Journal of Human Genetics (2011) 56, 97–98

www.nature.com/jhg

CORRESPONDENCE

Chromosome 17q21 SNP and severe asthma

Journal of Human Genetics (2011) **56,** 97–98; doi:10.1038/jhg.2010.134; published online 28 October 2010

Asthma is a complex disease that is influenced by poorly understood genetic and environmental factors.[1] A genome-wide association study (GWAS) of 994 cases and 1243 controls from the United Kingdom and Germany found strong associations ($P<10^{-12}$) between single-nucleotide polymorphisms (SNPs) at the 17q21 locus and childhood asthma using family and case–control panels.[2] The association between these SNPs and gene transcript levels in Epstein–Barr virus-transformed lymphoblastoid cell lines from the asthmatic children identified *ORMDL3* as a candidate gene for asthma. One of the SNPs, *rs7216389*, located within a highly conserved region containing an element homologous to the proinflammatory transcription factor C/EBPb, was associated to both asthma and *ORMDL3* transcript levels with the highest degree of statistical significance in the initial study (uncorrected $P=9\times10^{-11}$).[2]

The initial GWAS findings have subsequently replicated in a number of studies involving ethnically diverse populations, and the variants were reported to contribute to early-onset asthma and interacting to early-life environmental tobacco smoke exposures.[3]

A study published recently identified an association of *rs7216389* variant with disease severity in early-onset asthma.[4] In the study, asthmatic cases were stratified according to asthma severity and they were classified into mild, moderate and severe asthmatics following national and international guidelines. Severe asthmatics were not recruited through severe asthma clinics; however, the study proposed *rs7216389* as being involved in early-onset severe asthma.

Severe or 'difficult/therapy-resistant' asthma refers to asthma that is poorly controlled in terms of persistent symptoms, episodic exacerbations, and persistent and variable airway obstruction despite the use of high doses of inhaled corticosteroids, long-acting bronchodilators and short-acting β-agonists.[5] Studying individuals with an extreme phenotype can be very powerful when isolating the genetic determinants underlying a disease. Using this strategy, we have consequently examined the role of *rs7216389* in severe asthma.

The case group consisted of 397 severe asthmatic adults identified through specialist severe asthma clinics at two UK centers, Royal Brompton Hospital, London and the Glenfield Hospital, Leicester. Asthma was defined using the international GINA (Global Initiative for Asthma: http://www.ginasthma.com) guidelines and the ATS criteria for refractory asthma.[5] For 226 subjects, the age of onset of asthma was available. Childhood asthma onset was found in 114 samples and adulthood asthma onset in 112. The male-to-female ratio was 1:2, the mean age 48.95 years (s.d. 13.55) and mean immunoglobulin E (IgE) level (kU l^{-1}) 291.72 (s.d. 456.09). We derived 1429 previously genotyped healthy UK adult controls from the 1958 British Birth Cohort study. The 1958 British birth cohort includes 17 638 males and females with sex ratio 1:1 enrolled in the Perinatal Mortality Survey at the time of their birth during 1 week in March 1958 across England, Wales and Scotland.[6] A DNA collection was obtained during a follow-up in 2002–2004.[7] Genome-wide genotyping data from the Illumina HumanHap550 Beadarray on 1430 subjects were deposited by the Wellcome Trust Sanger Institute.[8]

Blood samples from cases were collected and DNA was extracted using whole blood DNA extraction protocols (Promega Wizard Genomic DNA purification kit, Promega, http://www.promega.com). TaqMan SNP Genotyping Assays (Applied Biosystems 7300 Real-Time PCR System, Applied Biosystems, http://www.appliedbiosystems.com, 40 cycles of 10 min at 95 °C, 15 s at 92 °C and 1 min at 60 °C) were used for the allelic discrimination (primer and probe sequences available upon request). Controls of known genotype were included.

Deviation from Hardy–Weinberg equilibrium (HWE) was calculated for the allele frequencies. Genotype and allele frequencies were compared between cases and controls by Fisher's exact test and logistic regression. Associations between the genotypes and IgE were also examined by Kruskal–Wallis test.

The genotyping success rate for *rs7216389* was 97%. No significant deviation from HWE was detected ($P>0.05$). The *rs7216389* SNP was found to be significantly associated with severe adult asthma (odds ratio (OR) 1.42, confidence interval (CI): 1.21–1.67, $P=1.8\times10^{-5}$) (Table 1). In our study the frequency

Table 1 Genotype frequencies and association test results for *rs7216389* with severe asthma, childhood-onset and adult-onset asthma susceptibility

	Genotype frequencies (N)				P	*OR (95% CI)*
	CC	*TC*	*TT*	*MAF*	*CC vs TC vs TT*	*T allele vs C allele*
Controls	0.283 (405)	0.487 (696)	0.229 (328)	0.47		
Severe asthmatics	0.179 (69)	0.519 (200)	0.301 (116)	0.56	1.8×10^{-5}	1.42 (1.21–1.67)
Childhood-onset asthmatics	0.089 (11)	0.482 (59)	0.429 (44)	0.67	4.5×10^{-6}	2.02 (1.53–2.68)
Adult-onset asthmatics	0.259 (29)	0.500 (56)	0.241 (27)	0.49	0.853	1.07 (0.78–1.47)

Abbreviations: CI, confidence interval; MAF, minimum allele frequency; OR, odds ratio.

of the T allele in the asthmatic adults was 56%, which is lower than that reported by Moffatt *et al.* (62%).[2] When the data were stratified according to the disease age of onset, a significant association between SNP *rs7216389* and severe asthma was reported only in the childhood-onset asthmatics (OR 2.02, CI: 1.53–2.68, $P=4.5\times10^{-6}$) (Table 1). Interestingly, the frequency of the T allele in cases of childhood onset was 67%, similar to the figure reported by Moffatt *et al.*[2] Adult onset of the disease did not show any significant associations for this SNP ($P=0.853$) and minor allele frequency (T allele) was 49%, which was comparable to the control group (47%) (Table 1). No associations were found between genotypes and IgE levels. The results highlight the differences in the genetic components of childhood and adulthood asthma onset.

Our results confirm the role of the *ORMDL3* genomic area as a locus conferring susceptibility to childhood-onset asthma of the most severe type of the disease. In combination with the recently published studies in ethnically diverse populations, it highlights the importance of the *ORMDL3* and other genes from the chromosome 17q21 region in the development of this complex disease. Further studies are required to investigate the functional role of this polymorphism and its involvement in early-onset asthma, which could contribute in elucidating the mechanisms underlying asthma and could be applied for therapeutic interventions.

ACKNOWLEDGEMENTS

This study was funded by the Wellcome Trust. We acknowledge use of genotype data from the British 1958 Birth Cohort DNA collection, funded by the Medical Research Council and the Wellcome Trust.

Aristea Binia[1], Nadia Khorasani[1], Pankaj K Bhavsar[1], Ian Adcock[1], Chris E Brightling[2], K Fan Chung[1], William OC Cookson[1] and Miriam F Moffatt[1]

[1]National Heart and Lung Institute, London, Imperial College, London, UK and [2]Institute of Lung Health, University of Leicester, Leicester, UK
E-mail: aristea.binia05@imperial.ac.uk

1 Weiland, S. K., Björkstén, B., Brunekreef, B., Cookson, W. O., von Mutius, E. & Strachan, D P Phase II of the International Study of Asthma and Allergies in Childhood (ISAAC II): rationale and methods. *Eur. Respir. J.* **24,** 406–412 (2004).
2 Moffatt, M. F., Kabesch, M., Liang, L., Dixon, A. L., Strachan, D., Heath, S. *et al.* Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* **448,** 470–473 (2007).
3 Bouzigon, E., Corda, E., Aschard, H., Dizier, M. H., Boland, A., Bousquet, J. *et al.* Effect of 17q21 variants and smoking exposure in early-onset asthma. *N. Engl. J. Med.* **359,** 1985–1994 (2008).
4 Halapi, E., Gudbjartsson, D. F., Jonsdottir, G. M., Bjornsdottir, U. S., Thorleifsson, G., Helgadottir, H. *et al.* A sequence variant on 17q21 is associated with age at onset and severity of asthma. *Eur. J. Hum. Genet.* **18,** 902–908 (2010).
5 American Thoracic Society. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am. J. Respir. Crit. Care Med.* **162,** 2341–2351 (2000).
6 Power, C. & Elliott, J. Cohort profile: 1958 British birth cohort (National Child Development Study). *Int. J. Epidemiol.* **35,** 34–41 (2006).
7 Strachan, D. P., Rudnicka, A. R., Power, C., Shepherd, P., Fuller, E., Davis, A. *et al.* Lifecourse influences on health among British adults: effects of region of residence in childhood and adulthood. *Int. J. Epidemiol.* **36,** 522–531 (2007).
8 van Heel, D. A., Franke, L., Hunt, K. A., Gwilliam, R., Zhernakova, A., Inouye, M. *et al.* A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. *Nat. Genet.* **39,** 827–829 (2007).