

3'. The antisense primer was 5'-AGGGAGCGTCTGCTGGCTG-3' for both alleles. The reaction volume was 10 µl, comprising 100 µM dNTP, 67 mM Tris-Cl, 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.01% Tween 20, 10% glycerol, 20 ng genomic DNA, 50 ng of each primer, and 1 U Taq polymerase. Cycling conditions were as follows: initial denaturation at 94°C for 5 min, 35 cycles at 94°C for 1 min, 63°C for 30 s, and 72°C for 30 s, followed by 72°C for 10 min. The accuracy of the AS-PCR typing was confirmed by sequencing 25 of the samples.

A total of 219 southern Chinese (Cantonese, Hokkien, and Teochew) and 73 Malay unrelated subjects were studied with informed consent. We found no association of the at-risk alleles, as defined by Mofatt & Cookson (1) (Fisher's statistic of between 0.20 and 2.35 and *P* values of between 0.354 and 0.902) with the asthma phenotype (Table 1). There was no association of TNF-308\*2/LTα NcoI\*1 haplotype with asthma in either race. For Malays, the frequency of TNF-308\*2 was extremely low although the incidence and severity of asthma in Malays is comparable to Chinese (3). Comparison showed both the at-

risk allele frequencies between asthmatics and controls in both race groups not to be significantly different (Z scores between 0.274 and 1.484, *P* values of >0.05).

In summary, we found no evidence of association of TNF haplotypes or alleles with asthma in the two main racial groups in our population. The failure to confirm the findings of Mofatt & Cookson (1) indicates that the susceptibility genes for asthma are probably different in our population. Another possible factor is the difference in recruitment, as their 413 subjects were from 88 nuclear families while all our subjects were unrelated. It should also be noted that another study on Caucasians by Albuquerque et al. (2) not only failed to confirm the association, but identified different at-risk alleles for each of the biallelic polymorphisms.

The TNF-308\*2 genetic variant has been linked with susceptibility to cerebral malaria (4) and worse outcome in patients with severe sepsis (5) and may therefore be a useful clinical marker. The AS-PCR assay we have developed for genotyping the TNF-α polymorphism is simpler and quicker than conventional allele-specific hybridization using TMAC (4), and

should be useful for studying individuals at risk of infectious or autoimmune diseases associated with TNF-α promoter variants and increased TNF-α secretion.

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## Vacuum cleaners and airborne dog allergen

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**Key words:** asthma; Can f 1; dog allergen; HEPA; vacuum cleaner.

● EFFECTIVE allergen avoidance carried out for a sufficiently long period may improve asthma control in sensitized individuals. Complete avoidance of dog allergens is all but impossible in areas with a high proportion of pet ownership, because the exposure may occur not only in homes with dogs, but also in homes without dogs

**Table 1.** Association of tumor necrosis factor genotypes with asthma

| Genotypes        | 1.1 | 1.2 | 2.2 | <i>P</i> value |
|------------------|-----|-----|-----|----------------|
| <b>TNF-308</b>   |     |     |     |                |
| Malays           |     |     |     | 0.358          |
| Asthmatics (38)* | 34  | 4   | 0   |                |
| Controls (36)    | 35  | 1   | 0   |                |
| Chinese          |     |     |     | 0.734          |
| Asthmatics (67)  | 49  | 18  | 0   |                |
| Controls (151)   | 115 | 36  | 0   |                |
| <b>LTα</b>       |     |     |     |                |
| Malays           |     |     |     | 0.354          |
| Asthmatics (38)  | 7   | 20  | 11  |                |
| Controls (35)    | 4   | 15  | 16  |                |
| Chinese          |     |     |     | 0.902          |
| Asthmatics (66)  | 13  | 38  | 15  |                |
| Controls (153)   | 30  | 84  | 39  |                |

\*Number in parenthesis indicates total number of subjects studied. At-risk alleles according to Mofatt & Cookson (1) are allele 2 for TNF-308 and allele 1 for LTα.

and in public places (1, 2). However, very high exposure to Can f 1 in dog-allergic asthmatics who keep dogs in their homes may be an important determinant of the severity of their asthma, and every effort should be made to reduce this exposure. Intensive vacuum cleaning may remove dust from carpets, reducing the overall size of the allergen reservoir. However, vacuum cleaners with

inadequate bags and exhaust filtration may actually produce an aerosol of allergen, and

vacuuming with such cleaners may induce acute asthma in allergic patients. The benefits of intensive vacuum cleaning to allergic patients have never been conclusively established.

The aim of this study was to investigate the effectiveness of different types of vacuum cleaners in preventing the leakage of Can f 1 into the air. Thirteen domestic vacuum cleaners were tested. These comprised the following:

- seven new vacuum cleaners with double-thickness bags and integral high efficiency particulate air (HEPA) filters (Miele S444I, Nilfisk GM210, Nilfisk Allergy Vac, Electrolux Excellio, Electrolux Airstream, AEG Vampyr, and Medivac)
- one new cyclone vacuum cleaner with HEPA filter (Dyson Absolute Plus)
- five previously used household vacuum cleaners without integral HEPA filters.

For testing vacuum cleaners for leakage of dog allergen, we used methodology similar to that found to be very effective in testing the effect of vacuum cleaners on the concentration and particle size distribution of airborne cat allergen (3). Experiments were performed in a clean, airtight, experimental chamber with a volume of 8.25 m<sup>3</sup>. For each test cycle, the walls of the chamber were cleaned with alcohol wipes. The air in the

chamber was then cleaned for 30 min with a HEPA filtration unit, after which a baseline air sample was collected for 30 min with the high-volume pump (60 l/min high-volume dust samplers – Rotheroe-Mitchell, Greenford, UK). Each vacuum cleaner was loaded with dust containing 20 mg of Can f 1 outside the chamber, and then placed inside, switched on, and left running for 30 min. Air was sampled at the same time as described above. The test cycle was repeated for each vacuum cleaner in turn.

The testing was repeated with the seven new vacuum cleaners with double-thickness bags and integral HEPA filters when they were 6 and 18 months old, during which time they had been in regular domestic use in homes. Maintenance of the machines, such as changing the filters, was the responsibility of the users, all of whom were provided with spare bags and filters. Each machine was fitted with a new bag, but the HEPA filter was not changed for the repeat testing at 6 and 18 months. This was done to minimize the effect of variability in allergen content of the bag.

After air sampling, each filter was cut into four pieces, and then placed into a syringe with 1 ml of 1% bovine serum albumin in phosphate-buffered saline with 0.1% Tween 20 (BSA PBS-T). After overnight extraction at 4°C, the extraction liquid was aspirated backward and forward several times through a three-way stop lock into a second syringe, and then transferred into a test tube and centrifuged at 3000 rpm for 30 min at 4°C. The supernatant was stored at -20°C.

A monoclonal/polyclonal antibody ELISA, as previously described (2), was used to measure Can f 1. The samples were assayed neat and at two-, and fourfold dilutions. For concentrations lying off the linear portion of the standard curve, the assays were repeated at an appropriate dilution.

The assay was quantitated with doubling dilutions of dog allergen standard (UVA 94/02) from 500 IU/ml to 1 IU/ml Can f 1. The UVA 94/02 (10 000 IU Can f 1/ml) was

substandardized against the WHO/IUIS International Reference Preparation of dog hair and dander (NIBSC 84/685), which contains 100 000 IU/ml Can f 1. One international unit is approximately 1 ng Can f 1 protein, and this value was used to calculate the results.

Baseline levels of airborne Can f 1 in the experimental room between individual tests were all below the limit of detection (<0.8 ng Can f 1/m<sup>3</sup>). Airborne Can f 1 levels were below the detection limit for all eight new vacuum cleaners (seven with integral HEPA filters; one cyclone) and two out of five old vacuum cleaners. However, three old vacuum cleaners leaked Can f 1 (recorded levels 2.5, 1.8, and 1.3 ng Can f 1/m<sup>3</sup>). After 6 months of domestic use, none of the eight previously new vacuum cleaners were found to leak detectable levels of Can f 1. However, after 18 months of regular domestic use, one did leak Can f 1 (Electrolux Airstream), despite having the vacuum cleaner bag replaced (recorded level of airborne Can f 1 1.8 ng/m<sup>3</sup>).

In conclusion, we found that:

- None of the vacuum cleaners which contained integral HEPA filters and double-thickness bags leaked Can f 1 when tested in the experimental room when brand new and after 6 months of domestic use.
- The only vacuum cleaner which leaked Can f 1 after 18 months of domestic use had not had the HEPA filter changed during this time.
- Sensitized patients should use vacuum cleaners with built-in HEPA filter and double-thickness bags, but it is important to service the machine regularly.

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## Occupational rhinitis caused by beech wood dust

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**Key words:** beech; occupational rhinitis; rhinomanometry; wood dust.

• DIFFERENT kinds of wood have been implicated in causing respiratory symptoms in workers who inhale their dust. It has been suggested that an allergic mechanism may be implicated in their pathogenesis, but an IgE-mediated sensitization has been described in a few studies only (1–5).

Beech (*Fagus sylvatica*) wood is frequently used in carpentry cabinet-making, and the paper industry. However, occupational diseases related to this wood have not been reported. This study describes a case of occupational rhinitis caused by IgE-mediated sensitization to beech wood dust. To our knowledge, no previous cases have been reported.

The 57-year-old patient had been working for 20 years as a carpenter. At work, he was

exposed to beech and iroko wood dust daily. Three years later, he started to present symptoms of rhinitis when he was working with beech wood. Such symptoms increased during the working week and improved during weekends, and they were not related to the inhalation of iroko wood dust. The patient had no history of atopy and had never smoked.

Beech and iroko dust wood extracts were prepared at a 1:40 w/p in 0.125 M ammonium bicarbonate by magnetic stirring for 15 h at 2–8°C and after centrifugation (10 000 rpm) for 30 min at 10°C. Then the preparation was dialyzed against distilled water with a dialysis membrane which retained molecules larger than 3.5 kDa, filtered through a 0.22-µm pore size membrane, and lyophilized. These extracts were supplied by C.B.F.

Leti (Barcelona, Spain).

Skin prick test (SPT) was performed with common commercial extracts and beech and iroko wood extracts at 2 mg/ml of dry weight. Histamine chlorhydrate (10 mg/ml) was used as a positive control and PBS as a negative control. A positive reaction was defined as a skin reaction showing a mean wheal diameter of at least 3 mm.

Specific IgE antibodies to beech and iroko wood were detected by ELISA (Leti, Barcelona, Spain) (6). In a CAP-System assay (Kabi-Pharmacia, Sweden), we determined IgE antibodies against the same battery tested in SPT. A positive result was defined as a value of CAP of ≥ class 2 or optical density (OD) of >0.100 in ELISA.

ELISA IgE inhibitions were done against beech wood and beech pollen (*F. sylvatica*).

Nasal provocation tests were performed with the extracts of iroko and beech wood measured by computerized active anterior rhinomanometry with a Rhinotest MP-500 rhinomanometer, according to the criteria of the Committee Report on Standardization of

Rhinomanometry (7). After spraying 0.2 ml of diluent, increasing concentrations of allergen were sprayed into both nostrils every 15 min. We considered the test positive when there was a decrease of more than 40% in nasal airflow from diluent values according to the criteria of Bachmann (8). To detect possible late response, nasal flow was measured 4 and 6 h after the last administration of allergen.

All tests were performed in five asymptomatic, exposed workers and three atopic, unexposed subjects.

The prick test with beech extract at 2 mg/ml gave an immediate positive reaction, with a wheal of 6×5 mm. No late response was observed. SPT was negative for common inhalant allergens as well as for an extract of iroko dust. The test was negative in all control subjects.

Total serum IgE was 140 IU/ml (Pharmacia Immuno-Cap System, Uppsala, Sweden). Specific IgE was positive for beech extract with the patient's serum (OD 0.108) and negative with a battery of common allergens and iroko wood. The control group had negative ELISA (OD 0.020–0.052) to beech wood dust. ELISA 50% IgE-binding inhibition values were 16.4 mg/ml dry weight for beech-wood extracts and 19.5 mg/ml for *F. sylvatica* pollen extract.

The nasal provocation with beech-wood extract was positive. We obtained an immediate response (46% decrease in nasal airflow) after administration of a 1/10 dilution of the original extract (2 mg/ml). No significant response was elicited in the nasal provocation test with iroko wood extract. Nasal challenge was negative in all control subjects.

The rhinitis suffered by our patient could be considered occupational rhinitis: after a sensitization period, symptoms occurred during each exposure to beech wood dust and improved during weekends. Type I hypersensitivity is strongly suggested by the positivity of SPT and by the demonstration of specific IgE antibodies (although at a low

### A case of occupational rhinitis caused by IgE-mediated sensitization to beech wood dust.