4)	113 (37.3)	537 (34.3)	938 (37.8)
	16-17 years	233 (21.4)	264 (28.6)	66 (26.6)	59 (19.5)	442 (28.3)	627 (25.3)
	>=18 years	134 (12.3)	196 (21.3)	43 (17.3)	53 (17.5)	226 (14.5)	386 (15.5)
Age at start regular smoking n (%)	<= 11 years	8 (0.8)	4 (0.5)	0 (0.0)	2 (0.7)	13 (0.8)	3 (0.1)
	12-13 years	47 (4.5)	29 (3.4)	17 (7.2)	23 (7.9)	69 (4.5)	122 (5.0)
	14-15 years	268 (25.9)	142 (16.8)	39 (16.5)	58 (19.9)	314 (20.3)	495 (20.3)
	16-17 years	383 (37.0)	264 (31.2)	83 (35.0)	86 (29.6)	587 (37.9)	824 (33.8)
	>=18 years	328 (31.7)	408 (48.2)	98 (41.4)	122 (41.9)	566 (36.5)	995 (40.8)
Total # years smoked	*M (SD)*	7.9 (6.4)	9.2 (7.8)	17.6 (12.7)	14.9 (11.1)	20.2 (13.4)	16.8 (12.2)
	n	113	102	1606	1946	1536	2440
Total # quit attempts	*M (SD)*	-	-`	2.1 (2.0)	2.3 (2.2)	2.6 (6.1)	2.4 (5.3)
	n	-	-	245	344	1526	2416
Former smokers							
Years since quitting smoking	*M (SD)*	-	-	4.1 (8.4)	5.2 (7.8)	17.6 (11.4)	17.6 (11.1)
	n	-	-	60	80	973	1442

Not all variables were available across all surveys within the research cohorts; total quit attempts and years since quitting smoking were only included in surveys of the 2000-2004 and 2009-2013 cohorts.

Table S2. Relationship characteristics for all spousal pairs, stratified by research cohort (1991-1997, 2000-2004 or 2009-2013)

		1991-1997	2000-2004	2009-2013
Duration of relationship	*M (SD)*	-	18.8 (14.4)	33.1 (11.2)
	n	-	1798	1919
Age difference	*M (SD)*	2.9 (2.7)	3.0 (2.7)	3.0 (2.6)
	n	1260	1822	2391

Not all variables were available across all surveys within the research cohorts, duration of relationship was only included in surveys of the 2000-2004 and 2009-2013 cohorts.

Chapter 5.

Associations between smoking and caffeine consumption in two European cohorts.

This chapter is based on:

Treur JL, Taylor AE, Ware JJ, McMahon G, Hottenga JJ, Baselmans BML, Willemsen G, Boomsma DI, Munafò MR* and Vink JM*. Associations between smoking and caffeine consumption in two European cohorts. Addiction, in press.
*shared last authorship

Abstract

Aims: To estimate associations between smoking initiation, smoking persistence and smoking heaviness and caffeine consumption, in two population-based samples from the Netherlands and the United Kingdom. **Design:** Observational associations between self-reported smoking behaviour and caffeine consumption. **Setting:** Adults from the general population. **Participants:** Participants from the Netherlands Twin Register (NTR: *n* = 21,939, mean age 40.8 [*SD* = 16.9], 62.6% female) and the Avon Longitudinal Study of Parents and Children (ALSPAC: *n* = 9,086, mean age 33.2 [*SD* = 4.7], 100% female). **Measurements:** Smoking initiation (ever vs. never smoking), smoking persistence (current vs. former smoking), smoking heaviness and caffeine consumption through coffee, tea, cola and energy drinks. **Findings:** After correction for age, gender (NTR), education and social class (ALSPAC), smoking initiation was associated with consuming on average 52.8 (95% *CI* = 45.6 to 60.0;NTR) and 59.5 (51.8 to 67.2; ALSPAC) mg more caffeine per day. Smoking persistence was also associated with consuming more caffeine (+57.9 [45.2 to 70.5]) and +83.2 [70.2 to 96.3] mg, respectively). Each additional cigarette smoked per day was associated with 3.8 (2.0 to 5.6; NTR) and 8.6 (7.0 to 10.1; ALSPAC) mg higher daily caffeine consumption in current smokers. Smoking was positively associated with coffee consumption and less strongly with cola and energy drinks. For tea, associations were positive in ALSPAC and negative in NTR. **Conclusions:** There appears to be a positive association between smoking and caffeine consumption in the Netherlands and the United Kingdom.

Introduction

Cigarette smoking is associated with higher consumption of coffee (43). In a sample of individuals from the United States (US) who never drank coffee, 4.8% of males and 8.1% of females were smokers, compared with 34.7% and 48.1% respectively in those who drank 6 or more cups of coffee per day (41). A positive correlation (r=0.13) was also reported between number of cigarettes smoked and level of total caffeine consumption among British smokers (42). Less is known about the association between smoking and the consumption of caffeinated drinks other than coffee. Klesges *et al.* (1994) found no differences in smoking behaviour between (caffeinated) tea drinkers and non-drinkers in a US-based sample (136). In two studies linking adolescent consumption of energy drinks to health behaviours, regular consumption of energy drinks (≥1 week) was associated with a higher odds of smoking initiation (137) and frequency of energy drink use was positively correlated (r ~0.2) with past 30-day frequency of cigarette use (138). Terry-McElrath *et al.* (2014) also found positive correlations of 0.12–0.23 between soft drink consumption and cigarette use, but no distinction was made between cola (which contains caffeine) and other soft drinks (which generally don't contain caffeine) (138).

Preferences for type of caffeinated drink, in particular coffee vs. tea, vary between countries. Most of the available literature on the association between smoking and caffeine use is based on populations from the US. The current study includes two European cohorts of which one is Dutch and one is British. While tea is the dominant drink in the United Kingdom (UK) (139), the Dutch are reportedly among the world's heaviest coffee drinkers (140). Such cultural differences may have an influence on the association between smoking behaviour and caffeine consumption, but so far this has not been investigated. Smoking prevalence does not differ much between the two countries with 26% of Dutch men and 20% of Dutch women being smokers in 2013 (141), compared with 22% and 17% of British men and women respectively in that same year (142).

The popularity of caffeine as a psychoactive substance, and the high burden of morbidity and mortality due to smoking, make it important to better understand their relationship. In order to achieve this aim, it is necessary to first explore in depth the associations among different aspects of smoking behaviour and the use of different types of caffeinated drinks. This will help to determine the importance of the relationship between smoking and caffeine and guide the development of future intervention or preventive studies. In this study, associations between self-reported smoking behaviour and caffeine consumption are investigated in the Netherlands Twin Register (N=21,939, the Netherlands) and the Avon Longitudinal Study of Parents and Children (N=9,086, UK). Data from these two samples are analysed separately, so that any cultural differences in the association between smoking and caffeine can be distinguished. There were two main research questions: **1**. Is the association between smoking and caffeine consumption consistent across different types of caffeinated drinks or is it

specific to coffee? **2**. Is the association between smoking and caffeine consumption consistent across two European countries with different patterns of caffeine consumption?

Methods

Study sample

Participants of two large population-based studies were included; the Netherlands Twin Register (NTR) and the Avon Longitudinal Study of Parents and Children (ALSPAC).

The NTR is an ongoing longitudinal study of Dutch twins and their family members, which was established in 1987. Adolescent and young adult twins were initially recruited through city council offices across the Netherlands. Over time, recruitment of additional twins and family members of twins (among which parents, siblings and spouses) has continued through several approaches. A fuller description of this population based study and its design can be found elsewhere (58). A total of 17,998 adult participants who completed the 10th survey of the NTR in 2013-2014 were included in the present investigation. This survey contained, among others, questions on smoking and the consumption of an extensive list of caffeinated and decaffeinated drinks. An additional 2,896 individuals were selected who did not participate in the 10th survey but completed the 5th NTR survey in 2000, which included questions on coffee consumption. Data from 60 additional individuals were added because they participated in a brief online survey specifically focussed on coffee consumption, sent out in 2012. Adding these three samples resulted in a total group of 21,939 individuals (mean age 40.8 [*SD* = 16.9], 62.6% female).

ALSPAC is a prospective cohort study into which 14,541 pregnant women residing in the county of Avon in the UK with expected delivery dates ranging between 1 April 1991 and 31 December 1992 were recruited. Ethics approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees. Since recruitment, regular follow-ups have been conducted consisting of self-report surveys and clinic visits of the mothers, their children and their partners. An extensive description of this study and its methods is available elsewhere (143, 144). Surveys containing questions on smoking and caffeinated and decaffeinated coffee, tea and cola consumption were sent to the mothers during pregnancy at 18 weeks gestation, 32 weeks gestation, and after delivery when the child was 2 months old, 47 months old, 85 months old, 97 months old and 145 months old. Data at all time-points were analysed but only the results from the time that the child was 47 months are presented (N=9,086, mean age mothers 33.2 [*SD* = 4.7], 100% female). This specific time-point was selected because of its large sample size and because smoking behaviour and caffeine use may be different during and immediately after pregnancy. To check if temporary, pregnancy-related changes in smoking and caffeine use affected their association, data from all time-points were analysed (see online supplementary material). The study website contains details of all the data that is available through a fully searchable data dictionary (www.bris.ac.uk/alspac/researchers/data-access/data-dictionary).

Smoking behaviour

Participants were classified as current smokers, former smokers or never smokers. In the NTR sample, smoking status was based on two questions: 'Have you ever smoked?', with answer categories 'No', 'A few times just to try', and 'Yes', and 'How often do you smoke now?', with answer categories 'I don't smoke regularly', 'I've quit smoking', 'Once a week or less', 'A few times a week', and 'Once a day or more'. Those who said 'Yes' when asked the first question and stated they currently smoked once a week or more were classified as current smokers, while those who said 'I've quit smoking' to the second question were classified as former smoker. When answers to these two questions were contradictory or missing, additional questions such as 'How many years have you smoked?' or 'At what age did you quit smoking?' determined classification. Current smokers were asked how many cigarettes they smoke on average per day (for daily users), or per week (for weekly users). In the ALSPAC sample, smoking status was determined by the open ended question 'About how many cigarettes do you smoke each day?'. Participants were classified as smokers when they reported smoking one cigarette per day or more (there was no question on weekly use). An individual was identified as a former smoker when they had reported smoking at one of the previous time-points, but reported not smoking in the current survey. Participants who said they smoked zero cigarettes per day and hadn't reported previously that they were smoking were classified as never smokers.

Caffeine consumption

In both samples, questions were asked about caffeinated coffee, tea (including green tea in NTR) and cola, while in the NTR an additional question on energy drinks was included. NTR participants were asked whether they drank each of these drinks daily, weekly or (almost) never. The average number of drinks per day (for daily use) or per week (for weekly use) was also obtained. In the case of weekly use, the number of drinks was divided by seven to get an estimate of average daily use. ALSPAC participants were asked how many cups of coffee and tea they currently drank (open format) separately for weekdays and weekends. The replies to these questions were recoded into one measure of daily use. For cola, there was a closed format question on number of drinks per week ('never or rarely', 'once every 2 weeks', '1 to 3 times a week', '4 to 7 times a week', 'once a day or more'), which was recoded to respectively 0, 0.5, 2, 5.5 and 7 per week. For all questions it was made clear that participants should report on caffeinated drinks only. Caffeine use was computed by weighting the drinks by their caffeine content. Caffeine content (in mg per serving) was set at 75 mg per cup of regular coffee, 65 mg per cup of espresso (only in NTR), 40 mg per cup of regular tea, 20 mg per cup of green tea (only in NTR), 10 mg per 100 ml of cola (a serving was specified as one can of 330 ml in ALSPAC and as one glass of 180 ml in NTR) and 80 mg per can of energy drink (only in NTR) (145). For an estimate of total daily caffeine use, the daily caffeine intake of all drinks was summed.

Statistical analyses

All regression analyses were performed in Stata (version 9.0; StataCorp LP, College Station, Texas) and corrected for family clustering by utilizing the robust cluster option in NTR data. This function takes information on family relatedness and uses it to correct for the correlation within families (i.e., clusters). Linear regression analyses were performed with daily caffeine consumption (mg per day) as the dependent variable. The independent variable was either smoking initiation (0=never smoking, 1=ever smoking) or smoking persistence (0=former smoking, 1=current smoking). Associations between smoking behaviour and the consumption of decaffeinated coffee (dichotomized into 0=non-users, 1=users) were also tested.

The association between cigarettes smoked per day (independent variable) and daily caffeine consumption (dependent variable) was investigated using linear regression analyses. For these analyses, non-smokers and non-caffeine users (or non-users of a specific drink when analysed individually) were excluded. This was to test whether an increase in cigarettes smoked per day is associated with an increase in caffeine consumption in those who consumed at least some caffeine to start with.

All analyses described here were done for total caffeine use, individual caffeinated drinks and decaffeinated coffee, both unadjusted and adjusted for age, educational attainment (ALSPAC in five categories: Secondary Education [CSE], Vocational, O level, A level and Degree; NTR in seven categories: primary school only, lower vocational schooling, lower secondary schooling, intermediate vocational schooling, intermediate/higher secondary schooling, higher vocational schooling and university), social class (only in ALSPAC in 6 categories: class I, class II, class II [non-manual], class III [manual], class IV and class V) and gender (only in NTR).

Results

Descriptive statistics

Table 1 depicts smoking status and daily caffeine use for 21,939 male and female NTR participants and 9,086 female ALSPAC participants. There were more current smokers in the British sample (22.9%) compared with the Dutch sample (14.9% for women and 17.6% for men), and the number of cigarettes smoked per day was higher in the British women (mean 12.6) compared with the Dutch smokers (10.6 in women and 11.4 in men). Men were more likely to be coffee drinkers (81.9%) than women (67.0% in Dutch women and 59.0% in British women), while for tea the opposite was true (60.9% in Dutch men compared to 75.5% in Dutch women and 78.4% in British women). On average, the Dutch consumed more coffee per day (in the total group, 2.2 cups in women and 3.8 cups in men) than the British women (1.8 cups). For tea, a higher consumption was found in the British (mean of 3.0 cups) compared with the Dutch (mean of 2.1 cups for women and 1.3 for men). Daily or weekly cola use was more common in the British (51.4%) compared with the Dutch sample (26.1% for women and 36.7% for men) while energy drinks were rarely consumed on a daily or weekly basis (4.4% in

Dutch women and 5.9% in Dutch men). Any consumption of decaffeinated coffee ranged from 8.9% to 18.8%.

Strong associations were found between smoking/caffeine use and educational attainment, social class and age (see Table 2). In the Dutch sample, older participants were more likely to have initiated smoking, while their odds of smoking persistence (being a current smoker rather than a former smoker) were lower. In the British sample, older age was associated with lower odds of both smoking initiation and smoking persistence. Older participants consumed more total caffeine, coffee and tea, but they consumed less cola and energy drinks. Participants with a higher educational attainment or a higher social class were less likely to have initiated smoking and less likely to still be smoking when smoking was initiated (smoking persistence). Higher educational attainment and social class were also associated with a lower consumption of all caffeinated drinks, except for tea in the Dutch sample where a higher education was associated with a higher consumption. These variables (age, gender, educational attainment and social class) were included in all analyses as covariates.

Table 1. Descriptive statistics on smoking behaviour and caffeine use in the *Netherlands Twin Register (NTR)* and the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

			NTR		ALSPAC
			Men	*Women*	*Women*
Age		Mean years (*SD*)	42.3 (17.5)	39.9 (16.5)	33.2 (4.7)
Smoking status		Never smoker (n[%])	4,463 (54.5%)	8,579 (62.4%)	4,966 (54.7%)
		Former smoker (n[%])	2,289 (27.9%)	3,120 (22.7%)	2,035 (22.4%)
		Current smoker (n[%])	1,440 (17.6%)	2,048 (14.9%)	2,085 (22.9%)
Number of cigarettes per day in smokers		Mean (*SD*)	11.4 (7.6)	10.6 (7.2)	12.6 (6.9)
Any caffeine use		N(%)	7,541 (97.2%)	12,189 (95.9%)	8,339 (95.0%)
Daily caffeine use total	*in total group*	Mean mg (*SD*)	334.4 (216.9)	240.0 (179.7)	260.9 (170.9)
Daily caffeine use total	*in users*	Mean mg (*SD*)	346.1 (211.3)	251.7 (175.9)	274.6 (164.2)
Any coffee use		N(%)	6,622 (81.9%)	9,106 (67.0%)	5,318 (59.0%)
Daily coffee use	*in total group*	Mean mg (*SD*)	280.2 (222.7)	166.3 (174.2)	135.3 (164.9)
		Mean cups (*SD*)	3.8 (3.0)	2.2 (2.3)	1.8 (2.2)
	in users	Mean mg (*SD*)	342.0 (198.5)	248.3 (157.9)	229.2 (156.7)
		Mean cups (*SD*)	4.6 (2.7)	3.3 (2.1)	3.1 (2.1)
Any tea use		N(%)	3,946 (60.9%)	8,498 (75.5%)	7,044 (78.4%)
Daily tea use	*in total group*	Mean mg (*SD*)	46.2 (68.4)	65.9 (81.3)	121.5 (106.0)
		Mean cups (*SD*)	1.3 (1.9)	2.1 (2.5)	3.0 (2.7)
	in users	Mean mg (*SD*)	75.8 (73.7)	87.3 (83.0)	155.1 (95.5)
		Mean cups (*SD*)	2.2 (2.0)	2.8 (2.5)	3.9 (2.4)
Any cola use		N(%)	2,375 (36.7%)	2,933 (26.1%)	4,596 (51.4%)
Daily cola use	*in total group*	Mean mg (*SD*)	6.1 (14.4)	3.9 (11.8)	4.7 (7.6)
		Mean servings (*SD*)	0.3 (0.8)	0.2 (0.7)	0.1 (0.2)
	in users	Mean mg (*SD*)	16.6 (19.7)	14.9 (19.3)	9.1 (8.5)
		Mean servings (*SD*)	0.9 (1.1)	0.8 (1.1)	0.3 (0.3)
Any energy drink use		N(%)	379 (5.8%)	500 (4.4%)	-
Daily energy drink use	*in total group*	Mean mg (*SD*)	2.1 (12.7)	1.8 (14.3)	-
		Mean servings (*SD*)	0.03 (0.2)	0.02 (0.2)	-
	in users	Mean mg (*SD*)	35.4 (39.9)	41.1 (54.4)	-
		Mean servings (*SD*)	0.4 (0.5)	0.5 (0.7)	-
Any decaffeinated coffee use		N(%)	575 (8.9%)	1,225 (10.9%)	1,697 (18.8%)
Daily decaffeinated coffee use	*in total group*	Mean cups (*SD*)	0.2 (0.9)	0.2 (0.8)	0.5 (1.3)
	in users	Mean cups (*SD*)	2.3 (2.1)	2.0 (1.7)	2.5 (1.8)

Participants with missing gender (n = 7, only in NTR) were excluded from this table. Any use = weekly or daily use

Table 2. Associations between covariates and smoking initiation, smoking persistence and daily caffeine consumption (in mg) in *the Netherlands Twin Register (NTR)* and the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

		Smoking initiation – *OR* (95% C)		**Smoking persistence – *OR* (95% C)**		**Total caffeine – *B* (95% C)**		**Coffee – *B* (95% C)**		**Tea – *B* (95% C)**		**Cola – *B* (95% C)**		**Energy drink – *B* (95% C)**
NTR	*N*		*N*		*N*		*N*		*N*		*N*		*N*	
Age	*21,843*	1.04 (1.03 to 1.04)	*8,849*	0.94 (0.94 to 0.94)	*17,640*	4.6 (4.4 to 4.8)	*21,583*	4.0 (3.9 to 4.2)	*17,640*	0.4 (0.4 to 0.5)	*17,640*	-0.1 (-0.1 to -0.1)	*17,640*	-0.1 (-0.1 to -0.1)
Gender	*21,939*	0.72 (0.68 to 0.76)	*8,897*	1.04 (0.96 to 1.14)	*17,731*	-94.5 (-100.4 to -88.5)	*21,677*	-113.8 (-119.2 to -108.5)	*17,731*	19.7 (17.4 to 22.1)	*17,731*	-2.2 (-2.6 to -1.8)	*17,731*	-0.2 (-0.7 to 0.2)
Education	*14,816*	0.55 (0.51 to 0.59)	*6,752*	0.77 (0.69 to 0.85)	*11,851*	-14.6 (-21.6 to -7.6)	*14,632*	-28.1 (-34.7 to -21.5)	*11,851*	11.0 (8.1 to 13.9)	*11,851*	-0.2 (-0.6 to 0.3)	*11,851*	-0.4 (-0.7 to -0.1)
ALSPAC	*N*		*N*		*N*		*N*		*N*		*N*		*N*	
Age	*8,960*	0.96 (0.95 to 0.97)	*4,062*	0.91 (0.90 to 0.92)	*8,864*	1.0 (0.2 to 1.7)	*9,095*	0.4 (-0.3 to 1.1)	*9,080*	0.9 (0.4 to 1.3)	*9,026*	-0.3 (-0.4 to -0.3)	-	-
Education	*8,798*	0.58 (0.53 to 0.64)	*3,970*	0.45 (0.40 to 0.52)	*8,721*	-15.1 (-22.4 to -7.7)	*8,942*	-7.5 (-14.5 to -0.5)	*8,929*	-4.3 (-8.8 to 0.2)	*8,883*	-2.0 (-2.4 to -1.7)	-	-
Social class	*7,442*	0.77 (0.70 to 0.84)	*3,304*	0.61 (0.53 to 0.71)	*7,371*	-3.0 (-10.8 to 4.7)	*7,542*	-1.2 (-8.7 to 6.3)	*7,538*	-0.6 (-5.3 to 4.2)	*7,498*	-1.5 (-1.8 to -1.1)	-	-

Regression analyses were performed with smoking initiation, smoking persistence, total caffeine use, coffee, tea, cola or energy drink as the dependent variable and age (continuous), gender (0 = male 1 = female), education (0 = low 1 = high) or social class (only in ALSPAC: 0 = low 1 = high) as the independent variable. For NTR low education = primary school only, lower vocational schooling, lower secondary schooling, intermediate vocational schooling or intermediate/higher secondary schooling and high education = higher vocational schooling or university; for ALSPAC low education = Secondary Education (CSE), vocational or O level and high education = A level or Degree. Low social class = class II (non-manual), class III (manual), class IV or class V and high social class = class I or class II.

Smoking initiation and caffeine use

In both the Dutch and the British sample, ever smokers consumed more caffeine than never smokers (respectively +52.8 mg [95% *CI* = 45.6 to 60.0] and +59.5 mg [95% *CI* = 51.8 to 67.2], Figure 1, left hand side). The same pattern was seen when exploring coffee use only. While Dutch ever smokers consumed less tea compared with never smokers (-16.1 [95% *CI* = -19.1 to -13.1]), results were opposite in the British sample (+12.3 [95% *CI* = 7.5 to 17.1]). Ever smokers consumed slightly more cola and energy drinks compared with never smokers (ranging from +0.5 [95% *CI* = 0.2 to 0.9] to +1.2 [95% *CI* = 0.8 to 1.7]).

Smoking persistence and caffeine use

Current smokers consumed more coffee, cola and energy drinks than former smokers, resulting in a higher overall caffeine intake (Figure 1, right hand side). Again, contrasting results were found for the consumption of tea, with Dutch current smokers using less (-17.1 [95% *CI* = -21.5 to -12.7]), while British smokers used more tea compared to former smokers (+9.9 [95% *CI* = 1.9 to 17.9]).

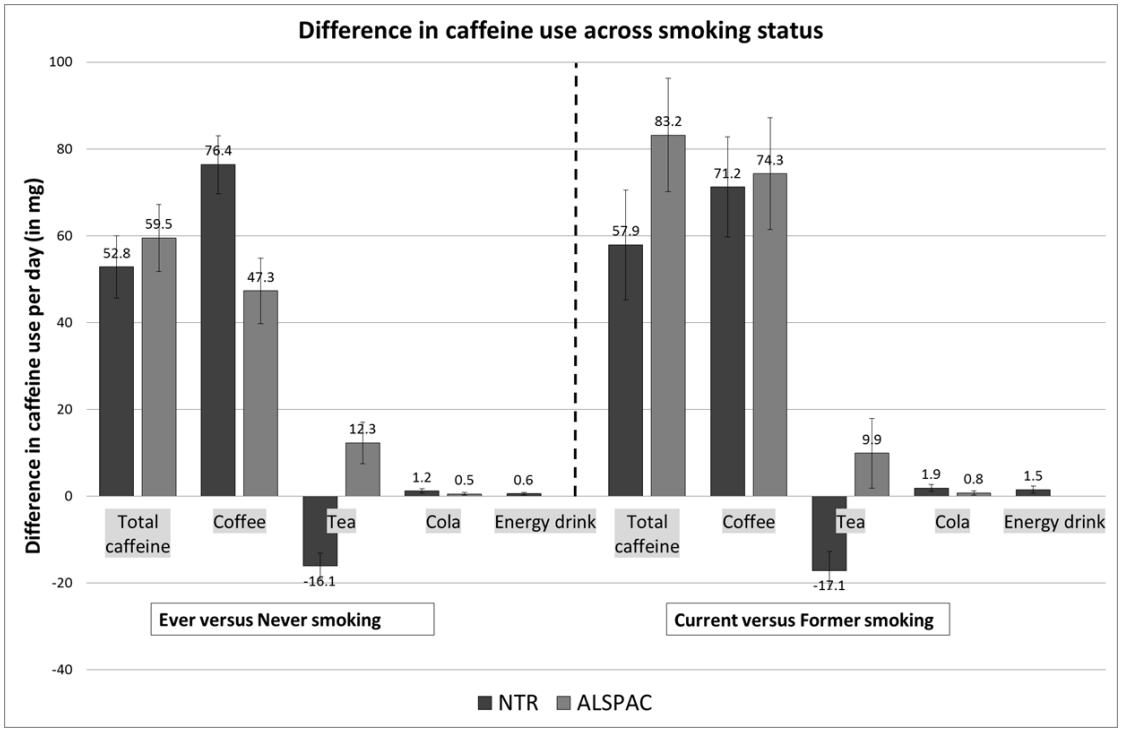

Figure 1. NTR = Netherlands Twin Register; ALSPAC = Avon Longitudinal Study of Parents and Children. For ever vs. never smoking the number of participants for each analysis in the NTR was 11,850 for total caffeine, 14,564 for coffee, 11,805 for tea, 11,805 for cola and 11,805 for energy drinks and in ALSPAC respectively 7,117, 7,278, 7,277, 7,233 and 0. For current vs. former smoking the number of participants in the NTR was 5,400 for total caffeine, 6,619 for coffee, 5,400 for tea, 5,400 for cola and 5,400 for energy drinks and in ALSPAC respectively 3,155, 3,226, 3,233, 3,210 and 0.

Smoking heaviness and caffeine use in current smokers

Each cigarette smoked per day was associated with an increased consumption of 3.7 mg [95% *CI* = 1.9 to 5.5] caffeine in the Dutch and 8.4 [95% *CI* = 6.9 to 10.0] in the British sample (Figure 2). Number of cigarettes per day was also positively associated with caffeine use through coffee and cola. For total caffeine and coffee, the effect was stronger in the British sample compared to the Dutch sample. While British smokers consumed more tea with every additional cigarette (+2.7 mg [95% *CI* = 1.7 to 3.8]), there was no association in Dutch smokers (+0.2 mg [95% *CI* = -0.7 to 1.1]). When grouping smoking heaviness into categories of <5, 5-9, 10-14, 15-19, 20-24 and 25+ cigarettes per day, a linear association was seen with total caffeine and coffee in the NTR (Figure S1) and with total caffeine, coffee and tea in ALSPAC (Figure S2). Due to the weaker association between number of cigarettes per day and cola use, linearity was less distinct when grouping smoking heaviness into categories. For ALSPAC, results at time-points other than the one described here (at 47 months after delivery) were very similar.

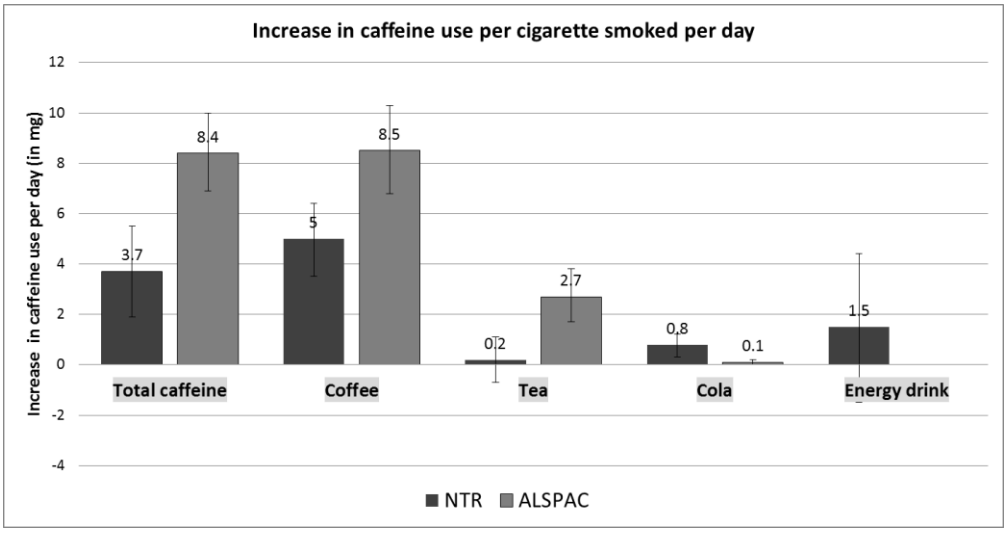

Figure 2. NTR = Netherlands Twin Register; ALSPAC = Avon Longitudinal Study of Parents and Children. For the NTR the number of participants for each analysis was 1,282 for total caffeine, 1,790 for coffee, 717 for tea, 402 for cola and 50 for energy drinks while for ALSPAC these numbers were respectively 1,408, 993, 1,125, 877 and 0.

Smoking heaviness was not associated with the consumption of decaffeinated coffee in ALSPAC, while smoking persistence and, at some time-points, smoking initiation was associated with a lower odds of consuming decaffeinated coffee (Tables S4-S6). In the NTR current smokers had a lower odds of decaffeinated coffee use compared with former smokers (Tables S10-S12).

Discussion

Smoking behaviour was strongly associated with caffeine consumption in two large population-based samples. This is the first time that the consumption of coffee, tea, cola and energy drinks and the association with smoking behaviour was investigated comparing data from two different countries: a 'coffee drinking' country (the Netherlands) and a 'tea-drinking' country (the United Kingdom).

The British participants of ALSPAC drank more tea than the Dutch participants of the NTR. While the Dutch drank more coffee than the British, this difference was less distinct. These findings, based on self-report, are in agreement with comparisons between the Netherlands and the UK based on historical and sales figures (139, 140). For total caffeine use, there was a strong and positive association with smoking initiation, smoking persistence and smoking heaviness. Similar associations were found when assessing coffee separately, consistent with earlier findings in populations from the United States (US) (41), the UK (42) and the Netherlands (146).

The first research question was whether the association between smoking and caffeine consumption is consistent across different types of caffeinated drinks, or specific to coffee. Smoking initiation and smoking persistence were associated with consuming more tea in the British sample, while the opposite was true in the Dutch sample. These results were not substantially altered after excluding green tea in the Dutch sample (data not shown). A possible explanation for this cultural difference is that tea consumption is very common in the UK, while in the Netherlands tea drinkers differ from non-drinkers. This explanation is supported by the fact that in the Dutch sample a higher education was associated with drinking more tea, while in the British sample neither education nor social class were associated with tea consumption. All analyses were corrected for these variables, but it may be that there were other, unmeasured covariates that made Dutch tea drinkers different from British tea drinkers. For instance, in a population-based cohort of ~40,000 Dutch individuals, a higher tea consumption (ranging from 0-1 cups per day to >5 cups per day) was not only associated with a lower prevalence of current smoking, but also with a reduction in alcohol consumption, BMI and total energy intake. In contrast, higher coffee consumption was accompanied by a higher prevalence of current smoking and higher alcohol consumption, BMI and total energy intake (146). High correlations between smoking, alcohol and cannabis have been reported previously (147-149). Our findings in Dutch and British individuals are in contrast with a previous US-based study that found no difference in smoking behaviour between tea drinkers and non-drinkers (136). This could be due to differences in the social patterning of tea use across these countries. In the US-based study, gender (male vs. female), a higher age and a higher education were associated with lower odds of being a (caffeinated) tea drinker. In contrast, a higher age and in the Dutch sample a higher education was associated with drinking more tea in the present study. These findings emphasize the need to study such behaviours in multiple (culturally distinct) populations. Small but consistent

positive associations were also found between cola/energy drink consumption and smoking initiation, smoking persistence and (for cola only) smoking heaviness. This supports previous research linking smoking to a higher consumption of soft drinks (138) and energy drinks (137) in adolescents. We have now replicated these findings for energy drinks and cola (which is caffeinated) specifically, and shown that it also applies to an adult population.

Overall our findings suggest an association between smoking and caffeine use that is consistent across the most commonly used caffeinated drinks. Except for tea, this association is also consistent across the Netherlands and the UK, answering the second research question. In the NTR the prevalence of current smoking was lower than in the general Dutch population. This (slight) bias is probably due to a relatively high proportion of highly educated participants (107). These differences were accounted for by correcting all observational analyses for educational attainment and in ALSPAC also for social class.

This study has some limitations that need to be considered. Most importantly, the two included samples are not entirely comparable and may not be generalizable to other populations. The Dutch sample contains men and women from twin-families, while the British sample consists of women only. By comparing less cooperative to highly cooperative families, the Dutch sample has previously found that data on health, personality and lifestyle were only mildly biased by non-response (107). In the present study we corrected for age, gender, educational attainment and social class, to minimize possible bias. As for the ALSPAC sample, it may be that mothers of young children adjust their smoking and/or caffeine use during or after pregnancy. For this reason, and because of sample size, we analysed data from 47 months after delivery. When analysing all time-points between 18 weeks gestation and 145 months after delivery, there were no major differences in the association between smoking and caffeine use. Also, our findings were not substantially altered when comparing the (female) ALSPAC sample to female NTR participants only, instead of including both male and female NTR participants and correcting for gender (data not shown). In line with previous studies of NTR and ALSPAC, we defined regular smoking as (minimally) *weekly* smoking for the NTR compared to *daily* smoking for ALSPAC. To check whether this discrepancy affected the comparability of our findings we re-analysed the NTR data such that only daily smoking was identified in both samples. The results of these analyses were not substantially different (data not shown).

Different mechanisms have been suggested to explain the strong association between smoking and caffeine. From previous work we know that both smoking and caffeine use are influenced moderately to strongly by genetic factors (26, 38). It could therefore be that smoking and caffeine are associated because they are influenced by the same genes. Evidence for shared genetic and environmental factors between smoking and caffeine use was indeed found in two US-based, twin studies (150-153). The subtle cultural differences found in the present study emphasize the need for bivariate twin studies in other populations. A second

explanation for the association between smoking and caffeine is a causal effect from caffeine use on smoking or vice versa. Experimental work in animal and human subjects has found evidence for causal effects in both directions. Smoking may causally increase caffeine use because nicotine in inhaled cigarette smoke increases the metabolism of caffeine (154-156). In the other direction, it was found that rats consuming caffeine in their drinking water self-administered significantly more nicotine than did controls. It was hypothesized that this was due to a pharmacokinetic interaction such that caffeine potentiates the reinforcing properties of nicotine (157-159). Causal effects need to be studied further, with one way of assessing causality being Mendelian randomization analysis (MR). This technique utilizes genetic variants associated with a certain trait as an instrument, or proxy, for that trait, thereby minimizing effects of confounding and reverse causation (160). To gain further insight into the association between smoking and caffeine, additional research utilizing the genetically informative methods described above and newly emerging methods is required. Here, we have identified an association between smoking behaviour and caffeine use. If this association is (partly) due to causal effects there could be important implications. For instance, knowledge of factors that causally increase or decrease the odds of quitting smoking would be valuable, since many smokers who attempt to quit fail (88, 89).

Supplement

Table S1. Associations between smoking initiation (ever vs. never smokers) and daily caffeine consumption (in mg) in the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

	18w gestation		**32w gestation**		**2 months**		**47 months**		**85 months**		**97 months**		**145 months**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
Total caffeine – *β* (95% CI)														
N	*12,566*	*9,441*	*10,353*	*8,628*	*7,485*	*5,932*	*8,777*	*7,117*	*7,701*	*6,283*	*6,947*	*5,831*	*4,141*	*3,583*
Never smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Ever smokers	70.9	55.4	70.6	53.9	66.9	54.0	66.2	59.5	68.6	59.0	63.1	53.2	50.1	44.5
	(65.3 to 76.5)	(49.4 to 61.5)	(64.8 to 76.4)	(47.8 to 60.1)	(58.8 to 75.0)	(45.1 to 62.9)	(59.1 to 73.2)	(51.8 to 67.2)	(61.0 to 76.2)	(50.7 to 67.4)	(55.2 to 71.0)	(44.7 to 61.8)	(40.6 to 59.6)	(34.3 to 54.7)
Coffee – *β* (95% CI)														
N	*12,689*	*9,500*	*10,815*	*8,990*	*7,873*	*6,187*	*9,006*	*7,278*	*7,901*	*6,427*	*7,185*	*6,021*	*4,730*	*4,039*
Never smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Ever smokers	45.8	38.7	44.1	35.6	51.3	39.7	51.1	47.3	55.8	47.7	55.5	48.8	47.7	39.9
	(40.9 to 50.6)	(33.4 to 44.1)	(39.2 to 48.9)	(30.3 to 40.9)	(43.9 to 58.7)	(31.7 to 47.7)	(44.4 to 57.9)	(39.8 to 54.8)	(48.2 to 63.3)	(39.3 to 56.0)	(47.7 to 63.3)	(40.3 to 57.2)	(39.1 to 56.4)	(30.7 to 49.1)
Tea – *β* (95% CI)														
N	*12,674*	*9,499*	*10,877*	*9,045*	*8,140*	*6,354*	*8,989*	*7,277*	*7,892*	*6,427*	*7,165*	*6,004*	*5,752*	*4,909*
Never smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Ever smokers	20.1	15.4	24.2	16.7	15.3	12.2	14.7	12.3	12.0	10.9	8.9	5.2	8.9	8.6
	(16.7 to 23.5)	(11.6 to 19.1)	(20.6 to 27.9)	(12.8 to 20.6)	(10.8 to 19.8)	(7.1 to 17.3)	(10.3 to 19.1)	(7.5 to 17.1)	(7.3 to 16.7)	(5.7 to 16.0)	(4.1 to 13.7)	(0.1 to 10.3)	(3.5 to 14.2)	(2.8 to 14.3)
Cola – *β* (95% CI)														
N	*12,639*	*9,473*	*10,720*	*8,921*	*7,973*	*6,235*	*8,934*	*7.233*	*7,825*	*6,371*	*7,076*	*5,929*	*6,198*	*5,220*
Never smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Ever smokers	4.9	1.8	2.7	1.5	0.9	0.7	1.0	0.5	0.6	0.2	0.7	0.2	0.7	0.4
	(3.9 to 5.9)	(0.8 to 2.8)	(2.1 to 3.2)	(0.9 to 2.1)	(0.5 to 1.3)	(0.2 to 1.1)	(0.6 to 1.3)	(0.2 to 0.9)	(0.3 to 1.0)	(-0.2 to 0.5)	(0.3 to 1.0)	(-0.2 to 0.6)	(0.4 to 1.1)	(0.1 to 0.8)

Linear regression analyses were performed with total caffeine use, caffeine use through coffee only, caffeine use through tea only or caffeine use through cola only as the dependent variable and smoking initiation (0 = never smoking 1 = ever smoking) as the independent variable. Unadj = unadjusted; adj = adjusted for age, educational attainment & social class (all continuous).

Table S2. Associations between smoking persistence (current vs. former smokers) and daily caffeine consumption (in mg) in the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

	18w gestation		**32w gestation**		**2 months**		**47 months**		**85 months**		**97 months**		**145 months**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
Total caffeine – *β* (95% CI)														
N	*6,341*	*4,502*	*5,179*	*4,139*	*3,467*	*2,699*	*3,970*	*3,155*	*3,281*	*2,603*	*3,126*	*2,522*	*1,773*	*1,505*
Former smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Current smokers	92.8	88.1	108.0	100.8	80.7	81.7	79.4	83.2	82.3	81.6	89.9	85.0	76.4	78.5
	(83.7 to 101.8)	(77.8 to 98.4)	(98.9 to 117.1)	(90.7 to 111.0)	(67.8 to 93.7)	(67.0 to 96.4)	(67.8 to 90.9)	(70.2 to 96.3)	(69.3 to 95.4)	(66.8 to 96.4)	(76.7 to 103.1)	(70.2 to 100.0)	(60.5 to 92.4)	(60.7 to 96.3)
Coffee – *β* (95% CI)														
N	*6,423*	*4,540*	*5,429*	*4,333*	*3,660*	*2,826*	*4,078*	*3,226*	*3,375*	*2,670*	*3,238*	*2,610*	*2,039*	*1,702*
Former smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Current smokers	59.4	66.4	68.1	70.5	74.1	77.1	65.5	74.3	70.0	72.7	73.8	72.0	81.9	74.4
	(51.3 to 67.5)	(57.1 to 75.8)	(60.2 to 76.1)	(61.5 to 79.5)	(61.8 to 86.3)	(63.5 to 90.8)	(54.2 to 76.9)	(61.4 to 87.2)	(56.8 to 83.3)	(57.8 to 87.7)	(60.3 to 87.3)	(56.8 to 87.3)	(67.0 to 96.8)	(57.8 to 91.1)
Tea – *β* (95% CI)														
N	*6,404*	*4,534*	*5,462*	*4,364*	*3,774*	*2,896*	*4,077*	*3,233*	*3,359*	*2,658*	*3,221*	*2,597*	*2,355*	*1,961*
Former smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Current smokers	26.0	19.5	35.6	25.9	14.1	9.7	14.0	9.9	10.1	6.5	16.9	14.5	10.9	8.5
	(20.7 to 31.4)	(13.2 to 25.7)	(29.8 to 41.4)	(19.4 to 32.3)	(6.8 to 21.3)	(1.2 to 18.1)	(6.8 to 21.2)	(1.9 to 17.9)	(2.2 to 18.0)	(-2.3 to 15.3)	(9.0 to 24.7)	(6.0 to 23.1)	(1.8 to 19.9)	(-1.3 to 18.3)
Cola – *β* (95% CI)														
N	*6,385*	*4,519*	*5,385*	*4,300*	*3,711*	*2,846*	*4,048*	*3,210*	*3,342*	*2,645*	*3,185*	*2,567*	*2,545*	*2,092*
Former smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Current smokers	6.6	2.6	3.1	1.4	1.7	0.8	1.4	0.8	1.4	0.5	1.4	0.7	1.5	1.0
	(5.0 to 8.2)	(0.8 to 4.4)	(2.2 to 3.9)	(0.4 to 2.3)	(1.0 to 2.3)	(0.1 to 1.6)	(0.9 to 1.9)	(0.2 to 1.3)	(0.8 to 1.9)	(-0.1 to 1.1)	(0.8 to 2.0)	(0.02 to 1.3)	(1.0 to 2.1)	(0.4 to 1.7)

Linear regression analyses were performed with total caffeine use, caffeine use through coffee only, caffeine use through tea only or caffeine use through cola only as the dependent variable and smoking persistence (0 = former smoking 1 = current smoking) as the independent variable. Unadj = unadjusted; adj = adjusted for age, educational attainment & social class (all continuous).

Table S3. Associations between number of cigarettes smoked per day and daily caffeine consumption (in mg) in smokers from the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

	18w gestation		**32w gestation**		**2 months**		**47 months**		**85 months**		**97 months**		**145 months**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
Total caffeine – *β* (95% CI)														
N	*2,320*	*1,464*	*2,065*	*1,499*	*1,591*	*1,085*	*1,937*	*1,408*	*1,541*	*1,121*	*1,226*	*914*	*692*	*559*
Number of cigarettes	6.0 (4.7 to 7.0)	5.2 (3.7 to 6.6)	6.8 (5.5 to 8.0)	5.9 (4.3 to 7.2)	7.0 (5.5 to 8.4)	6.0 (4.3 to 7.7)	8.1 (6.8 to 9.4)	8.4 (6.9 to 10.0)	7.2 (5.8 to 8.5)	6.6 (4.9 to 8.2)	7.1 (5.6 to 8.6)	7.3 (5.6 to 8.9)	6.9 (5.0 to 8.8)	7.2 (5.1 to 9.3)
Coffee – *β* (95% CI)														
N	*1,567*	*1,020*	*1,423*	*1,031*	*1,206*	*810*	*1,355*	*993*	*1,096*	*804*	*900*	*674*	*742*	*586*
Number of cigarettes	5.7 (4.3 to 7.0)	4.8 (3.2 to 6.5)	5.7 (4.4 to 7.1)	5.7 (4.1 to 7.3)	5.9 (4.2 to 7.5)	5.0 (3.0 to 6.9)	7.8 (6.3 to 9.3)	8.5 (6.8 to 10.3)	8.5 (6.8 to 10.1)	7.6 (5.6 to 9.6)	7.3 (5.5 to 9.2)	7.9 (5.9 to 10.0)	6.6 (4.7 to 8.5)	6.4 (4.2 to 8.5)
Tea – *β* (95% CI)														
N	*1,946*	*1,219*	*1,899*	*1,374*	*1,471*	*989*	*1,549*	*1,125*	*1,199*	*872*	*981*	*740*	*721*	*570*
Number of cigarettes	3.7 (2.9 to 4.4)	2.7 (1.8 to 3.6)	4.2 (3.3 to 5.0)	2.8 (1.8 to 3.8)	3.2 (2.4 to 4.1)	2.5 (1.5 to 3.5)	3.8 (2.9 to 4.7)	2.7 (1.7 to 3.8)	2.4 (1.5 to 3.4)	1.7 (0.7 to 2.8)	2.8 (1.8 to 3.7)	2.0 (1.0 to 3.1)	1.8 (0.7 to 2.8)	1.7 (0.6 to 2.8)
Cola – *β* (95% CI)														
N	*984*	*603*	*1,146*	*815*	*555*	*360*	*1,223*	*877*	*888*	*633*	*756*	*558*	*538*	*414*
Number of cigarettes	0.5 (0.1 to 0.9)	0.3 (-0.2 to 0.8)	0.4 (0.2 to 0.5)	0.1 (-0.1 to 0.4)	0.2 (-0.0 to 0.4)	0.1 (-0.1 to 0.3)	0.1 (-0.0 to 0.2)	0.1 (-0.0 to 0.2)	0.1 (0.1 to 0.2)	0.1 (0.0 to 0.2)	0.1 (-0.0 to 0.2)	0.1 (-0.1 to 0.2)	0.1 (0.0 to 0.2)	0.1 (-0.0 to 0.2)

Linear regression analyses were performed with total caffeine use, caffeine use through coffee only, caffeine use through tea only or caffeine use through cola only as the dependent variable and number of cigarettes as the independent variable. Non-caffeine users (or non-users of a specific beverage when analysed individually) were excluded. Unadj = unadjusted; adj = adjusted for age, educational attainment & social class (all continuous).

Table S4. Associations between smoking initiation (ever vs. never smokers) and decaffeinated coffee consumption (users vs. non-users) in the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

	Decaf coffee – *OR* (95% CI)													
	18w gestation		**32w gestation**		**2 months**		**47 months**		**85 months**		**97 months**		**145 months**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
N	*12,677*	*9,485*	*10,440*	*8,712*	*7,502*	*5,953*	*9,021*	*7,292*	*7,063*	*5,767*	*6,448*	*5,428*	*4,659*	*3,981*
Never smokers	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*
Ever smokers	0.74	0.97	0.82	0.96	0.77	0.94	0.77	0.84	0.7	0.74	0.68	0.75	0.71	0.75
	(0.67 to 0.81)	(0.87 to 1.08)	(0.75 to 0.90)	(0.87 to 1.06)	(0.69 to 0.86)	(0.83 to 1.06)	(0.69 to 0.86)	(0.74 to 0.94)	(-0.09 to 0.04)	(0.65 to 0.83)	(0.60 to 0.78)	(0.66 to 0.87)	(0.62 to 0.80)	(0.65 to 0.87)

Logistic regression analyses were performed with decaffeinated coffee consumption (0 = non-user 1 = user) as the dependent variable and smoking initiation (0 = never smoking 1 = ever smoking) as the independent variable. Unadj = unadjusted; adj = adjusted for age, educational attainment & social class (all continuous).

Table S5. Associations between smoking persistence (current vs. former smokers) and decaffeinated coffee consumption (users vs. non-users) in the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

	Decaf coffee – *OR* (95% CI)													
	18w gestation		**32w gestation**		**2 months**		**47 months**		**85 months**		**97 months**		**145 months**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
N	*6,414*	*4,530*	*5,260*	*4,222*	*3,446*	*2,698*	*4,088*	*3,235*	*3,034*	*2,409*	*2,942*	*2,379*	*1,993*	*1,665*
Former smokers	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*	*Ref*
Current smokers	0.56	0.71	0.69	0.78	0.58	0.73	0.70	0.77	0.73	0.78	0.75	0.80	0.62	0.56
	(0.48 to 0.66)	(0.58 to 0.86)	(0.60 to 0.79)	(0.67 to 0.92)	(0.49 to 0.69)	(0.59 to 0.89)	(0.59 to 0.83)	(0.63 to 0.93)	(0.60 to 0.88)	(0.62 to 0.97)	(0.62 to 0.92)	(0.63 to 1.01)	(0.50 to 0.76)	(0.44 to 0.72)

Logistic regression analyses were performed with decaffeinated coffee consumption (non-user = 0 user = 1) as the dependent variable and smoking persistence (0 = former smoking 1 = current smoking) as the independent variable. Unadj = unadjusted; adj = adjusted for age, educational attainment & social class (all continuous).

Table S6. Associations between number of cigarettes smoked per day and decaffeinated coffee consumption (users vs. non-users) in smokers from the *Avon Longitudinal Study of Parents and Children (ALSPAC)*

	Decaf coffee – *OR* (95% CI)													
	18w gestation		**32w gestation**		**2 months**		**47 months**		**85 months**		**97 months**		**145 months**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
N	*2,454*	*1,527*	*2,217*	*1,616*	*1,617*	*1,112*	*2,066*	*1,487*	*1,489*	*1,081*	*1,206*	*893*	*850*	*666*
Number of cigarettes	0.97	0.99	0.98	0.99	0.96	0.97	0.97	0.97	0.96	0.98	0.97	0.97	0.99	1.00
	(0.95 to 0.99)	(0.97 to 1.02)	(0.96 to 0.99)	(0.96 to 1.01)	(0.94 to 0.98)	(0.95 to 1.29)	(0.95 to 0.99)	(0.95 to 0.99)	(0.94 to 0.98)	(0.95 to 1.00)	(0.95 to 0.99)	(0.95 to 1.00)	(0.97 to 1.01)	(0.97 to 1.02)

Logistic regression analyses were performed with decaffeinated coffee consumption (0 = non-user 1 = user) as the dependent variable and number of cigarettes as the independent variable. Unadj = unadjusted; adj = adjusted for age, educational attainment & social class (all continuous).

Table S7. Associations between smoking initiation (ever vs. never smokers) and daily caffeine consumption (in mg) in the *Netherlands Twin Register (NTR)*

	Total caffeine – *β* (95% CI)		**Coffee – *β* (95% CI)**		**Tea – *β* (95% CI)**		**Cola – *β* (95% CI)**		**Energy drink–*β*(95% CI)**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
N	*17,736*	*11,805*	*21,682*	*14,584*	*17,736*	*11,805*	*17,736*	*11,805*	*17,736*	*11,805*
Never smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Ever smokers	108.8	52.8	120.3	76.4	-10.3	-16.1	0.3	1.2	1.0	0.6
	(102.6 to 114.9)	(45.6 to 60.0)	(114.7 to 125.9)	(69.7 to 83.0)	(-12.7 to -7.9)	(-19.1 to -13.1)	(-0.2 to 0.7)	(0.8 to 1.7)	(0.5 to 1.5)	(0.2 to 0.9)

Linear regression analyses were performed with total caffeine use, caffeine use through coffee only, caffeine use through tea only, caffeine use through cola only or caffeine use through energy drinks only as the dependent variable and smoking initiation (0 = never smoking 1 = ever smoking) as the independent variable. Unadj = unadjusted; adj = adjusted for age (continuous), educational attainment (continuous) & gender (0 = male 1 = female); analyses were corrected for family clustering by utilizing the robust cluster option in STATA.

Table S8. Associations between smoking persistence (current vs. former smokers) and daily caffeine consumption (in mg) in the *Netherlands Twin Register (NTR)*

	Total caffeine – *β* (95% CI)		**Coffee – *β* (95% CI)**		**Tea – *β* (95% CI)**		**Cola – *β* (95% CI)**		**Energy drink–*β*(95% CI)**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
N	*7,088*	*5,400*	*8,762*	*6,619*	*7,088*	*5,400*	*7,088*	*5,400*	*7,088*	*5,400*
Former smokers	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Current smokers	14.1	57.9	26.1	71.2	-19.0	-17.1	4.1	1.9	5.9	1.5
	(3.5 to 24.8)	(45.2 to 70.5)	(16.4 to 35.7)	(59.7 to 82.8)	(-22.6 to -15.4)	(-21.5 to -12.7)	(3.3 to 4.9)	(1.0 to 2.8)	(4.7 to 7.1)	(0.7 to 2.4)

Linear regression analyses were performed with total caffeine use, caffeine use through coffee only, caffeine use through tea only, caffeine use through cola only or caffeine use through energy drinks only as the dependent variable and smoking persistence (0 = former smoking 1 = current smoking) as the independent variable. Unadj = unadjusted; adj = adjusted for age (continuous), educational attainment (continuous) & gender (0 = male 1 = female); analyses were corrected for family clustering by utilizing the robust cluster option in STATA.

Table S9 . Associations between number of cigarettes smoked per day (CPD) and daily caffeine consumption (in mg) in smokers from the *Netherlands Twin Register (NTR)*

	Total caffeine – *β* (95% CI)		**Coffee – *β* (95% CI)**		**Tea – *β* (95% CI)**		**Cola – *β* (95% CI)**		**Energy drink – *β* (95% CI)**	
	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj	Unadj	Adj
N	*1,954*	*1,282*	*2,572*	*1,790*	*1,122*	*717*	*751*	*402*	*243*	*50*
CPD	6.1 (4.4 to 7.9)	3.7 (1.9 to 5.5)	6.5 (5.1 to 7.9)	5.0 (3.5 to 6.4)	-0.0 (-0.7 to 0.7)	0.2 (-0.7 to 1.1)	0.9 (0.6 to 1.3)	0.8 (0.3 to 1.2)	2.3 (0.8 to 3.8)	1.5 (-1.5 to 4.4)

Linear regression analyses were performed with total caffeine use, caffeine use through coffee only, caffeine use through tea only, caffeine use through cola only or caffeine use through energy drinks only as the dependent variable and number of cigarettes as the independent variable. Non-caffeine users (or non-users of a specific beverage when analysed individually) were excluded. Unadj = unadjusted; adj = adjusted for age (continuous), educational attainment (continuous) & gender (0 = male 1 = female); analyses were corrected for family clustering by utilizing the robust cluster option in STATA.

Table S10. Associations between smoking initiation (ever vs. never smokers) and decaffeinated coffee consumption (users vs. non-users) in the *Netherlands Twin Register (NTR)*

	Decaf coffee – *OR* (95% CI)	
	Unadj	Adj
N	*17,736*	*11,805*
Never smokers	*Ref*	*Ref*
Ever smokers	1.43 (1.29 to 1.58)	0.94 (0.80 to 1.06)

Logistic regression analyses were performed with decaffeinated coffee consumption (0 = non-user 1 = user) as the dependent variable and smoking initiation (0 = never smoking 1 = ever smoking) as the independent variable. Unadj = unadjusted; adj = adjusted for age (continuous), educational attainment (continuous) & gender (0 = male 1 = female); analyses were corrected for family clustering by utilizing the robust cluster option in STATA.

Table S11. Associations between smoking persistence (current vs. former smokers) and decaffeinated coffee consumption (users vs. non users) in the *Netherlands Twin Register (NTR)*

	Decaf coffee – *OR* (95% CI)	
	Unadj	Adj
N	*7,088*	*5,400*
Former smokers	*Ref*	*Ref*
Current smokers	0.58 (0.49 to 0.69)	0.80 (0.66 to 0.98)

Logistic regression analyses were performed with decaffeinated coffee consumption (0 = non-user 1 = user) as the dependent variable and smoking persistence (0 = former smoking 1 = current smoking) as the independent variable; unadj = unadjusted; adj = adjusted for age (continuous), educational attainment (continuous) & gender (0 = male 1 = female); analyses were corrected for family clustering by utilizing the robust cluster option in STATA.

Table S12. Associations between number of cigarettes smoked per day and decaffeinated coffee (users vs. non users) in smokers in the *Netherlands Twin Register (NTR)*

	Decaf coffee – *OR* (95% CI)	
	Unadj	Adj
N	*1,999*	*1,308*
Number of cigarettes	0.99 (0.97 to 1.01)	0.98 (0.96 to 1.01)

Logistic regression analyses were performed with decaffeinated coffee consumption (0 = non-user 1 = user) as the dependent variable and number of cigarettes as the independent variable. Unadj = unadjusted; adj = adjusted for age (continuous), educational attainment (continuous) & gender (0 = male 1 = female); analyses were corrected for family clustering by utilizing the robust cluster option in STATA.

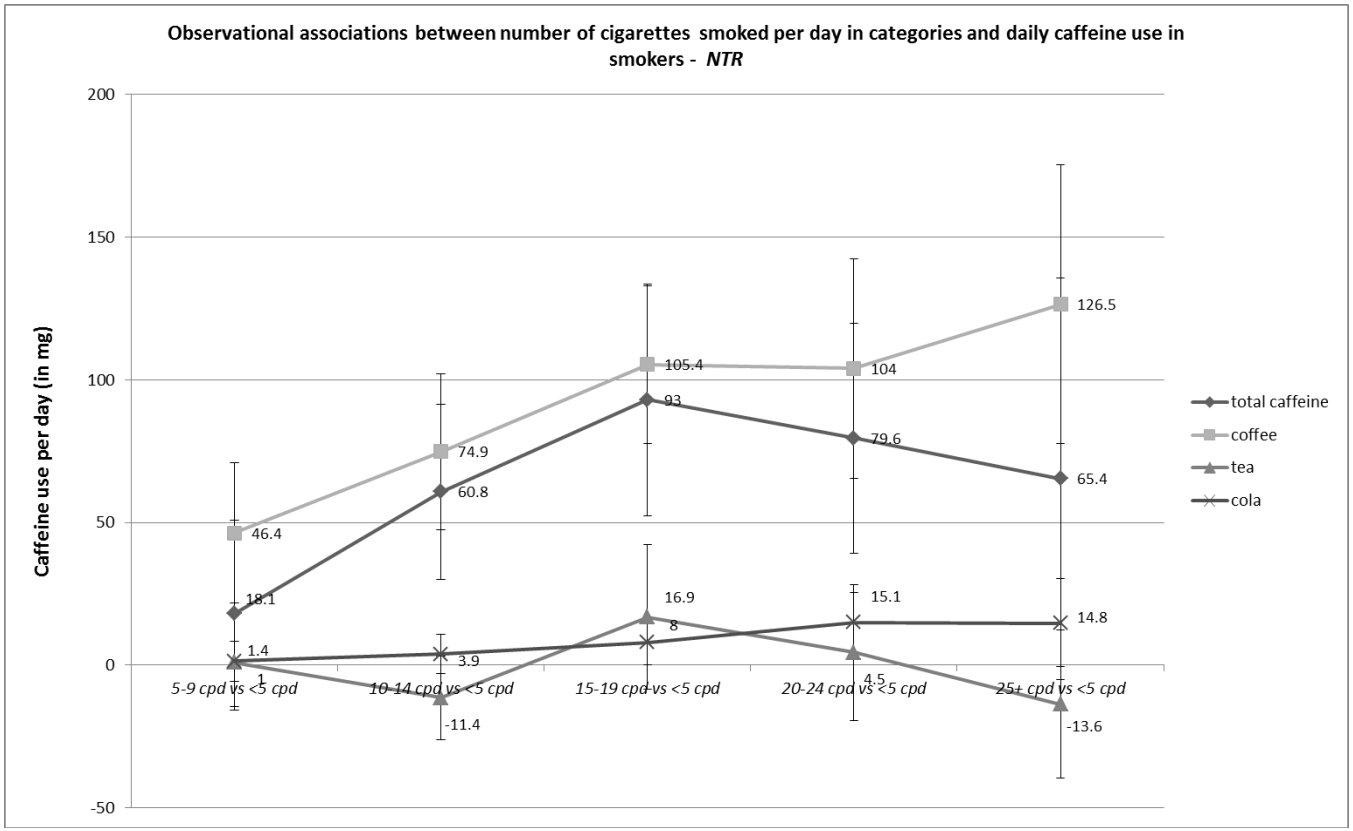

Figure S1. NTR = Netherlands Twin Register; cpd = cigarettes per day. The number of participants for each analysis was 1,282 for total caffeine, 1,790 for coffee, 717 for tea and 402 for cola. Energy drinks were not included due to the low number of users (n=50). Adjusted for age (continuous), educational attainment (continuous) & gender (0 = male 1 = female) and family clustering

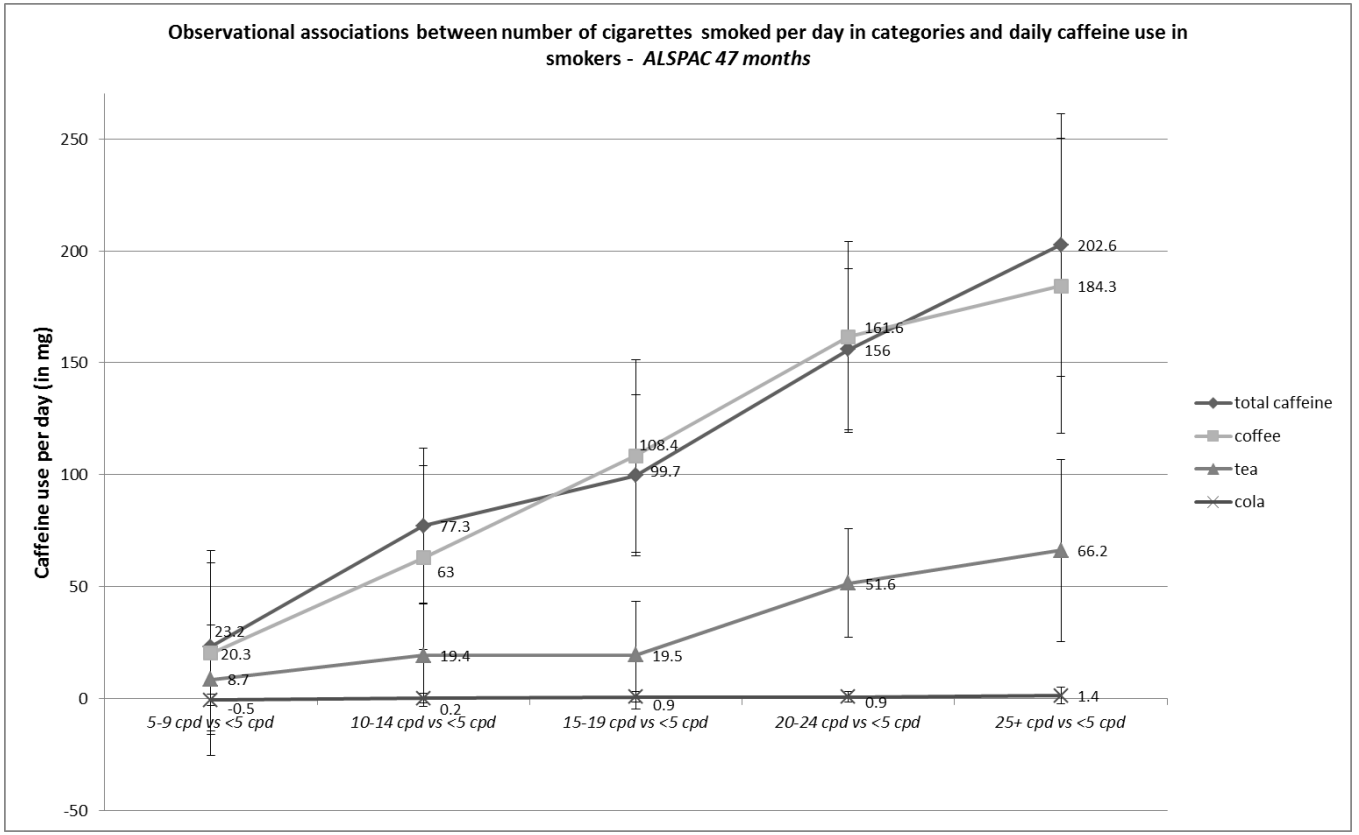

Figure S2. ALSPAC = Avon Longitudinal Study of Parents and Children; cpd = cigarettes per day. The number of participants for each analysis was 1,408 for total caffeine, 993 for coffee, 1,125 for tea and 877 for cola. Adjusted for age, educational attainment & social class (all continuous)

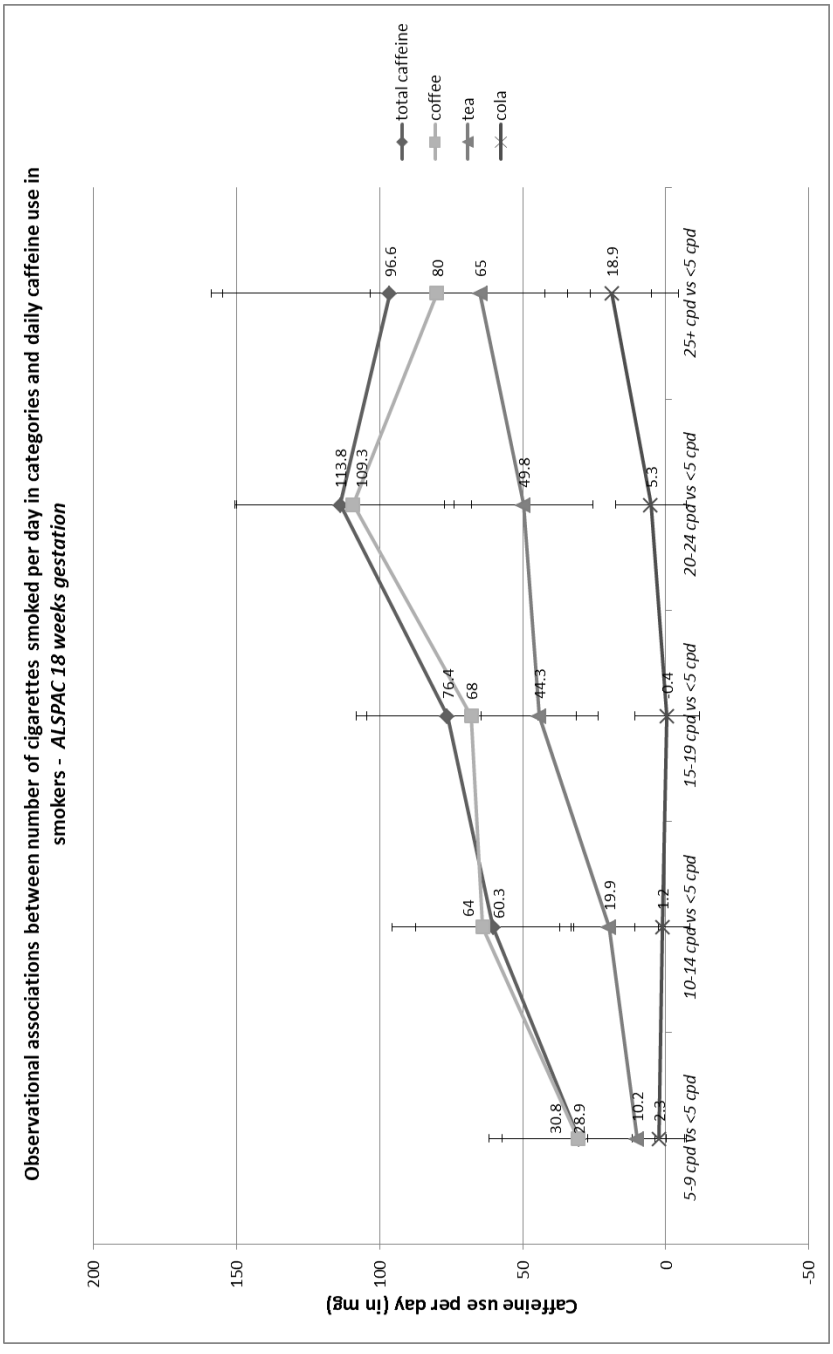

Figure S3. ALSPAC = Avon Longitudinal Study of Parents and Children; cpd = cigarettes per day. The number of participants for each analysis was 1,464 for total caffeine, 1,020 for coffee, 1,219 for tea and 603 for cola. Adjusted for age, educational attainment & social class (all continuous)

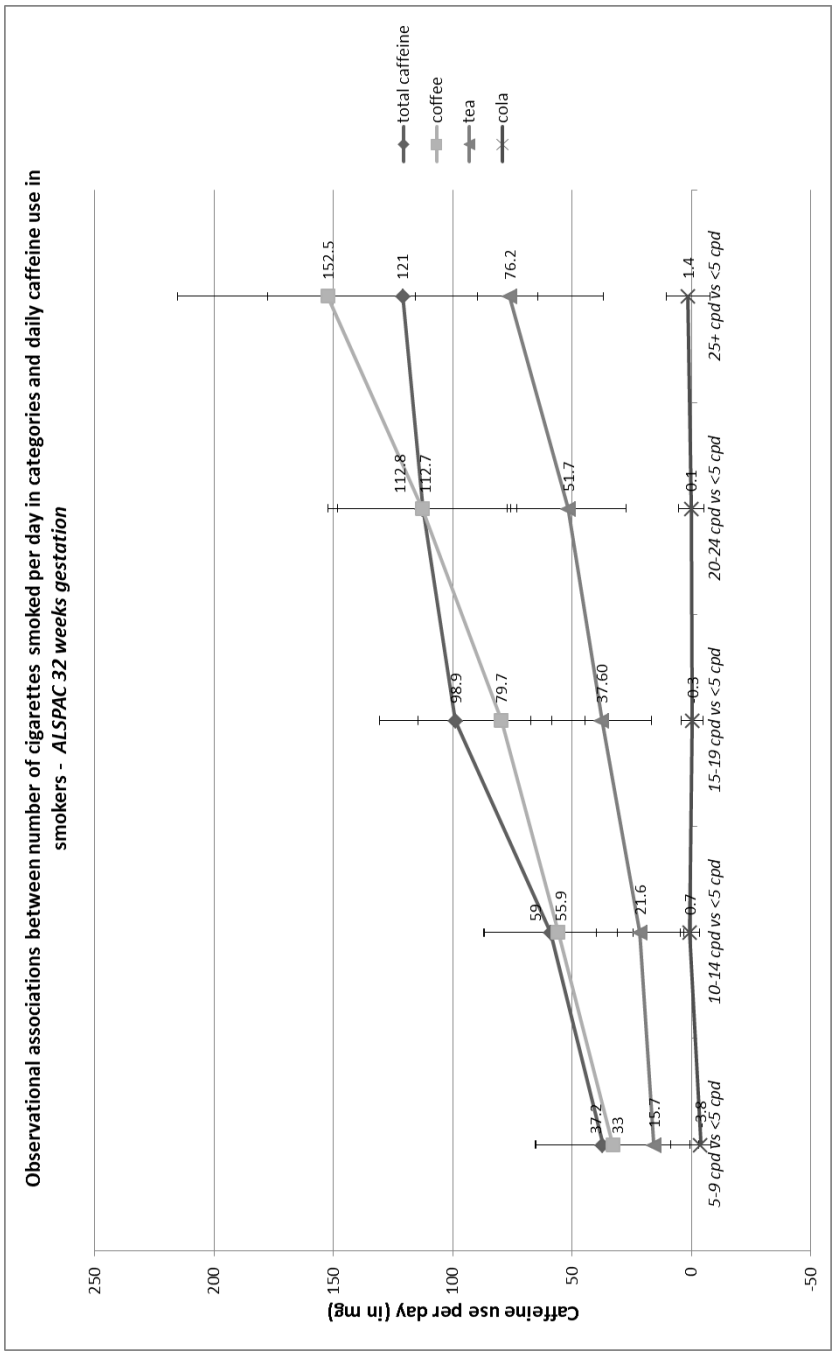

Figure S4. ALSPAC = Avon Longitudinal Study of Parents and Children; cpd = cigarettes per day. The number of participants for each analysis was 1,499 for total caffeine, 1,031 for coffee, 1,374 for tea and 815 for cola. Adjusted for age, educational attainment & social class (all continuous).

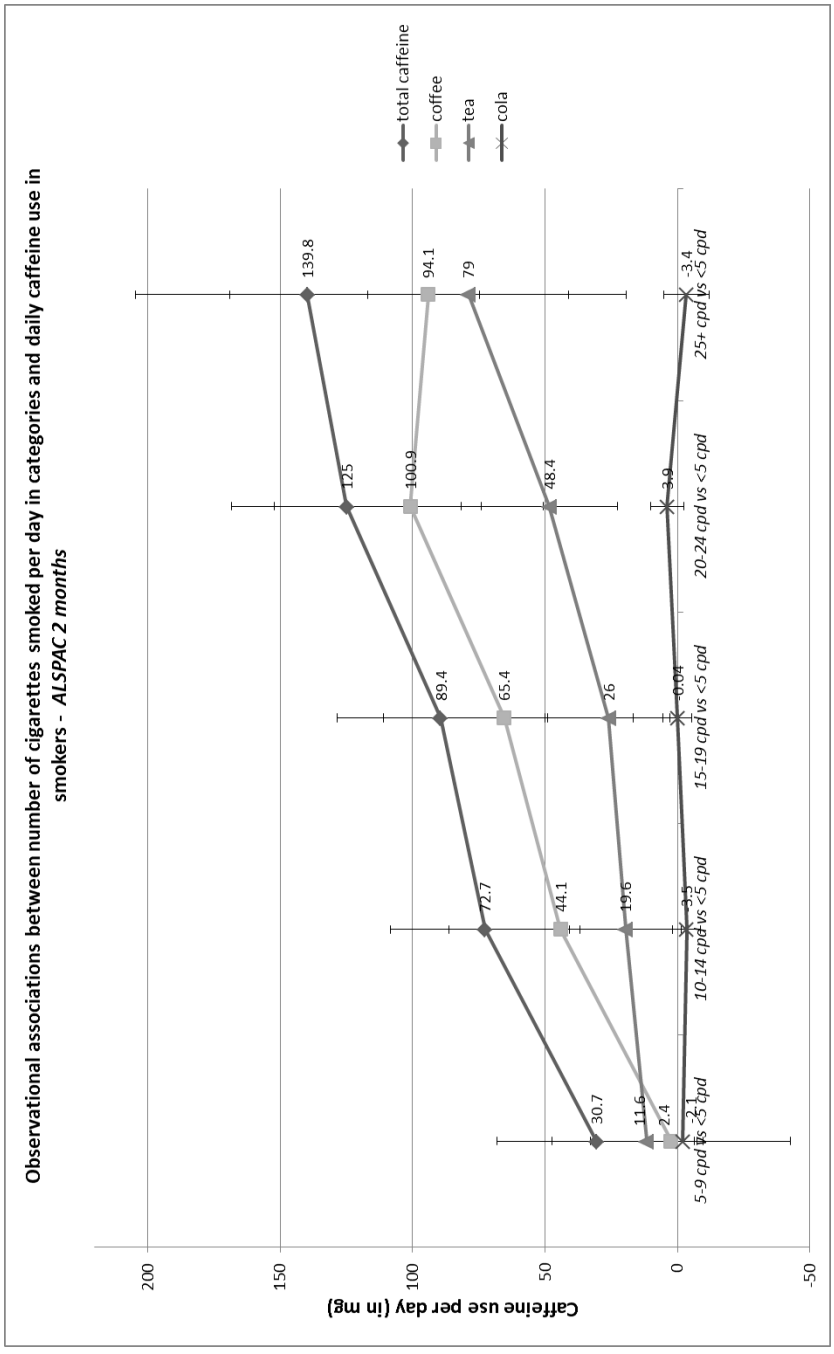

Figure S5. ALSPAC = Avon Longitudinal Study of Parents and Children; cpd = cigarettes per day. The number of participants for each analysis was 1,085 for total caffeine, 810 for coffee, 989 for tea and 360 for cola. Adjusted for age, educational attainment & social class (all continuous).

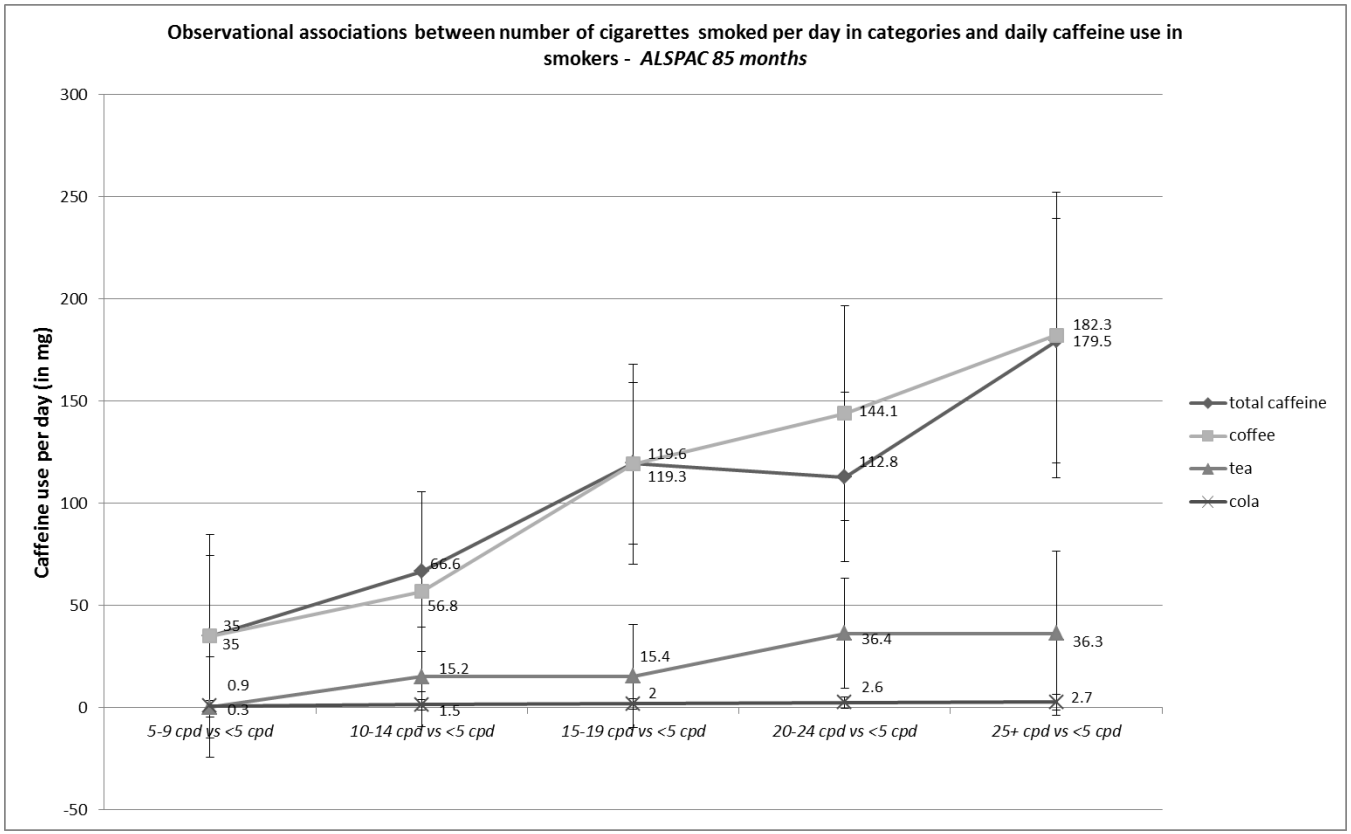

Figure S6. ALSPAC = Avon Longitudinal Study of Parents and Children; cpd = cigarettes per day. The number of participants for each analysis was 1,121 for total caffeine, 804 for coffee, 872 for tea and 633 for cola. Adjusted for age, educational attainment & social class (all continuous).

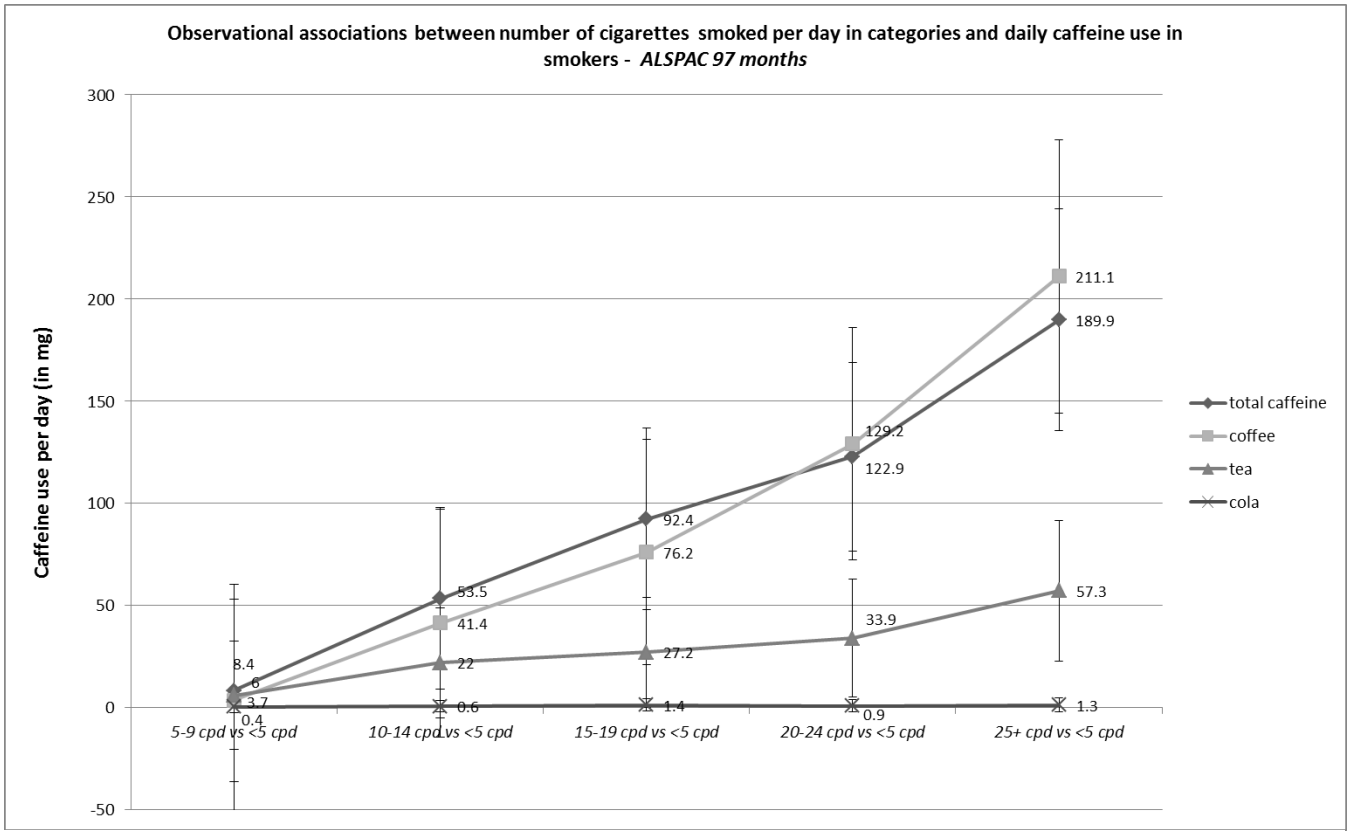

Figure S7. ALSPAC = Avon Longitudinal Study of Parents and Children; cpd = cigarettes per day. The number of participants for each analysis was 914 for total caffeine, 674 for coffee, 740 for tea and 558 for cola. Adjusted for age, educational attainment & social class (all continuous).

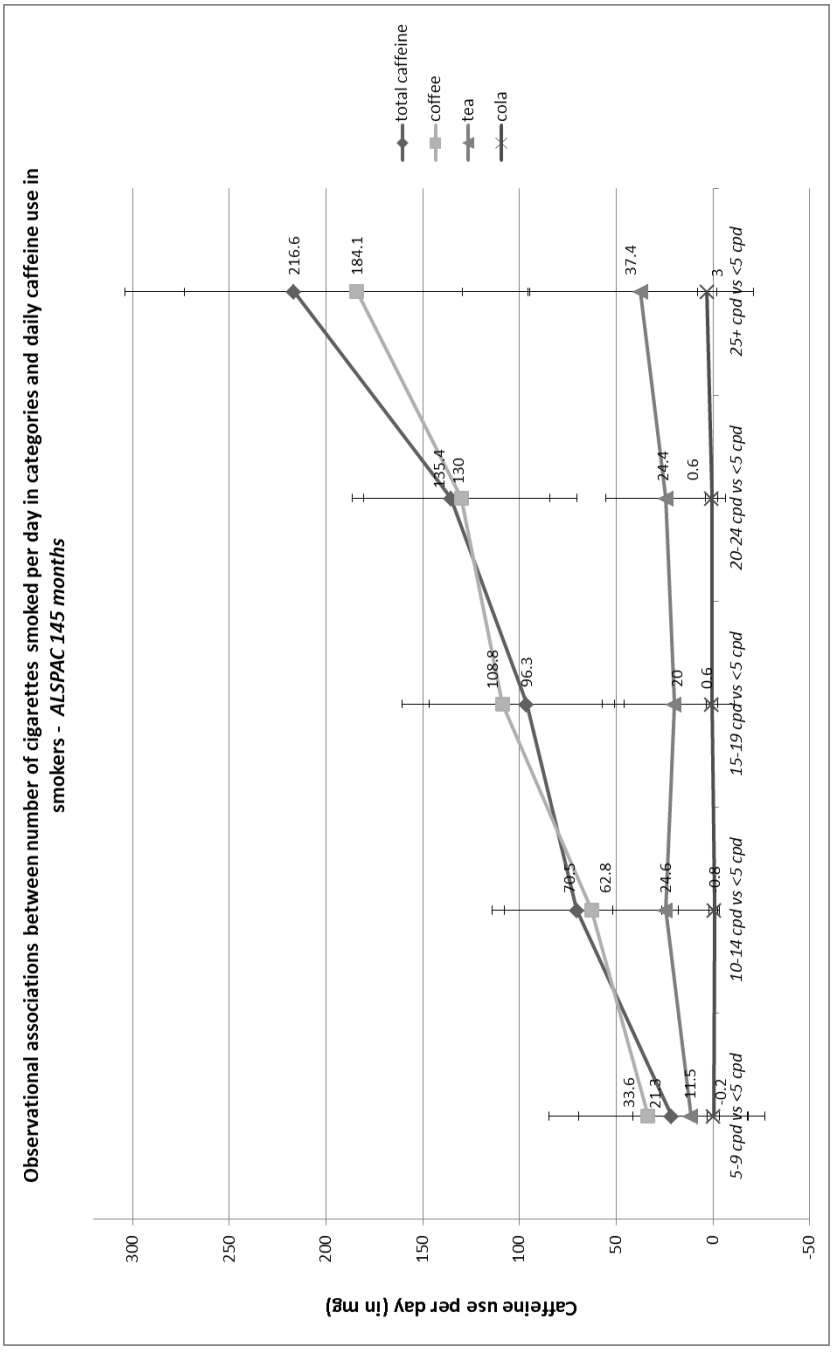

Figure S8. ALSPAC = Avon Longitudinal Study of Parents and Children; cpd = cigarettes per day. The number of participants for each analysis was 559 for total caffeine, 586 for coffee, 541 for tea and 414 for cola. Adjusted for age, educational attainment & social class (all continuous).

Chapter 8.

Smoking during adolescence as a risk factor for attention problems in adulthood.

This chapter is based on:

Treur JL, Willemsen G, Bartels M, Geels LM, van Beek JH, Huppertz C, van Beijsterveldt CE, Boomsma DI and Vink JM (2015). Smoking during adolescence as a risk factor for attention problems. Biological Psychiatry; 78(9):656-663

Abstract

Background: Cigarette smoking and attention-deficit/hyperactivity disorder (ADHD) are highly co-morbid. One explanation is that individuals with ADHD use cigarettes as 'self-medication' to alleviate their attention problems. However, animal studies reported that exposure to nicotine during adolescence influences the developing brain and negatively affects attention. This is the first human study exploring the effects of smoking during adolescence on attention problems. **Methods:** Longitudinal data on smoking and attention problems were available for 1,987 adult and 648 adolescent monozygotic (MZ) twin pairs from the Netherlands Twin Register. Twin pairs were classified as concordant/discordant for smoking and compared on attention problems. Within adult discordant pairs, the difference in attention problems between the smoking and never-smoking twins was first assessed cross-sectionally. In longitudinal analyses, the increase in attention problems from adolescence, when neither twin smoked, to adulthood was compared within discordant pairs. In subgroups with longitudinal data from childhood and adolescence, changes in smoking concordance and subsequent changes in attention problems were explored. **Results:** Adult twins who ever smoked, reported significantly more attention problems than their never-smoking co-twin. Longitudinal analyses showed a larger increase in attention problems from adolescence to adulthood in smoking twins than their never-smoking co-twin ($p<0.05$). In childhood/adolescence, smoking twins had more attention problems than their never-smoking co-twin, while scores were similar before smoking was initiated or after both twins started smoking (not significant in all groups). **Conclusions:** Results from this genetically informative study suggest smoking during adolescence leads to higher attention problem scores, lasting into adulthood.

Introduction

Attention-deficit/hyperactivity disorder (ADHD) is highly comorbid with smoking. Adolescents and young adults diagnosed with ADHD show significantly higher smoking rates compared with individuals without the disorder (7, 54, 56, 212-214). Approximately 40% of adults with ADHD smoke compared with 26% of the general population (54). A common disorder, ADHD has an estimated prevalence of around 5-6% in children (215, 216). In adults, the prevalence was 4.4% in a nationally representative household survey in the United States, based on diagnostic interviews (217); 6-7.4% of a Dutch population met the criteria for adult ADHD based on the ADHD index (218).

Several mechanisms may explain why smoking and ADHD are related. One hypothesis is that individuals with ADHD use cigarettes as 'self-medication' to alleviate their attention problems (55, 219). A 10-year follow-up study of young adolescents with and without ADHD demonstrated that ADHD is a significant risk factor for the development of substance use disorders and smoking (56). Kollins *et al*. (2005) showed that each additional ADHD symptom increases the likelihood of being a regular smoker (213).

Animal research has drawn attention to an alternative hypothesis: the direction of causality in the association between smoking and ADHD symptoms may also go from smoking to ADHD. In rats, nicotine exposure during adolescence causes diminished attentional performance, lasting into adulthood (8, 220). Although smoking can have an immediate positive effect on attention in adults with ADHD (221-223), exposure to nicotine may have detrimental long-term effects on the brain when it is still developing (224), especially on the prefrontal cortex (PFC). The PFC is involved in attention and impulse control (impulsivity) and it continues to develop into late adolescence and early adulthood. During this critical period, nicotine inhaled through tobacco smoke can affect the developing PFC, causing long-lasting changes in brain function (225). Epidemiologic studies in humans have also suggested a negative effect of smoking on attention (226-228). A functional magnetic resonance imaging study showed that prefrontal attentional network function was significantly reduced in young adult smokers (n = 15) compared with nonsmokers (n = 12) and that the extent of this reduction was related to the number of years smoked (229). These results support an effect of smoking on attentional performance, but cannot establish causality due to the cross-sectional design of the study. To date, there are no longitudinal human studies concerning the long-term effects of smoking during adolescence on attention problems.

The present study explores the effect of smoking on attention problems by employing the discordant monozygotic (MZ) co-twin design. This genetically informative design tests whether smoking causally leads to more attention problems by comparing the attention problem score of the twin who has smoked with that of his or her co-twin who has never smoked. Because MZ twins are genetically almost identical and grow up in the same family, the design corrects for confounding of genetic factors and shared family environment (165, 230-232). If the

association between smoking and attention problems is due only to genetic or shared environmental factors, one would expect the smoking and the never-smoking twin of a discordant MZ twin pair to score the same on attention. In contrast, if the association is causal, we expect that within-pair differences in smoking are associated with within-pair differences in attention problem score. In other words, the smoking twin should score significantly higher on attention problems compared with the never-smoking co-twin in MZ pairs discordant for smoking. We analyze data from different subsets of MZ twin pairs who took part in surveys spanning from childhood to adulthood. The attention problem scores were first compared cross-sectionally within adult MZ twin pairs discordant for smoking initiation. For a subsample of the discordant adult twin pairs with longitudinal data, the increase in attention problems from adolescence, when neither smoked, to adulthood (average follow-up 10 years) was compared. In adolescent MZ twin pairs with data at two ages, changes in smoking concordance and subsequent changes in attention problems were explored. If smoking causally increases attention problem scores, it is predicted that twins of a MZ twin pair will not differ in attention problems when both do not initiate smoking, whereas attention problems will be higher in the smoking twin than in the never-smoking twin when the twin pair becomes discordant for smoking.

Methods

Subjects

All participants are registered with the Netherlands Twin Register (NTR) (58, 82). The Young NTR consists of participants who were recruited as newborn twins from 1987 onward and their siblings who were included later on. At the ages of 3, 5, 7, 10 and 12 years of age, parents completed surveys about the development of the twins. At approximately 14, 16 and 18 years of age, the twins and their siblings completed surveys themselves. The Adult NTR comprises adolescent and adult twins and their family members who were recruited since 1990. From 1991 onwards, surveys are sent out to all participants of the adult NTR approximately every 2-3 years.

Data on attention problems and smoking were available for 20,824 adults (mean age, 42 years [*SD* = 15.6]; range 18-97) and 11,386 adolescents (mean age, 15 years [*SD* = 1.2]; range 8-18). Subgroups of MZ twin pairs were selected to measure the effects of smoking on attention problem scores (Figure 1).

For 1,987 adult MZ twin pairs (mean age, 34 years [*SD* = 13.8]), information on attention problems and smoking in adulthood was available from surveys sent in 2004-2005 or in 2009 (**group I**) (Table S1 in Supplement). Mean sum scores on attention problems were calculated for concordant pairs (731 pairs concordant ever smokers and 721 pairs concordant never smokers) and for 454 discordant pairs (one twin had smoked whereas the other had never smoked). For a subgroup of the 454 adult discordant twin pairs, information on attention

problems was also available at an age when the smoking twin had not yet started smoking (**group II;** *n* = 123 pairs).

In adolescents, information on attention problems and smoking at two ages was available for 648 MZ twin pairs. Their mean age was 15 years (*SD* = 0.9) at the first observation, and 17 years (*SD* = 0.6) at the second (**group** III) (Table S1 in Supplement). Two trajectories were explored: First, we looked at 71 pairs concordant for never smoking at age 15 years that became discordant at age 17 years because one of the twins started smoking (**group III-a**). Next, we studied 21 pairs who were discordant for smoking at age 15 years but became concordant at age 17 years because the never-smoking co-twin had started smoking (**group III-b)**. Finally, we selected all discordant MZ twin pairs who completed at least one survey during adolescence (mean age, 16 years [*SD* = 1.1]), and who had information on childhood attention problem scores reported by the mother at the ages 10 and 12 years (**group IV;** *n* = 123).

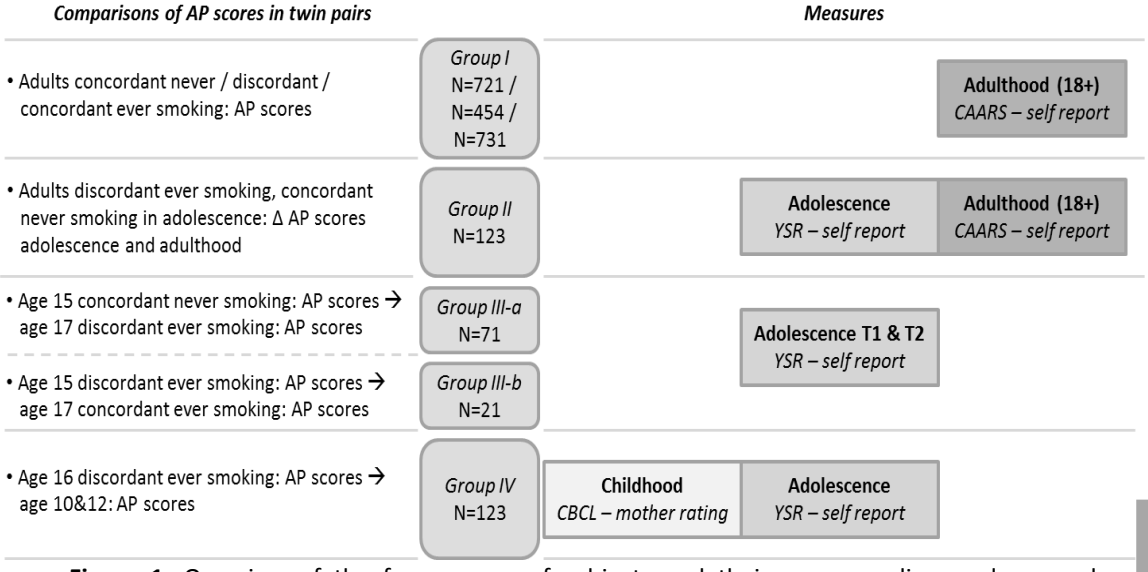

Figure 1. Overview of the four groups of subjects and their corresponding analyses and measurements. AP = attention problems; CAARS = Conners' Adult ADHD Rating Scales, attention-deficit/hyperactivity disorder index; YSR = Youth Self Report, rating scale on attention problems; CBCL = Child Behaviour Checklist, rating scale on attention problems; T1 = time-point 1; T2 = time-point 2.

Measures

Adult ADHD symptoms were measured by the ADHD index, taken from the Conners' Adult ADHD Rating Scales. The Conner's Adult ADHD rating Scales are self-report scales for adults consisting of 30 items that reflect DSM-IV ADHD symptom measures. A sum score of 12 core items makes up the ADHD index. Participants were asked to respond with 'never' (score = 0), 'once in a while' (score = 1), 'often' (score = 2) or 'very frequently' (score = 3) to statements such as 'I am always on the go as if driven by a motor' and 'I am easily distracted from what I

am doing by things I hear or see' (233). Childhood and Adolescent ADHD symptoms were measured by empirically based rating scales on attention problems from the Child Behaviour Checklist, completed by mothers, and the Youth Self Report, completed by twins. Both the Child Behavior Checklist and the Youth Self Report are part of the Achenbach System of Empirically Based Assessment (234-236) and consist of 9 statements such as 'I have trouble sitting still' and 'I have trouble concentrating or paying attention for long'. Answers on a 3-point scale—'not true' (score = 0), 'somewhat or sometimes true' (score = 1) or 'very true or often true' (score = 2)—were summed. Childhood ADHD symptoms (at 10 and 12 years of age) were rated by mothers on the nine items mentioned before plus two additional age-specific items (237). For Conners' Adult ADHD Rating Scales, Child Behavior Checklist, and Youth Self Report, the total distributions of sum scores on attention problems and hyperactivity were analyzed. Throughout this article, these sum scores are referred to as '*attention problem scores'.*

A dichotomous variable with the categories 'ever smoked' and 'never smoked' reflected smoking initiation. This variable was assessed by asking participants the following questions: 'Have you ever smoked' (answer categories 'no', 'a few times just to try' and 'yes') and 'How often do you smoke now' (answer categories 'I don't smoke regularly', 'I've quit smoking', 'once a week or less', 'a few times a week' and 'once a day or more'). Participants were classified as '*never smoked*' when they answered 'no' to the question 'Have you ever smoked'. When participants answered they had smoked before (answer categories 'a few times just to try' and 'yes'), they were classified as '*ever smoked*'. When the answer to the first question was missing but participants answered they had quit smoking or said they currently smoke ('once a week or less', 'a few times a week' and 'once a day or more'), they were also classified as '*ever smoked*'.

Data on methylphenidate (Ritalin) use collected by the NTR showed that 2.8% of twins had used methylphenidate (between 2000 and 2012). When looking only at the data collected in 2011, the prevalence was 3.9%, which is close to the 4.6% prevalence reported in Dutch children and youth 11-20 years old (238). Given these low numbers, methylphenidate use was not corrected for in the analyses.

Statistical analysis

The association between attention problems and smoking was separately determined in the overall population of 20,824 adults and 11,386 adolescents. Because there is ample evidence that sex and age are associated with attention problems (239), linear regression analysis was performed with a correction for sex (0 = male, 1 = female) and age (continuous) as follows: $Y = \beta_0 + \beta_1 X_{smoking} + \beta_2 X_{age} + \beta_3 X_{sex} + \varepsilon$, where Y is the attention problem score; β_0 is the intercept (i.e. the value of Y when all independent variables are 0); independent variables are $X_{smoking}$ (regression coefficient for smoking initiation; never vs. ever smoked), X_{age} (regression coefficient for age) and X_{sex} (regression coefficient for sex); and ε is the error term. Regression

analyses were corrected for family relatedness by using the robust cluster option in Stata (version 9.0; StataCorp LP, College Station, Texas).

In the discordant monozygotic (MZ) co-twin design, paired *t* tests were performed to determine whether attention problem scores differed significantly within twin pairs discordant for smoking. Analyses were carried out in IBM SPSS Statistics for Windows, Version 20 (IBM Corp, Armonk, New York). All analyses and the corresponding measures are depicted in Figure 1 and described subsequently.

The within twin pair difference was tested for discordant adult twin pairs (group I) both in the total group and for different ages at first cigarette in smokers (≤14 years old, 15-17 years old, or ≥18 years old). In the case of concordant twin pairs, a mean attention problem score was calculated for each pair (score twin 1 + score twin 2 / 2). The difference between the mean score in concordant twin pairs was tested with a *t* test for independent samples.

Longitudinal difference scores were calculated as the difference between attention problem scores at an adult age and at an adolescent age when the exposed twin had not yet smoked his/her first cigarette (group II). The mean difference between those two measurements was 10 years. We compared the difference score of the smoking twin and the never-smoking co-twin with a paired *t* test.

The difference in attention problem score between both twins of a twin pair (within-twin pair difference) was tested with a paired *t* test at 15 years old and 17 years old for twin pairs going from concordant never smoking at age 15 years to discordant at age 17 years and from discordant at age 15 years to concordant ever smoking at age 17 years (group III-a & group III-b). Finally, the within-twin pair difference in attention problem score was tested in adolescence (16 years old) and in childhood (10 years old and 12 years old) with a paired *t* test (group IV).

Results

Within the total sample of 20,824 adults, attention problem scores were significantly higher in ever smokers compared to never smokers (β = 0.636, 95% confidence intervals [*CI*] = 0.521-0.751, p<0.001). In 11,386 adolescents, the cross-sectional association between smoking and attention problem scores was even stronger (β = 1.127, *CI* = 1.018-1.237, p<0.001).

In adult MZ twin pairs, the mean attention problem score was higher for 731 concordant ever-smoking pairs (8.3) than for 721 concordant never-smoking pairs (7.7), with a difference of 0.60 (*CI* = 0.20-0.91, p<0.01). In concordant ever-smoking twin pairs, attention problem scores were lower when the age at which the twins smoked their first cigarette was higher, being respectively 8.4, 8.1 and 8.0 when age at first cigarette was ≤14 years old (n = 146), 15-17 years old (n = 126) and ≥18 years old (n = 39). Figure 2 shows mean attention problem scores

for the adult MZ twin pairs discordant for smoking. In the total sample (*n* = 454), the smoking twins scored 0.50 points higher than the co-twins who never smoked (*CI* = 0.06-0.88, p<0.05). In subgroups based on age at first cigarette, there was a similar pattern, with higher attention problem scores for the twins who initiated smoking compared to their never-smoking co-twins. When age at first cigarette for the smoking twins from discordant pairs was ≤14 years old, the mean attention problem score was higher than when the twins initiated smoking at 15-17 years old (difference of 1.28, *CI* = 0.22-2.35, p<0.05) and ≥18 years (1.04, *CI* = -0.13-2.20, p = 0.08). A similar pattern was seen when comparing the never-smoking twins from discordant pairs, although here both differences were not significant (smoking initiated at ≤14 years old vs. 15-17 years old showed a difference of 0.54, *CI* = -0.50-1.58, p = 0.31, and smoking initiated at ≤14 years old vs. ≥18 years old showed a difference of 1.07, *CI* = -0.05-2.19, p = 0.06).

For 123 adult MZ twin pairs discordant for smoking with longitudinal data, difference scores were calculated between adult age and the age at which the smoking twin had not yet smoked a first cigarette. When the smoking twin had not yet smoked a first cigarette, attention problem scores of both twins did not differ significantly. The attention problem score of the twin who started smoking increased more (increase of 4.4 points) compared with the co-twin who did not start smoking (increase of 3.5 points), (*CI* = 0.07-1.75, p<0.05).

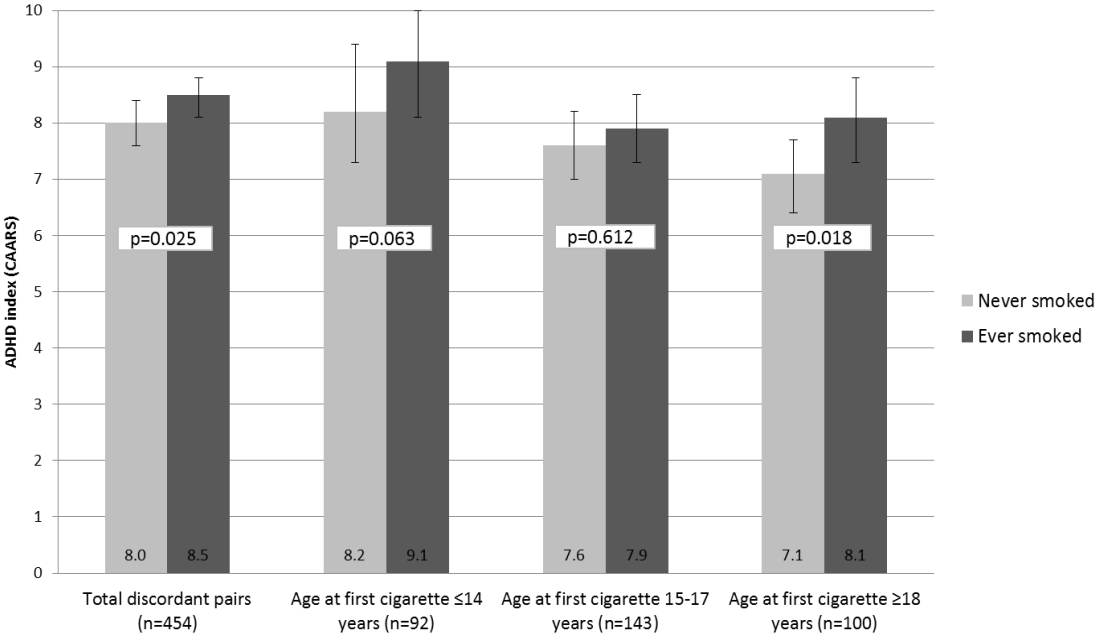

Figure 2. Mean attention problem scores (attention-deficit/hyperactivity disorder [ADHD] index) with 95% confidence interval error bars for adult monozygotic twin pairs discordant for smoking (group I). Data are shown for the total group and for different age groups, defined by the age at which the smoking twin from discordant monozygotic pairs smoked the first cigarette. *p* value for paired *t* test within monozygotic twin pairs.

In adolescent twin pairs going from concordant never smokers at age 15 years to being discordant for smoking at age 17 years, both twins scored similar on attention problems when neither smoked (see Figure 3). At age 17 years, the twins who had started smoking tended to score 0.59 points higher (*CI* = -0.07-1.25) on attention problems than the co-twins who did not smoke, but the difference was not significant (p = 0.08). Within 21 twin pairs discordant at age 15 years and concordant ever smoking at age 17 years, the smoking twins scored on average 1.05 points higher (*CI* = -0.40-2.50) than the never-smoking co-twins at age 15 years, but significance was not reached (p = 0.15). Twins scored equally high at age 17 years when both twins smoked. Of the 123 twin pairs discordant for smoking at age 16, the smoking twins scored on average 0.55 points higher (*CI* = 0.05-1.06) on attention problems than the never-smoking co-twin (p<0.05), whereas their attention problem score did not differ at ages 12 and 10 years when both twins were nonsmoking (Figure 4).

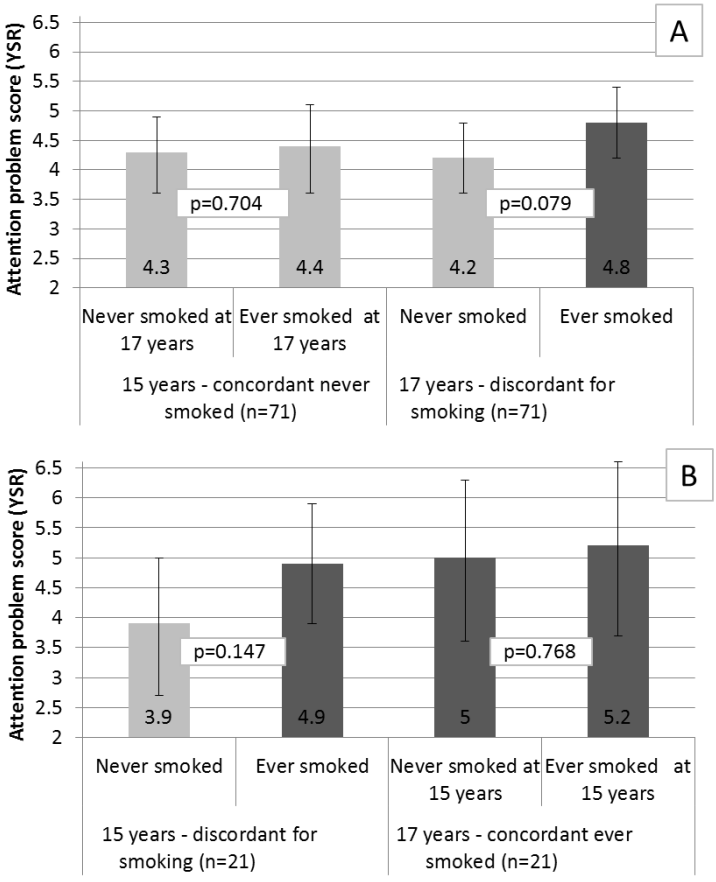

Figure 3. Mean attention problem scores (Youth Self Report [YSR]) with 95% confidence interval error bars for adolescent monozygotic twin pairs changing in concordance. (**A**) Going from concordant never smoking at 15 years to discordant for smoking at 17 years (group III-a). (**B**) Going from discordant at 15 years to concordant ever smoking at 17 years (group III-b). *p* value for paired *t* test within monozygotic twin pairs.

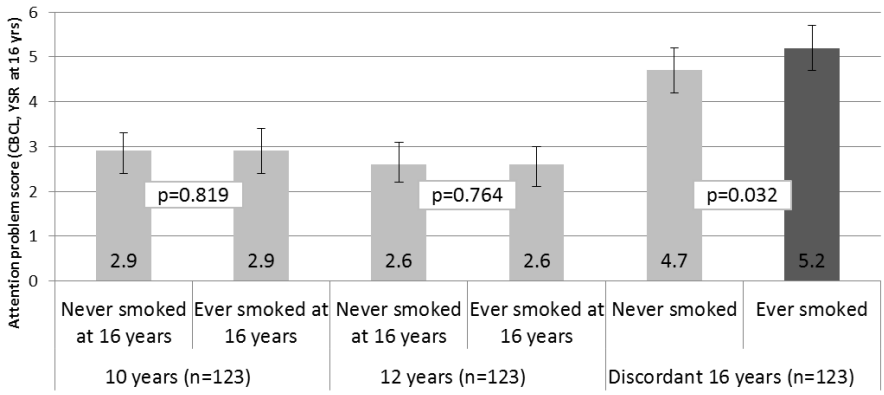

Figure 4. Mean attention problem scores with 95% confidence interval error bars for adolescent monozygotic twin pairs when they were discordant for smoking at 16 years (Youth Self Report [YSR]) and when they were 12 and 10 years old (Child Behaviour Checklist [CBCL]) (group IV). Scores at age 16 are from self-ratings, and scores at ages 10 and 12 are rated by the mother of the twins. *p* value for paired *t* test within monozygotic twin pairs.

Discussion

The present study implies that adolescent smoking leads to higher attention problem scores, with the effects lasting into adulthood. This is the first time that this finding has been reported in humans based on longitudinal data spanning from childhood to adulthood. Our results provide further support for the hypothesis that smoking affects the brain and thereby increases attention problems, as suggested in animal studies (8, 220).

As shown by earlier work of the NTR, the ADHD phenotype seems to be less heritable in adults than in children (239). The effect of nicotine use on cognitive functioning is possibly one of the factors involved in the individual differences in ADHD symptoms observed in adults. Family members are more similar in their smoking behaviour because of genetic and shared environmental factors, but unique environmental factors can cause them (in particular MZ twins) to differ with regard to the initiation of smoking (26, 80, 81). Individual differences in the initiation of smoking could lead to higher attention problem scores for individuals who start smoking compared with non-smokers.

Although cigarette smoke contains many harmful components (240), animal research suggests nicotine is the causal agent in the relationship between smoking and attention problems (8, 220, 225, 241). Regarding the biological mechanism behind the negative effect of nicotine on attention, Counotte *et al.* (2011) point to the role of metabotropic glutamate levels (8). It is suggested that the exposure to nicotine during adolescence affects synaptic signaling mechanisms involving metabotropic glutamate signaling in the PFC. These specific mechanisms are important for plasticity and synaptic maturation, explaining the effect on cognitive functioning (241). When Counotte *et al.* (2011) exposed rats to nicotine during

adulthood instead of during adolescence, there were no long-term consequences for attentional performance. In the present study, the smoking twins of the discordant adult twin pairs scored higher on attention problems than the never-smoking co-twins, even when smoking was initiated at ≥18 years old. This finding could be explained by the fact that the development of the PFC is delayed during adolescence compared with other cortical areas and development continues into young adulthood (225, 242, 243). In our own data, adult smoking twins scored on average 0.98 points higher (*CI* = 0.02-1.93) than their never-smoking co-twins when smoking was initiated at 18-20 years ($p<0.05$, n = 80), whereas the within-twin pair difference was 0.79 points (*CI* = -1.27-2.84) when smoking was initiated at 21-22 years old (p = 0.42, n = 14), and only 0.17 points (*CI* = -2.22-2.56) when smoking was initiated at ≥23 years old (p = 0.88, n = 12). Given the small sample sizes, these results need to be interpreted with caution. Further research should determine whether smoking has an effect on attention problems only when occurring while the brain is still developing or also when smoking is initiated later on in life. The minimum legal age to smoke or purchase cigarettes varies worldwide from 15-21 years (244). In the Netherlands, the legal age for smoking has been raised from 16 to 18 years since January 1, 2014, in accordance with recommendations by the European Union (245). Of the 28 European Union states, 5 still have a minimum age limit of 16 years (246). Given the results of the present study, it seems important that the legal age for smoking be raised to 18 years and preferably higher. In the city of New York, a new law was adopted raising the minimum age to smoke to 21 years (247).

When smoking was initiated at ≤14 years old (compared to 15-17 years old or ≥18 years old), both the smoking and the never-smoking twin of adult MZ twin pairs discordant for smoking scored higher on attention problems. Adolescents with ADHD are not only more likely to initiate smoking (56) but also are more likely to do so at a younger age (212, 248). Because MZ twins are genetically almost identical and ADHD symptoms are heritable (239), genetic factors causing the smoking twin to score higher on attention problems are also present in the never-smoking co-twin, causing him or her to score higher as well. Because the smoking twins score even higher on attention problems than their never-smoking co-twin, an additional causal effect of cigarette smoking is suggested on top of a possible genetic vulnerability.

The brain is also vulnerable to tobacco smoke during childhood. Max *et al*. (2013) reported that exposure to secondhand smoke (measured by self-report and cotinine level) was significantly associated with a higher chance of ADHD in children 4-15 years old, after controlling for sociodemographics, maternal smoking during pregnancy, and preschool attendance (249). Exposure to nicotine can occur through maternal smoking during prenatal development (in utero). Genetically informative studies demonstrated that prenatal nicotine exposure significantly increased the risk of ADHD and conduct problems in young children (250, 251). The above-described studies indicate that exposure to tobacco smoke can result in more attention problems, even when it is through secondhand smoking or prenatal exposure. In the case of secondhand exposure to tobacco smoke, exposure levels are usually a lot lower

than when a person smokes him of herself (0.03-0.18 nmol/mL vs. 7.92-39.99 nmol/mL total cotinine, respectively (252)), suggesting that even a low exposure can have an effect on ADHD scores. In the present study, exposure to nicotine through smoking was assessed by asking participants if they ever smoked before. Attention problem scores for individuals who replied with 'No', 'A few times just to try' and 'Yes' when asked 'Have you ever smoked before?', were 7.76, 8.40 and 8.08 in 20,824 adults and 4.45, 5.25 and 5.90 in 11,386 adolescents. As shown by these scores, individuals who stated they had smoked a few times already had elevated attention problem scores. Further work needs to establish at what quantity nicotine negatively affects cognitive functioning in humans. To determine whether the effect is restricted to attention, the association of smoking with the separate dimensions of the ADHD phenotype, should also be investigated.

Despite the strong design, the present study has some limitations. Some subgroups of adolescent MZ twins were small with 71 and 21 complete twin pairs. Not many of the 1,987 adult and 648 adolescent MZ twin pairs were discordant for smoking. This finding is to be expected because smoking initiation and nicotine dependence are moderately to largely heritable (26, 80, 81), making it more likely that both twins of a MZ twin pair are similar in their smoking status. Although these particular groups were small, we still observed a trend. This trend was in line with the finding that in a larger group of 123 twin pairs discordant for smoking at age 16, attention problem scores were significantly higher for the smoking twin.

Twins who smoke may also differ from their never-smoking co-twin when it comes to the use of other substances and sociodemographics (3, 253). Educational achievement did not differ within twin pairs discordant for smoking in adults ($p = 0.98$) and adolescents ($p = 0.49$). Smoking twins more often drank alcohol at least two to four times a month compared to their never-smoking co-twin (75.5% vs. 67.8% in adults [$p<0.05$] and 58.5% vs. 46.8% in adolescents [$p<0.001$]) and more often initiated cannabis use (30.1% vs. 16.1% in adults [$p<0.01$] and 16.2% vs. 3.4% in adolescents [$p<0.01$]). When correcting for frequency of alcohol consumption, smoking twins still scored higher on attention problems than their never-smoking co-twin in 433 adult discordant pairs (difference of 0.43 points, *CI* = 0.01-0.84, $p<0.05$) and 94 adolescent discordant pairs (0.64, *CI* = 0.06-1.21, $p<0.05$). After correcting for cannabis initiation (yes/no), adolescent smoking twins continued to score higher on attention problems than their never-smoking co-twin in 118 pairs (0.54, *CI* = 0.03-1.05, $p<0.05$); for adults there were too few data on cannabis use. Although the smoking and never-smoking twins differed in alcohol and cannabis use, it appears this did not affect the results.

In conclusion, our analyses provide evidence for a negative effect of smoking on ADHD-related symptoms. This knowledge is of great importance since smoking is highly prevalent worldwide (61) and it is usually initiated during adolescence or young adulthood (13). Both smoking and ADHD are influenced by genetic factors. We have now shown that, besides existing individual differences due to genetic background, a person's score on attention problems can increase by

smoking. Previous studies have reported that adolescents with ADHD are more likely to initiate smoking (55, 56, 213, 219), making adolescents with preexisting ADHD an important target group for smoking prevention programs. When these adolescents initiate smoking, this could have an additional negative effect on their attention problems. Ongoing efforts toward preventing smoking are therefore recommended, particularly in adolescents or young adults with ADHD.

Supplement

Methods

In the adult twins of group I, age at first cigarette was determined by the question 'At what age did you smoke your first cigarette?', with answer categories '11 years or younger', '12 years', '13 years', '14 years', '15 years', '16 years', '17 years', '18 years or older' (obtained from surveys sent in 1993, 1995, 1997 & 2009). When participants answered this question in multiple surveys, but gave slightly different answers (with a maximum of 2 years difference), the lowest age was selected (16.5% of the cases). Age at first cigarette was coded as unknown when answers between surveys differed 3 or more years (5.6%). When information on the variable age at first cigarette was available, the attention problems score from before this age was used (21.1%). If age at first cigarette was not available but the smoking twin answered 'No' when asked 'Have you ever smoked?' in one of the adolescent surveys, the attention problems score from that time-point was used as the pre-smoking measure (remaining 78.9%).

Table S1. Socio demographic characteristics and concordance on smoking status

Adult MZ twins (Group I) n = 1,987 pairs / 3,974 individuals		**Adolescent MZ twins (Group III) n = 648 pairs / 1,296 individuals**	
Concordant never smoking, *n pairs* (%)	731 (36.8)	Concordant never smoking, *n pairs* (%)	500 (77.2)
Concordant ever smoking, *n pairs* (%)	721 (36.3)	Concordant ever smoking, *n pairs* (%)	76 (11.7)
Discordant, *n pairs* (%)	454 (22.8)	Discordant, *n pairs* (%)	63 (9.7)
Concordance missing, *n pair* (%)	81 (4.1)	Concordance missing, *n pairs* (%)	9 (1.4)
Age, M (*SD*)	33.8 (13.8)	Age, M (*SD*)	15.0 (0.9)
Sex, *n individuals* (%)		Sex, *n individuals* (%)	
Male	1,050 (26.4)	Male	414 (31.9)
Female	2,922 (73.6)	Female	882 (68.1)
Educational achievement, *n individuals* (%)		Educational achievement, *n individuals* (%)	
Low	567 (15.3)	Low	351 (33.5)
Intermediate	1189 (32.1)	Intermediate	293 (28.0)
High	1,943 (52.5)	High	403 (38.5)
Employment, *n individuals* (%)			
Fulltime (>32 hrs)	1,170 (35.3)		
Part time (≤32 hrs)	1,022 (30.8)		
No paid job	1,121 (33.8)		
In a steady relationship, *n individuals* (%)			
Yes	2,456 (65.9)		
No	1,273 (34.1)		

Concordance was missing if it could not be determined due to missing answers for one or both of the twins; to determine educational achievement the participants were asked about their current educational level, this variable had the following categories: primary school/lower vocational schooling (low), intermediate vocational/upper secondary school (intermediate) and upper vocational/university (high); the employment category 'No paid job' included the status of housewife/man, student and involuntarily unemployed.

Chapter 9.

Summary and Discussion

In this thesis, smoking and co-occurring addictive behaviours were investigated with the help of several genetically informative designs. Below I first summarize the most important results per chapter and then discuss these findings within a broader context.

Summary

In *chapter 3*, a simple question on smoking expectancy ('Do you think you will smoke in a year's time?') predicted future smoking behaviour in never and former smokers, but not in current smokers. This was tested by measuring smoking expectancy and smoking status at baseline, and then assessing smoking status again two years later. These analyses were corrected for a number of confounders among which age, gender, education, self-reported health and in the case of (former) smokers, (former) smoking frequency and quantity. Whether or not an individual predicted their future smoking behaviour correctly was partly heritable. Genetic factors explained 59% of the variation in the ability to predict future smoking in adolescents and 27% in adults. The remainder was explained by unique environmental factors in both adolescents and adults.

The aim of *chapter 4* was to elucidate the mechanism behind spousal resemblance for smoking. First, findings from previous studies were confirmed by showing that smoking behaviour of spouses correlates more than would be expected by chance. An individual who smokes was more likely to have a spouse who smokes as well, and vice versa. For both ever smoking and current smoking, spousal resemblance was higher for a more recent compared to a less recent cohort (cohorts: 1997-2000, 2000-2005 and 2009-2013). This increase was mostly driven by a rise in the number of couples in which neither smoked. A higher age of men was associated with a lower spousal resemblance for ever smoking. By utilizing data of twins and spouses, it was shown that the resemblance between spouses in smoking behaviour was most likely due to phenotypic assortment. Under phenotypic assortment, spouses select each other on phenotype and are therefore genotypically more similar than two randomly paired individuals. Since smoking is moderately to highly heritable this has consequences for the offspring of smoking parents, which will, on average, have a higher genetic risk of smoking.

In *Chapter 5*, observational associations between smoking behaviour and caffeine consumption through coffee, tea, cola and energy drinks were tested in a typical 'coffee-drinking country' (the Netherlands) and a typical 'tea-drinking country' (the United Kingdom). After correction for age, gender, education and social class, we found a positive association between smoking and caffeine use. This association was consistent across the two countries and for total caffeine, coffee and cola. For tea use, there was a negative association in the Dutch sample (smokers consumed less tea) and a positive association in the British sample (smokers consumed more tea). A higher age was associated with a higher consumption of total caffeine, coffee and tea but with a lower consumption of cola and energy drinks. Women consumed less total caffeine, coffee and cola than men. In the Dutch sample women consumed more tea than men while there was no association between gender and tea use in

the British sample. Finally, a higher educational level was associated with a lower consumption of total caffeine, coffee, cola and energy drinks. Again, in the Dutch sample a higher educational level was associated with a higher consumption of tea while there was no association in the British sample.

In *chapter 6*, explanations for the observational association between smoking and caffeine consumption as reported in chapter 5 were explored with three methods: bivariate twin modeling, LD-Score regression and Mendelian randomization analysis. The first two methods were utilized to estimate the correlation between genetic influences on smoking and genetic influences on caffeine consumption, while the third method was employed to explore causal effects. Results were remarkably consistent in showing that there was a considerable genetic correlation between smoking and caffeine consumption (*rg*=0.4-0.5). The positive observational association between smoking and caffeine consumption was mostly due to these correlated genetic factors. Mendelian randomization analysis provided no evidence for causal effects of smoking on caffeine consumption or of caffeine consumption on smoking, but this may have been due to a lack of power. These findings suggest that the initiation of smoking may be especially undesirable for individuals who use a lot of caffeine. Given their genetic susceptibility they are more likely to also smoke more heavily or to more easily become nicotine-dependent.

Chapter 7 focussed on the heritability of sugar consumption and the association with substance use. Consumption of sugar-containing drinks (e.g. soft drinks, coffee or tea with sugar) was measured, as was the use of five addictive substances (nicotine, alcohol, caffeine, cannabis and illicit drugs). By employing a bivariate twin model, it was tested whether sugar consumption (high vs. low consumption of sugar-containing drinks) and substance use (high vs. low substance use) were associated and whether this association was due to genetic and/or environmental factors. We found that sugar consumption was 48% heritable with the remaining variation being explained by unique environmental factors (52%). For substance use this was 62% and 38%, respectively. There was a moderate genetic correlation between sugar consumption and substance use (*rg*=0.24). Overall, these findings indicate that sugar consumption is influenced by genetic factors to a considerable degree and that neuronal circuits underlying the development of both addiction and obesity may be related. The unique environmental correlation was *re*=0.20, suggesting that there are also environments that influence both sugar consumption and substance use (e.g. social situations).

Finally, *chapter 8* describes a study that puts forward evidence for an adverse effect of smoking on attention problems. Such a causal association had been suggested in animal research, but there was no convincing evidence from human research yet. In this study, the discordant monozygotic (MZ) co-twin design was applied. This genetically informative design tests whether smoking causally leads to more attention problems by comparing the attention problem score of a twin who has smoked with that of his or her co-twin who has never

smoked. Because MZ twins are genetically almost identical and grow up in the same family, the design corrects for confounding of genetic factors and shared family environment. We found that in adult twin pairs discordant for smoking, the smoking twin had significantly more attention problems than their non-smoking cotwin. With longitudinal data it was shown that during adolescence, when neither of the twins smoked, this difference in attention problems did not yet exist. These results provide further support for the hypothesis that smoking causally increases attention problems, as suggested in animal studies.

Discussion

The results of this thesis corroborate with the large body of existing literature in showing that addictive behaviour (including smoking, caffeine use and high sugar consumption) is moderately to highly heritable. Gaps in the literature have also been addressed by focusing on the nature of the associations between different types of addictive behaviour, by studying the mechanisms underlying spousal resemblance for smoking and by exploring the (causal) effects of smoking on attention problems. Here I will discuss the most important findings of this thesis in a broader context and reflect on their possible implications.

Identifying groups at high risk of smoking

Smoking is one of the most harmful addictive behaviours when considering its contribution to morbidity and mortality (1). It is desirable to prevent the initiation of smoking as much as possible, especially since the heritability of nicotine dependence (75%) is much higher than that of smoking initiation (44%) (26). It is becoming increasingly clear that delivering treatment or preventive measures with a personalized approach is more effective than providing one generic program for all (108). In order to personalize preventive efforts in the field of smoking, the identification of risk groups may be useful. When individuals who are at high risk of smoking are identified, preventive measures can be either personalized or targeted so that those who are most vulnerable to smoking receive the highest possible benefit. One way of distinguishing individuals at high risk of smoking from those at lower risk is by enquiring about someone's expectations. Smoking expectancy, which was explored in chapter 3, reflects a single, simple question and is capable of predicting future smoking behaviour in never and former smokers. Measuring smoking expectancy could thus be a reliable and easy way of defining never smokers who are at risk of initiating smoking and former smokers who are at risk of relapsing. Similar single-item measures for identifying risk groups have been investigated in previous studies. Kotz, Brown, & West (2013) investigated the predictive value of the 'Motivation To Stop Scale' (MTSS), a single-item measure with seven answer categories, designed to predict which smokers will attempt to quit smoking in the future and which will not. The MTSS provided a strong and accurate prediction of quit attempts in current smokers (85). In another study, 'susceptibility to smoking' was measured in never-smoking adolescents. This single-item measure aimed to predict which adolescents would start smoking in the future and it was defined as not being able to rule out the idea of smoking one year later (dichotomous variable). Adolescents who were susceptible to smoking were much more likely

to initiate smoking than those who were not (79). A big advantage of smoking expectancy, as presented in this thesis, is that it can be applied to individuals of all smoking statuses. This is in contrast to the two other two single-item measures described here. However, after correction for confounders smoking expectancy was not able to predict future smoking status in current smokers. This poorer predictive value of smoking expectancy in smokers was mostly driven by incorrect expectancies of smokers who said they would 'certainly not' or 'probably not' smoke in a year's time, but who did still smoke two years later (see Table S1 in chapter 3). Such incorrect expectancies emphasize how difficult it is for smokers to stop smoking. It has been noted many times that most smokers attempting to quit will fail in remaining abstinent. One study showed that only 3%-5% of self-quitters (those quitting without treatment/help) achieved prolonged abstinence for 6-12 months after a quit attempt (88, 89). An explanation for the greater predictive value of the MTSS in smokers could be that its ability to predict quit attempts was tested, instead of prolonged abstinence as we tested (85). In conclusion, it is demonstrated in this thesis that a single-item measure can be useful when aiming to predict future smoking behaviour. Such information could be of use for prevention programs with the goal of preventing smoking initiation in youth. It may for instance be worthwhile to start off a school-based intervention program by assessing the risk of smoking with a question on smoking expectancy. Those at higher risk can then be given a personalized program, while all others receive a generic intervention.

Apart from asking people about their own views with single-item measures such as smoking expectancy, another indication for being at high risk of smoking can be derived from chapter 4 of this thesis. In that study, spousal resemblance for smoking was explored and it was found that such resemblance was due to phenotypic assortment. Under phenotypic assortment, spouses select each other based on their phenotype which means that the offspring of two smoking parents is at higher genetic risk of smoking (84). The heritability of nicotine dependence (75%) is higher than that of smoking initiation (44%) (26). Thus, the increased risk in children of smoking parents especially relates to their vulnerability to become dependent to nicotine after smoking is initiated. From this it follows that they can benefit most from programs aimed at preventing the initiation of smoking (when they do not start smoking, they cannot develop nicotine dependence). Such preventive programs may increase in effectiveness when the smoking status of parents is employed in order to identify high risk groups. After high risk adolescents have been identified, their personal views or expectations about smoking could also be incorporated. For instance, a child of two smoking parents who scores high on smoking expectancy (thus thinking it is likely that he/she will smoke in a year's time) would be at the high end of risk for smoking. An approach where prevention is personalized depending on the risk of smoking may be more (cost-)effective than the current method of delivering one, generic prevention program to all school-going youth. This is of particular importance given the disappointing effects of school-based interventions. For example, a Dutch school-based prevention program consisting of lessons on knowledge, attitudes and social influences had a positive effect on high-SES children only (254). Another

study showed that a Dutch school-based prevention program that is applied by ~75% of all secondary schools in the Netherlands was not effective at all (regardless of SES group) (255). These findings stress the need for more effective school-based approaches to prevent smoking. This may be achieved by identifying (high) risk groups and by applying more personalized approaches. Variation in the initiation of smoking is explained by the environment for 56% with most of this estimate consisting of common environment influences (51%). This includes the family environment and thus parents (26). Another advice would therefore be to involve parents (more) in the prevention of smoking. A recent Cochrane review study provided moderate quality evidence that family-based interventions have a positive effect on preventing smoking initiation in children and adolescents (256).

Genetic overlap between addictive behaviours

Results in chapters 6 & 7 demonstrated that the clustering of different addictive behaviours (smoking and caffeine use, substance use and sugar consumption) was for a considerable part due to genetic factors. This has previously also been shown for example for smoking, alcohol and caffeine use (157) and for the association between disordered gambling and smoking, alcohol and caffeine use (257). This thesis and the current literature thus indicate that certain genetic variants increase a person's risk of using several addictive substances and/or engaging in more than one addictive behaviour. Obvious candidates for such genetic variants are those that code for receptors of neurotransmitters that are involved in the brain's reward system, such as dopamine (258) or serotonin (259). Significant associations between genetic variants located in or near dopamine receptor genes or serotonin transporter genes and measures of alcohol use/dependence have been found through candidate gene studies and GWAS (260). For smoking initiation, coffee consumption and BMI, there is also evidence for association with a gene that affects the dopaminergic system. This gene (*BDNF* gene) codes for a neurotrophin that regulates the survival of dopaminergic neurons (33, 164, 206). In addition, a gene that codes for a protein that converts dopamine into norepinephrine (*DBH* gene), was associated with smoking cessation (33). When searching for genetic similarities between substance use and sugar consumption, genetic variants coding for opioid receptors may also be of interest. The opioid receptor gene *OPRM1* was associated with having higher preferences for sweet and fatty foods and measures of overeating and BMI, but also with dependence on alcohol, heroin and cocaine (261-264). Recently, another interesting finding was published. A genetic variant in the *CHRNA5-A3-B4* gene region, robustly associated with the number of cigarettes smoked per day in smokers, predicted an increased BMI and waist and hip circumference in non-smokers (207, 208). Together, the findings described here suggest that there are general genetic factors that influence the (in)ability to resist rewarding stimuli. However, much is still unknown about the exact genes that are involved in the risk of addictive behaviour and it is becoming increasingly clear that the development of both substance dependence and obesity is determined by a complex interplay of numerous environmental and genetic factors (265, 266). A next step would be to further asses which genetic variants are involved in the

development of addictive behaviour, and to what degree these variants overlap between the different kinds of behaviour.

A recent approach to estimating genetic correlations, which was also applied in chapter 6 of this thesis to data on smoking and caffeine use, is LD-Score regression (162, 163). This technique estimates the genetic correlation between two traits by utilizing effect-size estimates of all SNPs that are included in genome-wide association (GWA) meta-analyses. Briefly, the expected product for the Z scores of the association between a SNP and the two phenotypes is modelled as a function of the linkage disequilibrium (LD) the SNP has with all neighboring SNPs (i.e. the LD-score). An interesting application of LD-Score regression would be to test the genetic correlation between substance use and sugar consumption. In chapter 7 of this thesis we found a genetic correlation of 0.24 through bivariate twin modeling. It would be good to complement this analysis with a genetic correlation based on effect-size estimates from GWA meta-analyses. At the moment this is not possible because no GWAS on sugar consumption have been published. As an alternative for sugar consumption, summary statistics of GWAS on BMI could be utilized. As such it would be possible to study the overlap in genetic variants associated with BMI, which is causally increased by high sugar consumption (50), and substance use. A recent overview of LD-Score regression findings included a significant genetic correlation of 0.29 between BMI and cigarettes per day while SNPs for BMI and ever vs. never smoking correlated 0.20 (163). These results emphasize the importance of further research to the aetiology of high sugar consumption and the (genetic) overlap with other addictive behaviours.

For now, the most important conclusion is that individuals who are highly dependent on one substance, such as nicotine, are more likely to also be or become dependent on another, such as caffeine. From this it follows that individuals who are dependent on multiple substances probably have a high genetic susceptibility to addictive behaviour in general, and they may therefore find it more difficult to remain abstinent than others. It may also be that those wanting to quit using one (harmful) substance, could best switch to using another (less harmful) substance as a 'substitute'. This kind of harm reduction has for example been proposed for cannabis as an alternative to alcohol, prescription drugs and/or illicit drugs (267, 268). Under this assumption it would be easier to stop smoking when switching to the use of (large amounts of) caffeine. It is unlikely that this holds true for smoking and caffeine however, given the fact that caffeine consumption has been associated with failed smoking quit attempts and induced craving for cigarettes (43, 269-271). In chapter 7 the consumption of different combinations of substances (including smoking, alcohol, caffeine, cannabis and illicit drugs) were described (Figure S1). In a group of men and women who used two substances, the most common combination was smoking-alcohol, closely followed by alcohol-caffeine and smoking-caffeine. For those using three substances the most frequently occurring combination was smoking-alcohol-caffeine. A few studies explored associations between these often co-occurring substances in clinical samples and in some cases explored the relationship with

treatment outcomes. Men with both nicotine dependence and alcohol dependence were found to have higher levels of the Nicotine Dependence Syndrome Scale (NDSS) than men with nicotine dependence only (272). In a group of alcohol-dependent men and women, those who were current smokers and nicotine-dependent individuals had a greater severity of alcohol dependence than those who did not smoke/were not nicotine dependent (273). A final study measured caffeine consumption and family history of alcoholism in pregnant women, and tested the women's ability to reduce caffeine consumption during pregnancy. Interestingly, caffeine-dependent women with a family history of alcoholism were not able to reduce or eliminate caffeine use during pregnancy while caffeine-dependent women without a family history of alcoholism were able to do so (274). It is important that health professionals working in (clinical) practice are aware of such associations and the possibly underlying (genetic) mechanisms.

Causal effects of smoking

In chapters 6 & 8 the causal effects of smoking were explored. In chapter 8, the effect of smoking on attention problems was tested with the powerful discordant MZ co-twin design. The results pointed to a causal increase of attention problems due to smoking. It is the first time that such causality was indicated in human data and it emphasizes that smoking can have detrimental effects not only on physical, but also on mental health. As discussed in a commentary on our findings by London (2015), previous studies have provided evidence that there are differences between smokers and nonsmokers on many executive functioning domains, including attention problems but also cognitive impulsivity, working memory and risk taking during decision making (275). Future studies are needed to test whether these differences are also the result of smoking. The most obvious implication of these findings is that smoking initiation should be prevented or at least delayed as much as possible. One way of achieving this is by increasing the legal age at which someone is allowed to smoke or buy cigarettes. In the Netherlands, the legal age at which cigarettes (and alcohol) can be bought has been raised from 16 years to 18 years in 2014 (245), but our results imply that this may not be enough. Smoking twins still differed from their non-smoking co-twin if smoking was initiated at 18 years or older, implying that it is still detrimental for the developing brain at that age. An example of a stricter and possibly more suitable policy is that implemented in the city of New York, where a law raising the minimum age to smoke to 21 years was adopted in 2013 (247).

More studies are necessary to strengthen the evidence for a causal effect of smoking on attention problems, and thereby further assess the need of increasing the legal age of smoking. To obtain stronger causal inference from observational data, multiple (genetically) informative study designs can be and need to be applied (276). Besides the discordant MZ co-twin design, another way of testing causal effects of smoking is through Mendelian randomization analysis (MR). This technique employs genetic variants as a proxy, or an instrument for a particular trait, which reduces effects of confounding and reverse causation

(156). Future research could include MR analysis to test the effect of smoking on attention problems/ADHD symptoms. MR is increasingly being used to study presumed causal effects of smoking, among which the possible adverse effects of smoking on mental health (277). One example is the nature of the association between smoking and depressive and anxiety disorders. So far, research findings in this area were inconsistent. Some suggested that smoking causally leads to depression/anxiety (278) or the other way around that depression increases smoking (self-medication hypothesis) (279), while others concluded that the association arose from shared familial factors (230). When MR analysis was carried out in >120,000 individuals, there was no evidence for a causal effect of smoking heaviness on depression or anxiety (280). Another large MR study of >63,000 individuals also provided no evidence for a causal influence of smoking on depression, while a direct effect of smoking on psychotic conditions (e.g. schizophrenia) seemed likely (281). It would be suitable to perform similar MR analyses in order to test the causal effect of smoking on attention problems.

When reflecting on the causal effect that smoking may have on attention problems, an important group to consider is that of early adolescents who are diagnosed with ADHD and/or those who suffer from attention problems. In previous, longitudinal work it has been shown that youth diagnosed with ADHD are more likely to initiate regular smoking (56). This may be because these individuals are more impulsive and therefore more prone to experiment with cigarettes, or because they use cigarettes as a type of self-medication. In this thesis it has now been shown that the direction of causality can also go from smoking to attention problems. For adolescents who experience attention problems even before smoking is initiated, this effect of smoking may be most disadvantageous. It therefore seems justified to put more effort into preventing smoking in adolescents with ADHD/attention problems. Informing adolescents with ADHD better about the possible risks of smoking for attention problems might deter them from initiating smoking. In a qualitative study, 39 children and adolescents diagnosed with ADHD (aged 9-17 years) were interviewed about their experiences in everyday life related to the disorder. All participants described that they struggled with their symptoms and reported problems related to school and school achievements (282). Given the problems that youth with ADHD/attention problems themselves report, they may be more open to warnings about (relatively) short-term effects of smoking on attention problems, than they are to warnings about long-term risks such as lung cancer and cardiovascular disease.

In chapter 6, Mendelian Randomization was applied to study the association between smoking and caffeine use, and particularly to test if smoking causally influences caffeine use, or vice versa. No evidence for causal effects was found. As discussed in chapter 6, this may have been due to low power. When assuming that there are no causal effects, it would not be necessary to, for example, adjust caffeine consumption when trying to quit smoking. There is no consensus about the (causal) nature of the association between smoking and caffeine yet, however, since other studies did find evidence for causality. Some experimental and animal studies have suggested that smoking causally increases caffeine use (150-152) while others

reported that caffeine use causally increases smoking (153-155). In contrast, a recent study found a causal effect such that caffeine decreases the number cigarettes smoked per day (181). Overall, the evidence is inconclusive and more and larger (MR) studies are needed to figure out the causality in this relationship. Even though we did not find direct causal effects, there was a considerable overlap in the unique environmental influences on smoking and the unique environmental influences on caffeine use. These findings imply that some environments can evoke both the urge to smoke and the urge to consume caffeine. This information may be important when trying to quit smoking. Environments where one would normally consume caffeine are likely the same environments where one would normally smoke and may therefore best be avoided in the first stages of a quit attempt, when the risk of relapse is the highest (88, 89). When caffeine consumption and smoking of cigarettes often occur at the same time, this could evoke an indirect reciprocal interaction where the use of one substance acts as a cue to use the second substance (43). This line of reasoning is supported by research showing for example that having a coffee in a café or at home after lunch/dinner induced craving for cigarettes in adult current smokers (270, 271). It may be that this is only important for adults, because adolescent smokers who were measured 3 weeks after a quit attempt did not show a lower self-efficacy to stay quit after having consumed coffee (283).

Future research into novel addictive behaviours

The prevalence of smoking has steadily decreased over the past years. In 1991-1997, 65.8% of men and 56.8% of women had ever smoked, while 38.0% and 33.2%, respectively were current smokers. By 2009-2013 this had decreased to 47.7% and 41.8%, respectively for ever smoking and 15.6% and 15.3%, respectively for current smoking. Smoking prevalences in the NTR were somewhat lower than in the general Dutch population in 2014 where 60% of men and 50% of women had ever smoked, while 28% and 22%, respectively were current smokers (2). This slight bias is most likely due to a relatively high proportion of highly educated participants (107), for which we corrected throughout this thesis by including education as a covariate. Along with the decrease in smoking of regular cigarettes, there is currently a rise of 'novel' addictive behaviours such as the use of e-cigarettes and water pipe (also referred to as 'hookah' or 'shisha'). In future studies, it is therefore likely that the focus will shift more towards such traits. The debate on the pros and cons of e-cigarettes is still ongoing, with the biggest concerns being their potential health effects and the possibility that non-smokers will start using them (284-286). As for water pipe, users tend to underestimate, or are not aware of, the negative health effects (287, 288). In an analysis of data from the 2011-2014 National Youth Tobacco Surveys in the US, it was found that while the use of cigarettes is on the decline this is accompanied by increases in the use of e-cigarettes and water pipe. As a result, there was no change in overall use of tobacco-containing products, in spite of the decrease in cigarette smoking (289). In this thesis I present evidence for a causal effect of smoking on attention problems. Animal research has suggested that this causal effect works through nicotine that is inhaled through cigarette smoke (8). This would mean that while the use of e-

cigarettes and/or water pipe may be less detrimental when it comes to the long-term risks of developing cancer or cardiovascular disease, both may still have a detrimental effect on attention problems. This also emphasizes the need to better understand the aetiology of the use of products such as e-cigarettes and water pipe. As a first step it would be interesting to explore the heritability of such behaviours. Another important question to ask is whether the (genetic and environmental) risk factors for using e-cigarettes and water pipe are the same as the risk factors for using regular cigarettes. For a decisive answer on such questions more (twin) studies are necessary.

Another emerging and interesting area of research is the 'addictive' potential of particular nutrients such as sugar or of unhealthy foods. I looked at the heritability of sugar consumption and its overlap with substance use and found that sugar consumption was partly heritable (48%) and that there was a moderate genetic correlation with substance use. There is no scientific consensus yet about whether a particular nutrient such as sugar or other foods can be considered addictive (45, 189, 204, 205, 290). Although 'food addiction' is a relatively new topic, the addictive potential of other, non-substance related, behaviours such as gambling and gaming or internet use have been a investigated for some time. In participants of the NTR it was shown that compulsive internet use in adolescents was for 48% genetic in nature (291), while the heritability of pathological gambling was 50%-60% in American twins (292). The aetiology of the consumption of sugar and unhealthy foods and the role that environmental and genetic influences play, are becoming more and more important in today's society. A high consumption of sugar/unhealthy foods contributes greatly to the increase in overweight and obesity (49, 50). Therefore, the influence of genetic and environmental factors on such behaviours needs to be studied and it should be explored which genetic variants underlie the heritability for these traits and whether or not these are genes that are common to multiple addictive behaviours.

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Appendices.

APPENDIX I Items and scales included in NTR survey 10

Survey 10-O (developed for 65+ population)

A	**Biographical information**	First name – gender – date of birth – role within the family ('twin', 'triplet', 'quadruplet', 'partner of multiple', 'full brother/sister of multiple', 'half-brother/-sister of multiple with the same mother', 'half-brother/sister of multiple with the same father', 'adoptive/foster/step brother/sister of multiple', 'biological parent of multiple', 'biological child of multiple', 'other, namely…').
B	**Family situation**	Number of biological/non-biological children – Number of biological/non-biological grandchildren - marital status ('never been married/in a stable relationship', 'married/in a stable relationship since…[date]', 'widow/widower since…[date] ', 'divorced/broken relationship since…[date]', 'different, namely…') – ***if applicable:*** birth date and gender of partner – living situation ('alone' and/or 'with partner' and/or 'with child(ren)' and/or 'with twin sister/brother' and/or 'in an assisted living situation' and/or 'in a nursing home' and/or 'different, namely…')*. * based on Netherlands Kinship Panel Study (293)
C	**Coffee and tea**	Number of servings per day (when consumed daily) or per week (when consumed weekly) of the following beverages; coffee with caffeine, coffee without caffeine, espresso, black tea, green tea, herbal tea. When consumed rarely or never this could be indicated with 'I rarely/never consume this beverage' – takes sugar/sweeteners in coffee/tea ('yes, always', 'sometimes', 'no, never', 'not applicable')
D	**Other drinks**	Number of consumptions per day (when consumed daily) or per week (when consumed weekly) of the following drinks; diet coke with caffeine, normal coke with caffeine, diet coke without caffeine, normal coke without caffeine, diet soda, normal soda, diet fruit juice, fruit juice, diet energy drink with caffeine, energy drink with caffeine, diet energy shot with caffeine, energy shot with caffeine, sports drink without caffeine, chocolate milk (hot and cold), milk/butter milk/yoghurt drink, mineral or tap water, different, namely…, When consumed almost never possible to answer with 'I don't consume this drink regularly'.
E	**Alcohol use**	Alcohol initiation ('no', 'yes, a few times to try', 'yes') – ***if applicable:*** frequency of alcohol use in the past year ('never', 'monthly or less', '2-4 times per month', '2-3 times per week', 4-5 times per week', '6 times per week or daily') – number of glasses of beer/wine/liquor per week on average in the past year – reason for not drinking ('health reasons', 'religious beliefs', 'don't like alcohol', 'other, namely…')

F	**Sport and exercise**	Types of sport: name of sport, number of years played, number of months played per year, number of times per week and amount of minutes per time – Types of hobbies: name of hobby, number of times per week – how many hours and minutes do you cycle in an average week – how many hours and minutes do you go out for a walk in a normal week.
G	**Questions for women**	Age at onset menopause – how did menopause start ('naturally', 'artificially', 'I don't know') – urinary incontinence on a bar going from 1 till 10 (1 representing never and 10 always).
H	**Health**	How is your health ('poor', 'fair', 'reasonable', 'good', 'excellent') – memory problems ('no', 'sometimes, but it is not a problem', 'yes and it is a problem', 'yes and it is a serious problem') – length (in centimeters) and weight (in kilograms) – use of sedatives / tranquilizers ('no', 'yes, on doctor's prescription', 'yes, not on doctor's prescription') – ever needed help from a physical or manual therapist? ('never', 'yes, in the past, but not now', 'yes, now') – ever needed help from social work a mental health institution or a psychologist? ('never', 'yes, in the past, but not now', 'yes, now') – list of current conditions (diagnosed by a physician) accompanied by any prescription medicine taken for this condition – list of past conditions
I	**Wellbeing - 1**	Quality of life on a bar going from 1 till 10 (1 being the worst life you can imagine and 10 the best) – how often do you feel that you miss company ('almost never', 'sometimes', 'often') – how often do you feel excluded ('almost never', 'sometimes', 'often') – how often do you feel isolated from others ('almost never', 'sometimes', 'often')* amount of financial stress in the past year ('none/little', 'moderate', 'a lot') * Loneliness scale (294)
J	**Smoking**	Smoking initiation ('no', 'yes, a few times to try', 'yes') – current smoking frequency and quantity ('never been a regular smoker', 'used to smoke but quit', 'once a week or less', 'several times a week, not every day→ number of cigarettes per week', 'daily→number of cigarettes per day') – type of smoking material ('cigarettes, sometimes in combination with cigars/pipe tobacco', 'only cigars/pipe tobacco') – did your father /mother smoke? ('no', 'yes', 'I don't know').
K	**Religion**	Religious upbringing 'yes', 'no' – active member of a religious community at this moment ('no, I'm not religious', 'I am religious, but not actively involved in a community', 'yes, I am actively involved') – what is your religion.
L	**Wellbeing – 2**	Older Adult Self-Report adapted for ages 65+ (OASR (59)); 123 items, subscales: Internalizing (Anxious/Depressed, Withdrawn, Somatic Complaints), Externalizing (Aggressive Behaviour, Rule Breaking Behaviour, Intrusive) Thought Problems, Attention problems, answer categories: 'not at all', 'somewhat/sometimes', 'very much so/often'.

M	**Remarks**	Do you have a handicap, illness or injury that limits you in your daily life? ('no', 'yes, specification…') – have you experienced a special period in your life which caused you to answer the questions in this survey differently from what you would do normally (for example due to illness) 'no', 'yes, namely...' – room for comments about the survey

Survey 10-S ('standard', complete version of survey 10)

A	**Biographical information**	First name – gender – date of birth – role within the family ('twin', 'triplet', 'quadruplet', 'partner of multiple', 'full brother/sister of multiple', 'half-brother/-sister of multiple with the same mother', 'half-brother/sister of multiple with the same father', 'adoptive/foster/step brother/sister of multiple', 'biological parent of multiple', 'biological child of multiple', 'other, namely…').
B	**Family situation**	Birth date of parents - number of full/half/non-biological brothers/sisters – number of biological/non-biological children – marital status ('never been married/in a stable relationship', 'married/partnership/relationship equivalent to marriage since…[date]', 'widow/widower since…[date] ', 'divorced/ended relationship since…[date]', 'other, namely…') – ***if applicable:*** duration of stable relationship (in years and months) – ***if applicable:*** gender, birth date and birth country of partner – ***if applicable:*** partner is a multiple 'yes', 'no' – living situation ('I live alone' and/or 'I live with my partner/husband/wife' and/or 'I live with my child(ren)' and/or 'I live with my twin sister/brother' and/or 'I live with parents' and/or 'other, namely…')*. *Based on Netherlands Kinship Panel Study (293)
C	**Coffee and tea**	Number of servings per day (when consumed daily) or per week (when consumed weekly) of the following beverages; coffee with caffeine, coffee without caffeine, espresso, black tea, green tea, herbal tea. When consumed rarely or never this could be indicated with 'I rarely/never consume this beverage' – takes sugar/sweeteners in coffee/tea ('yes, always', 'sometimes', 'no, never', 'not applicable')
D	**Other drinks**	Number of servings per day (when consumed daily) or per week (when consumed weekly) of the following beverages; diet coke with caffeine, regular coke with caffeine, diet coke without caffeine, regular coke without caffeine, diet carbonated soft drink, regular carbonated soft drink, diet fruit juice/fruit drink, regular fruit juice/fruit drink, diet energy drink with caffeine, regular energy drink with caffeine, diet energy shot with caffeine, regular energy shot with caffeine, sports drink without caffeine, chocolate milk (hot and cold), milk/butter milk/yoghurt drink, mineral/tap water, other, namely… When consumed rarely or never this can be indicated with 'I rarely/never consume this beverage'.

E	**Alcohol use**	Alcohol initiation ('no', 'a few times, just to try', 'yes') – ***if applicable:*** age at first alcoholic drink – age at onset regular drinking – frequency of alcohol use in the past year ('not at all', 'once every month or less', '2-4 times a month', '2-3 times a week', 4-5 times a week', '6 times per week or daily') – number of glasses of beer/wine/spirits a week on average in the past year – number of days a week that you drank 1 or more glasses of alcohol in the past year ('0 days', '1 day', '2 days', '3 days', '4 days', '5 days', '6 days', '7 days')† – maximum number of glasses of alcohol you drank within a 24-hour period in the past year – 6 questions on problems related to alcohol use in the past year, answered with 'never', 'less than once a month', 'every month', 'every week' or '(almost) every day' (not being able to stop drinking once started, failed to do what was normally expected from you because of drinking, needing a first drink in the morning to get yourself going, not being able to remember what happened the night before because of drinking, number of times having had six or more drinks on one occasion, feeling guilt or remorse about your drinking)* – 5 questions on problems related to alcohol over your entire life, answered with 'no', 'yes, not in the past year', 'yes, in the past year' (other people being concerned about your drinking behaviour* **, you or someone else being injured as a result of your drinking*, feeling guilty about your drinking habits**, having the feeling you should cut down on drinking**, ever drank after getting up in the morning to calm down your nerves**). *AUDIT: The Alcohol Use Disorder Identification test (197) **CAGE: Cutting down, Annoyance by criticism, Guilty feelings, Eye-openers (295)
F	**Mood**	Personality Assessment Inventory-Borderline Features Scale (PAI-BOR (296)): 24 items, subscales: Affective Instability, Identity Disturbance, Negative Relationships, Self-Harm, answered with 'not at all true', 'somewhat true', 'mostly true' or 'very true'.
G	**Smoking**	Exposure to cigarette smoke in any of the following situations, and if so, how many years; at home when you were a child (until age 18)/at home as an adult/in other situations, namely… – did your mother smoke when she was expecting you? ('no', 'yes', 'I don't know') – smoking habits of your father, mother and/or partner ('non-smoker', 'ex-smoker', 'smokes now and again', 'smokes 1-10 cigarettes a day', 'smokes 10 or more cigarettes a day', 'I don't know', 'not applicable') - smoking initiation ('no', 'a few times, just to try', 'yes') – ***if applicable:*** type of smoking ('cigarettes, at times in combination with cigars/pipe tobacco', 'only cigars/pipe tobacco') – age at smoking initiation – current smoking frequency and quantity ('I've never been a regular smoker', 'I used to smoke but I quit', 'I smoke once a week or less', 'I smoke several times a week, not every day→number of cigarettes per week', 'I smoke every day→number of cigarettes per day') – age at onset regular smoking

		– total number of years smoked – number of serious attempts to quit smoking – use of nicotine replacements such as nicotine patches or gum when trying to quit ('no, never', 'yes, once or several times') – ***former smokers***: time since smoking cessation (in days/weeks/months or years) – ***smokers & former smokers, period of most heavy smoking***: quantity of cigarettes smoked on average a day – time between awakening and lighting first cigarette ('within 5 minutes', 'after 6-30 minutes', 'after 31-60 minutes', 'after more than 60 minutes') – difficulty to refrain from smoking in places where smoking is prohibited (e.g. train, plane, school, hospital) 'no', 'yes' – cigarette you would find hardest to give up 'first one in the morning', 'another one' – smoked more in the morning than during the rest of the day 'no', 'yes' – smoking when you were ill and spent the greater part of the day in bed 'no', 'yes'*. * FTND: Fagerström Test for Nicotine Dependence, 6 items, sum scores range between 0-10 (19)
H	**Sport and exercise**	Participates in sports regularly 'yes', 'no' – ***if applicable:*** name(s) of sport, number of years played, number of months played per year, number of times per week and amount of minutes per time – how good are you at sports, ranging from 0 'not good at all' to 8 'very good' – how many hours and minutes do you cycle in an average week – how many hours and minutes do you walk/have a welk in a normal week.
I	**Substance use**	Ever experimented with cannabis (hash/marijuana) 'yes', 'no' – ***if applicable:*** age at first use – number of times using cannabis in whole life ('1-2 times', '3-5 times', '6-10 times', '11-19 times', '20-39 times', '40 times or more') – frequency of cannabis use in period of most heavy use ('monthly or less', '2-4 times per month', '2-3 times per week', '4-5 times per week', '6 times per week or daily') – age in period of most heavy use – cannabis use in past year ('no', 'yes, incidental', 'yes, regular') – reason for cannabis use 'for pleasure', 'medicinal'. Ever tried any of the following substances: electronic cigarette with nicotine, water pipe (shisha/hookah), ecstasy, cocaine, amphetamine, ketamine, GHB, hallucinogenic mushrooms, opiates – ***if applicable:*** how old were you when you used for the first time, did you use in the past year and if yes, how many times in the past year.
J	**Self-assessment**	60 items on the subscales extraversion, neuroticism, agreeableness, conscientiousness & openness. Answer categories: 'disagree completely', 'disagree', 'neutral', 'agree', 'agree completely'*. *NEO five factor inventory (297)
K	**Health**	How is your health ('poor', 'fair', 'reasonable', 'good', 'excellent') – length (in centimeters) and weight (in kilograms) – use of sedatives / tranquilizers ('no', 'yes, on doctor's prescription', 'yes, not on doctor's prescription') – memory problems ('no', 'sometimes, but it is not a problem', 'yes and it is a problem', 'yes and it is a serious

		problem') – ever needed help from a physical or manual therapist? ('never', 'yes, in the past, but not now', 'yes, now') – ever needed help from social work a mental health institution or a psychologist? ('never', 'yes, in the past, but not now', 'yes, now') – list of current diseases/conditions – prescription medicine taken for current diseases/conditions, how often used and since when – list of past conditions – do you ever get headaches ('no, never', 'yes', 'I used to, but not any more') – frequency of headaches ('once a year of less', 'several times a year', 'about once a month', 'several times a month', 'about once a week', 'several times a week', 'almost continuously') – length of headaches ('shorter than 4 hours', '4 hours till 1 day', '1 to 3 days', 'longer than 3 days', 'it varies', 'almost continuously') – how often did you have mediocre or severe headache in your whole life ('0-4 times', '5-10 times', '11 times or more') – during the headaches, do you suffer from 'hypersensitivity to light', 'hypersensitivity to noise', 'nausea and/ or vomiting' ('yes', 'no') – how severe is the headache during most of the attacks ('light', 'mediocre', 'heavy') – aggravation of the headache by physical exercise 'yes', 'no' – is the headache usually on one side of the head 'yes', 'no' – specification of the type of headache ('throbbing or pounding', 'stabbing, pressing or pinching', 'different, namely…') – in the past year, did you suffer from back pain, neck pain, headache (no migraine), migraine, pain in abdomen or stomach, pain in joints of arms/hands/legs/feet, chest pain, toothache, pain in your face, pain somewhere else, namely… ('no', 'yes, once in a while', 'yes, a large portion of the time').
L	**Education and occupation**	Highest level of education of self, mother, father and partner (if any) on a scale ranging from 1 'elementary school' to 9 'post-graduate degree or PhD degree' – diploma /degree attained (self, mother, father and partner (if any), 'yes', 'no') – total number of years of education after elementary school – current occupation and current occupation partner ('paid work … hours per week', 'volunteer work … hours per week', 'student', 'stay-at-home mother/father, since …(year)', 'unemployed/seeking work, since…(year)', 'retired, since… (year)', 'disabled/unfit for work, since …(year)', 'other, namely…')*† *Question based on Netherlands Kinship Panel Study (293)
M	**Attention**	12 items on attention. Answer categories: 'never', 'once in a while', 'often', 'very often'* *CAARS index (233)
N	**Questions for women**	Pill/other use of hormonal contraception ('no', 'yes, I used to', 'yes, now') – ***if applicable:*** number of years using hormonal contraception – menopause started ('no', 'yes, naturally', 'yes, induced', 'I don't know') - ***if applicable:*** age at onset menopause – ever had a postnatal depression after pregnancy ('no', 'yes', 'not applicable') – ever suffered from striae during pregnancy ('no', 'yes', 'not applicable') – ever been diagnosed with endometriosis 'no',

		'yes' – did you undergo surgery for endometriosis – ***female twin pairs:*** who started menstruating first ('I did', 'my co-multiple/twin sister; triplet/quadruplet: name', 'I don't know/not applicable') – who reached menopause first ('I did', 'my co-multiple/twin sister; triplet/quadruplet: name', 'I don't know/not applicable').
O	**Wellbeing and stress - 1**	Quality of life on a bar going from 1 till 10 (1 being the worst life you can imagine and 10 the best) – frequency of stress at home in the past year ('never', 'once in a while', 'regularly', 'constant') – frequency of stress at work over past year (('never', 'once in a while', 'regularly', 'constant', 'not applicable') – satisfaction with own income ('dissatisfied', 'somewhat dissatisfied', 'not dissatisfied/not satisfied', 'somewhat satisfied', 'satisfied') – satisfaction with family income ('dissatisfied', 'somewhat dissatisfied', 'not dissatisfied/not satisfied', 'somewhat satisfied', 'satisfied') – amount of financial stress in the past year ('none/little', 'moderate', 'a lot') – answering 'strongly agree', 'agree', 'slightly agree', 'neither agree nor disagree', 'slightly disagree', 'disagree', 'strongly disagree', on the following questions; In most ways my life is close to my ideal - The conditions of my life are excellent – I am satisfied with my life – So far I have gotten the important things I want in life – I could live my life over, I would change almost nothing* - how often do you feel that you miss company ('almost never', 'sometimes', 'often') – how often do you feel excluded ('almost never', 'sometimes', 'often') – how often do you feel isolated from others ('almost never', 'sometimes', 'often').** * Life satisfaction (298) ** Loneliness scale (294)
P	**Religion**	Religious upbringing 'yes', 'no' – active member of a religious community at this moment ('no, I'm not religious', 'I am religious, but not actively involved in a religious community', 'yes, I am actively involved') – what is your religion.
Q	**Wellbeing - 2**	Adult self-report (the ASR(299)) ; 123 items, subscales: Internalizing (Anxious/Depressed, Withdrawn, Somatic Complaints), Externalizing (Aggressive Behaviour, Rule Breaking Behaviour, Intrusive) Thought Problems, Attention problems, answer categories: 'not at all', 'somewhat/sometimes', 'very much so/often'.
R	**Remarks**	Have you experienced a special period in your life which caused you to answer the questions in this survey differently from what you would do normally (for example due to illness) 'no', 'yes, namely...' – room for comments about the survey.
S	**Questions for twins**	Birth order ('firstborn', 'second born', 'I don't know') – twin brother/sister alive 'yes', 'no, age of death' – twin brother or sister – similarity to your twin as a kid as to face, hear color, skin tone and eye color ('not', 'somewhat', 'exactly', 'n.a.') – were you and your twin a spitting image as kids ('no', 'yes', 'n.a.') – were you and your twin sometimes confused by your parents ('no', 'yes', 'n.a.') - were you and your twin sometimes confused by other family members

		('no', 'yes', 'n.a.') – did strangers find it hard to keep you apart ('no', 'yes', 'n.a.') – are there big differences between you and your twin which surprise people in your surroundings (for example medical conditions or striking physical differences).
T	**Questions for triplets**	Birth order – names of all members of the triplet – gender of all members of the triplet – triplet brother/sister alive 'yes', 'no, age of death' – similarity to your triplet as a kid as to face, hear color, skin tone and eye color ('not', 'somewhat', 'exactly', 'not applicable') – were you and your triplet a spitting image as kids ('no', 'yes', 'not applicable') – were you and your twin sometimes confused by your parents ('no', 'yes', 'not applicable') - were you and your triplet sometimes confused by other family members ('no', 'yes', 'not applicable') – did strangers find it hard to keep you apart ('no', 'yes', 'not applicable') – are there big differences between you and your triplet which surprise people in your surroundings (for example medical conditions or striking physical differences).

The online version of this survey was tailored in such a way that certain questions were skipped based on three color codes. The questions that could be skipped are indicated in the table in different shades of grey; color code blue = survey 8 and/or the introductory (basislijst) survey was completed, color code green = survey 8 was completed, color code red = survey 9 was completed †question only included in batch 2 of survey 10-S

APPENDIX II Invitation letter inviting participants to complete NTR survey 10-O

VU VRIJE UNIVERSITEIT AMSTERDAM

Nederlands Tweelingen Register

DATUM	ONS KENMERK	WEBSITE:
Mei 2013	Antrlijst10	www.tweelingenregister.org/lijst10

E-MAIL	TELEFOON	FAX	BIJLAGE(N)
VragenlijstNTR10@vu.nl	020 598 8792	020 598 8832	Folder, vragenlijst(en), antwoordenveloppe(n)

Geachte heer/mevrouw xxxxx,

Namens het Nederlands Tweelingen Register (NTR), nodigen wij u hierbij van harte uit voor het invullen van de nieuwste **vragenlijst** in het langlopende onderzoek onder volwassen tweelingen en hun familieleden. De vragenlijst is dit keer wat korter dan u van ons gewend bent. Het deel over gedrag is speciaal ontwikkeld voor een **oudere doelgroep**.

Met het invullen van deze vragenlijst levert u een belangrijke bijdrage aan medisch wetenschappelijk onderzoek bij ouderen. Uw gegevens zijn strikt persoonlijk. Het NTR gaat vanzelfsprekend zeer zorgvuldig om met uw vertrouwelijke gegevens.

Bij voorbaat hartelijk dank voor uw bijdrage aan het onderzoek! De ingevulde vragenlijst kunt u (zonder postzegel) naar ons terugsturen in de bijgevoegde antwoordenvelop.

Met vriendelijke groet, namens het NTR-team,

Drs. Jorien Treur - Dr. Jacqueline Vink - Prof. Dr. Dorret Boomsma

FACULTEIT DER PSYCHOLOGIE EN PEDAGOGIEK

BEZOEKADRES
Van der Boechorststraat 1
1081 BT Amsterdam

POSTADRES
Van der Boechorststraat 1
1081 BT Amsterdam

APPENDIX III Brochure with information on NTR survey 10, accompanying the invitation letter

Wij krijgen vaak de vraag of mensen hun persoonlijke score kunnen krijgen. Wij bouwen daarom een 'portal' waarmee u in de toekomst kunt inloggen op uw eigen NTR-pagina en onder andere de scores kunt bekijken van bepaalde onderdelen uit de vragenlijst. Uw gegevens worden uiteraard zorgvuldig verwerkt. Wij bewaren persoonlijke gegevens (zoals naam en e-mailadres) apart van uw antwoorden op de vragenlijst. De onderzoeksresultaten zijn alleen toegankelijk voor onderzoekers en worden gebruikt in eigen NTR-onderzoeken en in samenwerkingen met collega-onderzoekers.

Contact

Nederlands Tweelingen Register

Meer informatie over ons onderzoek vindt u op:
www.tweelingenregister.org/lijst10

Indien u vragen heeft kunt u op maandag t/m vrijdag van 09.00-17.00 uur contact opnemen met Jorien Treur via:
telefoonnummer 020-598 8792
e-mail: VragenlijstNTR10@vu.nl

Indien u vragen heeft die u liever niet aan de onderzoeker zelf stelt is het mogelijk om een onafhankelijke arts te raadplegen:
Dr. Nils Lambalk, telefoonnummer: 020-444 0070.

Volg ons ook op Facebook en Twitter:
www.facebook.com/NederlandsTweelingenRegister
www.twitter.com/NTR_VU

"Ruim 25 jaar onderzoek levert veel inzicht! Blijven doorgaan..."

Het Nederlands Tweelingen Register is in 1987 opgericht voor het doen van medisch-wetenschappelijk onderzoek. Er is in 25 jaar tijd veel ontdekt en er zijn nieuwe interessante inzichten verkregen. Wat onveranderd is gebleven is dé centrale vraag: waarom verschillen mensen van elkaar wat betreft hun gezondheid, leefgewoonten en persoonlijkheid? Komt dit door erfelijke aanleg óf juist door de omgeving óf een combinatie van allebei?

Top-50 met interessante bevindingen & weetjes

In een kwart eeuw zijn baanbrekende onderzoeksresultaten behaald! Zo hebben de onderzoeken bij tweelingfamilies aan de basis gestaan van veel opmerkelijke resultaten. Dit geldt bijvoorbeeld voor de vondst van genetische varianten. Uit gegevens van vragenlijsten is duidelijk geworden dat maar liefst 70% van de verschillen tussen mensen in lichaamsgewicht en overgewicht een genetische oorzaak heeft. Vervolgens werden deze gegevens over lichaamsgewicht gekoppeld aan informatie over DNA-varianten. Het resultaat? Er zijn verschillende genen gevonden die lichaamsgewicht en in het bijzonder het ontwikkelen van overgewicht kunnen beïnvloeden.

Benieuwd naar nog meer interessante bevindingen & weetjes? Kijk voor de onderwerpen eens op:
www.tweelingenregister.org/25jaarntr/25-jaar-ntr-onderzoek

Lijkt uw broer, zus, partner of kind op u?

In dit onderzoek stellen wij vragen over leefgewoonten zoals sporten, roken, alcoholgebruik en het gebruik van cafeïne en gaan we na in hoeverre deze leefgewoonten samengaan met persoonlijkheid en gezondheid. In welke mate speelt erfelijke aanleg bij dit alles een rol? Daarom vergelijken we de antwoorden van familieleden. Ook de omgeving kan een belangrijke rol spelen en er is aandacht voor de interactie tussen genen en omgeving. Als u al vaker meedeed ziet u dat u deels dezelfde vragen krijgt als eerder. Dat stelt ons in staat om te kijken naar veranderingen tijdens de levensloop. Gaan mensen meer of minder drinken, roken of sporten als ze ouder worden? En zijn deze leefgewoonten voorspellend voor gezondheid op latere leeftijd? Spelen erfelijke factoren hierbij een rol?
De online vragenlijst hebben we zo flexibel mogelijk gemaakt. Als u antwoordt dat u niet rookt krijgt u uiteraard geen vervolgvragen over het aantal sigaretten dat u rookt. Uiteraard kunt u op elk moment stoppen (de antwoorden worden dan opgeslagen) en op een later tijdstip verder gaan.

Wie worden uitgenodigd?

Alle NTR-deelnemers van 18 jaar en ouder, zowel meerlingen als hun ouders, broers/zussen en levenspartner. Als u een meerling bent en uw familieleden hebben zelf geen vragenlijst ontvangen, dan kunnen zij zich aanmelden op: www.tweelingenregister.org/contact/aanmelden/familie-van-meerling
Als ouder van een tweeling ontvangt u mogelijk voor het eerst een vragenlijst over uzelf, in plaats van over uw kinderen. Als niet alleen meerlingen maar ook familieleden meedoen aan onderzoek, kunnen wij nog beter inzicht krijgen waarom mensen van elkaar verschillen wat betreft leefgewoonten (komt dat door genen of door omgeving?). Ook als u geen familie heeft die mee wil doen met het onderzoek is uw bijdrage heel waardevol voor ons.

APPENDIX IV Thank you card

Hartelijk dank voor uw deelname!

Wij stellen het zeer op prijs dat u heeft meegedaan aan het vragenlijst onderzoek naar gezondheid en leefgewoonten. U levert hiermee een belangrijke bijdrage aan medisch-wetenschappelijk onderzoek!

Namens het NTR-team,

Drs. Jorien Treur, Dr. Jacqueline Vink & Prof. Dr. Dorret Boomsma

Nederlands Tweelingen Register | Tel. 020-598 8787 | Email VragenlijstNTR10@vu.nl

Port Betaald
Port Payé
Pays-Bas
postnl

APPENDIX V Reminder card

Wij hebben uw vragenlijst nog niet ontvangen

Wij hopen dat u alsnog bereid bent om aan ons onderzoek mee te werken? U levert daarmee een belangrijke bijdrage aan medisch-wetenschap onderzoek! Als u de vragenlijst ondertussen al heeft ingevuld en teruggestuurd kunt u deze herinnering als niet verstuurd beschouwen en willen wij u hartelijk bedanken. Als u de vragenlijst niet (meer) heeft kunt u contact met ons opnemen via email of telefoon en dan sturen wij u graag een (nieuwe) vragenlijst toe.

Namens het NTR-team,

Drs. Jorien Treur, Dr. Jacqueline Vink & Prof. Dr. Dorret Boomsma

Nederlands Tweelingen Register | Tel. 020-598 8787 | Email VragenlijstNTR10@vu.nl

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Port Payé
Pays-Bas
postnl

APPENDIX VI Invitation letter inviting participants to complete NTR survey 10-S

VU VRIJE UNIVERSITEIT AMSTERDAM

Nederlands Tweelingen Register

DATUM	ONS KENMERK	WEBSITE:	
Oktober 2013	Antrlijst10	www.tweelingenregister.org/lijst10	
E-MAIL	TELEFOON	FAX	BIJLAGE(N)
VragenlijstNTR10@vu.nl	020 598 8792	020 598 8832	Folder

Geachte heer/mevrouw xxxxx,

Namens het Nederlands Tweelingen Register (NTR) nodigen wij u hierbij van harte uit voor het invullen van de nieuwste **online vragenlijst** in het langlopende onderzoek onder volwassen tweelingen en hun familieleden.

De vragenlijst staat op **www.tweelingenregister.org/lijst10** en u kunt inloggen met de volgende gegevens:
Gebruikersnaam: xxxxx
Wachtwoord: xxxxx

De online vragenlijst hebben we zo **flexibel** mogelijk gemaakt. Als u bijvoorbeeld antwoordt dat u niet rookt krijgt u uiteraard geen vervolgvragen over het aantal sigaretten dat u rookt. En als u enkele jaren geleden al heeft meegedaan krijgt u niet alle vragen opnieuw. Met het invullen van deze vragenlijst levert u een belangrijke bijdrage aan **medisch-wetenschappelijk onderzoek**.

Meer over ons onderzoek leest u in bijgesloten **folder**. Wij stellen het zeer op prijs als u de online vragenlijst wilt invullen vóór xxxxx**.** Vult u de vragenlijst liever op papier in? Als u op xxxxx de vragenlijst nog niet online hebt ingevuld dan sturen wij u automatisch een papieren versie toe, hier hoeft u verder niets voor te doen.

Bij voorbaat hartelijk dank voor uw bijdrage aan het onderzoek!
Met vriendelijke groet, namens het NTR-team,

Drs. Jorien Treur - Dr. Jacqueline Vink - Prof. dr. Dorret Boomsma

FACULTEIT DER PSYCHOLOGIE EN PEDAGOGIEK

BEZOEKADRES
Van der Boechorststraat 1
1081 BT Amsterdam

POSTADRES
Van der Boechorststraat 1
1081 BT Amsterdam

APPENDIX VII E-mail inviting participants to complete NTR survey 10-S

Geachte heer/mevrouw xxxxx,

Afgelopen week heeft u per post een uitnodigingsbrief van het Nederlands Tweelingen Register ontvangen voor de nieuwste (online) vragenlijst.
Wij zouden het zeer op prijs stellen als u deze vragenlijst wilt invullen!

Om het u gemakkelijk te maken sturen we u de link naar de vragenlijst en uw inloggegevens ook per e-mail toe. Klik op www.tweelingenregister.org/lijst10 om naar de vragenlijst te gaan, en vul uw login gegevens in:
Gebruikersnaam: xxxxx
Wachtwoord: xxxxx

Wij stellen het zeer op prijs als u de online vragenlijst wilt invullen vóór xxxxx. Het invullen neemt ongeveer 25 tot 45 minuten in beslag, en kan het beste op een PC, laptop of tablet gedaan worden (vanwege de lengte is de online vragenlijst wat minder geschikt voor mobiele telefoon).
Vult u de vragenlijst liever op papier in? Als u op xxxxx de vragenlijst nog niet online heeft ingevuld dan sturen wij u automatisch een papieren versie toe, hier hoeft u verder niets voor te doen.

Als u de uitnodigingsbrief (met meer informatie en een folder over het onderzoek) niet per post ontvangen heeft, horen we het graag van u via VragenlijstNTR10@vu.nl. Hier kunt u natuurlijk ook naar toe mailen met overige vragen over dit onderzoek.

Alvast hartelijk dank voor uw deelname!

Met vriendelijke groet,

Namens het Nederlands Tweelingen Register,
Jorien Treur

Jorien Treur MSc | PhD student
Dept. of Biological Psychology
VU VRIJE UNIVERSITEIT AMSTERDAM
Faculteit der Psychologie en Pedagogiek
T : (020) 598 3037 | E-mail : j.l.treur@vu.nl | Room: TR 2B-13
Van der Boechorststraat 1 , 1081 BT Amsterdam, The Netherlands

APPENDIX VIII Follow-up invitation letter inviting participants to complete NTR survey 10-S

VU VRIJE UNIVERSITEIT AMSTERDAM

Nederlands Tweelingen Register

DATUM	ONS KENMERK	WEBSITE:	
December 2013	Antrlijst10	www.tweelingenregister.org/lijst10	
E-MAIL	TELEFOON	FAX	BIJLAGE(N)
VragenlijstNTR10@vu.nl	020 598 8792	020 598 8832	vragenlijst, antwoordenveloppe

Geachte heer/mevrouw XXXXX,

Hierbij ontvangt u van ons de nieuwste vragenlijst in het langlopende vragenlijstonderzoek van het Nederlands Tweelingen Register. Wij hopen van harte dat u wilt deelnemen aan ons onderzoek.

Een aantal weken geleden hebben wij u een uitnodiging gestuurd om deze vragenlijst **online** in te vullen. Omdat veel mensen het prettiger vinden om de lijst op papier in te vullen sturen wij u nu een **papieren** versie toe. De ingevulde vragenlijst kunt u (zonder postzegel) naar ons terugsturen in de bijgevoegde antwoordenvelop.

Wanneer u deze vragenlijst toch liever online wilt invullen kan dit natuurlijk ook nog steeds. De vragenlijst staat voor u klaar op **www.tweelingenregister.org/lijst10** en voor de zekerheid sturen wij u nog een keer uw inloggegevens:
Gebruikersnaam: xxxxx
Wachtwoord: xxxxx

Als u de vragenlijst inmiddels al online hebt ingevuld, danken wij u heel hartelijk voor uw deelname en kunt u deze brief als niet verzonden beschouwen.

Op onze website **www.tweelingenregister.org/lijst10** staat extra achtergrondinformatie over het onderzoek, inclusief een pdf van de folder die u vorige keer heeft ontvangen. Wanneer u vragen heeft dan kunt u natuurlijk contact met ons opnemen per telefoon (020-598 8792) of per e-mail: VragenlijstNTR10@vu.nl

Met het invullen van deze vragenlijst levert u een belangrijke bijdrage aan **medisch-wetenschappelijk onderzoek**.

Bij voorbaat hartelijk dank voor uw bijdrage!
Met vriendelijke groet, namens het NTR-team,

Drs. Jorien Treur - Dr. Jacqueline Vink - Prof. dr. Dorret Boomsma

FACULTEIT DER PSYCHOLOGIE EN PEDAGOGIEK	BEZOEKADRES	POSTADRES
	Van der Boechorststraat 1 1081 BT Amsterdam	Van der Boechorststraat 1 1081 BT Amsterdam

APPENDIX IX Example report NTR portal

05/06/2014

Cafeïne inname en suikergebruik

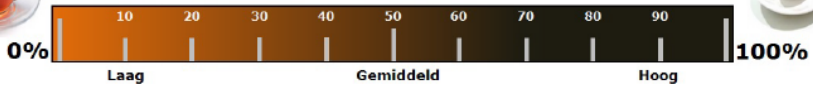
Deze rapportage is gebaseerd op een vragenlijst die u heeft ingevuld in 2013

Koffie en thee

Nederlanders drinken over het algemeen veel koffie. Er zijn echter wel individuele verschillen; de ene persoon drinkt bijvoorbeeld niet meer dan 2 koppen koffie per dag terwijl de ander minstens 8 koppen drinkt. Daarnaast vinden sommige mensen koffie helemaal niet lekker en drinken liever thee.

In onze vragenlijst vroegen wij om aan te geven hoeveel u van verschillende drankjes dronk. Voor elke deelnemer hebben wij een persoonlijke 'koffiescore' bepaald door het aantal koppen koffie per dag te delen door het totaal aantal koppen koffie én thee per dag. Hoe hoger dit percentage, hoe sterker de voorkeur voor koffie boven thee.

Uw persoonlijke koffiescore is: 83%



Iemand die alleen koffie drinkt en geen thee scoort 100% (koffieleut) en iemand die alleen thee drinkt en geen koffie scoort 0% (theeleut). In alle andere gevallen ligt de score ergens tussen deze twee uitersten. De mannen in onze studie dronken iets meer koffie dan de vrouwen: mannen hadden gemiddeld een koffiescore van 68%, terwijl de vrouwen gemiddeld een score van 59% hadden.

Cafeine

Cafeïne zit niet alleen in koffie en thee, maar ook in cola, chocolademelk en energiedrankjes. Daarom hebben we in de vragenlijst ook vragen gesteld over andere dranken. Op basis van uw antwoorden hebben we bepaald hoeveel cafeïne u per dag binnenkrijgt.

Per dag krijgt u 834 mg cafeïne binnen via de dranken die u drinkt.

Van de NTR deelnemers kregen de mannen gemiddeld 329 mg cafeïne binnen per dag, en de vrouwen 298 mg. Mensen die merken dat ze gevoelig zijn voor cafeïne kunnen zich het beste beperken tot maximaal 400 mg cafeïne per dag. Dat komt neer op bijvoorbeeld vijf koppen koffie of vier koppen zwarte thee.

Suikerhoudende dranken

Elke dag krijgen wij suiker binnen via ons voedsel. Wat veel mensen zich echter niet realiseren is dat we ook suiker binnen krijgen via de dranken die we drinken. Op basis van uw antwoorden op de vragenlijst hebben we berekend hoeveel suiker u, via deze dranken, per dag binnenkrijgt.

Per dag krijgt u 100 kcal aan suiker binnen via de niet-alcoholische dranken die u drinkt.

Van de deelnemers aan dit onderzoek krijgen de mannen per dag gemiddeld 205 kcal aan suiker binnen via dranken, en de vrouwen 137 kcal.

Naast de suiker die al standaard in dranken zit, gooien we er ook nog wel eens een schepje bij. Mannen blijken daarbij scheutiger dan vrouwen: 37% van de mannen gebruikt wel eens suiker in de thee of koffie, tegenover 17% van de vrouwen. Naast suiker wordt ook wel gebruik gemaakt van zoetjes (kleine pilletjes met een calorieloze zoetstof dat suiker kan vervangen): 22% van de mannen gebruikte wel eens zoetjes in de koffie of thee, tegenover 18% van de vrouwen.

Favoriete dranken

In de onderstaande tabel ziet u de wekelijkse consumptie voor elk van de soorten non-alcoholische drankjes waarnaar werd gevraagd in de vragenlijst. U ziet per soort drank het aantal glazen of koppen per week dat u zelf rapporteerde en daarnaast de gemiddelde inname voor mannen en vrouwen.

Dranken	Uw antwoord	Mannen	Vrouwen
1. Koffie	70	27.9	24.8
2. Thee	14	14.4	19.8
3. Water	28	8.7	16.2
4. Zuivel	7	7.5	7.2
5. Vruchtensap/fruitdrank	0	3.9	3.3
6. Cola	0	.9	.4
7. Frisdrank (0verig)	0	1.4	.8
8. Energie-/sportdrank	0	.1	0

NB: we hebben nog niet alle gegevens van dit onderzoek binnen, de gemiddelde cijfers van NTR deelnemers in deze rapportage kunnen dus nog wijzigen.

Nederlandse samenvatting.

Een genetisch informatieve studie naar verslavend gedrag met een focus op roken.

Introductie

Nicotine is een van de meest gebruikte verslavende middelen en levert, door het roken van sigaretten, een grote bijdrage aan morbiditeit (ziekte) en mortaliteit (sterfte). Wereldwijd veroorzaakt roken circa 6 miljoen sterfgevallen per jaar. Alhoewel het aantal mensen dat rookt in de afgelopen decennia sterk gedaald is in Nederland, rookte in 2014 nog steeds 28% van de (volwassen) mannen en 22% van de vrouwen. Een beter begrip van de oorzaken en gevolgen van roken kan helpen om dit aantal verder te doen dalen en hiermee de volksgezondheid te verbeteren. In dit proefschrift worden genetische en omgevingsinvloeden op verslavend gedrag onderzocht, met een nadruk op roken. Het is welbekend dat roken samenhangt met het gebruik van andere verslavende middelen zoals alcohol en cannabis. Er is echter veel minder duidelijkheid over de relatie tussen roken en cafeïnegebruik en de relatie tussen middelengebruik en het gebruik van suiker (door sommigen als potentieel verslavend beschouwd). Het onderzoeken van deze twee relaties is daarom een belangrijk onderdeel van dit proefschrift. Tevens wordt in dit proefschrift aandacht besteed aan de gevolgen van roken voor de geestelijke gezondheid. Dierproefonderzoek suggereert dat roken een causaal (oorzaak-gevolg) effect heeft, waarbij roken aandachtsproblemen vergroot. Dit is tot op heden nog niet in mensen aangetoond maar het kan worden getest met gegevens van tweelingen. Bij het onderzoeken van de verschillende thema's die hierboven worden beschreven, is gebruik gemaakt van gegevens welke zijn verzameld binnen het Nederlands Tweelingen Register.

Uit eerder onderzoek is gebleken dat opleidingsniveau sterk samenhangt met roken. Wanneer we de Nederlandse bevolking naar opleidingsniveau verdelen in 4 groepen (van laag naar hoog), is het percentage rokers 30%, 27%, 20% en 16%, respectievelijk. De meeste mensen beginnen met roken tijdens de adolescentie. Behalve opleidingsniveau zijn factoren die samenhangen met het beginnen met roken geslacht (jongens hebben een grotere kans om te beginnen met roken dan meisjes), het rookgedrag van vrienden, een afname van zelfeffectiviteit (of iemand gelooft dat hij/zij sterk genoeg is om niet te roken) en persoonlijkheidskenmerken zoals de mate van impulsiviteit. Na het beginnen met roken hangt het aantal sigaretten dat iemand rookt onder andere samen met opleidingsniveau, inkomen en de hoeveelheid ervaren stress in het dagelijks leven. Succesvol stoppen met roken is onder meer geassocieerd met een hoger opleidingsniveau, een hogere zelfgerapporteerde gezondheid en lager alcoholgebruik.

Genen spelen ook een belangrijke rol in rookgedrag. Met behulp van tweelingstudies is ontdekt dat verschillen tussen mensen in rookgedrag voor een aanzienlijk deel door genetische factoren worden verklaard. Het basisprincipe van een tweelingstudie is dat er twee soorten tweelingen zijn; eeneiige tweelingen (delen ~100% van hun genen en de gedeelde omgeving) en twee-eiige tweelingen (delen ~50% van hun genen en 100% van de gedeelde omgeving). Als eeneiige tweelingen meer op elkaar lijken dan twee-eiige tweelingen dan impliceert dat een invloed van genen. Als de correlatie tussen twee-eiige tweelingen groter is dan de helft van de correlatie tussen eeneiige tweelingen, suggereert dit een invloed van de

omgeving die de tweeling deelt (waaronder de familieomgeving). Met gegevens van Nederlandse tweelingen werd gevonden dat verschillen tussen mensen in het beginnen met roken voor 44% door genetische factoren kon worden verklaard. De overgebleven 56% werd verklaard door omgevingsfactoren (51% gedeelde omgeving en 5% unieke omgeving). Afhankelijkheid aan nicotine was voor een veel groter deel genetisch bepaald, namelijk 75%. De overige 25% bestond uit unieke omgevingsfactoren. Behalve onderzoek naar de relatieve invloed van genetica zijn er ook specifieke genetische varianten gevonden welke van invloed zijn op rookgedrag. Dit is bereikt met zogenaamde 'genoom-brede associatie studies' (GWAS). In dergelijke studies worden honderdduizenden SNPs ('single nucleotide polymorphisms') gemeten over het hele genoom. Een SNP is een enkele nucleotide in het DNA die 'polymorf' is, wat wil zeggen dat van deze SNP meer dan één variant te vinden is in de populatie. In GWAS wordt getest of mensen met een bepaalde eigenschap, zoals roken, vaker een specifieke variant bezitten dan mensen zonder die eigenschap. Met GWAS zijn inmiddels meerdere SNPs ontdekt welke met roken geassocieerd zijn. Degene met het grootste effect ligt in een gen dat codeert voor de nicotinereceptor; de verschillende varianten hebben invloed op de hoeveelheid receptoren in de hersenen.

Resultaten

In hoofdstuk 3 werd gevonden dat een simpele vraag over rookverwachtingen ('Denkt u dat u zelf over een jaar zult roken?'), een goede voorspeller was voor toekomstig rookgedrag in nooit rokers en ex-rokers, maar niet in huidige rokers. Met behulp van gegevens van een- en twee-eiige tweelingen werd daarnaast ontdekt dat de mate waarin iemand zijn of haar eigen rookgedrag kon voorspellen gedeeltelijk genetisch bepaald was. In adolescenten (14-18 jaar) werden verschillen tussen mensen in het voorspellen van toekomstig rookgedrag voor 59% bepaald door genetische factoren. In de groep volwassenen (18+ jaar) was dit 27%. De rest van de verschillen tussen mensen konden worden verklaard door unieke omgevingsfactoren (hierbij kan worden gedacht aan ervaringen op school of werk).

Uit eerder onderzoek was al gebleken dat levenspartners meer dan gemiddeld op elkaar lijken als we kijken naar rookgedrag. Hoofdstuk 4 beschrijft een studie naar de oorzaak van deze gelijkenis. Allereerst werd bevestigd dat iemand die rookt inderdaad een grotere kans heeft om een partner te hebben die ook rookt, en vice versa. Verder bleek dat partners meer op elkaar lijken wanneer meer recent verzamelde data werden geanalyseerd (er waren drie groepen: 2009-2013, 2000-2005 en 1997-2000). Dit laatste komt met name doordat er steeds minder mensen roken en er in de recentere groepen daardoor meer niet-rokende koppels zijn. Verder wees het onderzoek uit dat een fenomeen wat 'phenotypic assortment' heet, de gelijkenis tussen partners veroorzaakt. Dit wil zeggen dat partners elkaar (onder andere) selecteren op basis van rookgedrag. Omdat roken voor een aanzienlijk deel erfelijk bepaald is, betekent dit ook dat kinderen van rokende ouders, gemiddeld genomen, een hoger genetisch risico zullen hebben op roken.

Roken hangt sterk samen met het drinken van koffie, maar naar de relatie tussen roken en andere cafeïnehoudende dranken was tot op heden nog weinig onderzoek gedaan. Daarom werden in hoofdstuk 5 gegevens over rookgedrag en het gebruik van koffie, thee, cola en energiedranken geanalyseerd. Dit werd gedaan in een Nederlandse populatie en in een Engelse populatie, in samenwerking met de Universiteit van Bristol. De resultaten wezen uit dat mensen die ooit (regelmatig) gerookt hadden meer cafeïne gebruikten dan nooit rokers. Daarnaast gebruikten huidige rokers meer cafeïne dan ex-rokers. Deze relatie was consistent voor alle cafeïnehoudende dranken, behalve voor thee. Voor thee gold dat Nederlandse rokers er minder van gebruikten dan niet rokers maar Engelse rokers juist meer. Dit verschil heeft waarschijnlijk te maken met populatie specifieke culturele factoren welke het gebruik van thee beïnvloeden.

Hoofdstuk 6 beschrijft een studie waarin de oorzaak van de relatie tussen roken en cafeïnegebruik (zoals beschreven in hoofdstuk 5) werd onderzocht. Hiervoor werden drie verschillende methoden gebruikt: een bivariaat tweeling model, 'LD-Score regression' en Mendeliaanse randomisatie analyse. De eerste twee methoden maakten het mogelijk om een correlatie tussen de genetische risicofactoren voor roken en de genetische risicofactoren voor cafeïnegebruik te berekenen. De derde en laatste methode werd gebruikt om te testen of er een causaal effect was van roken op cafeïnegebruik, of van cafeïnegebruik op roken. De resultaten lieten een aanzienlijke genetische correlatie zien tussen roken en cafeïnegebruik (rg=0.4-0.5), maar leverden geen bewijs voor causale effecten. Dit suggereert dat mensen die (meer) roken vaak ook (meer) cafeïne gebruiken omdat ze genetische varianten hebben die ze gevoelig maakt voor beiden gedragingen.

In hoofdstuk 7 werd onderzocht of er genetische invloeden zijn op het consumeren van (veel) suiker. Daarnaast werd getest of deze genetische risicofactoren overlappen met genetische risicofactoren voor het gebruik van verslavende middelen. Suikerinname werd berekend door deelnemers te vragen naar hun dagelijkse consumptie van verschillende soorten dranken (waaronder frisdranken, fruitdranken en koffie/thee met suiker). Middelengebruik werd gemeten door te vragen naar rookgedrag, gebruik van alcohol, cafeïne, cannabis en hard drugs. Er bleek een aanzienlijke invloed van genetische factoren te zijn op het hebben van een hoge suiker inname (48%). De overige 52% van de verschillen tussen mensen werd verklaard door unieke omgevingsfactoren. Voor hoog middelengebruik was dit 62% voor genetische en 38% voor unieke omgevingsfactoren. Er was een bescheiden, maar significante, genetische correlatie tussen hoog suikergebruik en hoog middelengebruik (rg=0.24). Dit zou kunnen betekenen dat (genetisch bepaalde) biologische mechanismen die ten grondslag liggen aan de ontwikkeling van verslaving voor een deel overlappen met de mechanismen voor overgewicht.

Als laatste beschrijft hoofdstuk 8 een studie die bewijs levert voor een causaal effect van roken op aandachtsproblemen. In dierproefonderzoek werd al gevonden dat blootstelling van de ontwikkelende hersenen aan nicotine voor aandachtsproblemen kon zorgen, maar dit was

nog niet eerder in mensen aangetoond. In deze studie is gebruik gemaakt van discordante, eeneiige tweelingenparen. Dit zijn tweelingparen waarvan de een wel rookt en de ander niet. Eeneiige tweelingen zijn genetisch nagenoeg identiek en delen daarnaast een groot deel van hun (familie)omgeving. In deze studie werd gevonden dat de tweeling die rookte meer aandachtsproblemen vertoonde dan zijn of haar tweelingbroer of zus die niet rookte. Deze verschillen kunnen niet door genetische of gedeelde omgevingsfactoren worden veroorzaakt (omdat deze gelijk zijn voor eeneiige tweelingen). De verschillen in aandachtsproblemen bestonden nog niet toen de tweeling jonger was en geen van beiden rookte. Deze resultaten suggereren dat roken aandachtsproblemen verhoogd, zoals eerder in dierproefonderzoek werd aangetoond.

Conclusies en discussie

De verschillende studies in dit proefschrift bevestigen eerder onderzoek door te laten zien dat verslavend gedrag (waaronder roken, cafeïnegebruik en suikerinname) matig tot hoog genetisch bepaald is. Hiaten in de literatuur zijn geadresseerd door de aard van de relatie tussen verschillende verslavende gedragingen te onderzoeken, te bepalen waarom levenspartners op elkaar lijken voor rookgedrag en door te testen of roken een causaal effect heeft op aandachtsproblemen.

Een mogelijke implicatie van dit proefschrift verbeterde identificatie van hoogrisicogroepen. Gezien het feit dat een simpele vraag over rookverwachtingen een goede voorspeller kan zijn voor toekomstig rookgedrag kan deze vraag in de praktijk mogelijk gebruikt worden om mensen te identificeren die een hoog risico hebben op roken. Uit dit proefschrift blijkt verder dat levenspartners elkaar (onder andere) selecteren op basis van rookgedrag. Hieruit volgt dat kinderen van rokende ouders een hoger genetisch risico hebben op roken. Ook met deze informatie zouden hoogrisicogroepen geïdentificeerd kunnen worden. Voor kinderen waarvan beiden ouders roken is het bijvoorbeeld extra belangrijk dat zij niet beginnen met roken. De risicogenen die zij van hun ouders hebben gekregen kunnen voor hen de kans groter maken dat ze verslaafd raken aan nicotine.

In zowel hoofdstuk 6 als hoofdstuk 7 is onderzocht in hoeverre genetische factoren voor verschillende verslavende gedragingen met elkaar overlappen. In hoofdstuk 6 voor roken en cafeïnegebruik en in hoofdstuk 7 voor middelengebruik en suikergebruik. In beide gevallen bleek er een aanzienlijke genetische correlatie te zijn. Dit betekent dat er genetische varianten bestaan die het risico op meerdere verslavende gedragingen beïnvloeden. Dit zou kunnen komen doordat deze genetische varianten een effect hebben op iemands vermogen om belonende prikkels, zoals wordt ervaren bij het gebruik van verslavende middelen, te weerstaan. Voor de hand liggende kandidaten zijn genetische varianten die coderen voor receptoren van neurotransmitters die betrokken zijn bij het beloningssysteem in de hersenen. Voorbeelden van zulke neurotransmitters zijn dopamine en serotonine. Er is echter nog veel onduidelijk over de exacte genen die betrokken zijn bij het risico voor verslavend gedrag. Het

wordt steeds duidelijker dat dergelijke complexe eigenschappen het resultaat zijn van een samenspel aan genetische en omgevingsinvloeden.

Een thema dat op twee plekken in dit proefschrift terugkomt is het bestaan van (mogelijke) causale effecten van roken. In het geval van een causaal effect zou roken een bepaalde uitkomst veroorzaken. In hoofdstuk 8 werd bewijs geleverd voor een causale toename van aandachtsproblemen ten gevolge van het roken van sigaretten. Dit is een belangrijke conclusie en benadrukt het belang van het voorkomen van roken bij zoveel mogelijk mensen, of op z'n minst het uitstellen van het beginnen met roken tot een leeftijd waarop de ontwikkeling van de hersenen is voltooid. Een mogelijke manier om dit te bereiken is door het instellen van een leeftijdsgrens waaronder niet gerookt mag worden. In Nederland is deze grens per 1 januari 2014 van 16 naar 18 jaar verhoogd. De resultaten uit dit proefschrift suggereren dat deze verhoging misschien niet voldoende is. Mogelijk zou een leeftijdsgrens van 21 jaar gepaster zijn, zoals in 2013 bijvoorbeeld werd ingesteld in New York. Om het bewijs voor een causaal effect van roken op aandachtsproblemen te versterken, en daarmee de noodzaak van een hogere leeftijdsgrens, zijn meer studies nodig. Een veelbelovende techniek om causale effecten van roken te testen is Mendeliaanse randomisatie. Deze techniek gebruikt genetische varianten als instrument, of proxy, voor een bepaalde risicofactor en relateert die met een bepaalde uitkomst. Hiermee wordt het effect van zogenaamde 'confounders' (variabelen die zowel met de risicofactor als met de uitkomstvariabele geassocieerd zijn) geminimaliseerd. Het effect van roken op aandachtsproblemen zou in toekomstige studies kunnen worden getest door het meten van een genetische variant die sterk met roken geassocieerd is, en te testen of dragers van deze variant meer aandachtsproblemen hebben dan niet-dragers. In hoofdstuk 6 van dit proefschrift is gebruik gemaakt van Mendeliaanse randomistatie, om te testen of roken er voor zorgt dat mensen meer cafeïne gebruiken. De resultaten suggereren dat dit niet het geval was, maar replicatie is nodig in grotere populaties.

Naast de onderwerpen die in dit proefschrift staan beschreven zal toekomstig onderzoek zich in toenemende mate gaan richten op het gebruik van 'nieuwe' verslavende middelen. Zo is er in landen als Nederland en de Verenigde Staten een sterke toename te zien in het gebruik van waterpijp (ook wel 'shisha' genoemd) en komt het gebruik van elektronische sigaretten steeds meer voor. Er is nog veel onduidelijk over de risicofactoren voor het gebruik van deze middelen en de samenhang met het gebruik van sigaretten en andere verslavende middelen. Een ander nieuw thema in de recente literatuur is het idee dat voeding, of bepaalde voedingsmiddelen zoals suiker, 'verslavend' kunnen zijn. Een verbeterd inzicht in de factoren die ervoor zorgen dat mensen veel (suiker) eten/drinken is van groot belang gezien de forse toename van overgewicht en obesitas in de laatste tientallen jaren. Dit onderwerp werd ook behandeld in hoofdstuk 7 van dit proefschrift, waar genetische invloeden op hoog suikergebruik en de overlap daarvan met middelengebruik werden onderzocht. Er is echter meer onderzoek nodig op dit gebied. Onder meer naar verschillende voedingsaspecten zoals vetinname, eetpatronen en de hoeveelheid calorieën per dag. Ook is het nog onduidelijk

welke genetische varianten precies van invloed zijn op voeding.

Publication list.

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* Shared last authorship

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