Chapter 5

Maternal and child cytokine relationship is not altered by cytokine gene polymorphisms: A longitudinal study of young children in Indonesia

Manuscript in preparation for submission

Yenny Djuardi[1,2], Taniawati Supali[1], Heri Wibowo[1], Bas T Heijmans[3], Eline P Slagboom[3], Jeanine J Houwing-Duistermaat[4], Erliyani Sartono[2], Maria Yazdanbakhsh[2]

[1]Department of Parasitology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

[2]Laboratory of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

[3]Molecular Epidemiology, Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands

[4]Medical Statistics, Department of Medical Statistics and Bioinformatics, Leiden University Medical Center, Leiden, The Netherlands

Abstract

Background: The development of immune responses in early life is influenced by the interaction between environmental and genetic factors. Our previous study on young infants showed a close association between maternal and child's cytokine responses. We have now addressed the question how this association evolves over time and the contribution of genetic polymorphisms to this association.

Methods: TH_1 (IFN-γ), TH_2 (IL-5, IL-13), pro- (TNF-α) and anti-inflammatory (IL-10) cytokines in mitogen-stimulated whole blood culture were measured from mothers during pregnancy and from their children aged 2, 5, 12, 24, and 48 months. Cytokine gene polymorphisms were determined (or investigated) from blood samples of paired mothers and children.

Results: High production of maternal IL-10, TNF-α and IFN-γ was significantly associated with higher levels of the corresponding cytokines in their children at 2 months of age (baseline/T0), however these associations decreased in magnitude with time. Using an additive genetic model, maternal IFN-γ gene polymorphism, rs3181032, was associated with child cytokine levels at T0 (β: -0.30, 95%CI: -0.58, -0.01) but this relationship disappeared with time (β: 0.01, 95%CI: 0.002, 0.03). Maternal IL-10 rs4579758 was not associated with child's cytokine at T0, however this association changed over time to become positively and significantly associated child's cytokine (β: 0.005, 95%CI: 0.0002, 0.01). The child's genotype for rs13215091 had a significant effect on TNF-α (β: -0.01, 95%CI: -0.02, -0.004) at later ages but not at T0. In the final models including measured gene polymorphisms, maternal cytokines were the strongest determinant of child cytokines.

Conclusion: Independently from gene polymorphism, child's cytokine production was significantly associated with maternal cytokine production at 2 months of age. Maternal cytokine during pregnancy, which could be a proxy for environmental factors for the child showed its highest impact at early age, without any influence from genetic factors.

Keywords: cytokines, single nucleotide polymorphism, pregnant mother, early life

Introduction

There has been an increasing number of studies looking at immune responses in early life and its modulation by environmental factors, which may predispose an individual to certain diseases in later life. Several studies have shown parallels in cellular immune responses between mothers and their children [1–5]. The mechanisms behind this have not been elucidated. Moreover, all these studies were conducted in developed countries, where infectious diseases are better controlled while allergic and autoimmune diseases are increasingly affecting the population. Specifically, the relationship between in utero exposure, early immune profiles after birth and the development of immune responses in early childhood has not been examined comprehensively in populations where chronic parasitic infections are endemic. In this regard, our previous study in a helminth-endemic area found that maternal cytokines (IL-10, IFN-γ) were associated with infant's cytokines at the age of 2 months, even after taking into account several environmental factors including maternal parasitic infections [6], in agreement with the findings from the studies in industrialized countries where maternal and child cytokine responses appear to be tightly linked.

Interaction between environment and genetic factors will determine the phenotypic outcome of an individual. To our knowledge, there has been no study looking at the changing pattern of the mother-child cytokine relationship over time and whether genetic factors can modify this relationship. To investigate this, we measured child' cytokine responses to mitogen at 5 time points, starting around 2 months and up to 4 years of age and examined the relationship with maternal cytokine responses during pregnancy. We genotyped single nucleotide polymorphisms (SNPs) from mothers and children and asked whether they modified the cytokine relationship of mother and child.

Methods

Study population

This study is part of the longitudinal study of children living in a peri-urban area in Bekasi Distric, West Java province, Indonesia. Children were followed up at 5 time points: 2 (T0), 5 (T5), 12 (T12), 24 (T24) and 48 (T48) months of age. Among all participants, there were 126 pairs of pregnant

mothers in second or third trimester and their children who had both cytokine measurements and genotyping data.All mothers provided written informed consent for themselves and their children. This study was approved by the Ethical Committee Faculty of Medicine University of Indonesia.

Whole blood culture and cytokine measurement

Whole blood culture and stimulation was performed as described previously [6]. Briefly, heparinized venous blood was diluted 1:10 with RPMI-1640 medium (added with 1mM pyruvate and 2mM glutamate), followed by incubation with phytohaemmaglutinin (PHA; 2 µg/ml; Wellcome Diagnostics, Dartford, UK) in 37^{o}C and 5% CO_2. The concentrations of interleukin (IL)-10 and TNF-α were measured in day 1 supernatant, whereas IL-5, IL-13, IFN-γ were measured in day 6 supernatant. Supernatants were kept frozen at -20^{o}C and later on were thawed for the measurement with in-house multiplex bead-based assay (Luminex IS 100, Luminexcorp, Austin, TX, USA) for IL-10, TNF-α, IL-13 and IFN-γ , while ELISA was performed for measurement of IL-5 [6]. The detection limits for IL-10, TNF-α, IL-13, IFN-γ and IL-5 were 6.5 pg/ml, 1.7 pg/ml, 12.5 pg/ml, 3.6 pg/ml and 2 pg/ml, respectively. All cytokine levels below detection limit were given half of the threshold value.

DNA purification and genotyping

Genomic DNA was purified from 200 ul of whole blood samples from pregnant mothers and their children which was kept frozen at -20^{o}C, using QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The purified DNA was quantified using NanoDrop^{TM} 1000 Spectrophotometer (Thermo Fischer Scientific).

The source of cytokine gene polymorphisms for Indonesian living in West Java were derived from Malays population in Singapore Variation Genome Project (SGVP) database (http://www.statgen.nus.edu.sg/~SGVP)[7], with some additional single nucleotide polymorphisms (SNP)s which were not found in the database but were associated with phenotypes in Asian or other population. A set of gene polymorphisms spanning from 20 kb upstream and 10 kb downstream from each cytokine gene region were included in the genotyping.Pairwise tagging SNPs were obtained from Haploview's Tagger program (http://www.broad.mit.edu/mpg/tagger), with selection based on

minor allele frequency (MAF) $\geq$ 5% and r2 threshold = 0.8. In total there were 10 SNPs for IL-10, 4 SNPs for TNF-α, 6 SNPs for IFN-γ, 6 SNPs for IL-5, and 8 SNPs for IL-13. In addition to these cytokine genes, 4 SNPs of RAD50 genes were added since they occupy the region between IL-4, IL-5 and IL-13 genes (TH_2 cytokine locus) in chromosome 5q31. RAD50 gene encodes a DNA repair enzyme; it was recently found to have locus control region at its 3'end and is shown to be associated with asthma and eczema [8,9]. Genotyping of all SNPs were performed using Sequenom MassARRAY iPLEX Platform. Quality control was performed by including $\pm$ 10% of successfully genotyped samples and positive controls in the repeated measurement of failed samples. At the end all SNPs were genotyped successfully with call rates $\geq$ 90%. After exclusion of 2 mother-child pairs who had call rates < 95% and 5 pairs with Mendelian inconsistencies, data were available for a total of 119 pairs.

Statistical analysis

Cytokine levels displayed skewed distributions, therefore log (base 10) transformation was used for all cytokines except for IL-5 for which a square-root transformation was used. To investigate the association between mother and child's cytokine productions, we divided maternal cytokines based on median levels into high or low producer mothers [6] and by using this category we compared child cytokine levels at each time point. Minor allele frequency (MAF), deviations from Hardy-Weinberg Equilibrium (HWE) and pairwise linkage disequilibrium (LD) were calculated for each mother or child's SNP using Haploview software (http://www.broadinstitute.org).

First we modeled the association between maternal cytokines and child cytokines. Next we modeled the maternal cytokines together with maternal or child genotype. We used linear mixed models to study the effect of maternal cytokines and maternal or child genotypes over time on child cytokines. Using a likelihood ratio test, we tested whether the simplerfirst order autoregressive heterogenous (for IL-10, TNF-α) structrure could be used to model correlation over time instead of the unstructured (for IFN-γ, IL-5, IL-13) covariance structure. Each genotype was coded 0, 1, 2 for the increasing number of minor allele and all SNPs were tested using an additive genetic model. First we used model with main effects and one interaction term between time and SNP (i.e. linear change over time) for all SNPs. For significant SNPs, we continued with a larger model where time was included as a categorical variable to obtain more insight of the effect of the SNP over

time. Bonferroni corrections were done for multiple testing, where a *p* value was considered significant if < 0.0028. All models were adjusted for child gender as an *apriori* factor for child's cytokine responses [10]. The statistical analyses were performed using IBM SPSS version 20.

Result

Characteristics of mother-child pairs

The flow diagram of the entire study was described elsewere (*Djuardi et al*-**Chapter 2**). There were 119 mothers and children included in the present study, with 54% (64/119) children being males. Among 107 children with data on breastfeeding, 83 children (78%) received breastfeeding exclusively for 6 months, followed by 21 (19%) children who were partially breastfed (mixed with formula milk) and 3 (3%) children who were not breastfed. While all 119 mother and child pairs had genotype data, the number of children with cytokine data was different at each time point: 111 at T0, 95 at T5, 90 at T12, 88 at T24, 86 at T48. The median and interquartile range (IQR) of maternal cytokine levels were as follows: IL-10 (91.7, IQR:46.9 – 167.1 pg/mL), TNF-α (274.2, IQR:54.7 – 915.6 pg/mL), IFN-γ (152.5, IQR:43.7 – 425.5 pg/mL), IL-5 (859.8, IQR:295.0 – 1673.8 pg/mL) and IL-13 (243.5, IQR:75.7 – 627.2 pg/mL).

Minor allele frequency (MAF) and p-values for testing the null hypothesis that Hardy-Weinberg Equilibrium (HWE) holds forall SNPs of the mother-child pairs are shown in Supporting Table 1. All genotyped SNPs were in HWE with exception of the two IL-5 gene polymorphism, rs4143832 and rs17690122 (both in perfect linkage disequilibrium/r^2=1), which slightly deviated from HWE (p=0.039). The other SNPs which showed perfect LD were rs1878672 and rs1800896 in IL-10 gene. Several SNPs which were in high LD (r^2>0.8) as indicated in Supporting Table 1. The five SNPs which were in perfect LD or high LD were excluded from the analyses.

Association between maternal and child cytokines over time

Table 1 shows that high cytokine producer mothers were associated with higher child cytokine production at T0 or 2 months of age, this is true for IL-10 (estimate: 0.31; 95%CI: 0.19, 0.43), IFN-γ (estimate: 0.26; 95%CI: 0.03, 0.49) and to a lesser extent for TNF-α (estimate: 0.19; 95%CI: 0.02, 0.36). With increasing age, the mother-child relationship for IL-10 disappeared

(interaction between maternal IL-10 with time, p = 0.002). TH_2-type cytokine productions (IL-5, IL-13) were not significantly associated between mother and child at baseline or over time (Table 1).

Table 1. Effect of maternal cytokine on child's cytokine production over time.

	Estimate (95% CI)§	P value
IL10		
Maternal cytokine	0.31 (0.19, 0.43)	**<0.0001**
Time*maternal cytokine	-0.009 (-0.01, -0.003)	**0.002**
TNF-α		
Maternal cytokine	0.19 (0.02, 0.36)	**0.030**
Time*maternal cytokine	-0.006 (-0.01, 0.002)	0.146
IFN-γ		
Maternal cytokine	0.26 (0.03, 0.49)	**0.027**
Time*maternal cytokine	-0.009 (-0.02, 0.002)	0.093
IL-5		
Maternal cytokine	1.51 (-2.58, 5.60)	0.466
Time*maternal cytokine	0.15 (-0.04, 0.34)	0.121
IL-13		
Maternal cytokine	0.07 (-0.08, 0.23)	0.348
Time*maternal cytokine	0.0002 (-0.008, 0.009)	0.954

Time units are 2, 5, 12, 24 and 48 months. Bold: *p* value < 0.05
§adjusted for child gender. *interaction with time

Association between maternal cytokines or genetic factors and child's cytokines over time

IL-10 and TNF-α

The estimated effect of maternal IL-10 and maternal or child's genotype on child's IL-10 production are shown in Table 2. Here only genotypes which revealed significant associations are presented. In this model, at 2 months of age the estimates for mother-child cytokine relationship were similar to the estimates in the previous model without genetic factors (Table 2 & Figure 1A; estimate: 0.31; 95%CI: 0.18, 0.43), indicating that gene polymorphisms

Table 2. Effect of maternal cytokine, maternal and child's genotype on child's PHA-induced cytokine production over time.

Child cytokine	Variable	Estimate (95% CI)§	P value
IL10	Maternal cytokine	0.31 (0.18, 0.43)	**<0.0001****
rs4579758	Maternal genotype	-0.05 (-0.16, 0.05)	0.321
	Child's genotype	-0.009 (-0.11, 0.10)	0.866
	Time*maternal cytokine	-0.008 (-0.01, -0.003)	**0.004**
	Time*maternal genotype	0.005 (0.0002, 0.01)	**0.039**
	Time*child's genotype	0.001 (-0.003, 0.006)	0.616
TNF-α	Maternal cytokine	0.20 (0.03, 0.37)	**0.024**
rs13215091	Maternal genotype	-0.005 (-0.21, 0.20)	0.961
	Child's genotype	0.17 (-0.02, 0.37)	0.080
	Time*maternal cytokine	-0.006 (-0.01, 0.001)	0.108
	Time*maternal genotype	0.004 (-0.005, 0.01)	0.354
	Time*child's genotype	-0.01 (-0.02, -0.004)	**0.005**
IFN-γ	Maternal cytokine	0.26 (0.04, 0.48)	**0.023**
rs3181032	Maternal genotype	-0.30 (-0.58, -0.01)	**0.039**
	Child's genotype	0.26 (-0.10, 0.61)	0.152
	Time*maternal cytokine	-0.009 (-0.02, 0.001)	0.077
	Time*maternal genotype	0.01 (0.002, 0.03)	**0.024**
	Time*child's genotype	-0.0008 (-0.02, 0.01)	0.919
IL-5	Maternal cytokine	1.46 (-2.59, 5.51)	0.476
Rs4143832	Maternal genotype	4.32 (-1.27, 9.91)	0.129
	Child's genotype	-4.93 (-9.60, -0.27)	**0.038**
	Time*maternal cytokine	0.17 (-0.02, 0.36)	0.075
	Time*maternal genotype	-0.24 (-0.49, 0.02)	0.066
	Time*child's genotype	0.14 (-0.08, 0.35)	0.210
IL-5	Maternal cytokine	1.02 (-3.07, 5.10)	0.622
Rs739719	Maternal genotype	-2.10 (-5.76, 1.56)	0.259
	Child's genotype	4.17 (0.12, 8.23)	**0.044**
	Time*maternal cytokine	0.14 (-0.05, 0.34)	0.143
	Time*maternal genotype	-0.02 (-0.19, 0.15)	0.799
	Time*child's genotype	-0.02 (-0.21, 0.17)	0.849

§adjusted for child gender. Bold: p value < 0.05. ** significant after Bonferroni's correction (p<0.0028)

did not modify the relationship between maternal and child cytokines. This result was similar across all models of SNPs (data not shown). In contrast to maternal cytokine status, maternal or child's IL-10 gene polymorphism were not significantly associated with child's cytokine at T0. There was a tendency for children born to mothers with more minor alleles in rs4579758 to have significantly higher production of IL-10 over time (Table 2 & Figure 2A). Child's genotype had no effect on IL-10 production over time.

In a similar manner to IL-10 but weaker, the production of child's PHA-induced TNF-α at 2 months of age was associated with maternal cytokine producer status during pregnancy (Table 2 & Figure 1B; estimate: 0.20, 95%CI: 0.03, 0.37) with decreasing trend over time. Although at the beginning there was a positive trend for association between polymorphism of child's rs13215091 and TNF-α levels (Table 2 & Figure 2B; estimate: 0.17; 95%CI: -0.02, 0.37), the direction of association reversed over time with significantly stronger effect exerted by child's decreasing number of minor allele (estimate: -0.01; 95%CI: -0.02, -0.004). For the other 3 SNPs of TNF-α maternal and child s genotype did not show significant associations with child's cytokine at baseline and over time (data not shown).

IFN-γ, IL-5 and IL-13

The production of TH_1-type responses (IFN-γ) in children after adjustment for genotypes was associated with maternal cytokine producer status at baseline (Table 2 & Figure 1C; estimate: 0.26; 95%CI: 0.04, 0.48) and this association did not change over time (estimate: -0.009; 95%CI: -0.02, 0.001). Among 6 IFN-γ polymorphisms, the only genotype found to be significantly associated with child's cytokine was rs3181032 of mother, which is an IFN-γ gene polymorphism located in promoter region. Mothers with increasing number of this SNP's minor allele were more likely to have children producing lower IFN-γ levels at T0 (Table 2 & Figure 2C; estimate: -0.30; 95%CI: -0.58, -0.01), however over time the direction of the association reversed (estimate: 0.01; 95%CI: 0.002, 0.03).

Among all genotyped IL-5 SNPs, only 2 child's IL-5 polymorphisms, rs4143832 and rs739719, were associated with child's cytokine production at T0 (Table 2 & Figure 2D, E; estimate: -4.93; 95%CI: -9.60, -0.27 and estimate: 4.17; 95%CI: 0.12, 8,23, respectively) with no significant difference of slopes with time. None of IL-13 or RAD50 gene polymorphisms were associated with child's IL-13 or with IL-5 and IL-13 levels, respectively (data not shown).

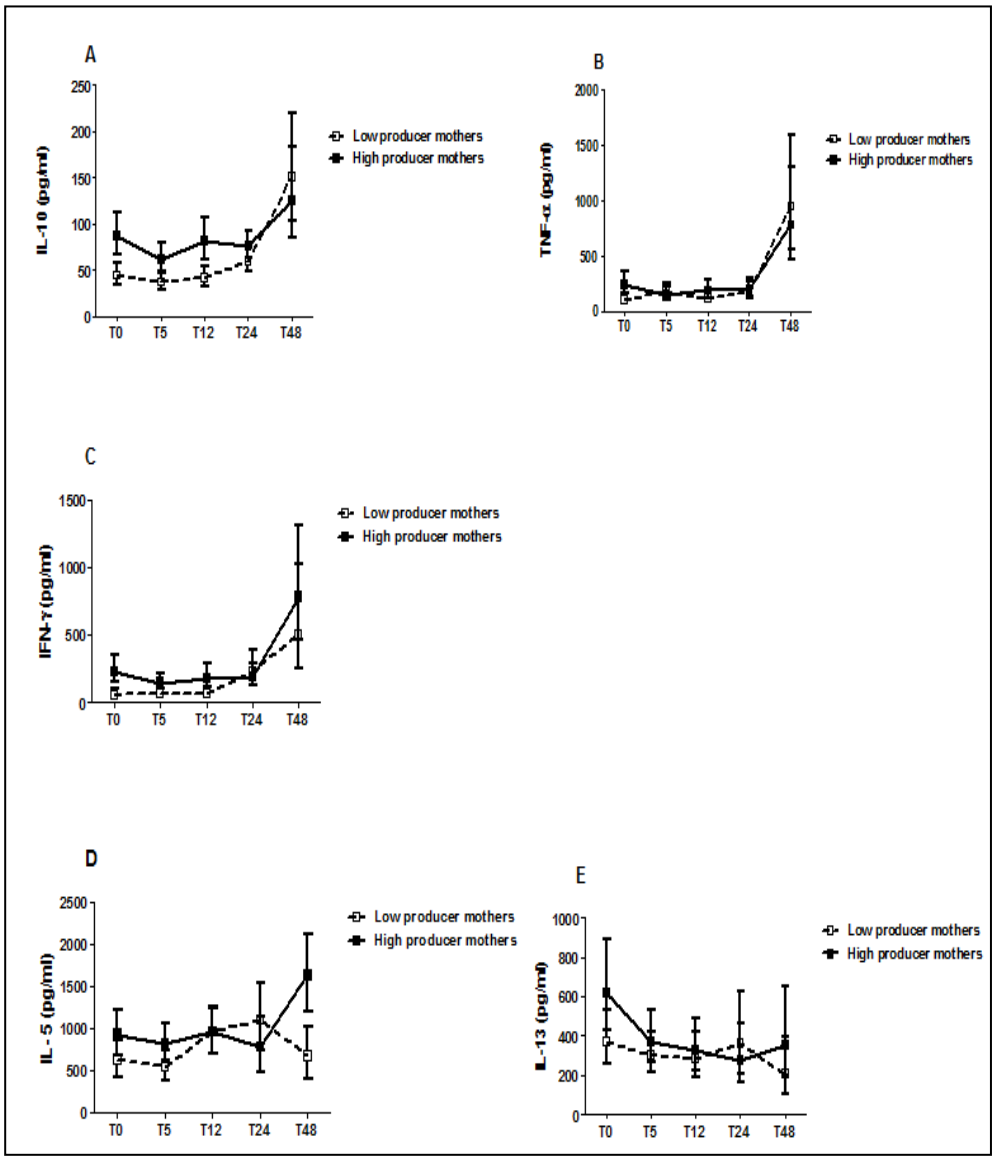

Figure 1. Child's PHA-induced cytokines over time, based on maternal cytokine producer status during pregnancy. Error bars represent geometric means of (**A**) IL-10, (B) TNF-α, (**C**) IFN-γ, (**D**) IL-5 levels, and mean of square of (**E**) IL-13 levelsof children at the age of 2 months (T0), 5 months (T5), 12 months (T12), 24 months (T24) and 48 months (T48). Closed dots are children born to high producer mothers, while open dots are children born to low producer mothers. All values were adjusted for mother and child's gene polymorphism, village of residence and child's gender.

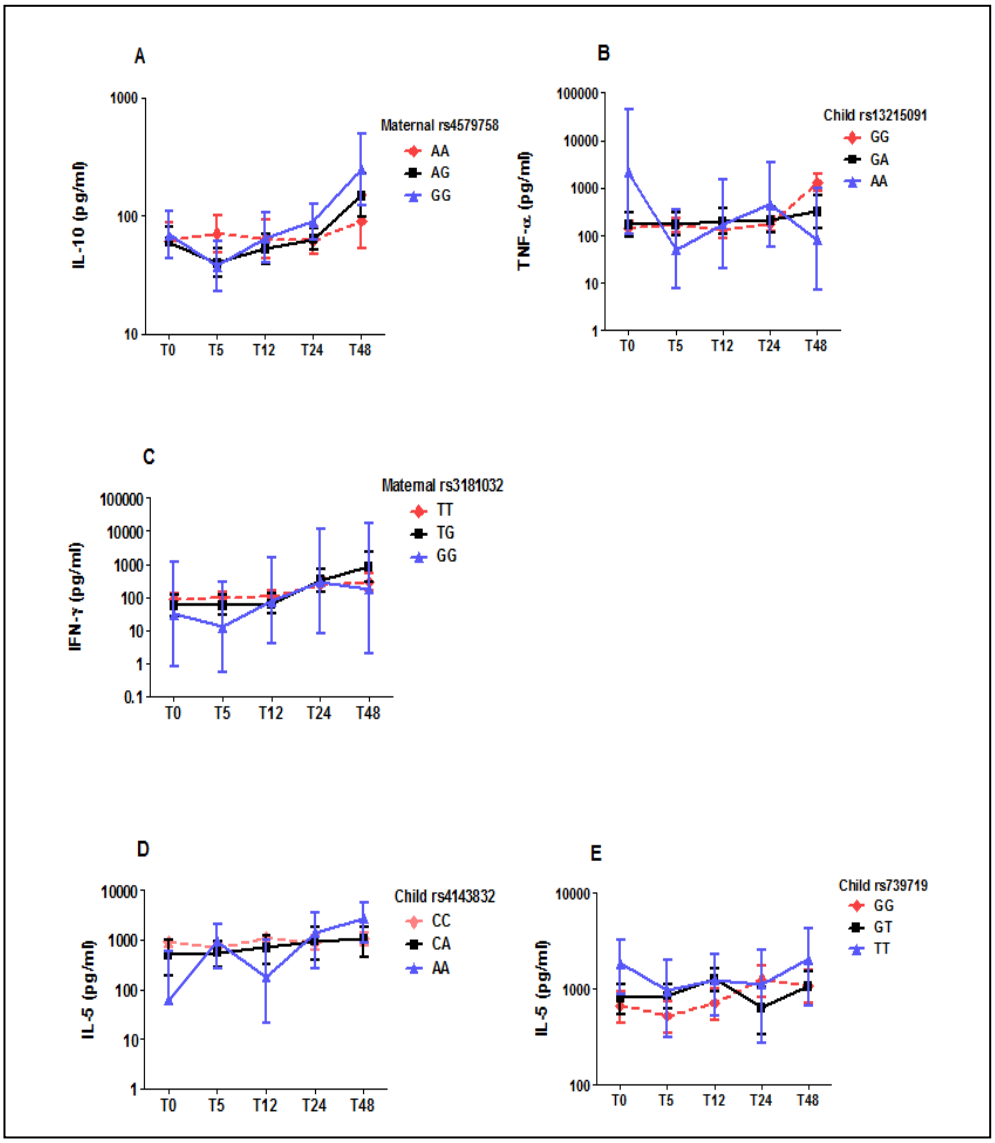

Figure 2. Child's PHA-induced cytokines over time, based on maternal or child genotypes. Only SNPs which showed significant associations in the multivariate models are shown.Error bars represent geometric means of (A) IL-10, (B) TNF-α, (C) IFN-γ and (D, E) IL-5 levels of children at the age of 2 months (T0), 5 months (T5), 12 months (T12), 24 months (T24) and 48 months (T48). Red dots/interrupted lines indicate the children having or born to mothers with major homozygote alleles, while black dots/solid lines for heterozygotes and blue dots and solid lines for minor homozygotes. All values were adjusted for maternal cytokines, village of residence and child's gender.

Discussion

In the present study we showed that genetic polymorphisms do not explain the strong association between maternal cytokine production during pregnancy with child's cytokine production in the first year of life. We also found that most of the mother-child cytokine relationships became weaker over time (IL-10, IFN-γ, and TNF-α) with the exception of IL-5. The association between maternal and child IL-5 production became significant when the child reached 4 years of age. These findings indicate that the strong association between cytokine responses of a pregnant mother and her child is not directly due to genetic factors but rather results from similar immune conditioning during gestational period extending into early childhood.

Earlier studies had found associations in cytokine production between mother (pre- or postpartum) and child at one or two time points, such as at birth in cord blood [1,2,5], at a time when infant was 3 months [3], 1 year [5,11] or 2 years of age [4]. The mother-child cytokine relationship was not always apparent directly after birth. For example, the birth cohort study by Halonen and coworkers showed no correlation of mitogen-stimulated IFN-γ and IL-13 between pregnant mother and fetus but instead with the child at 3 months of age [3]. Similarly, in another study with the lipopolysaccharides-induced IL-10 and TNF-α production, there was a significant association between pregnant mother and the child at the age of 1 year but not with cord blood [11]. Our results are in agreement with the previous two studies in that maternal IL-10 or IFN-γ production during pregnancy was positively associated with child's corresponding cytokines up to 1 year of age but with longer observation, the association was no longer present (Figure 1). This particular finding regarding IL-10 or IFN-γ may reflect intra uterine and nursing effect on child's developing immune system. While no trans-placental transfer of maternal cytokines in human are believed to occur [12]; the components of uterine microenvironment may modulate the fetal naïve immune cells. This might explain why maternal IL-10 during pregnancy showed the strongest association with child's IL-10. After birth, the maturating immune system of infants is believed to get compensations from breast milk which contains maternal humoral and cellular immune components including cytokines, chemokines and immune cells. Since the majority (97%) of the infants/children in our study was breastfed, breast milk may also contribute to the transfer of maternal immunological information to infants in this population. Both IL-10

and IFN-γ are present in breast milk [13,14]. In the case of IL-10 this is thought to be a continuation of immune regulation during gestation to avoid rejection of fetal allograft, while for IFN-γ the nursing can be the best way to complement the infant's immune system whose capacity to produce TH_1-type responses are less than in adults [15]. Interestingly, high levels of TNF-α are present in early milk but become almost undetectable after 1 month (reviewed in [14]). This might explain the weak association we observed in TNF-α production between mother and child in the first year of life. Interestingly, the pattern of mother-child relationship in IL-5 responses was different from other cytokines, in that the significant association was found only when the children reached 4 years of age.

It seems that at early age, the capacity of infant's immune cells to produce pro- and anti-inflammatory cytokines is more influenced by maternal cytokines, than gene polymorphisms which seem to have more influence at later age. On the other hand, we found that the production of TH_1-type cytokine at early age was independently associated with maternal cytokine and gene polymorphisms. Previous studies in twins showed that cytokines can have a low to high proportion of heritability, ranging from 30 – 75% for IL-10 [10,16,17], 40 – 85% for IFN-γ [10,18] and 17 – 80% for TNF-α [10,16–19]. The participants of these studies were adults. Our cohort study is unique in showing the effect of maternal cytokines on child cytokines in early age may overrule or mask the genetic effect during the maturation of child immune responses. This notion is supported by the finding in a twin study that the genetic effect on serum TNF-α increased with age [17].

In our previous study investigating factors that determined child cytokine production at 2 months of age, maternal TNF-α was not significantly associated with child TNF-α ($p=0.1$). However, the present study which analyzed a subset of whom there was both cytokine and genotype data showed a significant association ($p<0.05$). The discrepancy is not due to the method of analysis (child high/low producer status vs child continuous cytokine levels as the outcome), but rather in the different characteristics of participants. The participants from the present study were more often from Jati Karya village (71%), compared to the previous one (49%), which might contribute to the slight difference.

It is important to note that the significant associations between child cytokine production with SNPs detected in this study might also be caused by other unmeasured SNPs that were located in a larger distance but still in

LD. We realize that using single nucleotide analysis in this study may not entirely represent the genetic effect on child's cytokine responses since there are unmeasured genetic variations besides the measured SNPs, which may reveal a cytokine relationship between mother and child, such as copy number variation, haplotype, microsatellite alleles, as well as epigenetic processes (DNA methylation, histone modifications, small non-coding RNA). Nevertheless using the tagging SNPs we expected to limit the number of SNPs to be tested by covering those not genotyped which were in high LD with the tagging SNPs in the same gene [20].

Since the aim of the study was more focused on mother-child cytokine relationship, we consider the SNP analysis in relation to the child' cytokine responses exploratory. Therefore the weak associations ($p < 0.05$) found between genotypes and cytokine production before correction for multiple testing were not considered immediately as not significant. The confirmation of the gene association resultswould need replication with larger sample size in similar population/ race.

In conclusion, the close relationship of mother and child cytokine production, especially IL-10 and IFN-γ, was prominent in early life, before 1 year of age. This immunological relationship was independent of cytokine gene polymorphisms, suggesting that infant's cytokine responses were more influenced by the environment shared with the mother during intra uterine and breastfeeding period. Different cytokines can have different interaction with maternal cytokine and maternal/child genetic factors at certain time points, depending on the maturation of child immune responses and the challenges from the environment. Furthermore, whether the cytokine profile of children born to high or low producer mother is associated with clinical outcomes or only reflects physiological variation, needs to be further investigated.

Acknowledgement

We are very grateful to all families for their participation in the study and local health staff of Puskesmas Jati Sampurna for their assistance in blood collections. We also thank venipuncturists Sudirman and Heru, Erlinda Marthias for cytokine measurement and Dennis Kremer for his expertise and help in genotyping. This study was supported by The Netherlands Foundation for the Advancement of Tropical Research (W93-364 and W93-468), European Union Grant (MEST-CT-2005-020524-GALTRAIN) and Royal Netherlands Academy of Arts and Sciences (Ref.KNAW-05-PP-35).

References

1. Prescott SL, Taylor A, Roper J, Wahdan A, Noakes P, Thornton C, Dunstan J, Upham JW (2005) Maternal reactivity to fetal alloantigens is related to newborn immune responses and subsequent allergic disease. Clin Exp Allergy 35: 417-425.
2. Tse DB, Young BK (2012) Co-ordinate expression of Th1/Th2 phenotypes in maternal and fetal blood: evidence for a transplacental nexus. J Perinat Med 40: 165-170. 10.1515/jpm.2011.131 [doi];/j/jpme.2012.40.issue-2/jpm.2011.131/jpm.2011.131.xml [pii].
3. Halonen M, Lohman IC, Stern DA, Spangenberg A, Anderson D, Mobley S, Ciano K, Peck M, Wright AL (2009) Th1/Th2 patterns and balance in cytokine production in the parents and infants of a large birth cohort. J Immunol 182: 3285-3293. 182/5/3285 [pii];10.4049/jimmunol.0711996 [doi].
4. Larsson AK, Nilsson C, Hoglind A, Sverremark-Ekstrom E, Lilja G, Troye-Blomberg M (2006) Relationship between maternal and child cytokine responses to allergen and phytohaemagglutinin 2 years after delivery. Clin Exp Immunol 144: 401-408.
5. Lappalainen M, Roponen M, Pekkanen J, Huttunen K, Hirvonen MR (2009) Maturation of cytokine-producing capacity from birth to 1 yr of age. Pediatr Allergy Immunol 20: 714-725. PAI865 [pii];10.1111/j.1399-3038.2009.00865.x [doi].
6. Djuardi Y, Wibowo H, Supali T, Ariawan I, Bredius RG, Yazdanbakhsh M, Rodrigues LC, Sartono E (2009) Determinants of the relationship between cytokine production in pregnant women and their infants. PLoS One 4: e7711.
7. Teo YY, Sim X, Ong RT, Tan AK, Chen J, Tantoso E, Small KS, Ku CS, Lee EJ, Seielstad M, Chia KS (2009) Singapore Genome Variation Project: a haplotype map of three Southeast Asian populations. Genome Res 19: 2154-2162. gr.095000.109 [pii];10.1101/gr.095000.109 [doi].
8. Weidinger S, Gieger C, Rodriguez E, Baurecht H, Mempel M, Klopp N, Gohlke H, Wagenpfeil S, Ollert M, Ring J, Behrendt H, Heinrich J, Novak N, Bieber T, Kramer U, Berdel D, von BA, Bauer CP, Herbarth O, Koletzko S, Prokisch H, Mehta D, Meitinger T, Depner M, von ME, Liang L, Moffatt M, Cookson W, Kabesch M, Wichmann HE, Illig T (2008) Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. PLoS Genet 4: e1000166. 10.1371/journal.pgen.1000166 [doi].
9. Li X, Howard TD, Zheng SL, Haselkorn T, Peters SP, Meyers DA, Bleecker ER (2010) Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. J Allergy Clin Immunol 125: 328-335. S0091-6749(09)01735-7 [pii];10.1016/j.jaci.2009.11.018 [doi].
10. Hohler T, Reuss E, Adams P, Bartsch B, Weigmann B, Worns M, Galle PR, Victor A, Neurath MF (2005) A genetic basis for IFN-gamma production and T-bet expression in humans. J Immunol 175: 5457-5462. 175/8/5457 [pii].
11. Herberth G, Hinz D, Roder S, Schlink U, Sack U, Diez U, Borte M, Lehmann I (2011) Maternal immune status in pregnancy is related to offspring's immune responses and atopy risk. Allergy 66: 1065-1074. 10.1111/j.1398-9995.2011.02587.x [doi].
12. Aaltonen R, Heikkinen T, Hakala K, Laine K, Alanen A (2005) Transfer of proinflammatory cytokines across term placenta. Obstet Gynecol 106: 802-807. 106/4/802 [pii];10.1097/01.AOG.0000178750.84837.ed [doi].

13. Garofalo R, Chheda S, Mei F, Palkowetz KH, Rudloff HE, Schmalstieg FC, Rassin DK, Goldman AS (1995) Interleukin-10 in human milk. Pediatr Res 37: 444-449. 10.1203/00006450-199504000-00010 [doi].

14. Agarwal S, Karmaus W, Davis S, Gangur V (2011) Immune markers in breast milk and fetal and maternal body fluids: a systematic review of perinatal concentrations. J Hum Lact 27: 171-186.

15. Labbok MH, Clark D, Goldman AS (2004) Breastfeeding: maintaining an irreplaceable immunological resource. Nat Rev Immunol 4: 565-572. 10.1038/nri1393 [doi];nri1393 [pii].

16. Westendorp RG, Langermans JA, Huizinga TW, Elouali AH, Verweij CL, Boomsma DI, Vandenbroucke JP (1997) Genetic influence on cytokine production and fatal meningococcal disease. Lancet 349: 170-173. S0140673696064136 [pii].

17. Sas AA, Jamshidi Y, Zheng D, Wu T, Korf J, Alizadeh BZ, Spector TD, Snieder H (2012) The age-dependency of genetic and environmental influences on serum cytokine levels: a twin study. Cytokine 60: 108-113. S1043-4666(12)00181-0 [pii];10.1016/j.cyto.2012.04.047 [doi].

18. Stein CM, Guwatudde D, Nakakeeto M, Peters P, Elston RC, Tiwari HK, Mugerwa R, Whalen CC (2003) Heritability analysis of cytokines as intermediate phenotypes of tuberculosis. J Infect Dis 187: 1679-1685. JID20993 [pii];10.1086/375249 [doi].

19. Pantsulaia I, Trofimov S, Kobyliansky E, Livshits G (2002) Genetic and environmental influences on IL-6 and TNF-alpha plasma levels in apparently healthy general population. Cytokine 19: 138-146. S1043466602919599 [pii].

20. Howie BN, Carlson CS, Rieder MJ, Nickerson DA (2006) Efficient selection of tagging single-nucleotide polymorphisms in multiple populations. Hum Genet 120: 58-68. 10.1007/s00439-006-0182-5 [doi].

Supporting Table 1. Genotypes of 119 pairs of mothers and children

Gene	Polymorphism	Region	Position	Mother			Child			MAS
				N (%)	MAF	HWE, *P* value	N (%)	MAF	HWE, *P* value	MAF
IL-10	rs4579758									
	AA	intergenic	204997334	43 (36.4)	0.432	0.085	43 (36.1)	0.420	0.324	0.376
	AG			48 (40.7)			52 (43.7)			
	GG			27 (22.9)			24 (20.2)			
	rs4390174									
	AA	intergenic	204999074	47 (39.5)	0.395	0.240	48 (40.3)	0.374	0.699	0.287
	AG			50 (42.0)			53 (44.6)			
	GG			22 (18.5)			18 (15.1)			
	rs1878672*θ									
	CC	Intron	205010336	107 (89.9)	0.055	0.585	107 (89.9)	0.055	0.585	0.090
	CG			11 (9.3)			11 (9.3)			
	GG			1 (0.8)			1 (0.8)			
	rs1554286§									
	TT	Intron	205010856	54 (45.4)	0.307	0.261	62 (52.1)	0.294	0.306	0.376
	TC			57 (47.9)			44 (37.0)			
	CC			8 (6.7)			13 (10.9)			
	rs3021094									
	CC	Intron 1	205011575	34 (28.6)	0.437	0.247	34 (28.6)	0.454	0.745	0.449
	CA	5'upstream		66 (55.4)			62 (52.1)			
	AA			19 (15.0)			23 (19.3)			
	rs1800872§									
	AA	Intron	205013030	63 (52.9)	0.265	0.739	68 (57.2)	0.248	0.873	0.371
	AC	5'upstream		49 (41.2)			43 (36.1)			
	CC			7 (5.9)			8 (6.7)			
	rs1800896*θ									
	AA	5'upstream	205013520	107 (89.9)	0.054	0.585	107 (89.9)	0.055	0.585	0.090
	AG			11 (9.3)			11 (9.3)			
	GG			1 (0.8)			1 (0.8)			
	rs10494879θ									
	CC	Intergenic	205018827	103 (86.6)	0.071	0.918	105 (89.0)	0.059	0.672	0.108
	CG			15 (12.6)			12 (10.2)			
	GG			1 (0.8)			1 (0.8)			
	rs4072227									
	TT	Intergenic	205024181	56 (47.1)	0.328	0.443	56 (47.1)	0.315	1.000	0.315
	TC			48 (40.3)			51 (42.8)			
	CC			15 (12.6)			12 (10.1)			
	rs885334									

	GG									
	GA	Intergenic	205029039	32 (26.9)	0.483	1.000	33 (27.7)	0.487	0.618	0.466
	AA			59 (49.6)			56 (47.1)			
				28 (23.5)			30 (25.2)			
NFKBIL1	rs13215091									
	GG	3'downstream	31636669	95 (79.8)	0.109	0.822	87 (73.1)	0.143	1.000	0.169
	GA			22 (18.5)			30 (25.2)			
	AA			2 (1.7)			2 (1.7)			
TNF-α	rs2844482									
	CC	5'upstream	31647746	59 (49.6)	0.282	0.408	69 (58.0)	0.235	1.000	0.230
	CT			53 (44.5)			44 (37.0)			
	TT			7 (5.9)			6 (5.0)			
LTA	rs1800683									
	GG	5' UTR,	31648050	58 (48.7)	0.324	0.187	56 (47.1)	0.298	0.385	0.326
	GA	upstream,		45 (37.8)			55 (46.2)			
	AA	intron		16 (13.5)			8 (6.7)			
LTA	rs2239704									
	CC	5' UTR,	31648120	45 (37.8)	0.387	1.000	31 (26.1)	0.458	0.217	0.419
	CA	upstream,		56 (47.1)			67 (56.3)			
	AA	intron		18 (15.1)			21 (17.6)			
IFN-γ	rs10878763†									
	GG	3'downstream	66829965	98 (82.4)	0.088	0.761	96 (80.7)	0.105	0.738	0.148
	GT			21 (17.6)			21 (17.6)			
	TT			0 (0)			2 (1.7)			
	rs2069718									
	TT	Intron 3	66836429	47 (39.5)	0.378	0.811	46 (38.6)	0.399	0.308	0.327
	TC			54 (45.4)			51 (42.9)			
	CC			18 (15.1)			22 (18.5)			
	rs2069705									
	TT	5'upstream	66841278	31(26.1)	0.487	1.000	30 (25.2)	0.483	0.498	0.420
	TC			60 (50.4)			55 (46.2)			
	CC			28 (23.5)			34 (28.6)			
	rs3181032									
	TT	5'upstream	66842442	88 (74.0)	0.134	0.685	100 (84.0)	0.080	0.917	0.093
	TG			30 (25.2)			19 (16.0)			
	GG			1 (0.8)			0 (0)			
	rs10784683†									
	GG	Intron	66856790	97 (81.5)	0.092	0.687	97 (82.9)	0.085	0.824	0.125
	GA			22 (18.5)			20 (17.1)			
	AA			0 (0)			0 (0)			
	rs12146822									

	GG	?	66857933	47 (39.5)	0.395	0.240	42 (35.3)	0.412	0.864	0.453
	GA			50 (42.0)			56 (47.1)			
	AA			22 (18.5)			21 (17.6)			
IL-5	rs4143832*									
	CC	intergenic	131890876	92 (77.3)	0.118	1.000	94 (79.0)	0.126	**0.039**	0.101
	CA			26 (21.9)			20 (16.8)			
	AA			1 (0.8)			5 (4.2)			
	rs2706399									
	AA	3'UTR	131895601	77 (64.7)	0.189	0.710	68 (57.2)	0.227	0.173	0.258
	AG	Intergenic		39 (32.8)			48 (40.3)			
	GG			3 (2.5)			3 (2.5)			
	rs17690122*									
	AA	3'UTR	131895734	92 (77.3)	0.118	1.000	94 (79.0)	0.126	**0.039**	0.101
	AG	Intergenic		26 (21.9)			20 (16.8)			
	GG			1 (0.8)			5 (4.2)			
	rs743562									
	CC	3'downstream	131900282	59 (49.6)	0.282	0.408	51 (42.9)	0.340	0.950	0.343
	CT			53 (44.5)			55 (46.2)			
	TT			7 (5.9)			13 (10.9)			
	rs739719									
	GG	3'downstream	131900764	56 (47.1)	0.319	0.834	61 (51.3)	0.269	0.349	0.253
	GT			50 (42.0)			52 (43.7)			
	TT			13 (10.9)			6 (5.0)			
	rs2069812									
	TT	5'upstream	131907815	48 (40.3)	0.357	0.826	41 (34.5)	0.416	0.989	0.393
	TC	Intron		57 (47.9)			57 (47.9)			
	CC			14 (11.8)			21 (17.6)			
RAD50	rs17772565									
	CC	Intron	131980304	31 (26.0)	0.471	0.524	48 (40.3)	0.366	1.000	0.410
	CT	5'upstream		64 (53.8)			55 (46.2)			
	TT			24 (20.2)			16 (13.5)			
	rs17772583									
	AA	Intron 20	131981409	82 (68.9)	0.164	0.708	74 (62.2)	0.202	0.482	0.185
	AG	5'upstream		35 (29.4)			42 (35.3)			
	GG			2 (1.7)			3 (2.5)			
	rs2237060									
	AA	Intron 21	131998784	97 (81.5)	0.101	0.657	88 (73.9)	0.130	0.211	0.124
	AC			20 (16.8)			31 (26.1)			
	CC			2 (1.7)			0			
	rs2040704†									

	AA	Intron 22	132001076	72 (60.5)	0.214	0.648	68 (57.1)	0.244	1.000	0.180
	AG			43 (36.1)			44 (37.0)			
	GG			4 (3.4)			7 (5.9)			
IL-13	rs1881457θ									
	AA	Intronic	132020308	68 (57.1)	0.244	1.000	66 (55.5)	0.248	0.737	0.213
	AC	5'upstream		44 (37.0)			47 (39.5)			
	CC			7 (5.9)			6 (5.0)			
	rs1800925†θ									
	CC	Intronic	132020708	74 (62.2)	0.206	0.820	70 (58.8)	0.231	1.000	-
	CT	5'upstream		41 (34.4)			43 (36.1)			
	TT			4 (3.4)			6 (5.1)			
	rs2066960									
	CC	Intron 1	132022334	71 (59.7)	0.214	0.302	72 (60.5)	0.218	0.983	-
	CA			45 (37.8)			42 (35.3)			
	AA			3 (2.5)			5 (4.2)			
	rs2069744									
	CC	Intron 1	132022568	82 (68.9)	0.160	0.311	90 (75.6)	0.130	1.000	0.079
	CT			36 (30.3)			27 (22.7)			
	TT			1 (0.8)			2 (1.7)			
	rs1295686‡									
	GG	Intron 3	132023742	49 (41.2)	0.387	0.137	46 (38.7)	0.403	0.213	0.331
	GA			48 (40.3)			50 (42.0)			
	AA			22 (18.5)			23 (19.3)			
	rs20541‡									
	CC	Exon 4	132023863	47 (39.5)	0.395	0.240	42 (35.3)	0.412	0.864	0.343
	CT			50 (42.0)			56 (47.1)			
	TT			22 (18.5)			21 (17.6)			
	rs2069757									
	GG	Intron	132026312	70 (58.8)	0.235	0.979	73 (61.3)	0.210	0.732	0.135
	GA	3'downstream		42 (35.3)			42 (35.3)			
	AA			7 (5.9)			4 (3.4)			
	rs2243218									
	GG	3'UTR	132029923	91 (76.5)	0.118	0.330	91 (76.5)	0.122	0.925	0.057
	GA	5'upstream		28 (23.5)			27 (22.7)			
	AA			0			1 (0.8)			

dbSNP: the Single Nucleotide Polymorphism database, MAF: minor allele frequency, HWE: Hardy-Weinberg equilibrium, MAS: Malay in Singapore.
Pairwise linkage disequilibrium within Haploview:
* r^2=1 † ‡ r^2>0.8; rs 2040704 in RAD50 is in strong LD with rs1800925 in IL-13.
θ r^2 for mother >0.7, child >0.8. § r^2 for mother >0.8, child >0.7.