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Gender-specific effects of cytokine gene polymorphisms on childhood vaccine responses

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ABSTRACT

Cytokine gene polymorphisms affect vaccine responses and gender-specific effects are known for many phenotypes. Therefore, this study investigated gender-specific effects of cytokine gene polymorphisms on vaccine responses. In 263 2-year-old subjects selected for parental history of atopy, boys with IL-4 C-589T and IL-4R α I50V genotypes associated with atopy had increased Diphtheria Toxoid (DiphTox) and Tetanus Toxoid (TetTox) responses compared with the remaining alleles (IL-4 C-589T: DipTox $p = 0.01$, TetTox $p = 0.04$; IL-4R α I50V: DipTox $p = 0.04$, TetTox $p = 0.08$). Contrastingly, girls with IL-10 -592C genotypes associated with atopy had lower levels of DiphTox ($p = 0.03$) and TetTox ($p = 0.02$) responses compared with the remaining allele. Additionally, interaction effects were found for IL-4 C-589T ($p = 0.01$) and IL-4R α I50V ($p = 0.04$) polymorphisms. In conclusion, these findings support the interaction of primary genetic and modifying factors on vaccine responses and the importance of atopic genetics to these responses.

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1. Introduction

Childhood vaccine [1] responses are highly heritable and polymorphisms in cytokine genes have been associated with these responses [2,3]. However, genetic variants modulating vaccine responses do not operate independently of other inherited and non-genetic factors [2]. That gender may be one such factor is supported by: (1) the association of gender with vaccine responses [4–8], adverse effects of vaccination [9] and cytokine levels [10]. (2) Gender's influences on gene expression at X-chromosome and autosomal loci [11–14]. (3) Gender-specific effects of HLA alleles on antibody responses to diphtheria and measles vaccine [15] and of a cytokine gene on atopic [16–19] and non-atopic [20–22] phenotypes. We selected cytokine genes associated with atopy as they have been linked to altered vaccine responses [2,23] with the quality of these genetic associations being modified by non-genetic variables [2].

Additionally, considering that flexibility of cytokine gene expression may be greater earlier in development [24], relationships among gender, cytokine genes and vaccine responses would

be optimally analysed in early childhood. Therefore, in this study, we investigated a cohort of 2-year-olds, selected by parental history of atopy, to determine whether gender-specific effects on vaccine responses to diphtheria and tetanus toxoids were related to polymorphisms in cytokine genes associated with atopy.

2. Materials and methods

2.1. Population

Sera and extracted DNA were available on 202 children aged 2 years, born between July 1996 and July 1998, from a previously described Western Australian birth cohort recruited on the basis of parental history of atopy [25]. Parental smoking (PS) was defined by parental report, at 2-year-old questionnaires, of current cigarette smoking by either or both parents. This study was approved by the Ethics Committee of the Princess Margaret Hospital for Children.

2.2. Vaccines and vaccination schedules

At ages 2, 4, and 6 months, the subjects received DTPa (acellular pertussis) (InfanrixTM; GlaxoSmithKline (GSK) or DTPw (whole cell pertussis) (Triple AntigenTM; CSL), oral polio (Polio SabinTM; GSK) and intramuscular *Haemophilus influenzae* type b (Hib) (HibTITERTM; Wyeth-Ayerst) vaccines. A measles–mumps–rubella vaccine (MMRIITM; Merck, Sharp & Dohme or Priorix; GSK) was

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administered at age 12 months. At 18 months, a further dose of DTPa or DTPw and Hib vaccination was given. The last DTP vaccine prior to blood collection was DTPa in 44.5% of individuals. Three per cent of individuals never received whole cell DTPw prior to blood collection.

2.3. Measurement of vaccine specific antibody concentrations

Blood samples were obtained at 2 years of age (mean: 2 years 15 days, range: 1 year 335 days to 2 years 35 days) and serum was isolated and stored at -20°C . The levels of anti-diphtheria and anti-tetanus IgG were measured by ELISA. No diphtheria antibody vaccine response was less than 0.01 IU/ml, the generally accepted minimum level giving some degree of protection [26], and 1.4% of tetanus antibody levels were below the protective level of 0.15 IU/ml [27].

2.4. Genotyping

Genotyping was performed by PCR-RFLP (IL-4 C-589T, IL-4 G2979T, IL-4R α Q551R, IL-13 C-1112T, IL-10 A-592C, IL-12 promoter CTCTAA/GC and IL-12 β A1188C) and denaturing HPLC (IL-4R α I50V and IL-13 R130Q) in an automated system (Varian Helix-System[®]) using the method developed by Oefner and co-workers [28]. Single nucleotide polymorphisms (SNPs) in IL-4 C-589T (rs2243250) and G2979T (rs2227284), IL-4R α I50V (rs1805010) and Q551R (rs1801275) and IL-13 C-1112T (rs1800925) and IL-13 Arg130Gln (rs2054) were genotyped as previously described [29]. The CD-14 C-159T polymorphism was genotyped using the method of Baldini et al. [30]. The IL-10 A-592C (rs 1800872) SNP was genotyped using the following primers: F 5'-tct gtg cct cag ttt gct-3' and R 5'-taa ctt agg cag tca cct tag g-3' and digested with RsaI. The IL-12 promoter CTCTAA/GC polymorphism and the IL-12 β A1188C SNP were genotyped as previously described [16,31].

2.5. Statistics

Vaccine responses were skewed and therefore were log transformed to approximate a normal distribution, as previously described [32]. To accommodate the negative correlation between antibody response and the time interval of last vaccine dose and blood sampling, linear regression was applied to derive residual values, corrected for the interval. The adjusted levels of antibody response were calculated with means of log values of the antibody response plus the corresponding residual values, and were used for the further analysis. Given the low general frequency of alleles previously associated with atopy, models dominant for these alleles (IL-4 -589T [33], IL-4 2979T [34], IL-4R α I50 [35], IL-4R α 551R [36], IL-13 -1112T [37] and IL-13 130Q [38], CD-14 -159C [30], IL-10 -592C [18], IL-12 β GC/GC [16] and IL-12 β 1118C [39]) were analysed i.e. those genotypes containing at least one copy of the allele previously associated with atopy were compared to the remaining genotype. For example, the IL-4 589T allele has previously been associated with atopy and therefore the TT and CT genotypes were compared with the CC genotype. The interaction between genes and gender on the vaccine outcomes was investigated using a likelihood-ratio test and comparing an unrestricted (with interaction) and a restricted model (without interaction) [40]. Subsequently, interactions were explored by stratification for gender. A p -value of <0.05 was considered significant. Whether or not to adjust for multiple comparisons is debated; these adjustments may increase the chance of detection of type II errors (the probability of accepting the null hypothesis when the research hypothesis is true) whilst reducing the chance of type I errors (the probability of accepting the research hypothesis

when the null hypothesis is true). It has been suggested that describing what was done and why and discussing the possible interpretations of these findings should allow the reader to reach a reasonable conclusion [41,42]. Therefore in this study, no adjustments were made for multiple testing as our investigations were based on an *a priori* hypothesis and we have described all tests performed. Interactions were investigated using Incooled Stata version 9.0, all other analyses were performed using SPSS version 11.5.

3. Results

3.1. Genotypic and phenotypic effects

Genotypes were in Hardy-Weinberg equilibrium and genotype frequencies were similar to previous reports. Genotypic effects of the polymorphisms in IL-4, IL-4R α and IL-13 have been described previously [2]. The only significant association was between the IL-4 -589 CT/TT genotypes and increased diphtheria toxoid responses ($p=0.049$). The additional genotypes investigated in this study showed no association with vaccine responses. Gender was not associated with vaccine response.

3.2. Interaction effects

Comparison of the unrestricted and the restricted model was used to investigate the interaction between genes and gender on vaccine responses. For tetanus toxoid response, the following SNPs had a p -value <0.05 for the interaction, IL-4 C-589T ($p=0.01$) and IL-4R α I50V ($p=0.04$). Other possible trends included the IL-4 G2979T ($p=0.12$), and IL-10 C-592A ($p=0.13$) SNPs with tetanus toxoid responses and the IL4 C-589T SNP with diphtheria toxoid responses ($p=0.10$).

3.3. Genotypic effects after stratification by gender

We further investigated the effect of the four SNPs identified above on the antibody responses stratified by gender. In boys there was a significant difference ($p=0.011$) in levels of diphtheria toxoid responses, individuals with IL-4 -589T alleles (CT/TT), that have been associated with atopy, had increased responses compared with CC homozygotes, with no difference found in girls. The same pattern was found for tetanus toxoid responses ($p=0.042$), Fig. 1. Additionally, for the IL-4R α I50V SNP boys with genotypes with a V allele (IV/VV), that have been associated with atopy, had higher levels of diphtheria ($p=0.039$) and tetanus toxoid ($p=0.077$) responses compared with II homozygotes. In contrast, girls with IL10 A-592C with genotypes containing an allele that have been associated with atopy (AC/CC) had significantly lower levels of diphtheria ($p=0.028$) and tetanus ($p=0.018$) toxoid responses compared with AA homozygotes. No significant difference was found in boys, Fig. 2. In girls, the other genotypes associated with atopy showed non-significant reductions in tetanus toxoid responses compared to the remaining genotypes, Fig. 3.

As an interaction of PS and genotype on vaccine responses has been shown we adjusted for this by multivariate linear regression analyses with antibody response as the dependent variable and genotypes and parental smoke as independent variables. Significant differences remained for the above associations of IL-4 C-589T and IL-10 A-592C genotypes. The trend remained for the IL-4R α I50V SNP with increased diphtheria and tetanus toxoid responses; however, the results were not significant ($p=0.068$ and $p=0.087$, respectively). Sample size precluded stratification by PS within gender-genotype groups.

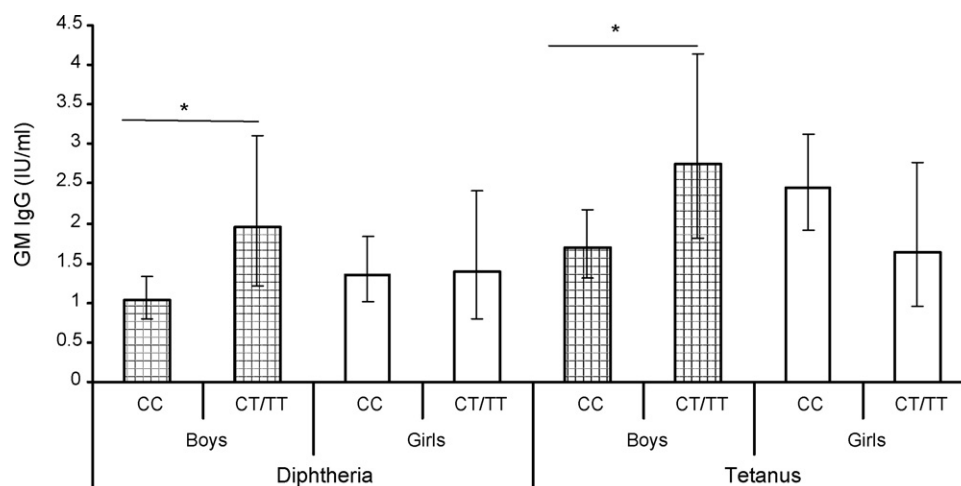


Fig. 1. Geometric mean (GM) IgG responses to diphtheria and tetanus toxoids by IL-4 C-589T genotype stratified by gender. Hashed boxes represent male gender. * $p < 0.05$.

4. Discussion

This is the first study to demonstrate gender-dependent effects of cytokine genes on vaccine responses and these findings are consistent with gender-dependent effects of HLA genes on diphtheria toxoid responses [15]. We demonstrated an increased antibody response in males and a decreased antibody response in females with genotypes associated with atopy. The effects reported herein are independent of the previously reported gene–parental smoking interaction [2] and further support the interaction of primary genetic and modifying factors on vaccine responses. The findings enhance the understanding of the role of genetic factors associated with atopy upon vaccine response.

Taken together, our findings show an increase in vaccine antibody response in boys with genotypes that have been associated with atopy and a reduction in these responses in girls with these genotypes. This apparent dichotomy of effect has previously been shown for the IL-4R α I50V polymorphism [17]. A gender differential in gene transcription is shared by many phyla [43] and this divergence is necessary as males and females use a similar genome to achieve distinct phenotypes. This results in different selective pressures between males and females, with a genetic variant producing a slight advantage in males not doing so in females or vice versa [43]. It has been suggested that gender-biased differences in

gene transcription may particularly occur in tissues with multifarious physiological roles [44] and gene-ontology analysis identified immune response genes were differentially expressed between males and females [44] and therefore could involve tissues of the immune system.

The influence of gender on the pathogenesis and expression of many diseases [45,46], including atopic disease [47] and gender-dependent differences in disease incidence and progression are thought to be due, at least in part, to the influence of sex hormones on gene expression [43]. Accordingly, sex steroids influence T cell populations, specific antibody production and proinflammatory mediators [48]. However, human sex-biased gene transcription may not be limited to hormone-dependent effects, as hormone-independent sex-specific transcriptional regulation has been shown in mammals [49] and gender-dependent genetic effects may be seen prior to the pubertal hormonal surge. Evidence in humans to support gender-dependent genetic effects includes differences in non-immune response gene expression in prepubertal children [50] and gender-dependent effects of polymorphisms in immune response genes, including IL-10 [20,21] on atopic [19,51–53] and non-atopic phenotypes [20,21] in prepubertal [16,50,54], peripubertal [16] and postpubertal individuals [18–22,52,53]. Gender-dependent effects of IL-10 promoter polymorphisms include the association of the –592A allele, as part of

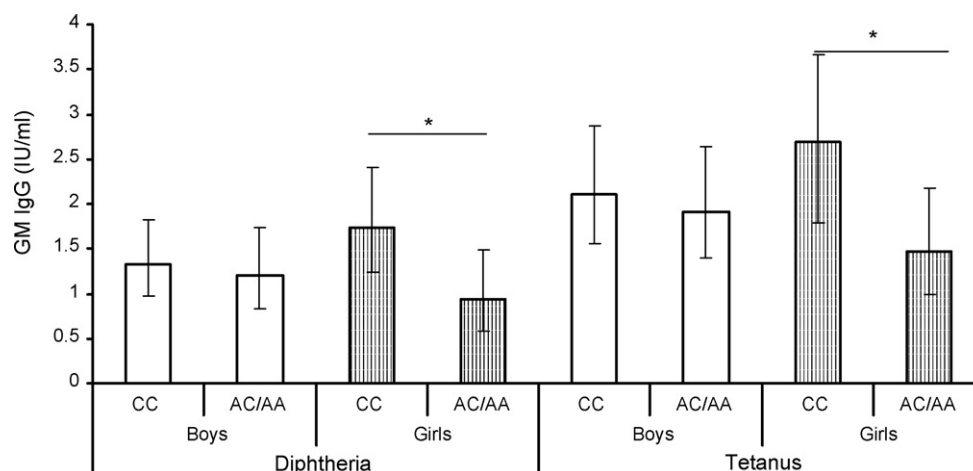


Fig. 2. Geometric mean (GM) IgG responses to diphtheria and tetanus toxoids by IL-10 A-592C genotype stratified by gender. Boxes with vertical lines represent female gender. * $p < 0.05$.

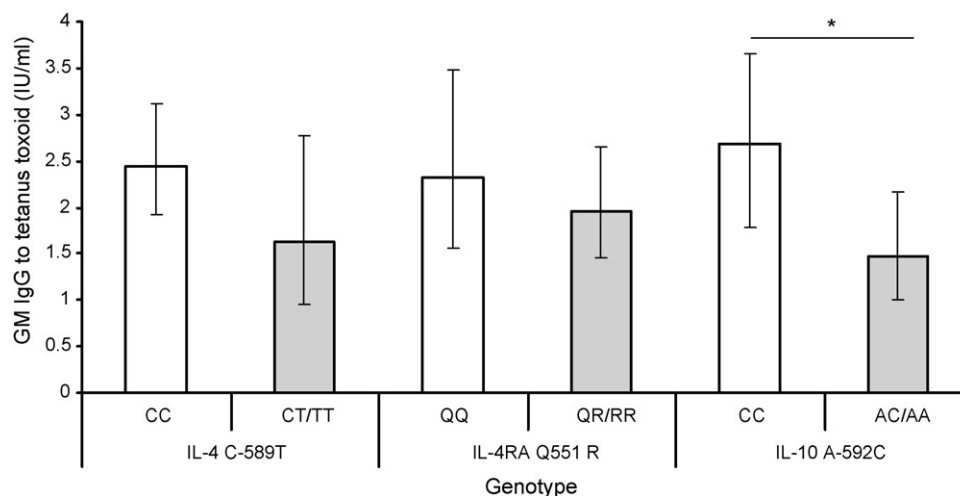


Fig. 3. Geometric mean (GM) IgG responses to tetanus toxoid in females categorised by genotype IL-10 A-592C genotype stratified by gender. Grey boxes represent genotypes that have been associated with atopy. * $p < 0.05$.

a haplotype, with increased IgE in male asthmatics [18], the -592A allele, with β -lactam allergy in atopic women [17], the -1082G allele with longevity in men [21], and with lower numbers of CD8 positive T cells in old men [55]. Furthermore, in twin studies of IL-10 production, female gender was associated with impaired responses [56]. IL-4R α I50 has been reported to be associated with predisposition to and protection from β -lactam allergy in atopic women and non-atopic men, respectively [17]. Additionally in animal studies, gender is a major determinant of immune response to infection, with disease severity proportional to local expression of IL-4 and IL-10 [57]. These observations support the biological plausibility of this study's findings, however, the clinical implications of our findings are unknown as the majority of antibody responses were above generally accepted minimum levels of protection for diphtheria and tetanus infection. However, no antibody level may confer absolute protection [26] and understanding the mechanisms underlying these observations may ultimately result in improved vaccine efficacy.

Epigenetic mechanisms have been proposed to modulate gender-dependent genetic effects [47]. In this context, it is noteworthy that methylation of CpG dinucleotides, a key epigenetic phenomenon regulating cytokine gene transcription [58], has a gender-differential [59]. Notably, promoters are enriched for CpG dinucleotides [60] and the majority of reports of gender-dependent genotype effects have been found in association with promoter polymorphisms [18–22,52,53]. Methylation is also integral to the prototypical gender-specific genetic process of X-inactivation [61]. Interestingly, X-inactivation is an example of monoallelic exclusion i.e. the process of expressing only one of two alleles of a gene and monoallelic expression has been implicated in regulation of cytokine genes [62,63]. Therefore, it is possible that gender may exert its effects through epigenetic influences and may be mediated through the mechanisms including methylation and monoallelic exclusion.

Neonatal tetanus is a major killer of newborns in developing countries; WHO estimated that approximately 400 000 cases occur annually [64]. Maternal passive immunisation is protective for neonatal tetanus, therefore, if the reduction in tetanus vaccine responses in girls with genotypes that have been associated with atopy persisted into childbearing years then infants of these mothers may be at particular risk. The risk to these particular infants, if they are exposed to PS, may be compounded by the demonstrated negative interaction of atopic genotypes with PS on vaccine

responses. These potential negative impacts might be disproportionately felt in developing countries given the frequency of some of the genotypes associated with lower responses to tetanus toxoids in females have been reported to be more common in developing countries [34,65].

This study has several limitations including: (1) without a healthy control group it is uncertain whether the demonstrated associations are specific to, or independent of, a high genetic risk of atopy. (2) No corrections were made for multiple testing. We elected not to make such corrections because (i) they can be overly conservative and may inflate type II errors and (ii) our analyses were based on an *a priori* hypothesis [41]. Instead, we have chosen to describe all statistical analyses carried out and to discuss the results. We agree with Rothman [42] that there is little to be gained by adjustment, provided that all analyses are described when the study is reported and there is a lot to be lost if anything other than the results of all hypothesis tests are reported. (3) Sample size precluded detailed investigation of the interaction of exposures with gender-dependent genetic effects.

In conclusion, gender-genotype interactions may influence vaccine response, and failure to consider this may have contributed to underreporting of these interactions, as many genetic association studies have controlled for gender rather than investigated its impact on genetic effects. Ultimately, understanding gender-specific genetic effects may yield improved vaccine responses and reduce the global infectious disease burden.

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