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BRIEF COMMUNICATION

Heritability estimates of innate immunity: an extended twin study

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Cytokines are key players in numerous inflammatory processes. Demonstration of a heritable component in the variation of cytokine production would indicate that simultaneous occurrence of conditions might be caused by a heritable inflammatory characteristic. We applied an extended twin study approach to assess heritability estimates of interleukin (IL)-1β, IL-1ra, IL-10, IL-6, and TNF-α production capacity after ex vivo stimulation with lipopolysaccharide. Cytokine production capacity was assessed in 42 monozygotic pairs, 52 dizygotic pairs, one trizygotic triplet, 33 single twins, and 83 additional siblings. Heritability estimates were derived from variance decomposition models using maximum likelihood estimation. For all cytokines, over 50% of the variance was genetically determined. IL-1ra and TNF-α had the lowest heritability estimate of 53%. Estimates for IL-6 and IL-10 were 57 and 62%, respectively. IL-1β had the highest estimate of 86%. We conclude that the production of cytokines is under tight genetic control.
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Numerous diseases are associated with an increased risk for other comorbid conditions. For example, patients with systemic lupus erythematosus have an increased risk of developing atherosclerotic disease,[1] prevalence of diabetes in patients with multiple sclerosis is three-five-fold increased compared to the general population,[2] and patients with cancer have an increased risk for developing neurological degenerations.[3] For all these diseases, inflammatory mechanisms have been implicated.[4–9] In addition, inflammation has also been postulated as explanation for the simultaneous occurrence of these conditions.

Cytokines are key players in numerous inflammatory processes. Upon recognizing an intrinsic or extrinsic danger signal, innate immune cells start to produce a variety of cytokines. The pattern of cytokine production is determined by the pathogen structure and the binding receptor through which signaling and activation of gene transcription takes place. Typically, activation of the innate immune system is not pathogen-specific, but depends on the binding of evolutionary conserved pathogen-associated molecular patterns.[10,11] Lipopolysaccharide (LPS) is one of such an evolutionary conserved pattern. After LPS activation through Toll-like receptor (TLR-) 4, innate immune cells immediately produce a variety of inflammatory mediators such as interleukin (IL-)1β, IL-1 receptor antagonist (ra), IL-6, IL-10, and tumor necrosis factor (TNF-) α.[12]

Although the genes coding for the various cytokines have been identified, association studies between production capacity of cytokines and polymorphisms in or around the various cytokine genes have given disappointing results.[13] This suggests that regulation of cytokine production might be influenced by other genes, for example, a regulatory single-nucleotide polymorphism (rSNP).[14,15] The unknown regulatory genes may be localized with linkage analysis, but, to our knowledge, linkage studies of cytokine production capacity have not been reported. Even the heritability of variation in cytokine production is largely unknown.

Identification and quantification of a heritable component in the variation of cytokine production would be the first line of evidence demonstrating that common disorders such as atherosclerosis, cancer, diabetes, and multiple sclerosis might share a heritable inflammatory characteristic. We applied an extended twin study approach to assess heritability estimates of IL-1β, IL-1ra, IL-10, IL-6, and TNF-α production capacity after *ex vivo* stimulation with LPS.

Characteristics of the 302 subjects included in the study are given in Table 1. The mean production capacity of IL-1β, IL-1ra, IL-6, IL-10, and TNF-α was not statistically different between zygosity groups or between twins and singleton siblings.

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Table 1 Characteristics of study participants

	Monozygotic twins (n = 90)*	*Dizygotic twins** (n = 128)*	*Siblings# (n = 84)*
No. of females (%)	39 (43%)	80 (63%)	44 (52%)
Mean age (years (s.d.))	40.1 (13.3)	37.4 (11.9)	39.2 (12.1)
Mean cytokine production (pg/ml (s.d.))			
Interleukin-1β ($n = 299$)	6215 (2819)	6361 (3017)	6180 (3268)
Interleukin-1ra ($n = 302$)	33 876 (9734)	33 324 (9479)	35 045 (10239)
Interleukin-6 ($n = 300$)	71 839 (24843)	75 198 (26487)	75 944 (30280)
Interleukin-10 ($n = 302$)	2266 (703)	2225 (849)	2343 (731)
Tumor necrosis factor-α ($n = 301$)	7422 (2863)	8327 (3986)	7284 (3683)

All 302 participants were recruited from a larger study on the genetics of adult brain function.[16] The monozygotic twins includes six single twins and 42 twin pairs, the dizygotic twins include 24 single twins and 52 twin pairs. The siblings include the third subject from one trizygotic triplet. Cytokine production capacity was assessed with an *ex vivo* whole blood assay.[17] Blood samples were drawn in the morning before 1100 hours to minimize circadian variation. Heparinized whole blood was diluted five-fold with RPMI-1640, stimulated with 10 ng/ml LPS, and then incubated for 24 h at 37°C and 5% CO_2. After centrifugation, the supernatants were stored at −80°C until assaying for the various cytokines using standard ELISA techniques according to the manufacturer's guidelines (Central Laboratory of the Blood Transfusion Service, Amsterdam, The Netherlands). Supernatants of subjects were randomly distributed over ELISA plates in order to spread laboratory variation equally between monozygotic and dizygotic twins.

*Includes 6 single twins and 42 twin pairs.

**Includes 24 single twins and 52 twin pairs.

#Includes the third subject from the trizygotic triplet.

Using Structural Equation Modeling as implemented in Mx, in which effects of age and sex can be estimated while taking into account the dependency of the data (ie the family structure), we found that production of IL-10 and TNF-α declined significantly with increasing age (−8.9 pg/ml ($P = 0.04$) and −52.0 pg/ml ($P = 0.03$) per increasing year of age, respectively). Men had significantly higher production capacity for IL-1β (1519 pg/ml, $P < 0.01$), IL-6 (8295 pg/ml, $P = 0.01$), and TNF-α (1162 pg/ml, $P < 0.01$).

Figure 1 shows scatterplots of cytokine production capacity of all possible pairs of family members for monozygotic twin pairs and for dizygotic twin and sibling pairs as a function of age and sex. Monozygotic twins have less variation within pairs as compared to dizygotic twins and sibling pairs. Table 2 indeed shows that the correlation coefficients for all cytokines are higher for monozygotic twin pairs than for dizygotic twin and sibling pairs. This suggests that heritability plays a role in the LPS-induced cytokine response. Results from the variance decomposition models confirmed that for all cytokines, over 50% of the variance is genetically determined (Table 2). IL-1ra and TNF-α had the lowest estimate (53%), while IL-1β had the highest estimate (86%). The remaining variation in LPS-induced cytokine response was attributable to nonshared environmental variance. Common environmental variance did not significantly contribute to the observed variance in any of the LPS-induced cytokine responses.

Until now, only two smaller studies had assessed heritability estimates for TNF-α[20] and IL-10.[20,21] We now demonstrate that between-subjects variability in production levels of the five cytokines has a genetic background of more than 50%. For these cytokines, functional polymorphisms have been described. However, most of these polymorphisms only explain a small part of the total variation in cytokine production.[13] Recently, both Tokuhiro *et al*[14] and Helms *et al*[15] demonstrated that a distant rSNP is important in affecting a DNA site known to be associated with the autoimmune diseases psoriasis and rheumatoid arthritis. We hypothesize that a similar mechanism might play a role in the regulation of the innate immune response. Since innate cytokine gene transcription depends on innate signaling pathways activated by binding pathogen patterns and recognizing receptor-complexes, this may involve a single or multiple rSNPs of genes encoding proteins of this signaling cascade.

We found the strongest heritability estimates for LPS-induced IL-1β. IL-1β is a highly inflammatory cytokine for which the margin between clinical benefit and immunopathology is very narrow in humans.[22] Hence, tight regulation of the production of this cytokine is essential. Moreover, Toll-like receptors and IL-1 receptors belong to a broader family of receptors sharing a conserved cytosolic region termed the Toll-IL-1 receptor (TIR) domain.[23] The high heritability of especially LPS-induced IL-1β suggests that components of the intracellular signaling mechanisms shared by TIRs explain the tight genetic control of the innate cytokine response.[24]

Our findings are in line with the notion that patients with certain diseases have increased risk for other comorbid conditions. For example, Marrosu *et al*[2] reported that in families with genetic inheritance of multiple sclerosis, type I diabetes is three to five times more prevalent than in families without a history of multiple sclerosis. We noted that this observed association might have an immunogenetic explanation,[25] since it is known that IL-10 production plays a crucial role in both multiple sclerosis and diabetes.

We deliberately randomly distributed twins and siblings over the various ELISA plates. In this way, variation between ELISA plates was equally distributed over the monozygotic and dizygotic twins. Moreover, the mean cytokine production capacity was equal for twins and singletons. This finding indicates that twins do not have an unusual cytokine production pattern which could have been responsible for our high heritability estimates.

We do not know whether LPS is the right stimulus to estimate innate immunity. However, cytokine production

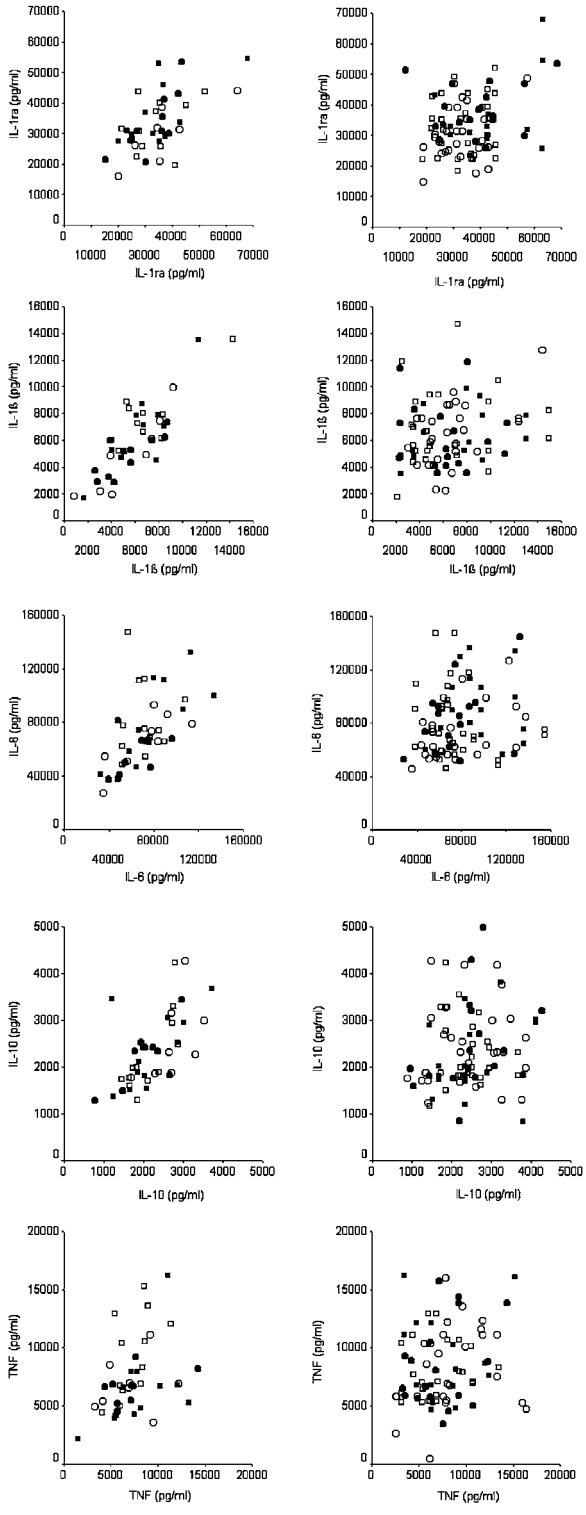

Figure 1 Scatterplots of cytokine production capacity of all nonindependent related pairs of family members, ordered across zygosity (monozygotic twins *vs* dizygotic twins or siblings). LPS-induced production capacity of IL-1β, IL-1ra, IL-6, IL-10, and TNF-α. Monozygotic twins are plotted in the left column, dizygotic twins and siblings are plotted in the right column. Squares are males, dots are females, open symbols are pairs under the median age of 35 years, solid symbols are pairs above the median age of 35 years. Dizygotic twins with siblings were included in the plots if the difference in age was less than 5 years. Opposite sex pairs were excluded from the plots.

Table 2 Correlation coefficients and heritability estimates for the various cytokines

Cytokine	*Monozygotic twins*	*Dizygotic twins and siblings*	*Heritability (95% CI)*
Interleukin-1β	0.86	0.32	0.86 (0.76–0.92)
Interleukin-1ra	0.58	0.20	0.53 (0.32–0.70)
Interleukin-6	0.59	0.25	0.57 (0.36–0.73)
Interleukin-10	0.67	0.24	0.62 (0.41–0.76)
Tumor necrosis factor-α	0.55	0.25	0.53 (0.30–0.70)

Correlations were corrected for age and sex where found statistically significant (IL-10 and TNF-α were corrected for age, interleukin-1β, interleukin-6, and TNF-α were corrected for sex). Heritability analysis proceeded in several stages. First, the distribution of each cytokine was visually inspected for outliers, which were subsequently eliminated from the analyses (three outliers for IL-1b, two for IL-6, and one for TNF-α). Then, for each cytokine, a saturated statistical model was built, consisting of 11 estimated parameters: three grand means (mean cytokine levels of monozygotic twins, dizygotic twins, and siblings), two covariates (age, sex), three variances (for monozygotic twins, dizygotic twins, and siblings), and three covariances (for monozygotic twin pairs, dizygotic twin pairs, and siblings). Next, the full model was reduced to a restricted model, the variance decomposition model, which included only parameters that contributed significantly to the model. All analyses were carried out using the software package Mx.[18] The observed interindividual variation in cytokine production was decomposed into additive genetic variation [A], common environmental variation shared by family members [C], and nonshared environmental variation [E].[19] By definition, common environmental variation [C] includes all environmental sources of variation that twins and siblings from the same family share, while nonshared environmental variation [E] is the environmental variation that is unique for an individual which is typically not shared with other family members. For monozygous twin pairs, similarities of additive genetic [A] and shared environmental influences [C] were fixed at 100%. For dizygotic twin pairs and nontwin sib pairs, similarity of additive genetic influences [A] was fixed at 50%. If the saturated models indicated no difference in covariation between dizygotic twin pairs and sib pairs, similarity in common environmental influences [C] was fixed at 100%. Similarity in nonshared environmental influences [E] was fixed at 0% for all family members. This component also includes measurement error. The total variance is A+C+E. The expectation for the covariance between monozygotic twins is A+C, and the expectation for the dizygotic twins and sib pairs is $\frac{1}{2}A + C$. Heritability (h^2) is calculated as the proportional contribution of genetic variation to the total observed variation: A/(A+C+E).

is not pathogen specific and now it appears to be a heritable characteristic. Moreover, we have previously shown that the assay as performed here strongly associates with outcomes of and susceptibility for various inflammatory diseases at young and old age, even though these diseases are not LPS mediated.[7,20,26–28]

In conclusion, our data provide strong evidence for a genetic basis for numerous inflammatory mediated diseases. Future linkage analyses in this extended twin panel using this intermediate phenotype will be set up to identify genomic regions important for LPS-induced cytokine production capacity. This will be followed by

fine mapping and association analyses within those regions. By the identification of the actual allelic variants influencing cytokine production, we will further our understanding of the underlying genetic mechanism of inflammatory mediated diseases.

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References

1 Roman MJ, Shanker BA, Davis A *et al.* Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. *N Engl J Med* 2003; **349**: 2399–2406.
2 Marrosu MG, Cocco E, Lai M, Spinicci G, Pischedda MP, Contu P. Patients with multiple sclerosis and risk of type 1 diabetes mellitus in Sardinia, Italy: a cohort study. *Lancet* 2002; **359**: 1461–1465.
3 Albert ML, Darnell RB. Paraneoplastic neurological degenerations: keys to tumour immunity. *Nat Rev Cancer* 2004; **4**: 36–44.
4 Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. *J Clin Pathol* 2003; **56**: 481–490.
5 Ross R. Atherosclerosis. An inflammatory disease. *N Engl J Med* 1999; **340**: 115–126.
6 Libby P. Inflammation in atherosclerosis. *Nature* 2002; **420**: 868–874.
7 de Jong BA, Schrijver HM, Huizinga TWJ *et al*. Innate production of interleukin-10 and tumor necrosis factor affects the risk of multiple sclerosis. *Ann Neurol* 2000; **48**: 641–646.
8 Mathis D, Vence L, Benoist C. Beta-cell death during progression to diabetes. *Nature* 2001; **414**: 792–798.
9 Coussens LM, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860–867.
10 Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001; **1**: 135–145.
11 Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001; **2**: 675–680.
12 Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995; **13**: 437–457.
13 Haukim N, Bidwell JL, Smith AJ *et al.* Cytokine gene polymorphism in human disease. *Genes Immun* 2002; **3**: 313–330.
14 Tokuhiro S, Yamada R, Chang X *et al.* An intronic SNP in a RUNX1 binding site of *SLC22A4*, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat Genet* 2003; **35**: 341–348.
15 Helms C, Cao L, Krueger JG *et al.* A putative RUNX1 binding site variant between *SLC9A3R1* and *NAT9* is associated with susceptibility to psoriasis. *Nat Genet* 2003; **35**: 349–356.
16 Posthuma D. *Genetic variation and cognitive ability.* PhD Thesis. Vrije Universiteit Amsterdam. Print Partners Ipskamp, Enschede: 2002.
17 van der Linden MW, Huizinga TWJ, Stoeken DJ, Westendorp RGJ. Determination of tumor necrosis factor-alpha and interleukin-10 production in whole blood stimulation system: assessment of laboratory error and individual variation. *J Immunol Methods* 1998; **21**: 63–71.
18 Neale MC. *Mx: Statistical Modeling*, 3rd edn. Richmond VA, 1997.
19 Neale MC, Cardon LR. *Methodology for Genetic Studies of Twins and Families*. Kluwer Academic Publishers: Dordrecht, 1992.
20 Westendorp RGJ, Langermans JA, Huizinga TW *et al.* Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 1997; **349**: 170–173.
21 Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Hohler T. Differential regulation of interleukin-10 production by genetic and environmental factors—a twin study. *Genes Immun* 2002; **3**: 407–413.
22 Dinarello CA. Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist. *Int Rev Immunol* 1998; **16**: 457–499.
23 O'Neill L. The Toll/interleukin-1 receptor domain: a molecular switch for inflammation and host defence. *Biochem Soc Trans* 2000; **28**: 557–563.
24 Wasserman SA. Toll signalling: the enigma variations. *Curr Opin Genet Dev* 2000; **10**: 497–502.
25 de Craen AJM, Huizinga TWJ, Westendorp RGJ. Multiple sclerosis and type 1 diabetes in Sardinia. *Lancet* 2002; **360**: 1253.
26 van der Linden MW, Westendorp RGJ, Sturk A, Bergman W, Huizinga TW. High interleukin-10 production in first-degree relatives of patients with generalized but not cutaneous lupus erythematosus. *J Investig Med* 2000; **48**: 327–334.
27 Westendorp RGJ, van Dunne FM, Kirkwood TB, Helmerhorst FM, Huizinga TWJ. human fertility and survival. *Nat Med* 2001; **7**: 873.
28 van den Biggelaar AHJ, de Craen AJM, Gussekloo J *et al.* Inflammation underlying cardiovascular mortality is a late consequence of evolutionary programming. *FASEB J* 2004; **18**: 1022–1024.