

Is toll-like receptor 6 or toll-like receptor 10 involved in asthma genetics – or both?

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Key words: asthma; genetics; linkage disequilibrium; TLR6; TLR10.

The genetics of bronchial asthma is highly complex, approximately 70 genes having been implicated by genetic association studies so far. The identification of susceptibility genes for asthma is hampered by genetic heterogeneity, incomplete penetrance, high phenocopy rate, epistatic effects and interactions with environmental factors. Yet another complicating factor lies in the structure of the genome itself, as exemplified by recent association studies with toll like receptor (TLR) genes.

The TLRs are involved in the innate immune system. They recognize so-called pathogen-associated molecular patterns (PAMPs) and initiate the production of various pro-inflammatory cytokines and chemokines. So far 10 different TLR have been identified in humans. Common polymorphisms within either *TLR10* (1) and *TLR6* (2, 3) have been independently found to be in association with bronchial asthma in different populations. However, it is important to realize that both genes are located at no more than 58 kb distance on chromosome 4p14; thus, apparent association with one gene might just be determined by proximity to the other gene.

In order to determine whether *TLR10* or *TLR6*, or even both genes, are involved in asthma genetics, we per-

formed genotyping of polymorphisms within both genes on asthmatics and controls by restriction fragment length polymorphisms. The asthmatic population included 322 children with doctor-diagnosed asthma and proven bronchial hyperreactivity as defined as a fall in forced respiratory volume in one second by at least 15% on provocation with histamine or exercise using standard protocols. The controls were 270 randomly chosen individuals [for more details on population's recruitment see (4)]. The study was approved by the local Ethic Committee. Two polymorphisms within *TLR6* and seven within *TLR10* were genotyped (see Table 1 for their identification numbers, as well as their position on chromosome 4 and within the respective gene). Thus, all three polymorphisms previously described in association with asthma were included in our study. The frequency of the polymorphisms in both populations is also shown in Table 1. Statistical analyses of single polymorphisms were conducted by the Armitage's trend test as implemented in de Finetti. None of the polymorphisms showed associated with bronchial asthma (see Table 1 for the *P*-values). However,

haplotype analyses spanning both genes by FAMHAP [see (4) for more information] revealed association with asthma ($P = 0.0065$). The weak association might not be surprising due to the above mentioned complexity of asthma genetics.

However, another finding might be important in regard to previous association studies: calculation of linkage disequilibrium (LD) between all polymorphisms by Arlequine revealed very tight LD. In fact, all polymorphisms in *TLR6* and *TLR10* previously found in association with asthma were in total LD in controls and asthmatics ($P = 0.00000$ for all possible pairs).

The strong LD between both genes as found by us is supported by a study on prostate cancer in the Swedish population. Here, partially different polymorphisms within both genes were typed, but again *TLR6* and *TLR10* polymorphisms were in tight LD (5). Finally, tight relation of both genes is demonstrated by recent data obtained within the International Hapmap Project (<http://www.hapmap.org>). Thus, it is not possible to distinguish which one of the neighbouring genes (or perhaps even

Genetic studies cannot distinguish between association of polymorphisms within *TRL6* and/or *TRL10* with asthma.

Table 1. All investigated polymorphisms in both genes, their position on chromosome 4 and within the genes. The allelic frequency in asthmatics and controls, and *P*-values for association by the Armitage's trend test are given

Polymorphism	Gene	Position on chromosome 4	Position within the genes	Frequency		
				Asthma	Controls	<i>P</i> -value
rs5743794	TLR6	38 655 293	Promotor	0.788	0.776	0.634
rs5743810		38 652 916	S249P	0.407	0.396	0.723
rs4274855	TLR10	38 600 037	Non-coding exon	0.726	0.736	0.677
rs10856839		38 599 802	Non-coding exon	0.814	0.826	0.609
rs11096957		38 599 057	N241H	0.617	0.617	0.999
rs11096956		38 598 746	P344P	0.755	0.752	0.911
rs11096955		38 598 673	I369L	0.634	0.633	0.981
rs11466657		38 598 360	I473T	0.969	0.955	0.261
rs4219009		38 597 455	I775?	0.802	0.780	0.364

both genes) is involved in asthma genetics on the basis of our current knowledge. Care must be taken when interpreting association results of single genes, and when interpreting association studies with a candidate gene, other genes in the genomic neighbourhood that are possibly in linkage disequilibrium with that gene should be considered, too. This task should become much more feasible once the haplotype pattern and linkage disequilibrium 'islands' of the genome are known (6).

This project was supported by grants from the Deutsche Forschungsgemeinschaft (DFG HE 3123/3-1 and HE 3123/4-2). The recruitment of asthmatic children was partially supported by a grant from the NIH (NIH R01 HL66533-01).

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Accepted for publication 29 January 2006

Allergy 2006; 61:649–650

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DOI: 10.1111/j.1398-9995.2006.01098.x

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Increased personal respirable endotoxin exposure with furry pets

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Key words: asthma; endotoxin; personal monitoring; pets.

We recently reported on the utility of using small portable monitors to more precisely measure particulate (1) and endotoxin (2)

exposure levels and relate these to asthma control. Utilizing these personal monitors to measure exposure, at the same

time monitoring pet contact, we report on the consequences of pet ownership and animal play on endotoxin exposure in children with asthma.

Ten schoolchildren aged 6–13 years were monitored over a 1-month period in January–February 2000. Personal monitoring was performed as previously described (1). Ethical and scientific approval for this study was obtained from the National Jewish Center and RTI International's Institutional Review Boards before initiation of recruitment.

Families completed a questionnaire before the study began describing demographic characteristics including pet

ownership. During the monitoring period, children also filled out questionnaires describing whether and how long they had played with cats or dogs. Filters were weighed to quantify PM_{2.5} before and after monitoring following the procedure of Lawless and Rodes (3). Endotoxin measurements and statistical analysis was performed as previously described (1, 4).

The median personal endotoxin exposure level was 0.06 EU/m³ [25th–75th interquartile range (IQR) = 0.10 EU/m³]. The median personal PM_{2.5} exposure level was 12.54 µg/m³ (IQR = 18.64 µg/m³). There was no significant correlation between endotoxin and PM_{2.5} exposures ($r = -0.103$, $P = 0.36$). Four households reported owning and living with furry pets, three with dogs and one with a cat (all indoors). Two children without pets reported playing with furry animals on single days and a third reported animal play on 2 days during the personal monitoring interval. Estimated playtime ranged from 10 to 30 min.

Figure 1 summarizes the relationship between furry animal ownership and/or play and personal endotoxin exposure levels. Children living in a household with dogs or a cat were exposed to significantly higher levels of endotoxin compared with those not owning pets (median 0.13 vs 0.04 EU/m³, $P = 0.02$). In this comparison no change in overall PM_{2.5} exposure was observed. In children not owning pets, there was a trend ($P = 0.08$) towards higher exposure on days that they reported playing with furry animals (median 0.07 vs 0.04 EU/m³) although PM_{2.5} exposure did not change. There was no further increase in endotoxin exposure levels on days when children living with pets reported playing with them compared with days when they did not (median 0.13 vs 0.13 EU/m³, $P = 0.61$).

There was a trend towards higher exposures if nonpet owning children played with a furry animal on a particular day. However, if one lived in a household with dogs or cats, exposures were similar whether or not children reported playing with pets suggesting that pet-containing households carry a near-maximum exposure load. This relationship was not observed with total PM_{2.5}, confirming that the association was as a

Children who live with pets are exposed to higher endotoxin levels independent of pet play.