

## Original article

# Longitudinal study on the relationship between cat allergen and endotoxin exposure, sensitization, cat-specific IgG and development of asthma in childhood – report of the German Multicentre Allergy Study (MAS 90)

**Background:** Controversial data have emerged regarding the question whether cat exposure in childhood favours or decreases the risk of sensitization and allergic airway disease. In a prospective birth-cohort study, we assessed the association between longitudinal cat allergen exposure, sensitization (immunoglobulin E, IgE), IgG antibody (ab) levels to cat and the development of asthma in children up to the age of 10 years.

**Methods:** Of 1314 newborn infants enrolled in five German cities in 1990, follow-up data at age 10 years were available for 750 children. Assessments included yearly measurements of specific serum IgE to cat and at age 6 and 18 months, 3, 4 and 10 years measurement of cat allergen Fel d 1 in house dust samples.

Additionally, Fel d 1-specific IgG ab were determined in 378 serum samples of 207 children. Endotoxin exposure in mattress dust was measured in a subgroup of 153 children at age 10 years. From age 4 years on, International Study of Asthma and Allergy in Childhood (ISAAC) questionnaires were completed yearly in order to assess the prevalence of wheeze and asthma.

**Results:** Serum IgG-levels to cat showed a large variation, however, intraindividually values showed rather constant concentration over a longer time period. The IgG levels at school-age correlated with cat allergen exposure during the first 2 years of life. Specific IgE to cat was clearly associated with wheeze ever, current wheeze and bronchial hyperresponsiveness (BHR), this was also observed for children with specific IgE ab to cat ( $>0.35$  kU/l) plus IgG levels above 125 U/ml. A large percentage of very highly exposed children showed high IgG but no IgE responses to cat, however, not all highly exposed children were found to be protected from sensitization. Children with IgG but without IgE ab to cat showed the lowest prevalence of wheeze ever and current wheeze despite high cat allergen exposure, however, this trend did not achieve significance. While homes of cat owners showed higher Fel d 1 concentrations than homes without cats, homes of cat owners were not found to have higher endotoxin levels in carpet dust samples than homes without cats.

**Conclusions:** We could confirm that high cat allergen exposure in a cohort with lower community prevalence of cats is associated with higher serum IgG and IgE levels to cat in schoolchildren. Sensitization to cat allergen (IgE) is a risk factor for childhood asthma. While exposure to cat allergen during infancy is associated with sensitization (IgE), only in the very highly exposed children the likelihood of sensitization (IgE) is decreased and high IgG levels to cat without IgE were associated with low risk of wheeze. However, cat-specific IgG ab levels did not protect children with IgE-mediated sensitization from wheeze.

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Key words: cat allergen exposure; childhood asthma; sensitization and immunoglobulin G antibodies to cat.

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**Abbreviations:** IgG ab, immunoglobulin G antibodies; Fel d 1, major allergen of cat (*Felis domesticus*); MAS, Multicentre Allergy Study; FEV<sub>1</sub>, forced expiratory volume after 1 s; ISAAC, International Study of Asthma and Allergy in Childhood.

It is still unclear how early life-exposure to pets is related to children's risk of developing allergic sensitization and asthma. Controversial data have been published during the last 5 years regarding the potential harmful or

favourable effect of pet exposure in households (1–8). Taking together all the information published to date, there is clear evidence that sensitization to cat is a risk factor for asthma in childhood and adulthood (7–12). However, the British Isle of Wight study and the German Multicentre Allergy Study (MAS) did not find differences in sensitization rates between families with and without pet keeping (9, 11) and effects can be modulated by the community prevalence of cats (13). Pet and pet allergen exposure in later life is associated with immunoglobulin (Ig)E-mediated sensitization and respiratory symptoms (5, 6, 8, 14, 15), and the apparent protective effect of current pet ownership in some studies could be due to selective avoidance or a 'healthy pet keeper effect' (4, 6).

It also remains unclear whether avoidance of pet allergens early in infancy and childhood (during the first 1–2 years of life) can specifically prevent allergic sensitization (16) or even reduce the risk for allergic airway disease. Some studies suggest that pet keeping during the first year of life can protect against sensitization and asthma at school-age probably via the induction of tolerance (2). Similar findings were reported in another Swedish investigation (7). While sensitization to cat was strongly associated with asthma, living with a cat was inversely related both to having a positive skin test to cat and incidence of doctor-diagnosed asthma. The proposed protective effect of pet keeping may be caused by the induction of tolerance following very high exposure and the induction of IgG<sub>4</sub> as suggested by Platts-Mills et al. (17). Furthermore, pet keeping may also increase the indoor exposure to bacterial compounds such as endotoxin [i.e. lipopolysaccharides (LPS) from Gram-negative bacteria] (18). This may favour a shift in the immune system from a T-helper 2 cell (Th2) to a T-helper 1 cell (Th1) type pattern. However, there are also reports that the effects of pet allergen and endotoxin exposure are independent and contrary (19). High-dose allergen exposure may induce an increase in IgG and IgG<sub>4</sub> antibodies (ab) with a decrease in IgE ab (20, 21), meaning a modified Th2 response (17).

Previous reports from the German MAS showed an increased risk for sensitization to cat at age 7 years among children who were highly exposed to cat allergen during the first years of life (11, 22). While sensitization to cat was related to asthma, there was no relation between asthma and cat allergen exposure at that age. Although exposure to cat was lower in the MAS compared with the American population-based cross-sectional study of schoolchildren (17), the current study was designed to analyse serological evidence for a potential induction of tolerance and a modified Th2-response in MAS children being highly exposed to cat allergen. The results for IgG ab to cat were related to sensitization, exposure to cat and endotoxin, the risk of wheeze, asthma and bronchial hyperresponsiveness (BHR).

## Methods

### Study design

In the German Multicenter Allergy Study (MAS 90), 1314 newborns were recruited in five German cities during 1990. A detailed description of study subjects and methods is given elsewhere (11). Of 7609 infants born in six obstetric departments of West Germany of five cities (Berlin, Munich, Mainz, Düsseldorf, Freiburg) between January and December 1990, 2053 filled in an extended questionnaire regarding parental atopic diseases and were invited to participate. About 22% of the mothers and 21% of the fathers were classified atopic according to the questionnaire. About 64% of all families invited, participated in the study: 499 infants with double-positive family history and/or cordblood-IgE values above 0.9 kU/l were recruited as infants at risk and 815 infants with neither of these risk factors participated as infants at random risk.

The study was approved by the local ethics committee. Parents gave informed consent. The cohort children were followed up at the age of 1, 3, 6, 12 and 18 months and from then on yearly within 3 months of the child's birthday up to the age of 10 years. If parents agreed, blood samples of the children were collected. At the age of 7 and 10 years, lung function was assessed. At age 7, histamine challenge was performed in order to assess bronchial responsiveness. For this paper, clinical and epidemiological data at ages 7 and 10 years were chosen for evaluation only of 207 children with complete information of serum IgG and IgE to cat.

### Parental questionnaire

At each follow-up visit, parents gave structured interviews to a study doctor focussing especially on asthmatic or atopic symptoms of the child. The International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire was used for the assessment of asthma (23). Parents were asked whether the child had ever had a 'wheezing or whistling noise in the chest while breathing' and whether the child had wheezed in the past 12 months prior to the interview. 'Current wheeze' was defined as at least one episode of wheezing during the last 12 months prior to the interview. Parents were also asked whether a doctor had ever diagnosed asthma, hay fever or eczema.

### Serum IgE

Serum samples were obtained from the children at birth, and at 1, 2, 3, 5, 6, 7 and 10 years of age. As described previously, total serum IgE and specific serum IgE ab to hen's egg, cow's milk, soy and wheat and to four inhalant allergens (house dust mite, cat dander, mixed grasses and birch pollen) were determined by CAP-RAST-FEIA (Pharmacia, Freiburg, Germany). Serum samples were available for 768 children at age 7 and 572 children at age 10 years. Sensitization to a specific allergen was defined as a concentration of  $\geq 0.35$  kU/l ( $\geq$ CAP class 1). Serological data of a subgroup of children at age 7 and 10 years were chosen for evaluation in this paper ( $n = 207$  and  $n = 115$ , respectively).

### Serum IgG assays

Serum samples collected at age 5 ( $n = 54$ ), 7 ( $n = 207$ ) and 10 ( $n = 115$ ) years were analysed for IgG to cat allergen. Low number of cases was due to limited serum amounts. About 22% samples of highly exposed children (exposure at age 6 and 18 months) were selected ( $> 1000$  ng/g Fel d 1) and 78% of low or median exposed children ( $< 1000$  ng/g Fel d 1) were randomly chosen, meaning

a slight oversampling of high exposure (total cohort: 13% being exposed to > 1000 ng Fel d 1/g). The IgG ab to the purified allergen Fel d 1 were measured with a radioimmunoprecipitation assay in Charlottesville, University of Virginia, Asthma and Allergy Center, VA, USA. A level of > 125 U/ml was considered as significant. A good correlation between specific IgG and IgG<sub>4</sub> to cat ( $r = 0.71$ ;  $P < 0.001$ ) in sensitized and nonsensitized individuals was reported previously (17).

#### Cat allergen analysis in house dust

Dust samples were obtained from carpets and mattresses within the children's homes and assayed for cat allergen Fel d 1 as described elsewhere (11, 22). At age 6 months, 18 months, 3, 4, 5 and 7 years, carpet dust samples from the whole cohort and at age 10 years, carpet and mattress dust samples in the Berlin subsample were analysed. In this analysis, data of allergen quantification at age 6 months was chosen.

#### Measurement of endotoxin

At age 10 years, 153 dust samples from mattresses of children from Berlin were collected in endotoxin-free vials.

Endotoxin content of all dust samples was determined by a kinetic Limulus assay (24) as described by Waser et al. (25) in the laboratory of the Institute for Occupational and Environmental Medicine of the University of Munich. Briefly, each filter was extracted by rapid shaking with endotoxin-free water (7.5 ml) for 1 h. From a diluted (1 : 1000 till 1 : 10 000) aliquot, 100 µl were added to a microtitre-plate well (96-wells; Nunc, Roskilde, Denmark) and assayed with LAL reagent (kinetic-QCL, Cambrex BioScience, Verviers, Belgium) at 37°C. A standard calibration curve (50, 5, 0.5 and 0.005 EU/ml) was performed on each plate. Optical density at 405 nm was measured by an automatic reader (PowerWave, MWG Biotech, Ebersberg, Germany). The intra-assay variability was < 5%, whereas the interassay variability was < 20%. Results are expressed in EU/mg.

#### Bronchial histamine challenges

Bronchial histamine challenge using the reservoir method as described elsewhere (26) was performed in 645 children aged 7 years. Provocation tests were performed in a titrated manner starting with 0.5 mg/ml histamine dihydrochloride (Merck, Darmstadt, Germany). Provocation was stopped if the highest concentration (8 mg/ml) was tolerated or if a 20% fall of forced expiratory volume after 1 s (FEV<sub>1</sub>) was induced. For the multivariate analysis, a threshold level of 0.6 mg/ml was chosen for the definition of BHR yes/no.

#### Statistics

Statistical analysis was performed with SPSS (version 8.0) and SAS (version 8.02). Chi-square tests for trend were used to analyse links

between exposure to cat allergen in the home and sensitization or ab responses (IgG). Exposure groups were formed by dividing the patient samples into tertiles. For better comparability with a previously published study, tertiles from that publication are also shown (17). Logistic regression analyses were used to distinguish associations with asthma, wheeze and BHR.

#### Results

Complete data on specific serum IgE and IgG to cat and completed ISAAC questionnaires were available for 207 children at age 7 and for 115 children at 10 years of age, additional information on Fel d 1 exposure at age 6 months in 195 of 207 children. At age 7 years, additional information on BHR was available for 166 children. The Fel d 1 levels in carpet dust samples were determined for all these 323 children at age 6 and/or 18 months and Fel d 1 and endotoxin concentrations in only 153 carpet and mattress dust samples at age 10 years (Berlin subsample). Median and arithmetic mean values for cat allergen levels at different times are shown in Table 1. Endotoxin levels in mattress dust varied from 0 to 187 EU/mg. There was no correlation between Fel d 1 and endotoxin in mattress dust. Homes with cats were found to have significantly higher Fel d 1 concentrations in carpet dust. However, no difference for endotoxin levels at age 10 years could be found (median Fel d 1 levels 42 ng/g without cat vs 16 000 ng/g with cat,  $P < 0.0001$ ; endotoxin 19 EU/mg without cat vs 23.9 EU/mg with cat,  $P = 0.28$ ). Similar findings were made for dog ownership (median endotoxin levels 19 vs 21.5 EU/mg,  $P = 0.82$ ).

Median levels of serum IgG to Fel d 1 were below detection limit at most of the time-points. Arithmetic mean concentrations ranged from 989 U/ml at age 5 years to 1300 U/ml at age 10 years. Maximum values were 15 300 and 80 000 U/ml, respectively. Intraindividually, IgG-values correlated at age 5, 7 and 10 years ( $r = 0.6$ , data not shown).

The IgG serum ab to cat at age 5, 7 and 10 correlated positively with the level of exposure at age 6 months, 18 months and 3 years, which was most pronounced in the group without specific IgE to cat (Fig. 1A, B; data for IgG at age 7 years and exposure at 6 months of age shown). Serum IgG and cat allergen exposure also correlated well at age 10 years (data not shown). Early cat allergen exposure during the first 2 years of life correlated with IgE responses to cat at age 5 and 7 years,

Table 1. Fel d 1 concentrations (ng/g of dust) in dust samples at different times in children with complete information on serum immunoglobulin (Ig)G and IgE concentrations at 7 years of age

| Age (sample site)               | 6 months (carpet)<br>( <i>n</i> = 195) | 18 months (carpet)<br>( <i>N</i> = 168) | 3 years (carpet)<br>( <i>N</i> = 141) | 4 years (carpet)<br>( <i>n</i> = 108) | 5 years (carpet)<br>( <i>n</i> = 155) | 10 years (mattress)<br>( <i>n</i> = 29) |
|---------------------------------|----------------------------------------|-----------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|-----------------------------------------|
| Median and 25th/75th percentile | 210 (80/800)                           | 180 (63/465)                            | 50 (20/210)                           | 80 (20–300)                           | 90 (30–410)                           | 170 (20–586)                            |
| Minimum/maximum                 | 0/400 000                              | 0/40 000                                | 0/65 000                              | 0/27 000                              | 0/19 000                              | 0/18 170                                |
| Arithmetic mean (SD)            | 8335 (36 270)                          | 8941 (40 623)                           | 1931 (7705)                           | 1377 (4397)                           | 1128 (3022)                           | 1802 (4323)                             |

but not at age 10 years. Cat allergen exposure at later ages did not correlate with serum IgE responses. The higher the exposure to cat at age 6 months, the higher the

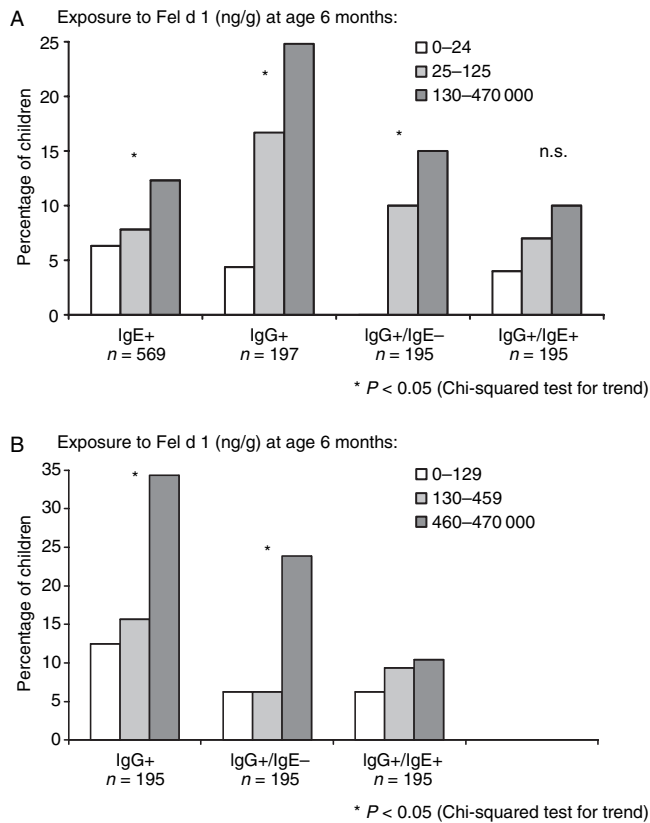


**Figure 1.** (A) Immunoglobulin (Ig)E to cat ( $>0.35$  kU/l) and IgG to Fel d 1 ( $>125$  U/ml) at age 7 years in different cat allergen exposure groups (Fel d 1, age 6 months). Exposure groups in tertiles of the total Multicentre Allergy Study (MAS) cohort with available information on Fel d 1 exposure at age 6 months ( $n = 948$ ). The IgE values for whole cohort ( $n = 569$ ), IgG-values for subgroup ( $n = 207$ ). (B) IgE to cat ( $>0.35$  kU/l) and IgG to Fel d 1 ( $>125$  U/ml) at age 7 years in different cat allergen exposure groups (Fel d 1, age 6 months). Tertiles of the subgroup with complete information on IgG to Fel d 1 ( $n = 207$ ) were used.

**Table 2.** Cross-sectional association of IgG and IgE to Fel D 1 with the outcome of wheeze and BHR at the age of 7 and 10 years: unadjusted odds ratios (OR) and 95% confidence intervals (CI); unadjusted, complete cases for all immunological variables, i.e. only subjects with IgE, IgG and total IgE not missing – equal sample sizes as in Table 3)

|                 | <i>n/N</i> | Wheeze ever |            |                 | Current wheeze |            |                 | BHR  |            |                 |
|-----------------|------------|-------------|------------|-----------------|----------------|------------|-----------------|------|------------|-----------------|
|                 |            | OR          | 95% CI     | <i>P</i> -value | OR             | 95% CI     | <i>P</i> -value | OR   | 95% CI     | <i>P</i> -value |
| Age 7 years     |            |             |            |                 |                |            |                 |      |            |                 |
| IgE ≥ 0.35      | 29/207     | 5.22        | 2.30–11.87 | <0.001          | 6.42           | 2.70–15.28 | <0.001          | 4.00 | 1.48–10.79 | 0.006           |
| IgG ≥ 125       | 48/207     | 1.98        | 0.99–3.95  | 0.053           | 1.85           | 0.82–4.16  | 0.136           | 2.16 | 0.93–5.03  | 0.074           |
| Total IgE ≥ 100 | 79/207     | 2.80        | 1.49–5.27  | 0.001           | 5.77           | 2.52–13.23 | <0.001          | 2.51 | 1.18–5.34  | 0.016           |
| Age 10 years    |            |             |            |                 |                |            |                 |      |            |                 |
| IgE ≥ 0.35      | 43/120     | 5.48        | 2.35–12.79 | <0.001          | 5.00           | 1.91–13.10 | 0.001           |      |            |                 |
| IgG ≥ 125       | 51/120     | 2.43        | 1.09–5.40  | 0.030           | 2.79           | 1.10–7.08  | 0.030           |      |            |                 |
| Total IgE ≥ 100 | 69/120     | 10.22       | 3.31–31.56 | <0.001          | 26.83          | 3.47–207.2 | 0.002           |      |            |                 |

Ig, immunoglobulin; BHR, bronchial hyperresponsiveness.

percentage of children with elevated serum IgG to cat and the higher the sensitization rate at age 7 years (Fig. 1). The latter relation was less pronounced. Using the tertiles from a previous study (ref. 17), 50% of the highly exposed children with measurement of IgG to cat showed only IgG but no IgE response to cat (data not shown). The highest risk for sensitization (IgE) plus detectable IgG ab was found in the medium exposure group (1700–22 900 ng Fel d 1/g), when choosing the tertiles of a previously published study (17, data not shown). Similar results were seen for Fel d 1 exposure at 18 months (data not shown).

The IgE to cat was clearly associated with wheeze ever, current wheeze and BHR at age 7 years (Tables 2 and 3). However, at age 10 cat sensitization (IgE) was only associated with wheeze if IgG was present, while total IgE remained strongly associated with wheeze. When children were classified into four groups by IgE ab to cat and IgG ab to Fel d 1, the risk of wheezing was strongest in those with IgE and IgG ab (Table 4), OR 6.2,  $P < 0.0001$ . The children with IgG ab but no IgE ab had the lowest prevalence of wheezing of any group, indeed out of 26 children in this group at age 7 years only one reported current wheezing, however, results did not achieve significance (Table 4).

## Discussion

We observed that in highly exposed children the likelihood of IgE-mediated sensitization to cat and high IgG levels to cat is increased. High levels of cat-specific IgG ab without IgE were associated with low risk of wheeze (not significant). However, cat-specific IgG ab levels did not protect children with IgE-mediated sensitization from wheeze.

Serum IgG responses to inhalant allergens can be observed in allergic and nonallergic individuals with exposure (27, 28 and unpublished data from MAS). A large proportion of specific serum IgG ab in allergic and nonallergic individuals are of the IgG<sub>4</sub> isotype (17), therefore only serum IgG to cat was determined in this

Table 3. Cross-sectional association of IgG and IgE to Fel D 1 with the outcome of wheeze and BHR at the age of 7 and 10 years: adjusted odds ratios (OR) and 95% confidence intervals (CI; mutually adjusted for other immunological variables in the table)

|                      | n/N    | Wheeze ever |            |         | Current wheeze |            |         | BHR  |           |         |
|----------------------|--------|-------------|------------|---------|----------------|------------|---------|------|-----------|---------|
|                      |        | OR          | 95% CI     | P-value | OR             | 95% CI     | P-value | OR   | 95% CI    | P-value |
| Age 7 years          |        |             |            |         |                |            |         |      |           |         |
| IgE $\geq$ 0.35      | 29/207 | 4.04        | 1.42–11.55 | 0.009   | 4.99           | 1.48–16.80 | 0.009   | 2.57 | 0.75–8.74 | 0.132   |
| IgG $\geq$ 125       | 48/207 | 0.86        | 0.35–2.11  | 0.743   | 0.50           | 0.15–1.61  | 0.244   | 1.22 | 0.43–3.43 | 0.705   |
| Total IgE $\geq$ 100 | 79/207 | 1.86        | 0.91–3.81  | 0.089   | 4.06           | 1.62–10.14 | 0.003   | 1.88 | 0.83–4.29 | 0.132   |
| Age 10 years         |        |             |            |         |                |            |         |      |           |         |
| IgE $\geq$ 0.35      | 43/120 | 2.78        | 0.96–8.06  | 0.059   | 1.84           | 0.57–5.95  | 0.311   |      |           |         |
| IgG $\geq$ 125       | 51/120 | 0.91        | 0.33–2.56  | 0.862   | 1.19           | 0.38–3.73  | 0.767   |      |           |         |
| Total IgE $\geq$ 100 | 69/120 | 6.52        | 1.93–21.99 | 0.003   | 18.74          | 2.26–155.1 | 0.007   |      |           |         |

Ig, immunoglobulin; BHR, bronchial hyperresponsiveness.

Table 4. Cross-sectional association of IgG with/without IgE to Fel D 1 with the outcome of wheeze and BHR at the age of 7 and 10 years: prevalence, odds ratios (OR) and 95% confidence intervals (CI; reference category: –IgG, –IgE)

|              | n/N     | Wheeze ever |            |         | Current wheeze |            |         | BHR  |            |         |
|--------------|---------|-------------|------------|---------|----------------|------------|---------|------|------------|---------|
|              |         | OR          | 95% CI     | P-value | OR             | 95% CI     | P-value | OR   | 95% CI     | P-value |
| Age 7 years  |         |             |            |         |                |            |         |      |            |         |
| –IgG, –IgE   | 152/207 | 1.00        | –          | –       | 1.00           | –          | –       | 1.00 | –          | –       |
| +IgG, –IgE   | 26/207  | 0.63        | 0.20–1.96  | 0.425   | 0.28           | 0.04–2.19  | 0.225   | 1.72 | 0.56–5.27  | 0.342   |
| –IgG, +IgE   | 7/207   | 2.60        | 0.56–12.20 | 0.225   | 5.25           | 1.09–25.29 | 0.039   | 7.22 | 1.14–45.82 | 0.036   |
| +IgG, +IgE   | 22/207  | 6.07        | 2.35–15.68 | <0.001  | 5.83           | 2.22–15.34 | <0.001  | 3.61 | 1.14–11.46 | 0.029   |
| Age 10 years |         |             |            |         |                |            |         |      |            |         |
| –IgG, –IgE   | 59/120  | 1.00        | –          | –       | 1.00           | –          | –       |      |            |         |
| +IgG, –IgE   | 18/120  | 0.94        | 0.23–3.87  | 0.932   | 0.44           | 0.05–3.83  | 0.455   |      |            |         |
| –IgG, +IgE   | 10/120  | 4.70        | 1.14–19.35 | 0.032   | 1.75           | 0.31–9.97  | 0.528   |      |            |         |
| +IgG, +IgE   | 33/120  | 5.64        | 2.14–14.84 | <0.001  | 5.44           | 1.89–15.65 | 0.002   |      |            |         |

Ig, immunoglobulin; BHR, bronchial hyperresponsiveness.

study. It remains unclear whether IgG<sub>4</sub> ab, which in the literature of the late-1980s were considered as blocking ab, are an epiphenomenon or have a functional role in the mechanism of desensitization and tolerance. There is more evidence, which during immunotherapy and the application of high allergen dosage or during constantly high exposure to cat allergen certain T-cell clones are activated, for example, T-reg cells or Th1-cells (29, 30).

Our results confirm the findings of an American and a Swedish cross-sectional study of schoolchildren that IgG levels to cat correlate with exposure (17, 31). The range of exposure in our study was also wide, however, maximum and median exposure levels are more than 10-fold lower compared with the American study. While the community prevalence of cats in the USA, Australia and New Zealand ranges between 50 and 60% of the families, community prevalence of cats in Germany ranges between 30 and 40% of the families. In the German MAS, 30% of the families kept furred pets. The results of our study confirm the dose–response link between early cat allergen exposure and specific sensitization up to the age of 7 years. At age 10, however, the domestic exposure was not related to the allergic immune response. This is in keeping with observations that children have intensive cat

allergen exposure at school and in homes of their friends (14), which may be more important for the later status of sensitization to cat allergens. At age 10, cat sensitization was only associated with current wheeze if IgG to cat was present, suggesting ongoing exposure in these children rather than a certain induction of tolerance like suggested by Hesselmar et al. (2).

In the MAS, high prevalence of IgG ab to cat without IgE was associated with the lowest prevalence of wheeze, but due to low number of cases and lack of significance this should be interpreted with caution. This is in accordance with the findings of the American study (17) and with the observations from occupational medicine and immunotherapy that frequent and high allergen contacts can induce IgG and IgG<sub>4</sub> responses to specific antigens without any allergic symptoms, e.g. in beekeepers (20, 32). However, those children with high IgE and IgG ab to cat allergen Fel d 1, showed the highest prevalence of wheeze, contradicting the hypothesis that IgG to inhalant allergens reflect a status of tolerance and favouring the thesis, which IgG and IgE responses can run in parallel. The observation that IgG<sub>4</sub>-ab do not directly mediate protection from asthma was also reported by Hesselmar et al. (31).

In the MAS, it appears that although in some children high exposure to cat induces high Fel d 1-specific IgG ab, being associated with a low risk of wheeze and BHR, in others high cat allergen exposure was associated with IgE-mediated sensitization and concomitant high IgG-levels to major cat allergen and an increased risk for asthma. The latter was especially apparent in families with positive family history for atopy. While sensitization (IgE) was only related to early exposure, IgG responses were related also to later exposure. The Fel d 1 exposure and IgG responses to Fel d1 were not correlated with endotoxin exposure at age 10 years.

As only a subgroup of the MAS children were analysed in this investigation one could suspect biasing. Although, in the subgroup analysed the prevalence of 'current wheeze' was slightly higher than in the whole study population [15% (33 of 207) vs 10% (93 of 939) of children at age 7 and 8.4% (66 of 782) at age 10], the prevalence of sensitization to cat and 'wheeze ever' were similar. However, there was a slight oversampling of highly exposed children (Fel d 1 > 1000 ng/g; 22% in the subgroup vs 13% in the whole cohort) in order to have a better comparability with the previously published American study (17).

The controversial data regarding the potential harmful or protective role of pet allergen exposure may be due to different exposure factors-like community prevalence of pets. Svanes et al. presented results from participants of the European Community Respiratory Health Survey (ECRHS) analysing the association between pet keeping and adult asthma and hay fever (13). Although information about pet keeping early in childhood was given retrospectively in a structured interview, the large number of cases ( $n = 18\,530$ ) makes the analysis reliable. Interestingly, those authors found that the effect of pet keeping in childhood varied according to the type of pet and the presence of cats in the community, i.e. in the wider environment. Cats owned in childhood were associated with more asthma in sensitized adults who grew up in areas with low community prevalence to cats. Dogs owned in childhood appeared to protect against adult allergic disease but promote nonallergic asthma. The first finding could be a proof of concept suggested by Platts-Mills et al. (2, 17, 30) that continuous high exposure in a community can induce tolerance. The second finding could be an effect of LPS-exposure favouring a Th1-response early in life but later in life promoting an inflammation in the lungs of susceptible individuals. The observation that very low and very high concentrations of the cat allergen, Fel d 1 can be protective in terms of specific sensitization was reported by Custovic et al., showing a 'bell-shaped curve' (33) correlating exposure and sensitization rates in adults. However, exposure was much higher in that study (>1000 µg/g Fel d 1) compared with our study. Therefore, in communities with lower Fel d 1 (median below 500 ng/g of dust) avoidance of pets may still be the correct recommendation for families with atopic predisposition.

The findings of farming communities, where a reduced risk of developing atopy among children raised on farms with livestock and thus with contact to high concentrations of endotoxin was observed (34–36), cannot be directly transferred to the urban population. It is known from occupational medicine and from longitudinal studies that endotoxin can favour bronchial inflammation (19, 37). The levels of endotoxin in the homes of Berlin were much lower than in farming communities (38) and not associated with the risk for asthma or atopy (sensitization). There was no difference between endotoxin concentrations in homes with and without cats, and with and without dogs, in contrast to reports from other authors (18). Therefore, pet owners in the German MAS were not exposed to higher levels of LPS, which is thought to favour Th1-responses.

Our findings show two different groups of individuals, one developing high IgG responses to cat allergen without sensitization (IgE) and respiratory symptoms, the other with sensitization (IgE), IgG and wheeze. It remains unclear, which mechanism induces tolerance towards pet allergens in highly exposed children. Either, additional unknown exposure factors modulate immune responses early in life or genetic factors influencing gene-environment interaction are responsible for the diverse responses to high cat allergen exposure, explaining the controversial reports in different epidemiological surveys.

## Authors contributions

Susanne Lau, Renate Nickel, Christoph Grüber, Bodo Niggemann were responsible for the planning and performing of the German Multicentre Allergy Study. Renate Nickel and Christoph Grüber and Susanne Lau were responsible for the clinical part of the study. Bodo Niggemann was responsible for the lung function testing and measurement of BHR. Sabina Illi was responsible for statistical evaluation. Ulrich Wahn rose the funding by the German Ministry of Research and Technology and is the principal investigator of the MAS. Thomas Platts-Mills and David Riposo were responsible for the IgG measurement. Susanne Lau supervised measurement of serum IgE and indoor allergen exposure and wrote the manuscript. Thomas Platts-Mills helped to improve the manuscript.

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