ORIGINAL RESEARCH LETTER

Cholinergic synapse pathway gene polymorphisms associated with allergen-induced late asthmatic responses

To the Editor:

Allergen inhalation challenge triggers well-defined airway responses in mild, allergic asthmatics. Some individuals develop only an isolated early response (early responders (ERs)) characterised by acute airway smooth muscle constriction immediately following allergen inhalation. Others develop a late response (dual responders (DRs)) that begins 3–4 h later, resulting in prolonged reduction of airway function, associated with cellular infiltration, inflammation and hyperresponsiveness of the airways [1]. It is not well understood how certain individuals are protected from developing a late response. Our previous research identified novel RNA transcripts in peripheral blood that are predictive of asthmatics who could develop a late response. Our findings pointed towards the presence of inherent differences underlying molecular mechanisms that predispose asthmatic individuals to the late response [2].

A genetic contribution to asthma aetiology has been well documented. Genetic polymorphisms have previously been shown to influence pharmacological responses in asthma [3]. To our knowledge, no studies have addressed the role of genetic variation in the allergen-induced late-phase asthmatic response. Several physiological and genetic data suggest asthmatic symptoms may be significantly modulated by the central nervous system. Changes at the level of parasympathetic neuronal control of airway smooth muscle have been shown to increase bronchoconstriction in response to vagal stimulation, leading to airway hyperresponsiveness [4]. Importantly, the cholinergic pathway mediated by the parasympathetic neurotransmitter, acetylcholine, is a predominant neurogenic mechanism contributing to bronchoconstriction in asthma [5]. We hypothesised that cholinergic pathway gene polymorphisms could play a potential role in regulating late-phase asthmatic responses after allergen inhalation.

We recruited 17 ER (six male and 11 female) and 38 DR research participants, all of whom were nonsmokers, with stable, mild allergic asthma and free of other lung diseases. The participants were of Caucasian ethnicity, and were between 18 and 55 years of age (mean±SD age: ER 28.47±8.88 years, DR1 30.73±12.94 years and DR2 28.47±11.58 years). Subjects who had forced expiratory volume in 1 s (FEV_1) >70% of predicted and baseline methacholine PC_{20} (provocative concentration causing a 20% fall in FEV_1) of <16 mg·mL^{-1}, and who developed an isolated early response (⩾20% fall in FEV_1 <2 h after allergen inhalation) or dual response (early response plus a late response: ⩾15% fall in FEV_1 3–7 h after allergen inhalation) were studied. The allergens used include: ragweed, fungus, cat, grass, horse and house dust mite. The study population of DRs was split into two groups: DR1 (six male and 13 female) elicited only the dual response while DR2 (eight male and 11 female) had intermediate phenotypes (*i.e.* elicited different responses during repeated allergen inhalation challenge) [2]. We utilised group 1 (17 ERs and 19 DRs (DR1)) data for our original analysis and group 2 (17 ERs and 19 DRs (DR2)) to validate the results.

Blood (5 mL) donated by each participant was utilised for DNA extraction using standard kits (Qiagen, Hilden, Germany). A total of 140 single-nucleotide polymorphisms (SNPs) with minor allelic frequency >10%, located <50 000 bp upstream of the transcription start site and downstream of the 3′ untranslated region of eight genes were selected from the cholinergic synapse KEGG (Kyoto Encyclopedia of Genes and

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Genomes) pathway for analysis: *ADCY3, AKT3, CACNA1S, CHRM3, CHRNB2, GNB1, GNG4* and *KCNQ4*. Genotyping was performed using Axiom SNP arrays (Affymetrix, Santa Clara, CA, USA).

With the exception of two SNPs in *CHRM3* in the DR1 group, all other SNPs satisfied Hardy–Weinberg equilibrium in both groups. A dominant genetic analysis model was adopted to assess genotype frequency distribution. Chi-squared analysis showed that the genotype and allele frequency of 28 out of 140 SNPs were significantly different between the ER and DR1 groups. This was validated in the DR2 cohort: 19 (68%) out of 28 SNPs showed a significant difference between the groups. Most of these polymorphic variants within each gene were in strong linkage disequilibrium and there were no significant differences in the haplotype frequencies between the two groups in either cohort.

The cumulative effect/accumulation of minor alleles was analysed using unweighted (linear sum of minor alleles divided by total SNPs) and weighted approaches (sum of minor alleles weighted by their individual odds ratio and scaled by total SNPs). The unweighted (Uw) cumulative minor allele content (Uw-cMAC) and weighted cumulative minor allele content (w-cMAC) values of the two groups were compared using Mann–Whitney U-tests. The mean Uw-cMAC of DR1 (Uw-cMAC 25.4) was significantly higher than that of ERs (Uw-cMAC 16; p=0.001, z-score 2.99) and this was reproducible in DR2 group (Uw-cMAC 25; p=0.002, z-score 2.8) (figure 1a). Mean w-cMAC of DR1 (w-cMAC 24.16), but not DR2 (w-cMAC 19.47), was significantly higher than that of ERs (w-cMAC 12.18; p=0.001, z-score −3.40) (figure 1b).

Cholinergic pathway genes (*CHRM1* and *CHRM3*) have been implicated as important susceptibility loci for asthma in Japanese and Mexican populations [6, 7]. Furthermore, the association of lung sensory neurons with hyperresponsive airways was shown by studies that examined the transient receptor potential (*TRP*) gene in asthma. A recent study that examined airway hyperreactivity with a genetically silenced *TRP* gene in a murine model of asthma showed that ablation of vagal sensory neuronal cells abolishes hyperreactive bronchoconstrictions, even in the presence of a full lung inflammatory response [8]. A strong association between *TRPA1* gene polymorphisms and childhood asthma has also been demonstrated [9].

The nicotinic acetylcholine receptor signalling mediates calcium influx *via* voltage-gated calcium channels (CACNA1S) that have been demonstrated to regulate airway smooth muscle contractility. In addition, the genes for G-protein coupled receptor signalling, such as *ADCY3, AKT3, GNB1, GNG4* and *KCNQ4,* although involved in plethora of signalling pathways, have been previously associated with asthma by candidate-gene, genome-wide and epigenome-wide association studies [10–14]. Together, these genes, constituting the cholinergic synapse pathway, may mediate convergent signalling and thereby predispose an individual to a specific asthma phenotype. To our knowledge, ours is the first study showing an association of cholinergic pathway genes in allergen-induced late-phase responses. Although our small sample size limits the possibility of multiple comparisons, the rigorous clinical phenotypic characterisation and reproducibility of our findings in an additional group of DRs adds to the strength of the current study.

It is established that the early response results from IgE-mediated mast cell activation, release of mediators and subsequent bronchial smooth muscle cell constriction. In contrast, in addition to release of mediators, the late response is associated with increased airway responsiveness and inflammation [15]. The findings are consistent with our hypothesis that isolated ERs may be genetically protected from developing severe

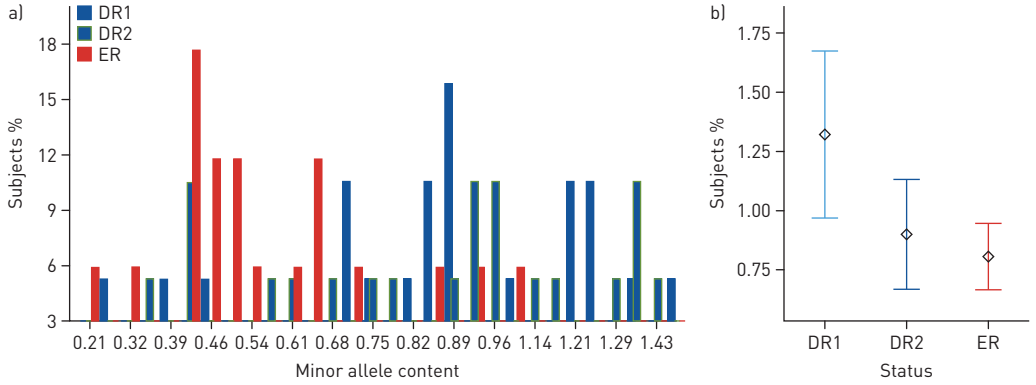

FIGURE 1 a) Minor allele distribution and b) mean differences in the weighted cumulative minor allele content (w-cMAC) between early responders (ERs) and dual responders (DRs). Error bars represent 95% confidence intervals.

forms of airway responsiveness, while additional mechanisms could be involved in precipitating a more complex phenotype of asthma such as the late response.

For the first time, enrichment of minor allele cholinergic synapse pathway genes was demonstrated to be one of several mechanisms contributing towards late-phase responses observed in DRs. The cholinergic synapse pathway has the potential to be a therapeutic target for inflammatory phenotypes of asthma. Further elucidating its role in differentiating asthmatic responses would be a worthwhile strategy for the treatment and management of asthma.

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