Genetics of occupational asthma

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Purpose of review

To discuss gene association studies conducted in workers diagnosed with occupational asthma.

Recent findings

Human leukocyte antigen studies conducted in European workers have defined major histocompatibility complex class II alleles and haplotypes associated with diisocyanate asthma. Recently, certain glutathione S-transferase genotypes (e.g. the GSTM1 null genotype) and N-acetyltransferase genotypes associated with slow acetylation phenotypes have been reported to be associated with diisocyanate asthma. Genotype combinations of IL-4 receptor-α and CD14 single nucleotide polymorphisms (SNPs) were significantly associated with diisocyanate asthma, but only in workers exposed to hexamethylene diisocyanate. A recent genome-wide association study (GWAS) conducted in Korea identified several SNPs of the *α-T-catenin* gene that were significantly associated with diisocyanate asthma.

Summary

Although candidate gene association studies have yet to identify reliable predictors of occupational asthma, future investigations including GWAS studies may identify high-risk genotypes allowing identification of workers at risk.

Keywords

diisocyanate, genetic, genotype, occupational asthma

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Introduction

Occupational asthma and work-exacerbated asthma (i.e. exacerbation of preexisting asthma) are the most common of all occupational lung disorders [1]. Occupational asthma is defined as reversible airways obstruction and airway hyperresponsiveness caused by a condition or substance encountered at work. Classically, occupational asthma may be induced by sensitization to chemicals or high molecular weight (HMW) protein allergens. Natural protein allergens encountered at work, derived from animal or plant sources, induce classic IgE-mediated sensitization after months or years of exposure to a causative substance [2]. Typically, affected workers experience symptoms of allergic rhinitis and conjunctivitis while at work preceding or coexisting with immediate onset asthmatic responses beginning within minutes after ambient exposure to an occupational allergen. In these cases, sensitization is easily demonstrated by skin prick testing or measuring serum allergen-specific IgE. However, confirmation of occupational asthma requires demonstration of decrements in lung function (e.g. force expiratory volume (FEV) 1) associated with exposure to the suspected causative agent.

Reactive chemicals like diisocyanates are potent chemical respiratory sensitizers. Diisocyanates remain among the major causes of occupational asthma in chronically exposed workers [1]. Toluene diisocyanate (TDI), methylene diphenyl diisocyanate (MDI) and hexamethylene diisocyanate (HDI) are most commonly implicated. Although workers exposed to HMW allergens can be easily monitored by skin testing for development of sensitization, there are no valid immunologic tests to identify workers sensitized to isocyanates. In addition, investigators have been unable to clearly define underlying immune mechanisms, risk factors and predictors of diisocyanate asthma. In many respects, diisocyanate asthma resembles allergic asthma in that a latency period of asymptomatic exposure precedes onset of symptoms, only small numbers of exposed workers are affected, and immediate and/or late asthmatic responses are elicited by very low levels of exposure (e.g. 5 ppb) [1]. Thus, in part due to the lack of progress in this area, there has been considerable interest in identifying markers of genetic susceptibility to diisocyanate asthma and to other chemical causes of occupational asthma. There is very little published on genetic markers of occupational asthma caused by occupational protein allergens.

Asthma and occupational asthma are complex polygenic disorders that present unusual challenges for genetic studies. A low incidence of occupational asthma limits

the ability to recruit large study populations for genetic association studies. Nevertheless, the ability to define chemically induced occupational asthma phenotypes precisely by specific inhalation challenge (SIC) testing is a clear advantage over studies of allergic asthma in which intermediate phenotypes (e.g. airway responsiveness to methacholine) are relied upon. Defining proper control groups for assessing genetic associations with confirmed diisocyanate asthma is another challenge. For example, asymptomatic exposed or symptomatic exposed workers in whom occupational asthma has been excluded have been employed as comparator groups in different studies.

Finally, as with any complex disease, it is difficult to identify optimal methodological approaches. In the case of occupational asthma, and specifically a rare condition like diisocyanate asthma in which numbers of participants are limited, candidate gene association approaches have been employed primarily but not exclusively. This article will review published genetic studies of occupational asthma investigating genetic polymorphisms associated with: innate immunity and immunoregulation; cytokines; antioxidant and transferase; and genome-wide association studies (GWASs).

Genes associated with immunoregulation and innate immunity

Genes associated with antigen presentation have been studied almost exclusively in diisocyanate-exposed workers. Several investigators have examined associations between human leukocyte antigen (HLA) class II haplotypes and/or alleles and isocyanate-induced asthma. Here, *HLA class II* gene products are hypothesized to regulate immune responsiveness to chemical antigens. In 1994, Bignon *et al.* [3] performed HLA typing using PCR and PCR-restriction fragment length polymorphism (RFLP) methods to identify HLA class II DQA1, DQB1, DPB1 and DRB alleles. Workers with diisocyanate asthma vs. exposed asymptomatic controls exhibited a higher frequency of DQB1∗0503 and of an allelic combination DQB1∗0201/0301. On the contrary, the allele DQB1∗0501 0501 and the DQA1∗0101-DQB1∗0501-DR1 haplotype were increased among exposed healthy controls vs. affected workers, suggesting a genetic protective effect. Mapp *et al.* [4], in a subsequent study of TDI workers, reported that allele frequencies of DQA1∗0104 and DQB1∗0503 were increased significantly in occupational asthma compared with asymptomatic workers, whereas DQA1∗0101 and DQB1∗0501 alleles were reduced in workers with TDI asthma. Balboni *et al.* [5] later identified a positive association of diisocyanate asthma with single amino acid substitutions at residue 57 of aspartic acid in DQB1 ∗ 0503 and negative association with valine in DQB1 ∗ 0501. A US study [6] employing smaller sample sizes of isocyanate workers with and without occupational asthma failed to identify between-group differences in HLA-DR or HLA-DQ alleles. A Korean study performed in 84 workers with TDI asthma and two asymptomatic comparator groups failed to show significant associations of occupational asthma with individual HLA-DR or HLA-DQ allele frequencies. However, the HLA DRB1∗1501-DQB1∗0602-DPB1∗0501 haplotype was significantly associated with TDI asthma [7•]. A study [8] conducted in Europe failed to demonstrate significant associations between HLA class I alleles and TDI-induced asthma. Hur *et al.* [9] reported an association between a HLA class II haplotype and serum-specific IgG antibody measured in MDI-exposed workers. All the latter studies are inadequately powered; larger studies in different background populations are needed to further evaluate inconsistent findings.

HLA association studies have been conducted in workers exposed to red cedar wood dust and acid anhydride chemicals used in plasticizers. DRB1 and DQB1 HLA class II alleles and DRB1-DQB1 haplotypes were studied in 56 wood workers with confirmed occupational asthma and 63 healthy exposed sawmill workers. Red cedar asthma workers had a higher frequency of HLA DQB1∗0603 and DQB1∗0302 alleles compared with healthy workers and a reduced frequency of the DQB1∗0501 allele. The DRB1∗0401-DQB1∗ 0302 haplotype was increased and the DRB1∗0101-DQB1∗0501 haplotype was reduced among workers with red cedar asthma [10]. HLA genotyping conducted in 52 workers with confirmed specific IgE to at least one acid anhydride antigen and 73 referents revealed a significantly positive association [odds ratio (OR) 3.0; 95% confidence interval (CI) 1.2–7.4] between HLA class II allele DQB1(∗)0501 within DQ5 and acid anhydride sensitization [11].

Jeal *et al.* [12] performed HLA typing for DRB1 and DQB1 loci in 109 laboratory workers sensitized to rat proteins and 397 referents. HLA-DR7 was significantly associated with sensitization (OR 1.82), respiratory symptoms at work (OR 2.96) and sensitization combined with symptoms (OR 3.8), whereas workers with HLA-DR3 were less likely to be sensitized.

Pacheco *et al.* [13] assessed laboratory animal workers for *Toll-like receptor* (*TLR*) gene markers associated with innate immune responses. *TLR4/8551* and *TLR4/8851* variants were evaluated in 335 laboratory animal researchers exposed concomitantly to endotoxin and laboratory animal proteins. Workers with a *TLR4 8851 T* variant exhibited reduced responsiveness to endotoxin and were at higher risk for atopy and laboratory animal sensitization.

Cytokine genes

Murine models of TDI-induced respiratory inflammation are associated with a mixed Th1/Th2 cytokine response [14], but may be primarily dependent on expression of

Th2 cytokines including IL-4 and IL-13 [15]. Although bronchial biopsies of workers with confirmed diisocyanate asthma demonstrate activated T cells, cellular IL-4 expression is transient [16,17]. Unlike nonoccupational asthma, there are few studies examining cytokine genes in worker populations. Beghe *et al.* [8] failed to identify positive or negative associations of a TNFα A308G polymorphism with occupational asthma in 142 patients with TDI-induced asthma in comparison with 50 asymptomatic exposed participants.

Bernstein *et al.* [18] evaluated polymorphisms of *Th2 cytokine* genes of interest previously implicated as modifiers of nonoccupational asthma and/or allergic sensitization. In this study, 62 workers with diisocyanate asthma confirmed by SIC and 75 symptomatic workers without diisocyanate asthma (SIC-negative) were genotyped for three single nucleotide polymorphisms (SNPs) associated with *IL4RA*, one with IL-13 and a CD14 promoter SNP. Although individual SNP alleles were not predictive of diisocyanate asthma, specific genotypes and genotype combinations were significantly associated with diisocyanate asthma, but only among HDI-exposed workers. Among HDI-exposed workers, the wild *IL4RA* (I50V) II genotype was more likely among those with diisocyanate asthma (OR 3.3, $P = .01$). Similarly, associations were found between diisocyanate asthma and the genotype combinations of *IL4RA* (I50V) II and IL-13 (R110Q) RR (OR 4.1, $P = .01$); *IL4RA* (I50V) II and CD14 (C159T) CT (OR 5.2, $P = .002$); and *IL4RA* (I50V) II, IL-13 (R110Q) RR and CD14 (C159T) CT (OR 6.4, $P = .01$). Because significant relationships were observed only in HDI and not in all isocyanate workers, gene–environment (i.e. HDI exposure) and gene–gene interactions may modify expression of diisocyanate asthma.

Antioxidant-associated and transferase enzyme-associated genes

Glutathione (GSH) is a major lung-reducing agent and antioxidative protein and glutathione S-transferases (GSTs) conjugate GSH and isocyanate ligands. Thus, genes associated with the mu (M), theta (T), and pi (P) classes of the GST isoenzyme superfamily have been investigated as genetic predictors of diisocyanate asthma. There is human data suggesting that GSTs genotype variants could modify biotransformation of isocyanates and excretion of metabolic products [19]. GSH conjugation directly inhibits in-vitro binding of diisocyanates with albumin [20]. Piirila *et al.* [21] first evaluated 109 workers with diisocyanate asthma and 73 asymptomatic workers for SNPs in the *GSTM1*, *GSTM3*, *GSTP1* and *GSTT1* genes. Lack of the *GSTM1* gene (null genotype) was significantly associated with a 1.9-fold risk of diisocyanate-induced asthma. The same group subsequently extended this study to investigate N-acetyltransferase (NAT) genotypes is an expanded population of 182 workers exposed to either HDI, TDI or MDI [22]. The presence of a slow acetylator NAT1 genotype was significantly associated with a 2.5-fold risk of diisocyanate asthma in workers exposed to any isocyanate and this was greatest among those workers exposed to TDI (OR 7.7, 95% CI 1.2–52). In a study of 131 TDI-exposed workers (92 with diisocyanate asthma and 39 who were asymptomatic), Mapp *et al.* [23] reported a nonsignificant trend that the GSTP1 Val/Val homozygous genotype was lower and perhaps modified risk for diisocyanate asthma (OR 0.23, $P = .074$).

Genome-wide association studies

GWAS of nonoccupational asthma have been conducted in a variety of large populations of different ethnic backgrounds. Only one GWA study has been reported of workers with occupational asthma. Kim *et al.* [24••] genotyped 84 patients with TDI asthma and 263 unexposed controls using two large GeneChip arrays consisting of 500 000 SNPs. After excluding SNPs with minor allele frequencies or those not in Hardy–Weinberg equilibrium, several SNPs of the *α-T-catenin* (*CTNNA3*) gene were identified to be significantly associated with diisocyanate asthma. α-T-Catenin is a molecule involved in E-cadherin-mediated cellular adhesion, but the functional significance of this finding is unknown.

Conclusion

Candidate gene association studies have yet to identify reliable predictors of occupational asthma. This may be in part due to inadequately powered studies having low case frequencies of occupational asthma syndromes and the intrinsic limitations of candidate gene methodological approaches. Future investigations including GWAS studies may be able to identify high-risk genotypes. In the future, gene expression profiling in human cells or with biologic specimens obtained from exposed workers with occupational asthma may assist in identification of biologically relevant genes, proteins and cytokines/chemokines [25,26].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 151–153).

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