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ORIGINAL RESEARCH

The Contribution of the Functional *IL6R* Polymorphism rs2228145, eQTLs and Other Genome-Wide SNPs to the Heritability of Plasma sIL-6R Levels

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Abstract The non-synonymous SNP rs2228145 in the *IL6R* gene on chromosome 1q21.3 is associated with a wide range of common diseases, including asthma, rheumatoid arthritis, type 1 diabetes and coronary heart disease. We examined the contribution of this functional *IL6R* gene polymorphism rs2228145 versus other genome-wide SNPs to the variance of sIL-6R levels in blood plasma in a large population-based sample (N ~5,000), and conducted an expression QTL analysis to identify SNPs associated with *IL6R* gene expression. Based on data from 2,360 twin families, the broad heritability of sIL-6R was estimated at 72 and 51 % of the total variance was explained by the functional SNP rs2228145. Converging findings from GWAS, linkage, and GCTA analyses indicate that additional variance of sIL-6R levels can be explained by other variants in the *IL6R* region, including variants at the 3′-end of *IL6R* tagged by rs60760897 that are associated with *IL6R* RNA expression.

Keywords Soluble interleukin-6 receptor · Inflammation · Heritability · GWAS · Gene expression · eQTL

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Introduction

The interleukin-6 receptor (IL-6R) forms part of the ligand-receptor complex that mediates the activities of interleukin-6 (IL-6). So-called classical IL-6 signaling occurs in hepatocytes and some leukocyte subtypes, which express a trans-membrane form of IL-6R on their surface (membrane-bound IL-6R, or mIL-6R; Rose-John et al. 2006). A second type of IL-6 signaling called IL-6 trans-signaling is

capable of stimulating a variety of different cell types as it is mediated by a soluble form of the IL-6R (soluble IL-6R, or sIL-6R; Mackiewicz et al. 1992; Taga et al. 1989). IL-6 trans-signaling allows cells lacking mIL-6R to respond to IL-6, as long as they express the trans-membrane signal transducer protein gp130 on their surface (which is thought to be expressed ubiquitously; Saito et al. 1992). On target cells, the complex of IL-6 and IL-6R binds to two molecules of gp130, thereby activating several signal transduction pathways (Taga and Kishimoto 1997), leading to cellular responses such as proliferation, differentiation and inflammatory processes. IL-6 trans-signaling plays a key role in several autoimmune diseases and inflammatory conditions, including asthma (Doganci et al. 2005), rheumatoid arthritis (Kotake et al. 1996), chronic inflammatory bowel disease (Atreya et al. 2000), some types of cancer (e.g. multiple myeloma; Becker et al. 2004; Stephens et al. 2012) and peritonitis (Hurst et al. 2001).

Two isoforms of sIL-6R have been identified in blood plasma of healthy individuals that are generated through different mechanisms (Jones et al. 2001; Muller-Newen et al. 1996). The majority of sIL-6R is thought to be produced by a process called shedding, referring to the proteolytic cleavage of mIL-6R and subsequent release of the ligand-binding ectodomain into the extracellular space (Müllberg et al. 1993). A second isoform is produced by translation of an alternatively spliced mRNA lacking a 94-bp sequence coding for part of the transmembrane domain (Horiuchi et al. 1994; Lust et al. 1992) that anchors the receptor to the cell membrane. The process of shedding is affected by a non-synonymous SNP (Asp358Ala or rs2228145 (A > C), previously also known as rs8192284) that occurs within the region encoding the proteolytic cleavage site, in exon 9 of the IL-6R gene, *IL6R*, on chromosome 1q21.3 (Müllberg et al. 1994). The SNP causes a striking difference in IL-6R concentrations between carriers of different alleles, with reduced concentrations of mIL-6R and increased concentrations of sIL-6R in carriers of the minor allele (C) (Ferreira et al. 2013; Galicia et al. 2004; Lourdusamy et al. 2012; Melzer et al. 2008; Rafiq et al. 2007). Although some previous studies found no association of rs2228145 with overall *IL6R* RNA expression (IL6R Genetics Consortium and Emerging Risk Factors Collaboration. 2012) or expression of the RNA transcript encoding mIL-6R (Ferreira et al. 2013), a positive association has been reported for the rs2228145 C allele and expression level of the alternatively spliced mRNA (Ferreira et al. 2013; Stephens et al. 2012), and we and others found a negative association between the rs2228145 C allele and overall expression level (Revez et al. 2013).

Variants in *IL6R* are associated with the risk of a wide spectrum of common diseases, with the rs2228145 C allele increasing susceptibility to asthma (Ferreira et al. 2011), and decreasing susceptibility to other diseases including rheumatoid arthritis (Eyre et al. 2012), coronary heart disease (IL6R Genetics Consortium and Emerging Risk Factors Collaboration. 2012), and type 1 diabetes (Ferreira et al. 2013). Two consortia reported a protective effect of the C allele on the risk of coronary heart disease and emphasized the potential of tocilizumab, a monoclonal antibody against IL-6R used for treatment of chronic inflammatory disease, as a novel therapeutic strategy to prevent cardiovascular disease (IL6R Genetics Consortium and Emerging Risk Factors Collaboration. 2012; The IL-6R Mendelian Randomisation Analysis (IL6R MR) Consortium 2012). Growing interest in the role of *IL6R* in complex disease and in therapeutic strategies targeting the IL-6R pathway highlight the value of novel insights into genetic determinants of IL-6R level. The associations with disease reported for multiple variants in the *IL6R* gene are generally ascribed to LD with rs2228145. Two recent studies showed that some additional variance in sIL-6R level is explained by other SNPs within *IL6R* (Ferreira et al. 2013; Revez et al. 2013), but it is unknown how much of the variance of sIL-6R levels in total is due to variants other than rs2228145 that remain to be identified.

We describe a series of analyses conducted in a population-based sample of ~5,000 Dutch individuals (Supplementary Table S1) aimed at evaluating the contribution of the known functional polymorphism rs2228145 to the variation in sIL-6R levels, and the contribution of other genetic variants within the *IL6R* region and the rest of the genome. We analyzed plasma sIL-6R levels in a large sample of twins and their family members to estimate the total heritability of sIL-6R, and conducted GWA, linkage, and eQTL analyses to identify additional genetic variants influencing sIL-6R level. Using the data from twin families and the classic biometrical model, we estimate the broad heritability of sIL-6R level to be 72 % and show that rs2228145 accounts for 51 % of the total variance of sIL-6R level. Results from linkage analysis corroborate this and indicate that genetic variation within the *IL6R* region on chromosome 1 explains 69 % of the variation in sIL-6R, of which 19 % is due to genetic variation other than rs2228145. Results from eQTL analysis point towards a role of genetic variants at the 3′-end of *IL6R* contributing to the levels of sIL-6R and *IL6R* RNA in blood. In passing, we provide empirical evidence that different methods based on the same corpus of genetic theory, including the classic biometrical model, the twin-family model, linkage analysis at the *IL6R* locus, and genome-wide SNP sharing in unrelated individuals (GCTA) all converge to the same conclusion.

Materials and methods

Subjects

Plasma sIL-6R level data were available for 8,929 participants from the Netherlands Twin Register (NTR;

Boomsma et al. 2006), of which 5,945 individuals also had genome-wide SNP data. Data from an additional 1,966 participants from the Netherlands Study of Depression and Anxiety (NESDA (Penninx et al. 2008)) were included in the eQTL analysis. Individuals using anti-inflammatory medication or medication influencing the HPA (Hypothalamic–Pituitary–Adrenal)-axis (NTR: N = 426/4.8 %, NESDA: N = 538/26 %) at the time of blood sampling, or with a sIL-6R level >100.000 pg/mL (N = 6/0.07 % of NTR subjects) were excluded from all analyses. For a detailed description of the characteristics of the subjects included in each analysis, see Supplementary Methods. NTR and NESDA studies were approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the US Office of Human Research Protections (IRB number IRB-2991 under Federal-wide Assurance-3703; IRB/institute codes, NTR 03-180, NESDA 03-183). All subjects provided written informed consent.

Blood sampling

Blood sampling procedures have been described previously (Spijker et al. 2004; Willemsen et al. 2010). In short; venous blood samples were drawn in the morning after an overnight fast. For RNA measurement, heparinized whole blood from NESDA en NTR participants was transferred within 20 min of sampling into PAXgene Blood RNA tubes (Qiagen) and stored at −20 °C. For sIL-6R measurement, EDTA plasma tubes were collected from NTR participants, and were centrifuged for 20 min at 2000×*g* at 4 °C. EDTA plasma, buffy coat and red blood cells were harvested and aliquoted (0.5 ml), snap-frozen in dry ice, and stored at −30 °C.

sIL-6R level

sIL-6R level was measured in EDTA plasma samples (diluted 1:100) using the Quantikine Elisa Human IL-6 sR assay of R&D systems. The inter-assay and intra-assay coefficient of variation were <15 %. In all analyses, sIL-6R level was adjusted for sex and age by inclusion of covariates or by analysis of residualized levels. sIL-6R level was on average higher in males (4.25×10^{-8} g/mL) compared to females (4.14×10^{-8} g/mL) and increased with age [1 SD of age (14 years) was associated with an increase in sIL-6R level of 0.17×10^{-8} g/mL].

Genotype data

DNA extraction has been described before (Boomsma et al. 2008). Genotyping was done on multiple chip platforms, for several partly overlapping subsets of participants. The following platforms were used: Affymetrix Perlegen 5.0, Illumina 370, Illumina 660, Illumina Omni Express 1 M and Affymetrix 6.0. After array specific data analysis, genotype calls were made with the platform specific software (Genotyper, Beadstudio). In total, genotype data were available for 12,133 subjects from NTR and NESDA. The extensive genotyping quality control steps and imputation procedures are described in the Supplementary Methods. All analyses were performed on 1000G-imputed data (phase I Interim release All panel (sequence data freeze 23/11/2010), release June 2011, https://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_interim.html.

IL6R expression

RNA extraction (Spijker et al. 2004; Willemsen et al. 2010) and expression QC procedures have been described in detail previously (Jansen et al. 2014). PAXgene tubes were shipped to the Rutgers University Cell and DNA Repository (RUCDR), USA. RNA was extracted using Qiagen Universal liquid handling system (PAXgene extraction kits following the manufacturer's protocol). Total RNA was measured by spectroscopy (Trinean DropSense) to determine purity and concentration while RNA fidelity was measured by the Agilent Bioanalyzer analysis. RNA samples were hybridized to Affymetrix U219 array plates (GeneTitan), which contain 530,467 probes, each 25 bases in length. Array hybridization, washing, staining, and scanning were carried out in an Affymetrix GeneTitan System following the manufacturer's protocol. Non-uniquely mapping probes (hg19) and probes containing a polymorphic SNP based on snp137 (UCSC) were removed. Expression values were obtained using RMA normalization implemented in Affymetrix Power Tools (APT, v 1.12.0). Probes targeting *IL6R* (Supplementary Table S4) were selected for analysis.

Statistical analysis

The heritability of sIL-6R level in extended twin families

To estimate the broad- and narrow-sense heritability of sIL-6R and to examine the contribution of rs2228145, genetic structural equation models were fitted in Mx (Neale et al. 2006) to sIL-6R data from mono- and dizygotic twins, siblings and parents. The model included additive genetic influences (A), non-additive genetic influences (D), sibling-shared environmental influences (C) and unique environment (E).These methods are outlined in full in the Supplementary Methods.

Variance explained by chromosome-wide SNPs and SNPs in the IL6R region using GCTA

The variance in sIL-6R level explained by all SNPs was estimated in GCTA (Genome-wide Complex Trait Analysis (Yang et al. 2011a, b)) separately for each chromosome. For a full description of the methods, see Supplementary Methods.

GWA analysis

GWA analysis was performed in PLINK (Purcell et al. 2007) on dosage data. PLINK accounts for familial relations by performing a stratified association analysis with clusters based on family id using the—family option. This option implements generalized estimating equations with an independence model (Dobson 2002) and robust standard errors obtained using the sandwich correction for the family clustering (Williams 2000). The analyses included one randomly selected twin of each MZ pair.

Biometrical model

The variance due to additive effects (V_A) and dominance effects (V_D) of rs2228145 were estimated by applying the biometrical model (Falconer 1960) to the allele frequency estimates and the mean sIL-6R level corresponding to each genotype group. According to biometrical model, V_A and V_D are calculated as follows:

$$V_A = 2pq\ [a\ + d\ (q - p)]^2$$

$$V_D = (2pqd)^2$$

where p is the frequency of allele 1, q is the frequency of allele 2, a is the genotypic value; half the distance between the mean phenotype level of the two homozygotes, and d is the dominance deviation; deviation of the mean phenotype level of heterozygotes from the midpoint of the two homozygotes.

Mean sIL-6R level per rs2228145 genotype, corrected for age and sex, were obtained in SPSS version 19. Allele frequency estimates were obtained with Sib-pair ("http://genepi.qimr.edu.au/staff/davidD/#sib-pair") using the best linear unbiased estimator (BLUE) option, which accounts for familial relatedness (McPeek et al. 2004).

Heritability explained by rs2228145

To assess the contribution of rs2228145 to the heritability of sIL-6R level, models were fitted to the sIL-6R data from twin families with and without adjustment of sIL-6R levels for rs2228145 genotype. In the model with adjustment for rs2228145, sIL-6R level was modeled as follows:

$$\text{sIL-6R level} = \alpha + \beta_{age} \times \text{age} + \beta_{sex} \times \text{sex} + \beta_{rs2228145} \times \text{rs2228145 genotype} + \varepsilon$$

where α is the intercept, age is the age at blood sampling (z-score), sex is coded as 0 for males and 1 for females, $\beta_{rs2228145}$ is the additive effect of rs2228145, rs2228145 genotype is the observed genotype at rs2228145 (coded as 0, 1, 2—corresponding to the number of minor alleles) and ε is the residual.

When rs2228145 genotype is not accounted for, the effect of this SNP on the resemblance of sIL-6R level among family members is included in the total genetic influences (A and D, see Supplementary Methods). When the effect of rs2228145 is incorporated (by estimating $\beta_{rs2228145}$), sIL-6R levels are adjusted for rs2228145 genotype and the variance of residual levels (ε) is partitioned into unmeasured genetic and environmental factors (A, C, D and E). Thus, the total broad heritability (H^2) of sIL-6R can be written as:

$$H^2(\text{sIL-6R}) = a^2 + d^2 = a^2_{rs2228145} + a^2_{residual} + d^2_{rs2228145} + d^2_{residual}$$

where a^2 and d^2 are the proportions of the variation in sIL-6R level due to total additive genetic and non-additive genetic effects, as estimated from the twin-family data, $a^2_{residual}$ and $d^2_{residual}$ represent all remaining unmeasured additive and non-additive genetic effects that are not captured by rs2228145 (expressed as a proportion of the total variation in sIL-6R level): these components were estimated in a model that included adjustment of sIL-6R levels for SNP effects. $a^2_{rs2228145}$ and $d^2_{rs2228145}$ are the proportion of the total variation in sIL-6R that can be explained by additive and non-additive genetic effects of rs2228145, which can be inferred from the difference between the variance estimates from the total heritability model (without correction for rs2228145) and the variance estimates from the model with adjustment for rs2228145.

Combined linkage and association analysis

Analysis of linkage while simultaneously modeling association, as suggested by Fulker et al. (1999) was conducted in QTDT (Abecasis et al. 2000). The data came from nuclear families of which both parents and offspring had data on genome-wide SNPs and sIL-6R level, where the offspring consisted of DZ twins or non-twin sib-pairs, or a single MZ twin + sibling(s). The analysis was performed for all imputed SNPs with a MAF >0.2 in the *IL6R* gene ± 10 MB (*IL6R* gene location build 37/hg19, chr1: 154377669–154441926), leading to a selection of 13,751 SNPs (chr1: 144377669-164441926). IBD probabilities were estimated in Merlin (Abecasis et al. 2002) using

multipoint estimation, which takes into account the correlated structure of markers and is therefore suited for dense SNP data (Abecasis and Wigginton 2005). To create a centiMorgan (cM) map of the region, cM distances between SNPs were inferred from base-pair distances following the assumption that a distance of 1 million base-pairs between SNPs corresponds to a distance of 1 cM. The analysis was performed on residual sIL-6R levels after taking out the effects of all covariates (Supplementary Methods). First, the evidence for linkage was evaluated and next, these results were compared to the results obtained when modeling linkage and association simultaneously (using the—at option to specify the total association model, which is not a TDT test). The test for linkage while simultaneously modeling association involves comparing H0: sIL-6R $= \mu + \beta$SNP and Variance (sIL-6R) $= V_E + V_G$ against H1: sIL-6R $= \mu + \beta$SNP and variance (sIL-6R) $= V_E + V_G + V_A$; where μ is the intercept (corrected for covariates), V_E is the non-shared environmental variance, V_G is the additive polygenic variance, which is estimated from the phenotypic covariance of relatives following the assumption that on average 50 % of V_G is shared among first degree relatives. V_A is the additive major gene effect, which represents the additive effect of linkage to a major gene and is based on the pi-hat measure derived from the IBD matrix of relatives. H0 is the null-hypothesis. H1 is the alternative hypothesis.

eQTL analysis

Inverse quantile normal transformation was applied to the individual probe data to obtain normal distributions. For each SNP-probe combination, a linear mixed model was fitted with expression level as dependent variable, and with fixed effects: genotype, sex, age, body mass index, smoking status, technical covariates (covering e.g. plate and well differences (Jansen et al. 2014)), three principle components (PCs) from the genotype data (Supplementary Methods) and five PCs from the transformed expression data. Random effects included family ID and zygosity to account for family and twin relations (Visscher et al. 2004). Cis-eQTLs are expression-associated SNPs with a distance <1 Mb to the gene, and trans-eQTLs are the complementary set of SNPs. In an initial analysis of genome-wide SNPs, no trans-eQTLs were observed. The cis-eQTL analysis yielded 36 (N probes) × 2,731(N SNPs) = 98,316 tests. To correct for multiple testing, a conservative *P* value threshold of 0.01/98,316 $\sim 1 \times 10^{-7}$ was applied. The conditional eQTL analysis was performed using the same model and *P* value threshold on expression data that had been residualized for the effect of rs7512646 in advance. To examine the relationship between sIL-6R level and *IL6R* expression level, the Pearson correlation between sIL-6R level and expression level was computed for all probes.

Results

The heritability of sIL-6R level in extended twin families

To examine the overall contribution of genetic and environmental influences to the variation in sIL-6R level, genetic structural equation modeling was performed on sIL-6R level data of 4,980 subjects from 2,360 twin families. This approach allowed us to estimate the variance due to total heritable genetic effects (broad-sense heritability) and additive genetic effects (narrow-sense heritability). Based on the pattern of phenotypic correlations for sIL-6R level among monozygotic (MZ) twins, dizygotic (DZ) twins, siblings, and parent-offspring pairs (Table 1), the broad-sense heritability of sIL-6R level was estimated at 72 % ($H^2 = (V_A + V_D)/V_{total} = (0.89 + 0.08)/1.35$) and the narrow-sense heritability was estimated at 66 % ($h^2 = V_A/V_{total} = 0.89/1.35$). The remaining variance (28 %) was ascribed to environmental factors not shared among family members (unique environment: $e^2 = V_E/V_{total} = 0.38/1.35$).

Variance explained by chromosome-wide SNPs and SNPs in the *IL6R* region using GCTA

We next used GCTA on 1000Genomes-imputed SNP data to examine how much of the variance in sIL-6R level can be explained by all SNPs in the *IL6R* region (*IL6R* ± 10 MB), how much by other SNPs on chromosome 1 and how much by each of the other chromosomes (Table 2, Supplementary Figure S1) based on the similarity across SNPs among 2,875 unrelated subjects. This method gives insight into the total contribution of additive genetic effects tagged by all genotyped and imputed SNPs together, providing an estimate of the total variance that could be identified by GWAS on this set of SNPs, given sufficient power to detect individual SNP effects. SNPs in the *IL6R* region with a MAF >0.001 (*N* SNPs = 42,268) together explained 54.7 % (SE = 2.5 %) of the variance of sIL-6R levels, while the rest of chromosome 1 did not contribute to the variance. Some additional variance was captured by SNPs on chromosome 2 (5.6 %, SE = 2.8). When the analysis was repeated with inclusion of related subjects (including siblings, DZ twins and parent-offspring pairs, total *N* subjects = 4,846) the estimate for chromosome 2 was somewhat lower (2.8 %, SE = 1.6), whereas otherwise highly similar results were obtained (Table 2).

Table 1 Familial correlations of sIL-6R level

	Complete pairs	Correlations, no correction for SNP effects[d]		Correlations, correction for rs2228145[e]	
	N	r	95 % CI	r	95 % CI
Monozygotic twins					
MZ male twins	208	0.805	(0.7607–0.8388)	0.560	(0.4530–0.6416)
MZ female twins	520	0.684	(0.6421–0.7207)	0.358	(0.2852–0.4248)
Male siblings					
DZ male twins	93	0.407	(0.2083–0.5566)	0.249	(0.0258–0.4324)
Male sibs[a]	126	0.243	(0.0882–0.3859)	0.271	(0.1033–0.4129)
Female siblings					
DZ female twins	216	0.373	(0.2627–0.4688)	0.258	(0.1445–0.3612)
Female sibs[b]	313	0.298	(0.1784–0.4018)	0.121	(0.0116–0.2250)
Opposite–sex siblings					
DZ opposite-sex twins	224	0.317	(0.1884–0.4282)	0.153	(0.0191–0.2782)
Opposite–sex sibs[c]	337	0.428	(0.3276–0.5121)	0.208	(0.0748–0.3250)
Parent–offspring					
Mother–daughter	342	0.335	(0.2543–0.4076)	0.180	(0.0975–0.2581)
Mother–son	204	0.438	(0.3434–0.5174)	0.292	(0.1700–0.3969)
Father–son	171	0.360	(0.2626–0.4447)	0.202	(0.0708–0.3190)
Father–daughter	314	0.316	(0.2311–0.3915)	0.188	(0.0928–0.2748)
Spouses					
Mother–Father	374	0.118	(0.0269–0.2075)	0.247	(0.1563–0.3317)

Note that the correlations of sIL-6R levels adjusted for the effect of rs2228145 provide information about the proportions of residual variance due to genetic and environmental effects. The proportion of the total phenotypic variance that is due to genetic effects beyond rs2228145 is reflected in the difference between the correlations with and without adjustment for rs2228145 genotype

MZ monozygotic, *DZ* dizygotic, *CI* Confidence interval

[a] Non-twin brother–brother pairs and pairs of brother-male twin

[b] Sister-sister and Sister-female twin

[c] Sister-brother, brother-female twin and sister-male twin

[d] Correlation from a saturated model with covariates age and sex

[e] Correlation from a saturated model with covariates age, sex and rs2228145 genotype

GWA analysis

GWA analysis of sIL-6R level was conducted on imputed SNP data from 4,846 subjects. The genomic inflation factor indicated no effect of population stratification ($\lambda = 1.015$). 680 Genome-wide significant hits were found ($P < 5 \times 10^{-8}$), which were all located on chromosome 1q21.3 (significant hits; chr 1: 153389207–154697624), except for four SNPs (MAF 0.01–0.09) on chromosomes 5, 8 and 20 (Supplementary Table S2). The top SNPs ($P < 1 \times 10^{-176}$) were located within *IL6R* and were all in LD with rs2228145.

Biometrical model

The minor (C) allele of rs2228145 occurred at a frequency of 0.39. To estimate how much of the variance in sIL-6R level is explained by rs2221845, we applied the classic biometrical model to the data. The average sIL-6R level was 5.698 (10^{-8} g/mL) in individuals homozygous for the minor allele (CC), 4.418 in heterozygotes (AC), and 3.238 in individuals with the AA genotype, giving an overall mean level of 4.17. The observed variance of sIL-6R levels in the total sample was 1.35. Applying the biometrical model to these data, it follows that:

$$\begin{aligned} a &= 0.5 \times (\text{sIL-6R}_{CC} - \text{sIL-6R}_{AA}) \\ &= 0.5 \times (5.698 - 3.238) = 1.23 \end{aligned}$$

$$\begin{aligned} d &= \text{sIL-6R}_{AC} - (\text{sIL-6R}_{AA} + a) \\ &= 4.418 - (3.238 + 1.23) = -0.05 \end{aligned}$$

$$\begin{aligned} V_A &= 2pq\,[a + d\,(q - p)]^2 \\ &= 2 \times 0.39 \times 0.61 \\ &\quad \times [1.23 - 0.05 \times (0.61 - 0.39)]^2 \\ &= 0.71 \end{aligned}$$

Table 2 Variance of sIL-6R level explained by chromosome-wide SNPs estimated using GCTA

Chromosome	N SNPs	Unrelated subjects (N = 2,875)		Unrelated and related subjects (N = 4,846)	
		V_G/V_P	SE	V_G/V_P	SE
1 *IL6R* region	42,268	0.547	0.025	0.533	0.019
1 Rest	584,291	0.003	0.025	0.000	0.015
2	692,964	0.056	0.028	0.028	0.016
3	590,258	0.000	0.024	0.009	0.014
4	605,730	0.000	0.023	0.002	0.013
5	541,738	0.000	0.023	0.005	0.013
6	530,265	0.017	0.021	0.030	0.014
7	483,988	0.000	0.021	0.009	0.013
8	447,709	0.001	0.021	0.010	0.013
9	350,386	0.000	0.021	0.000	0.012
10	418,731	0.010	0.021	0.017	0.013
11	410,817	0.000	0.014	0.005	0.009
12	393,609	0.001	0.019	0.010	0.011
13	307,137	0.003	0.017	0.007	0.011
14	265,369	0.000	0.017	0.000	0.010
15	227,463	0.013	0.017	0.000	0.009
16	242,058	0.006	0.018	0.002	0.010
17	202,299	0.014	0.017	0.000	0.010
18	234,349	0.002	0.018	0.000	0.010
19	158,942	0.003	0.014	0.001	0.008
20	180,019	0.016	0.016	0.007	0.010
21	109,887	0.000	0.013	0.000	0.007
22	105,190	0.003	0.012	0.010	0.008

SE standard error

$$V_D = (2pqd)^2 = (2 \times 0.39 \times 0.61 \times -0.05)^2 = 5.66 \times 10^{-4}$$

Thus, the allelic effect was almost completely additive, which illustrates that the average sIL-6R level of heterozygous individuals lies perfectly in the middle of the levels of the two homozygous groups (Fig. 1a).

Heritability explained by rs2228145

The data from twin families allowed us to examine how much of the heritability is explained by rs2228145, and how much genetic variance is left after adjusting sIL-6R levels for rs2228145. The contribution of rs2228145 to the heritability of sIL-6R level is illustrated by the drop of the correlations of sIL-6R level among family members after correcting sIL-6R level for rs2228145 genotype (Table 1). When the allelic effect of rs2228145 on sIL-6R level was taken into account in the twin family model (Table 3), the total variance of sIL-6R level dropped from 1.35 to 0.66 (difference = 0.69), illustrating that this SNP accounted for 51 % of the total variance in sIL-6R level ($H^2_{SNP} = 0.69/1.35 = 0.51$). Residual genetic effects not tagged by rs2228145 ($V_{G\text{-residual}} = 0.26$) accounted for 19 % of the total variance of sIL-6R ($H^2_{residual} = 0.26/1.35 = 0.19$).

The contribution of other additive SNP effects in the *IL6R* region

When we repeated the analysis of unrelated subjects in GCTA while correcting for the effect of rs2228145, SNPs in the *IL6R* region on chromosome 1 still explained 5.6 % of the total variance of sIL-6R level (SE = 1.8 %, $P = 6.0 \times 10^{-5}$). This suggests that part of the residual heritability that was estimated based on twin family analysis can be explained by other variation in the *IL6R* region that is tagged by genotyped and imputed SNPs.

Combined linkage and association analysis

In the GWA analysis of sIL-6R levels, highly significant hits were found across the *IL6R* region, which may include SNPs that merely capture the effect of rs2228145 (through LD) and SNPs tagging other causal variants. To search for genetic variants that explain additional variation in sIL-6R levels beyond the effect of rs2228145, we simultaneously modeled linkage and association using data from 355 nuclear families (total N subjects = 1,254) for SNPs in the *IL6R* region (*IL6R* ± 10 MB, Fig. 2a). This region covers all genome-wide significant GWA hits on chromosome 1. If the linkage test is not significant while simultaneously modeling association for a SNP (while the linkage test is significant when association is not modeled simultaneously), this indicates that the respective SNP is in high LD with a causal variant. On the other hand, the linkage signal will not be fully impaired when association is modeled for SNPs that are in lower LD with a causal variant, or when multiple causal variants in partial LD contribute to variation in sIL-6R levels.

In a model that included linkage (V_A, additive variance attributable to the locus estimated using IBD), additive polygenic variance (V_G, estimated from the familial resemblance in sIL-6R following the assumption that on average 50 % of V_G is shared among first degree relatives) and unique environment (V_E), but not incorporating association, the linkage signal around rs2228145 explained 69 % (V_A/V_{total}) of the total variation in sIL-6R levels ($X^2 = 34.12$, df = 1, $P = 5 \times 10^{-9}$) and V_G was estimated at 0 (Table 3). Comparison of the estimate of variance explained by linkage at *IL6R* (69 %) to the broad-

Table 3 Variance of sIL-6R level due to rs2228145, total heritability of sIL-6R level, and estimates from linkage analysis

Genotype	Frequency[a]	sIL-6R level (10^{-8} g/mL)[b]	Genotypic Value	Frequency × sIL-6R	(Deviation from μ)^2	Frequency × squared deviation
Observed variance due to rs2228145 genotype						
CC	$p^2 = 0.15$	5.698	+a	$0.15 \times 5.698 = 0.87$	$(5.698 - \mu)^2 = 2.32$	$0.15 \times 2.32 = 0.35$
AC	$2pq = 0.48$	4.418	d	$0.48 \times 4.418 = 2.10$	$(4.418 - \mu)^2 = 0.06$	$0.48 \times 0.06 = 0.03$
AA	$q^2 = 0.37$	3.238	−a	$0.37 \times 3.238 = 1.20$	$(3.238 - \mu)^2 = 0.88$	$0.37 \times 0.88 = 0.33$
Total			$\mu = 0.87 + 2.10 + 1.20 = 4.17$		$V_{SNP} = 0.35 + 0.03 + 0.33 = 0.71$	
						(95 % CI 0.68–0.74)

	$V_{sIL\text{-}6R}$	V_A	V_D	V_C	V_E	V/V_{total}	h^2	d^2	c^2	e^2
Extended twin family model estimates										
ACDE model[c]	$V_{total} = 1.35$	0.89	0.08	0.00	0.38	1.00	0.66	0.06	0.00	0.28
ACDE + rs2228145[d]	$V_{residual} = 0.66$	0.24	0.02	0.01	0.39	$V_{residual}/V_{total} = 0.49$	0.18	0.01	0.01	0.29
Effect of rs2228145[e]	$V_{SNP} = 0.69$	0.65	0.06	−0.01	−0.01	$V_{SNP}/V_{total} = 0.51$	0.48	0.05	0.01	0.01

	$V_{sIL\text{-}6R}$	$V_{A\text{-}linkage}$	$V_{A\text{-}polygenic}$	V_D	V_C	V_E	V/V_{total}	h^2	d^2	c^2	e^2
Linkage analysis estimates											
Linkage modeled at rs2228145	$V_{total} = 1.39$	0.96	0	–	–	0.43	1	0.69	–	–	0.31
Linkage + association modeled at rs2228145	$V_{residual} = 0.67$	0.26	0	–	–	0.41	$V_{residual}/V_{total} = 0.48$	0.19	–	–	0.30

V_{total} is the total (phenotypic) variance of age- and sex-adjusted sIL-6R levels. V_{SNP} is the variance of sIL-6R level attributable to rs2228145, V_{SNP}/V_{total} is the Proportion of total (phenotypic) variance sIL-6R level explained by rs2228145, $V_{residual}/V_{total}$ is the Proportion of total (phenotypic) variance sIL-6R level not explained by rs2228145, V_A is the Additive genetic variance, V_D is the Non-additive genetic variance, V_C is the Sibling-shared environmental variance, V_E is the Unique environmental variance, $h^2 = V_A/V_{total}$, $d^2 = V_D/V_{total}$, $c^2 = V_C/V_{total}$, $e^2 = V_E/V_{total}$

$V_{A\text{-}linkage}$ is the variance due to linkage, which is based on the pi-hat measure at rs2228145, derived from the IBD matrix of relatives

$V_{A\text{-}polygenic}$ is the variance due to all additive genetic effects that are not captured within the linkage component (i.e. additive genetic variance that is not linked to the *IL6R* locus), which was estimated from the phenotypic covariance of relatives following the assumption that on average 50 % of $V_{A\text{-}polygenic}$ is shared among first degree relatives

μ is the mean sIL-6R level in the population, estimated from the genotype frequencies and corresponding sIL-6R levels for each genotype group

[a] p is the minor allele frequency = 0.39 and q is the major allele frequency = 0.61

[b] Mean sIL-6R level, corrected for age and sex, for each genotype group

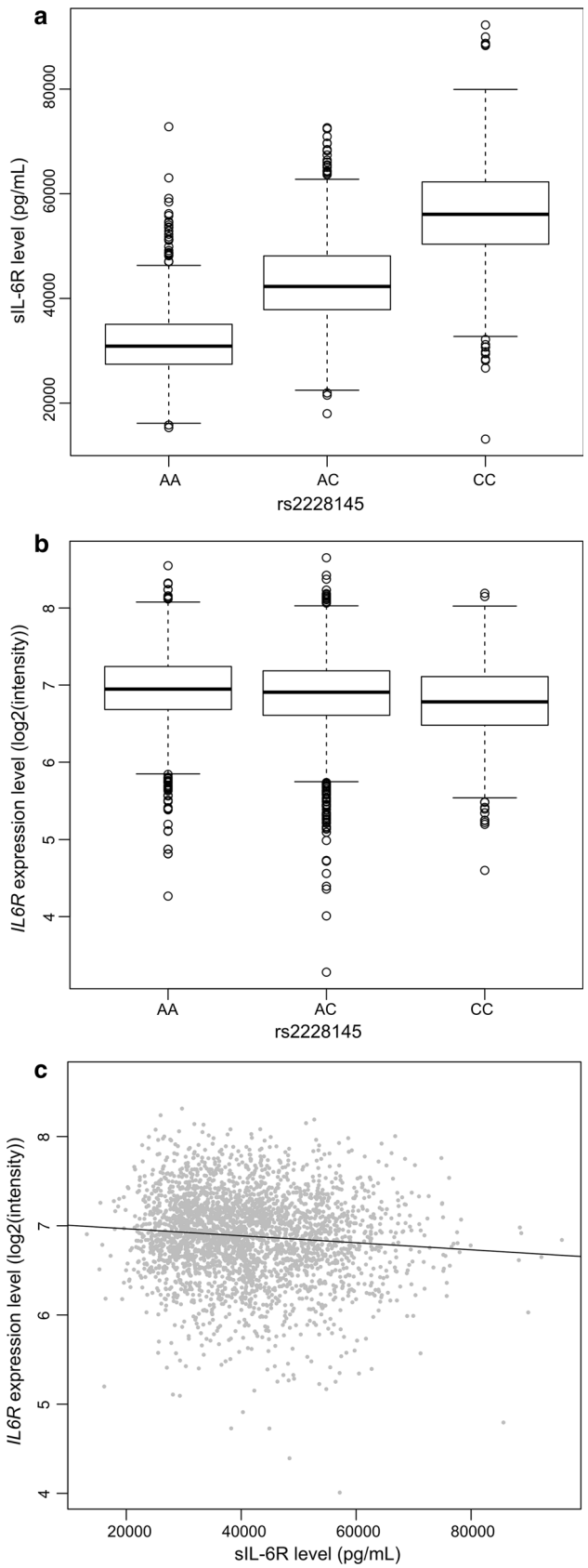
[c] ACDE model without SNP effects, in which V_{total} is decomposed into V_A, V_D, V_C, and V_E

[d] Model in which the additive effect of rs2228145 on sIL-6R level is modeled, and the residual variance of sIL-6R ($V_{residual} = V_{total} - V_{SNP}$) is decomposed into V_A, V_C, V_D and V_E

[e] The effect of rs2228145 was inferred from the difference between model [c] and model [d]

sense heritability (72 %) and narrow-sense heritability (66 %) estimated by the twin family model suggests that the entire narrow-sense heritability of sIL-6R level and nearly the entire broad-sense heritability of sIL6-R level can be captured by modeling the covariance of sIL6-R level among relatives as a function of IBD-sharing at the *IL6R* locus.

When the allelic effects of SNPs on the means were incorporated in the model (i.e., association was added), 19 % of the total variance in sIL-6R level (V_A/V_{total}) was still explained by the linkage component when modeling association at rs2228145 (similar to the estimate of residual heritability from the twin family model), although this signal was borderline significant only ($X^2 = 4.13$, df = 1, $P = 0.042$). Significant linkage was initially observed across the entire region, but when association was modeled, linkage only dropped to the level of borderline significance for SNPs in high LD with rs2228145 ($r^2 > 0.93$ among 21 SNPs with $P > 0.01$) indicating that rs2228145 explains most, though not the entire linkage signal. The linkage signal was also attenuated when modeling association for SNPs within an LD block at the 3′-end of *IL6R* containing the *IL6R* 3′-UTR and the adjacent genes *SHE* and *TDRD10* (location indicated in Fig. 2b). This region contains both SNPs in low LD ($r^2 \sim 0.1$) with rs2228145 and SNPs in high LD with rs2228145. When we conducted



◀**Fig. 1** Associations between sIL-6R level, *IL6R* expression and rs2228145 genotype. **a** Boxplots of plasma sIL-6R level (pg/mL) for each rs2228145 genotype. **b** Boxplots of *IL6R* expression level in blood as measured by expression probe 582_132 for each rs2228145 genotype ($P = 3.14 \times 10^{-19}$). C: Scatterplot of sIL-6R level (pg/mL) versus *IL6R* expression level for probe 582_132 ($r = -0.092$, $P = 1.42 \times 10^{-6}$)

a conditional GWAS to test for association between chromosome 1 SNPs and sIL-6R levels after taking out the effect of rs2228145 (by including this SNP as a covariate in the model), significant associations were still observed for SNPs within *IL6R* including SNPs at the 3′-end (Supplementary Table S3). These results support the presence of additional causal variants influencing sIL-6R level in the *IL6R* gene and 3′-end.

eQTL analysis

To identify genetic variants related to *IL6R* RNA expression levels, eQTL analysis was performed on data from 4,467 subjects for 36 probes (Supplementary Table S4) measuring *IL6R* RNA. A total of 341 significant associations (Supplementary Table S5) were found for 5 of these probes (4 probes targeting exon 7 and 1 probe targeting the 3′-end). These associations involved 179 SNPs that were all in *cis* (SNP positions; chr1q21.3: 154395125–154521584). The most significant association was between rs7512646 and expression probe 582_132 targeting exon 7 ($P = 2.84 \times 10^{-22}$). This SNP (intronic) is in high LD with rs2228145 ($r^2 = 0.94$) and rs2228145 itself showed significant associations with all four probes targeting exon 7. Probe 308_15 targeting the 3′-end was the only probe outside exon 7 with significant eQTLs (total N associated SNPs = 92, top hit = rs4072391 located in the *IL6R* 3′-UTR, $P = 1.32 \times 10^{-11}$, r^2 with rs2228145 = 0.142). Of the 680 significant hits identified in the GWAS of sIL-6R, 157 SNPs were significantly associated with *IL6R* expression level. For all of these SNPs, the allele associated with higher sIL-6R levels was associated with lower *IL6R* expression level.

Relationship between rs2228145, *IL6R* RNA and sIL-6R levels

As a measure of the relationship between plasma sIL-6R levels and the abundance of *IL6R* RNA in whole blood, the correlation between sIL-6R level and expression level was computed for all probes targeting *IL6R* transcripts (Supplementary Table S6). All five expression probes with significant eQTL hits showed small but statistically significant negative correlations with sIL-6R level (e.g. probe 582_132: $r = -0.092$, $P = 1.42 \times 10^{-6}$, Table 4). The

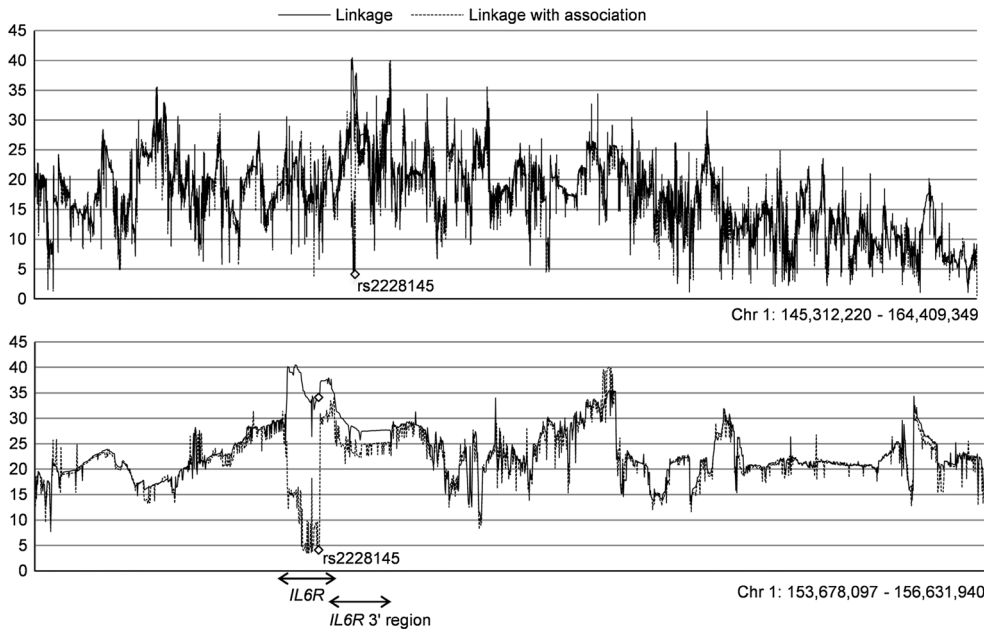


Fig. 2 Results from combined linkage and association analysis of the *IL6R* region on chromosome 1. The *solid black line* connects the X^2 values from the linkage test at each SNP without modeling SNP association and the dotted black line connects the X^2 values from the linkage test at each SNP when association is simultaneously modeled. The analysis was performed for all imputed SNPs with a MAF >0.2 in *IL6R* ± 10 MB, chr1 (build 37): 145312220-164409349 (x-axis, **a**). **b** Zooms into the region from basepair position 153678097 to 156631940. The *arrows* mark the location of the *IL6R* gene, and the location of an LD block stretching across the 3′-UTR of *IL6R* and adjacent genes SHE and TDRD10, where significant hits were found in the eQTL analysis for SNPs in partial LD with rs2228145 ($r^2 \sim 0.1$)

negative correlations indicate that across individuals, higher sIL-6R level was associated with lower overall *IL6R* expression (Fig. 1c). Correlations between *IL6R* RNA and sIL-6R levels became non-significant when expression levels were corrected for the effect of rs2228145 (e.g., probe 582_132: r = 0.019, $P = 0.314$, Table 4), which indicates that at the population level, the relationship between *IL6R* expression and sIL-6R level can be explained by the effect of rs2228145. Indeed, the minor C allele of rs2228145 was associated with higher sIL-6R level and with lower *IL6R* expression level (Fig. 1). When computed separately within rs2228145 genotype classes, correlations between IL6R expression and sIL-6R levels were not significant (Table 4).

Taken our eQTL design, we could not distinguish between alternative *IL6R* transcripts but observed a significant negative association between the rs2228145 C allele and total levels of *IL6R* RNA. In another eQTL analysis based on expression in peripheral blood (N = 1,469; Fehrmann et al. 2011), one of the probesets targeted *IL6R* exon 9, which is spliced out from the alternative RNA transcript that is presumed to directly code for sIL-6R. For this probeset, a negative relation between the minor allele of rs4845623 (r^2 with rs2228145 = 0.93) and expression level was observed ($P < 1 \times 10^{-7}$). We also downloaded the exon-specific expression data from HapMap lymphoblastoid cell lines (GEO accession nr = GSE9703, $N = 162$) and corresponding genotype data (Zhang et al. 2009). In this dataset, no significant association between rs2228145 and exon 9 expression level was found (possibly due to the small sample size). When we computed the ratio of exon 9 expression level over mean *IL6R* transcript expression, we found that the proportion of *IL6R* transcripts containing exon 9 decreased with each copy of the rs2228145 C allele ($P < 0.01$, Supplementary Figure S2), suggesting that rs2228145 is associated with the ratio of normal and alternatively spliced transcripts. Note that if the rs2228145 C allele has differential effects on alternative RNA transcripts, a negative association with total RNA levels (as assessed in our own study) will emerge if the increasing effect on levels of the alternative splice variant (Ferreira et al. 2013; Stephens et al. 2012) is smaller compared to the decreasing effect on other transcripts.

Conditional eQTL analysis

When the eQTL analysis was conducted after adjusting expression levels for the effect of the most significant eQTL SNP from the initial analysis (rs7512646), significant associations (Supplementary Table S7) were observed between probe 308_15 (targeting the 3′-end of *IL6R* transcripts) and 80 SNPs located in an LD block covering the

Table 4 Correlation between sIL-6R level and *IL6R* expression level for all probes with significant eQTL hits

IL6R probe	Probe location		Overall correlation (N = 2727)	Correction for rs2228145 (N = 2727)	Genotype AA (N = 991)	Genotype AC (N = 1278)	Genotype CC (N = 458)
323_134	Exon 7	r	−0.086	0.001	0.032	0.015	−0.023
		P value	7.48×10^{-6}	0.955	0.312	0.601	0.618
201_497	Exon 7	r	−0.054	0.011	0.047	0.009	0.002
		P value	0.005	0.577	0.136	0.755	0.974
582_132	Exon 7	r	−0.092	0.019	0.024	0.005	−0.063
		P value	1.42×10^{-6}	0.314	0.449	0.857	0.178
202_497	Exon 7	r	−0.071	0.005	0.072	−0.016	−0.017
		P value	1.94×10^{-4}	0.791	0.023	0.569	0.724
308_15	3′-end	r	−0.055	−0.003	0.031	−0.043	−0.088
		P value	0.004	0.894	0.335	0.124	0.059

r is the Pearson correlation between sIL-6R level (corrected for age and sex) and expression level (corrected for technical covariates, age and sex). Colum 5 only is based on expression levels that were additionally corrected for the additive allelic effect of rs2228145. Columns 6–8 show the correlations between sIL-6R level and expression level computed separately within rs2228145 genotype classes. Correlations for all *IL6R* expression probes are given in Supplementary Table S6

3′-UTR of *IL6R* and the adjacent 3′ region. This region was also highlighted by the analysis of linkage and association of sIL-6R levels (location indicated in Fig. 2b). The most significantly associated conditional eQTL SNPs with a *P* value of 1.89×10^{-9} were rs60760897, rs60255122, rs61698846 and rs61275241 (Located in *TDRD10*, r^2 with rs2228145 = 0.133, MAF = 0.21), followed by rs4072391 ($P = 2.10 \times 10^{-9}$, located in the *IL6R* 3′-UTR, r^2 with rs2228145 = 0.142; these SNPs are in high LD with each other and for each of the SNPs, the minor allele was associated with higher expression level and with lower sIL-6R level (*P* value (GWAS) for rs60760897 = 1.49×10^{-121}, and *P* value (conditional GWAS) for rs60760897 = 8.04×10^{-4}). All the initially observed associations for exon 7 expression probes were no longer significant after correcting for the most significant eQTL, suggesting that genetic variation tagged by rs2228145 and variation tagged by rs60760897 (*IL6R* 3′ region) are both independently associated with *IL6R* expression level.

Discussion

Based on the analysis of three different types of familial relations in extended twin families (monozygotic twins, sibling pairs/dizygotic twins, and parent-offspring pairs), the broad heritability of sIL-6R level was estimated at 72 % and the narrow-sense heritability was estimated at 66 %. Linkage analysis closely recaptured this, with 69 % of the variance in sIL-6R levels explained by all variation in the *IL6R* region that is captured by IBD. Both estimates are similar to the heritability of sIL-6R levels reported by a previous study conducted in middle-aged male twins ($h^2 = 0.68$) (Raggi et al. 2010). The estimate of variance explained by additive SNP effects in the *IL6R* region in unrelated subjects (GCTA) was slightly lower at 54.7 %, which suggests that the heritability of sIL-6R cannot be fully ascribed to additive effects of currently genotyped and imputed SNPs. Whereas the variance explained by all SNPs in unrelated subjects (in which LD extends over relatively short distances) specifically provide information about effects tagged by a given set of SNPs under an additive allelic model, linkage and twin analyses, which are based on IBD-sharing among close relatives, capture more variation contributing to the similarity of family members. Estimates from linkage may include variation in a region that is not (fully) captured by additive SNP effects, such as rare variants segregating in families, structural variation and effects of interacting loci (epistasis). Overall, the three different methods show the expected convergence.

After establishing the heritability, we examined the contribution of the functional *IL6R* polymorphism rs2228145, a known candidate SNP for sIL-6R (Galicia et al. 2004; Lourdusamy et al. 2012; Melzer et al. 2008; Rafiq et al. 2007; Reich et al. 2007), to the population variance in plasma sIL-6R levels. Using the classic biometrical model and twin family analysis, we showed that rs2228145 explained 51 % of the total variance of sIL-6R level, corresponding to 71 % of the broad heritability (51/72 = 0.71). The estimate of variance explained by this SNP in our sample is comparable to an estimate previously reported for subjects of European descent (49 % explained by rs2228145 (Reich et al. 2007)), and is larger compared to several other previous reports (33 % explained by rs2228145 in African Americans (Reich et al. 2007), 29 % explained by rs2228145 (Ferreira et al. 2013), 30 % explained by rs4129267; a SNP in LD (Revez et al.

2013), and 20 % explained by rs4537545; another SNP in LD (Rafiq et al. 2007)). The variation between studies might be related to differences in the lab protocol (e.g. differences related to sIL-6R detection assay and dilution of samples) and to differences between study populations. Whereas our study and the studies by Reich et al. (2007) and Rafiq et al. (2007) were conducted on population-based cohorts, the studies by Ferreira et al. (2013) and Revez et al. (2013) included patient populations. In contrast to all other studies, we excluded individuals using anti-inflammatory medication, which could have led to a slightly healthier population compared to previous studies, and it could be hypothesized that the variance explained by rs2228145 may vary with health status, as the levels of sIL-6R may rise 2–3 fold within a person during inflammation (Scheller et al. 2013). Although the allele frequencies of rs2228145 differ between individuals of European versus African descent, variation related to ancestry only seems explain the different estimates observed in the study by Reich et al. (2007), as all other studies focused on individuals of European descent and reported similar allele frequencies for rs2228145.

Functional studies have indicated that amino-acid mutations at the position encoded by rs2228145 can influence the production of sIL-6R through shedding of membrane-bound receptors (Müllberg et al. 1994). The effects of rs2228145 and other SNPs on *IL6R* expression are less well characterized. To gain insight into the regulatory impact of genetic variation on *IL6R* expression, we studied the relation between *IL6R* expression level and genome-wide SNPs. We found two clusters of SNPs within the *IL6R* region that were associated with expression level, one of which included rs2228145, suggesting that this SNP also influences *IL6R* expression, though the signal may also come from another causal variant in LD. A previous analysis of gene expression in multiple samples and tissues revealed no significant association between rs2228145 genotype and overall expression of *IL6R* RNA transcripts (possibly due to limited sample size; IL6R Genetics Consortium and Emerging Risk Factors Collaboration. 2012). Two other studies found that the rs2228145 C allele was associated with higher levels of an alternative *IL6R* mRNA splice variant (Ferreira et al. 2013; Stephens et al. 2012), which lacks a 94-bp sequence encoding part of the trans-membrane domain that anchors the membrane-bound IL-6R to the cell membrane and is presumed to directly code for the sIL-6R. In our study and in the study by Revez et al. (2013), the rs2228145 C allele was associated with lower overall *IL6R* RNA level and our analysis of the HAPMAP expression data suggested that rs2228145 (or a variant in LD) has an effect on the relative abundance of alternative *IL6R* RNA transcripts.

Though the opposite effects of rs2228145 on sIL-6R levels and overall *IL6R* expression level may appear contradictory, this finding is not unexpected given that rs2228145 has a strong effect on alternative splicing. As the C allele is associated with increased splicing of exon 9 (Stephens et al. 2012), this allele is likely to decrease the level of the full-length RNA transcript. It can also be hypothesized that rs2228145 may have secondary effects on *IL6R* expression by impacting on feedback systems that control *IL6R* expression. In hepatocytes, a positive feedback circuit has been identified through which increased membrane-bound receptor-mediated classic IL-6 signaling triggers microRNA (miRNA)-mediated regulatory pathways that stimulate increased *IL6R* expression(Hatziapostolou et al. 2011). The rs2228145 C allele has been shown to impair classical IL-6 signaling, as indicated by reduced levels of mIL-6R and decreased IL-6 responsiveness of CD4+ T cells and monocytes from C allele carriers (Ferreira et al. 2013). As the rs2228145 C allele weakens classical IL-6 signaling, positive feedback on *IL6R* expression may be reduced in individuals with the C allele, leading to lower RNA expression levels compared to individuals with the A allele. The associations between SNPs and variation in RNA and soluble IL-6 receptor abundance observed in our study provide guidance for functional studies into the mechanistic relationships among polymorphisms in *IL6R*, *IL6R* RNA expression and IL-6 receptor levels.

While the variance in sIL-6R levels that can be explained by rs2228145 is very large compared to single SNP effect sizes generally observed for quantitative traits, our study showed that other genetic effects also make an important contribution to the heritability of sIL-6R levels. Linkage analysis and analysis of the variance explained by chromosome wide SNP effects in unrelated subjects (GCTA) indicated that the remaining heritability of sIL-6R level appears to be primarily accounted for by other variation in the *IL6R* region on chromosome 1. Linkage analysis showed that genetic effects in this region not tagged by rs2228145 account for 19 % of the total variance in sIL-6R levels. We therefore tried to identify other SNPs in the *IL6R* region that explain additional variance in this clinically important soluble cytokine receptor.

Evidence for association with sIL-6R level and *IL6R* expression was seen for SNPs located in the *IL6R* 3′ region including the *IL6R* 3′-UTR, which is an important region of regulatory control. Genetic variation in the 3′-UTRs of genes can affect transcript levels in several ways (Kwan et al. 2008), including effects on mRNA stability, translation efficiency, and by affecting regulatory control by miRNAs. Previous studies have shown that the *IL6R* 3′-UTR contains binding sites for several miRNAs and that *IL6R* transcript levels are (down)regulated by binding of these miRNAs to their 3′-UTR target in *IL6R* mRNA (Gong et al. 2012; Hatziapostolou et al. 2011; Jia et al. 2012; Zhu et al. 2010). Alterations in components of this

regulatory pathway have been reported in cancer tissues (Gong et al. 2012; Hatziapostolou et al. 2011; Jia et al. 2012; Zhu et al. 2010) and in synovial fibroblasts from patients with rheumatoid arthritis (de la Rica et al. 2013). Our novel finding that SNPs in the *IL6R* 3′ region are associated with *IL6R* expression level generates novel hypotheses about the role of genetic variation in regulatory pathways of *IL6R* expression, and into the contribution of such pathways to complex disease susceptibility.

SNP rs4129267 (r^2 with rs2228145 = 0.97) has been identified as a risk variant for asthma; the minor allele that increases sIL-6R level is associated with increased asthma risk (OR 1.09; Ferreira et al. 2011). We looked at the effect of the top 3′ eQTL hits for *IL6R* (rs60760897 and SNPs in LD ($r^2 >= 0.3$)) on asthma in data from the Australian Asthma Genetics Consortium (Ferreira et al. 2011) (AAGC, $N = 2{,}110$ cases, $N = 3{,}857$ controls) and the GABRIEL consortium (Moffatt et al. 2010) ($N = 10{,}365$ cases, $N = 16{,}110$ controls). rs60760897 was not associated with asthma risk (Tables S9 and S10) but a suggestive association signal was found for SNPs in modest LD ($r^2 = 0.46$) with rs60760897 (rs4478801; GABRIEL: OR = 0.95, $P = 0.014$ and AAGC: OR = 0.94, $P = 0.045$). The minor G allele of rs448801 that showed a trend of lower asthma risk in the AAGC and GABRIEL study was associated with lower sIL-6R level in our study (GWAS $P = 1.27 \times 10^{-145}$) and with higher *IL6R* expression (eQTL $P = 4.87 \times 10^{-8}$).

Compared to previous reports that common SNPs together generally explain less than 50 % of the total variation of complex traits (Yang et al. 2011b), it seems remarkable that a single common variant in the *IL6R* gene alone accounts for such a large part of the variation in sIL-6R levels. What makes the level of circulating sIL-6R different from other quantitative traits such as height and BMI? An important factor is that sIL-6R can be produced through two mechanisms (receptor cleavage/shedding and translation of an alternatively spliced mRNA), and that the *IL6R* SNP rs2228145 has a major impact on both mechanisms. In contrast, it seems likely, although this is actually unknown, that many complex traits result from the integration of numerous different processes that each make a small contribution to the endpoint, in which case there could be many genetic variants in different pathways that each contribute to a small portion of the total variation in the trait.

In conclusion, we have shown that sIL-6R levels are highly heritable and that variants in *IL6R* other than the well-known functional SNP make an important contribution to the heritability. Our findings shed novel light on the effects of rs2228145 and SNPs at the *IL6R* 3′-end on *IL6R* expression. At the same time, we demonstrated that results from different methods based on the classic biometrical model are in agreement and converge to the same conclusion.

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Conflict of interest All authors declare that they have no conflict of interest.

Informed Consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration of 1975, as revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

References

Abecasis GR, Wigginton JE (2005) Handling marker–marker linkage disequilibrium: pedigree analysis with clustered markers. Am J Hum Genet 77(5):754–767

Abecasis GR, Cardon LR, Cookson WO (2000) A general test of association for quantitative traits in nuclear families. Am J Hum Genet 66(1):279–292

Abecasis GR, Cherny SS, Cookson WO, Cardon LR (2002) Merlin-rapid analysis of dense genetic maps using sparse gene flow trees. Nat Genet 30(1):97–101

Atreya R, Mudter J, Finotto S, Mullberg J, Jostock T, Wirtz S, Schutz M, Bartsch B, Holtmann M, Becker C, Strand D, Czaja J, Schlaak JF, Lehr HA, Autschbach F, Schurmann G, Nishimoto N, Yoshizaki K, Ito H, Kishimoto T, Galle PR, Rose-John S, Neurath MF (2000) Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo. Nat Med 6(5):583–588

Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A, Burg J, Strand S, Kiesslich R, Huber S, Ito H, Nishimoto N, Yoshizaki K, Kishimoto T, Galle PR, Blessing M, Rose-John S, Neurath MF (2004) TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. Immunity 21(4):491–501

Boomsma DI, de Geus EJ, Vink JM, Stubbe JH, Distel MA, Hottenga JJ, Posthuma D, van Beijsterveldt TC, Hudziak JJ, Bartels M, Willemsen G (2006) Netherlands twin register: from twins to twin families. Twin Res Hum Genet 9(6):849–857

Boomsma DI, Willemsen G, Sullivan PF, Heutink P, Meijer P, Sondervan D, Kluft C, Smit G, Nolen WA, Zitman FG, Smit JH, Hoogendijk WJ, van Dyck R, de Geus EJ, Penninx BW (2008) Genome-wide association of major depression: description of samples for the GAIN Major Depressive Disorder Study: NTR and NESDA biobank projects. Eur J Hum Genet 16(3):335–342

de la Rica L, Urquiza JM, Gomez-Cabrero D, Islam AB, Lopez-Bigas N, Tegner J, Toes RE, Ballestar E (2013) Identification of novel markers in rheumatoid arthritis through integrated analysis of

DNA methylation and microRNA expression. J Autoimmun 41:6–16

Dobson A (2002) An introduction to generalized linear models. Chapman & Hall/CRC, London

Doganci A, Eigenbrod T, Krug N, De Sanctis GT, Hausding M, Erpenbeck VJ, Haddad E, Lehr HA, Schmitt E, Bopp T, Kallen KJ, Herz U, Schmitt S, Luft C, Hecht O, Hohlfeld JM, Ito H, Nishimoto N, Yoshizaki K, Kishimoto T, Rose-John S, Renz H, Neurath MF, Galle PR, Finotto S (2005) The IL-6R alpha chain controls lung CD4+ CD25+ Treg development and function during allergic airway inflammation in vivo. J Clin Invest 115(2):313–325

Eyre S, Bowes J, Diogo D, Lee A, Barton A, Martin P, Zhernakova A, Stahl E, Viatte S, McAllister K, Amos CI, Padyukov L, Toes RE, Huizinga TW, Wijmenga C, Trynka G, Franke L, Westra HJ, Alfredsson L, Hu X, Sandor C, de Bakker PI, Davila S, Khor CC, Heng KK, Andrews R, Edkins S, Hunt SE, Langford C, Symmons D, Concannon P, Onengut-Gumuscu S, Rich SS, Deloukas P, Gonzalez-Gay MA, Rodriguez-Rodriguez L, Arlsetig L, Martin J, Rantapaa-Dahlqvist S, Plenge RM, Raychaudhuri S, Klareskog L, Gregersen PK, Worthington J (2012) High-density genetic mapping identifies new susceptibility loci for rheumatoid arthritis. Nat Genet 44(12):1336–1340

Falconer DS (1960) Introduction to quantitative genetics. Ronald Press Co, New York

Fehrmann RS, Jansen RC, Veldink JH, Westra HJ, Arends D, Bonder MJ, Fu J, Deelen P, Groen HJ, Smolonska A, Weersma RK, Hofstra RM, Buurman WA, Rensen S, Wolfs MG, Platteel M, Zhernakova A, Elbers CC, Festen EM, Trynka G, Hofker MH, Saris CG, Ophoff RA, van den Berg LH, van Heel DA, Wijmenga C, Te Meerman GJ, Franke L (2011) Trans-eQTLs reveal that independent genetic variants associated with a complex phenotype converge on intermediate genes, with a major role for the HLA. PLoS Genet 7(8):e1002197

Ferreira MA, Matheson MC, Duffy DL, Marks GB, Hui J, Le SP, Danoy P, Baltic S, Nyholt DR, Jenkins M, Hayden C, Willemsen G, Ang W, Kuokkanen M, Beilby J, Cheah F, de Geus EJ, Ramasamy A, Vedantam S, Salomaa V, Madden PA, Heath AC, Hopper JL, Visscher PM, Musk B, Leeder SR, Jarvelin MR, Pennell C, Boomsma DI, Hirschhorn JN, Walters H, Martin NG, James A, Jones G, Abramson MJ, Robertson CF, Dharmage SC, Brown MA, Montgomery GW, Thompson PJ (2011) Identification of IL6R and chromosome 11q13.5 as risk loci for asthma. Lancet 378(9795):1006–1014

Ferreira RC, Freitag DF, Cutler AJ, Howson JM, Rainbow DB, Smyth DJ, Kaptoge S, Clarke P, Boreham C, Coulson RM, Pekalski ML, Chen WM, Onengut-Gumuscu S, Rich SS, Butterworth AS, Malarstig A, Danesh J, Todd JA (2013) Functional IL6R 358ala allele impairs classical IL-6 receptor signaling and influences risk of diverse inflammatory diseases. PLoS Genet 9(4):e1003444

Fulker DW, Cherny SS, Sham PC, Hewitt JK (1999) Combined linkage and association sib-pair analysis for quantitative traits. Am J Hum Genet 64(1):259–267

Galicia JC, Tai H, Komatsu Y, Shimada Y, Akazawa K, Yoshie H (2004) Polymorphisms in the IL-6 receptor (IL-6R) gene: strong evidence that serum levels of soluble IL-6R are genetically influenced. Genes Immun 5(6):513–516

Gong J, Zhang JP, Li B, Zeng C, You K, Chen MX, Yuan Y, Zhuang SM (2012) MicroRNA-125b promotes apoptosis by regulating the expression of Mcl-1, Bcl-w and IL-6R. Oncogene 32(25):3071–3079. doi:10.1038/onc.2012.318

Hatziapostolou M, Polytarchou C, Aggelidou E, Drakaki A, Poultsides GA, Jaeger SA, Ogata H, Karin M, Struhl K, Hadzopoulou-Cladaras M, Iliopoulos D (2011) An HNF4α-miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. Cell 147(6):1233–1247

Horiuchi S, Koyanagi Y, Zhou Y, Miyamoto H, Tanaka Y, Waki M, Matsumoto A, Yamamoto M, Yamamoto N (1994) Soluble interleukin-6 receptors released from T cell or granulocyte/macrophage cell lines and human peripheral blood mononuclear cells are generated through an alternative splicing mechanism. Eur J Immunol 24(8):1945–1948

Hurst SM, Wilkinson TS, McLoughlin RM, Jones S, Horiuchi S, Yamamoto N, Rose-John S, Fuller GM, Topley N, Jones SA (2001) Il-6 and its soluble receptor orchestrate a temporal switch in the pattern of leukocyte recruitment seen during acute inflammation. Immunity 14(6):705–714

IL6R Genetics Consortium and Emerging Risk Factors Collaboration (2012) Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. Lancet 379(9822):1205–1213

Jansen R, Batista S, Brooks AI, Tischfield JA, Willemsen G, van Grootheest G, Hottenga JJ, Milaneschi Y, Mbarek H, Madar V, Peyrot W, Vink JM, Verweij CL, de Geus EJ, Smit JH, Wright FA, Sullivan PF, Boomsma DI, Penninx BW (2014) Sex differences in the human peripheral blood transcriptome. BMC Genom 15(1):33

Jia HY, Wang YX, Yan WT, Li HY, Tian YZ, Wang SM, Zhao HL (2012) MicroRNA-125b functions as a tumor suppressor in hepatocellular carcinoma cells. Int J Mol Sci 13(7):8762–8774

Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM (2001) The soluble interleukin 6 receptor: mechanisms of production and implications in disease. FASEB J. 15(1):43–58

Kotake S, Sato K, Kim KJ, Takahashi N, Udagawa N, Nakamura I, Yamaguchi A, Kishimoto T, Suda T, Kashiwazaki S (1996) Interleukin-6 and soluble interleukin-6 receptors in the synovial fluids from rheumatoid arthritis patients are responsible for osteoclast-like cell formation. J Bone Miner Res 11(1):88–95

Kwan T, Benovoy D, Dias C, Gurd S, Provencher C, Beaulieu P, Hudson TJ, Sladek R, Majewski J (2008) Genome-wide analysis of transcript isoform variation in humans. Nat Genet 40(2):225–231

Lourdusamy A, Newhouse S, Lunnon K, Proitsi P, Powell J, Hodges A, Nelson SK, Stewart A, Williams S, Kloszewska I, Mecocci P, Soininen H, Tsolaki M, Vellas B, Lovestone S, Dobson R (2012) Identification of cis-regulatory variation influencing protein abundance levels in human plasma. Hum Mol Genet 21(16):3719–3726

Lust JA, Donovan KA, Kline MP, Greipp PR, Kyle RA, Maihle NJ (1992) Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor. Cytokine 4(2):96–100

Mackiewicz A, Schooltink H, Heinrich PC, Rose-John S (1992) Complex of soluble human IL-6-receptor/IL-6 up-regulates expression of acute-phase proteins. J Immunol 149(6):2021–2027

McPeek MS, Wu X, Ober C (2004) Best linear unbiased allele-frequency estimation in complex pedigrees. Biometrics 60(2):359–367

Melzer D, Perry JR, Hernandez D, Corsi AM, Stevens K, Rafferty I, Lauretani F, Murray A, Gibbs JR, Paolisso G, Rafiq S, Simon-Sanchez J, Lango H, Scholz S, Weedon MN, Arepalli S, Rice N, Washecka N, Hurst A, Britton A, Henley W, van de Leemput J, Li R, Newman AB, Tranah G, Harris T, Panicker V, Dayan C, Bennett A, McCarthy MI, Ruokonen A, Jarvelin MR, Guralnik J, Bandinelli S, Frayling TM, Singleton A, Ferrucci L (2008) A genome-wide association study identifies protein quantitative trait loci (pQTLs). PLoS Genet 4(5):e1000072

Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S, von ME, Farrall M, Lathrop M, Cookson WO (2010) A large-scale, consortium-based genome wide association study of asthma. N Engl J Med 363(13):1211–1221

Müllberg J, Schooltink H, Stoyan T, Gunther M, Graeve L, Buse G, Mackiewicz A, Heinrich PC, Rose-John S (1993) The soluble

interleukin-6 receptor is generated by shedding. Eur J Immunol 23(2):473–480

Müllberg J, Oberthur W, Lottspeich F, Mehl E, Dittrich E, Graeve L, Heinrich PC, Rose-John S (1994) The soluble human IL-6 receptor. Mutational characterization of the proteolytic cleavage site. J Immunol 152(10):4958–4968

Muller-Newen G, Kohne C, Keul R, Hemmann U, Muller-Esterl W, Wijdenes J, Brakenhoff JP, Hart MH, Heinrich PC (1996) Purification and characterization of the soluble interleukin-6 receptor from human plasma and identification of an isoform generated through alternative splicing. Eur J Biochem 236(3): 837–842

Neale MC, Boker SM, Xie G, Maes HH (2006) Mx: statistical modeling. Department of Psychiatry, Virginia Commonwealth University, Richmond

Penninx BW, Beekman AT, Smit JH, Zitman FG, Nolen WA, Spinhoven P, Cuijpers P, De Jong PJ, Van Marwijk HW, Assendelft WJ, Van Der MK, Verhaak P, Wensing M, De GR, Hoogendijk WJ, Ormel J, van Dyck R (2008) The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res 17(3):121–140

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81(3):559–575

Rafiq S, Frayling TM, Murray A, Hurst A, Stevens K, Weedon MN, Henley W, Ferrucci L, Bandinelli S, Corsi AM, Guralnik JM, Melzer D (2007) A common variant of the interleukin 6 receptor (IL-6r) gene increases IL-6r and IL-6 levels, without other inflammatory effects. Genes Immun 8(7):552–559

Raggi P, Su S, Karohl C, Veledar E, Rojas-Campos E, Vaccarino V (2010) Heritability of renal function and inflammatory markers in adult male twins. Am J Nephrol 32(4):317–323

Reich D, Patterson N, Ramesh V, De Jager PL, McDonald GJ, Tandon A, Choy E, Hu D, Tamraz B, Pawlikowska L, Wassel-Fyr C, Huntsman S, Waliszewska A, Rossin E, Li R, Garcia M, Reiner A, Ferrell R, Cummings S, Kwok PY, Harris T, Zmuda JM, Ziv E (2007) Admixture mapping of an allele affecting interleukin 6 soluble receptor and interleukin 6 levels. Am J Hum Genet 80(4):716–726

Revez JA, Bain L, Chapman B, Powell JE, Jansen R, Duffy DL, Tung JY, Penninx PM, Visscher PM, de Geus EJ, Boomsma DI, Hinds DA, Martin NG, Montgomery GW, Ferreira MA (2013) A new regulatory variant in the interleukin-6 receptor gene associates with asthma risk. Genes Immun 14(7):441–446

Rose-John S, Scheller J, Elson G, Jones SA (2006) Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. J Leukoc Biol 80(2):227–236

Saito M, Yoshida K, Hibi M, Taga T, Kishimoto T (1992) Molecular cloning of a murine IL-6 receptor-associated signal transducer, gp130, and its regulated expression in vivo. J. Immunol. 148(12): 4066–4071

Scheller J, Garbers C, Rose-John S (2013) Interleukin-6: From basic biology to selective blockade of pro-inflammatory activities. Semin, Immunol

Spijker S, van de Leemput JC, Hoekstra C, Boomsma DI, Smit AB (2004) Profiling gene expression in whole blood samples following an in vitro challenge. Twin Res. 7(6):564–570

Stephens OW, Zhang Q, Qu P, Zhou Y, Chavan S, Tian E, Williams DR, Epstein J, Barlogie B, Shaughnessy JD Jr (2012) An intermediate-risk multiple myeloma subgroup is defined by sIL-6r: levels synergistically increase with incidence of SNP rs2228145 and 1q21 amplification. Blood 119(2):503–512

Taga T, Kishimoto T (1997) Gp130 and the interleukin-6 family of cytokines. Annu Rev Immunol 15:797–819

Taga T, Hibi M, Hirata Y, Yamasaki K, Yasukawa K, Matsuda T, Hirano T, Kishimoto T (1989) Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. Cell 58(3):573–581

The Interleukin-6 Receptor Mendelian Randomisation Analysis (IL6R MR) Consortium (2012) The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis. Lancet 379(9822):1214–1224

Visscher PM, Benyamin B, White I (2004) The use of linear mixed models to estimate variance components from data on twin pairs by maximum likelihood. Twin. Res. 7(6):670–674

Willemsen G, de Geus EJ, Bartels M, van Beijsterveldt CE, Brooks AI, Estourgie-van Burk GF, Fugman DA, Hoekstra C, Hottenga JJ, Kluft K, Meijer P, Montgomery GW, Rizzu P, Sondervan D, Smit AB, Spijker S, Suchiman HE, Tischfield JA, Lehner T, Slagboom PE, Boomsma DI (2010) The Netherlands Twin Register biobank: a resource for genetic epidemiological studies. Twin Res. Hum. Genet. 13(3):231–245

Williams RL (2000) A note on robust variance estimation for cluster-correlated data. Biometrics 56(2):645–646

Yang J, Lee SH, Goddard ME, Visscher PM (2011a) GCTA: a tool for genome-wide complex trait analysis. Am J Hum Genet 88(1): 76–82

Yang J, Manolio TA, Pasquale LR, Boerwinkle E, Caporaso N, Cunningham JM, de Andrade M, Feenstra B, Feingold E, Hayes MG, Hill WG, Landi MT, Alonso A, Lettre G, Lin P, Ling H, Lowe W, Mathias RA, Melbye M, Pugh E, Cornelis MC, Weir BS, Goddard ME, Visscher PM (2011b) Genome partitioning of genetic variation for complex traits using common SNPs. Nat Genet 43(6):519–525

Zhang W, Duan S, Bleibel WK, Wisel SA, Huang RS, Wu X, He L, Clark TA, Chen TX, Schweitzer AC, Blume JE, Dolan ME, Cox NJ (2009) Identification of common genetic variants that account for transcript isoform variation between human populations. Hum Genet 125(1):81–93

Zhu LH, Liu T, Tang H, Tian RQ, Su C, Liu M, Li X (2010) MicroRNA-23a promotes the growth of gastric adenocarcinoma cell line MGC803 and downregulates interleukin-6 receptor. FEBS J 277(18):3726–3734