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ORIGINAL ARTICLE

# Fungal DNA in dust in Swedish day care centres: associations with respiratory symptoms, fractional exhaled nitrogen oxide (FeNO) and C-reactive protein (CRP) in serum among day care centre staff

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**Abstract**
*Purpose* To study associations between fungal DNA in day care centres, fractional exhaled nitric oxide (FeNO) and inflammatory markers in day care centre staff.
*Methods* Totally, 62 staff (90 %) from five day care centres in Sweden participated. All were females. Settled dust was collected and analysed for five sequences of fungal DNA by quantitative PCR. Levels of FeNO (NIOX MINO 50 ml/min) and serum levels of eosinophilic cationic protein, myeloperoxidase (MPO) and high-sensitivity C-reactive protein in blood (HsCRP) were measured. Dynamic spirometry was performed, and dyspnoea was measured. Biomarkers and dyspnoea ratings were log-transformed, and associations were analysed by linear mixed models, adjusting for age, atopy, smoking, body mass index (BMI), ETS and dampness/mould at home.
*Results* Geometric mean (GM) for FeNO was 15.3 ppb, 6 % were smokers, 14 % were obese, 31 % were overweight and 18 % had atopy. GM concentration was $2.16 \times 10^5$ cell equivalents (CE)/g for total fungal DNA, 2310 CE/g for *Aspergillus/penicillium* (Asp/Pen) DNA, 17 CE/g for *Aspergillus versicolor* DNA and 14 CE/g dust for *Streptomyces* DNA. FeNO was associated with total fungal DNA ($p = 0.004$), Asp/Pen DNA ($p = 0.005$) and *Streptomyces* DNA ($p = 0.03$). HsCRP was associated with total fungal DNA ($p = 0.03$) and BMI ($p = 0.001$). Dyspnoea was associated with Asp/Pen DNA ($p = 0.04$). Subjects with ETS at home had lower lung function ($FEV_1$) ($p = 0.03$), and those with dampness/mould at home had lower MPO ($p = 0.03$).
*Conclusion* Fungal contamination in day care centres, measured as fungal DNA, can be a risk factor for airway inflammation, and CRP is associated with BMI.

**Keywords** FeNO · CRP · Respiratory symptoms · Fungal DNA · Day care centre · Mould

## Introduction

Day care centres are important indoor environments for children as well as for day care centre staff. There is a trend that more and more preschool children stay in day care centres. In Sweden, 83 % of all preschool children attended day care centres in 2010 (Anonymous 2010), and in Singapore, more than 90 % of the children attend day care centres (Zuraimi et al. 2007). There is some information about the indoor environment in day care centres. Measurements of formaldehyde, organic pesticides, lead, particular matter (PM), allergens and endotoxin have been taken, as reviewed by Bröms et al. (2006). Some studies have reported inadequate ventilation flow in day care centres, with $CO_2$ levels often exceeding 1000 ppm (Daneault et al. 1992; Ferng and Lee 2002; Mendes et al. 2014), while other studies found lower $CO_2$ levels (Cai et al. 2009; Roda et al. 2011).

Building dampness and indoor mould growth is an important indoor exposure with implications for allergy and respiratory health, associated with incidence of asthma (Quansah et al. 2012; Tischer et al. 2011a) as well as rhinitis (Jaakkola et al. 2013). Moreover, World Health Organisation (WHO) concluded that there is sufficient evidence

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to conclude that occupants of damp or mouldy buildings have an increased risk of respiratory symptoms, respiratory infections and exacerbation of asthma (WHO 2009). One nationwide study on the indoor environment in day care centres in Sweden found that 39 % of the Swedish day care centres had a history of dampness, water leakage or mould growth (Bröms et al. 2006). In a Finnish day care centre study, 70 % had water damage and 17 % had mould odour (Ruotsalainen et al. 1995).

There is a need for more health studies measuring mould in indoor environments by molecular methods (Norbäck and Cai 2015; Tischer et al. 2011b). Detection and quantification of selected indoor fungi are now possible by using quantitative polymerase chain reaction (qPCR or sometimes called real-time PCR) (Haugland et al. 2004; Vesper et al. 2005). Measurements of fungal DNA in day care centres have been taken in Swedish day care centres (Cai et al. 2009, 2011a). Associations were found between levels of total fungal DNA and reports on dampness and mould growth and indoor odour by the local director of the day care centres (Cai et al. 2009). Moreover, levels of total fungal DNA were elevated in certain type of buildings with an increased risk of dampness problems ("risk constructions") (Cai et al. 2011a).

There are few epidemiological studies on associations between the indoor environment in day care centres and respiratory health. One study from Singapore reported lower prevalence of asthma, allergy and respiratory symptoms in children attending naturally ventilated, as compared to mechanically ventilated day care centres (Zuraimi et al. 2007). Another study found a higher prevalence of wheeze in children attending day care centres with higher levels of $CO_2$ (Carreiro-Martins et al. 2014). One old study from Denmark found an association between formaldehyde levels in day care centres and mucous membrane irritation, headache and tiredness in day care centre staff (Olsen and Døssing 1982). Moreover, one study from Finland (Ruotsalainen et al. 1995) and two from Taiwan (Li et al. 1997a, b) found higher levels of respiratory illness and sick building syndrome (SBS) symptoms among day care centre staff in day care centres with dampness and indoor mould. Finally, one study from Taiwan found association between levels of *Aspergillus* species in air and SBS symptoms in day care centre staff (Li et al. 1997b). We found no previous epidemiological studies on associations between fungal DNA levels in day care centres and respiratory symptoms or clinical signs of inflammation in preschool children or day care centre staff.

The main aim was to study associations between fractional exhaled nitric oxide (FeNO) and serum levels of selected biomarkers of inflammation in day care centre staff and levels of five fungal DNA sequences in settled dust in day care centres in mid-Sweden. These five DNA sequences have been used in two previous epidemiological studies on associations between mould levels in schools and respiratory health (Cai et al. 2011b; Simoni et al. 2011). Moreover, we studied association between the clinical data and potential confounders [age, smoking, atopy, body mass index (BMI), environmental tobacco smoke at home (ETS) and dampness/mould at home].

## Materials and methods

### Study population and medical investigation

Five day care centres were randomly selected in Uppsala, a city with 200, 000 inhabitants situated in mid-Sweden. The local government at Uppsala provided us with contact lists to the local administration. All staff ($N = 70$) were invited, and 62 participated initially (90 %). The researchers informed all invited staff at meetings and collected participants' signed consent forms, followed later by investigations. All medical investigations were performed in compliance with the relevant laws and institutional guidelines for this kind of studies in April 2005, and the study was performed on May 2005. WHO regulation of clinical studies came in 2006. Informed consent was obtained from all the participants. The protocol of the study was approved by the Regional Ethical Committee of Uppsala University (2005/65).

All participants ($N = 62$) answered a general questionnaire on occupation, asthma, allergies, other chronic diseases diagnosed by a doctor, demographic data and the current home environment. Each subject was tested at the beginning of the study to detect allergy to common allergens through a skin prick test (ALK Abello SA, Madrid, Spain) for birch, timothy, mug worth, cat, dog, horse, house dust mites (*D. pteronyssinus* and *D. farinae*) and mould (*Cladosporium* and *Alternaria*). Normal saline solution and histamine were used as negative and positive controls. After 20 min, the wheal diameter was measured by adding the largest diameter and its perpendicular diameter and dividing the sum with two. The mean wheal diameter for each control and allergen was calculated. The test result was considered as positive if the mean wheal diameter was more than or equal to 3 mm.

Each volunteer answered a medical symptom questionnaire and was invited to the clinical test three times, with a 2-week interval. All 62 participants fulfilled the study, but 3–4 persons each time could not participate in all medical tests for various reasons. The study was a part of a crossover intervention study, testing health effects of two types of buckwheat cookies (Wieslander et al. 2011). For

this indoor environment study, clinical data from all three investigations were used since for each participant the three tests were balanced with respect to the dietary intervention (one test prior to the intervention, one with one type of cookie and a third test with the other type of cookie in a randomised sequence).

Information on current symptoms of breathlessness (dyspnoea) was obtained from a questionnaire. Answers were given on a 100-mm visual analogue rating scale (VAS scale) based on the Borg scale (Nihlen et al. 1998). The scale has endpoints graded from "no perceived symptoms at all" (0), to "unbearable symptoms" (100). In addition, it has fixed points with verbal expressions at certain points of the line, 7 means "hardly any", 22 "a few", 50 "many", 78 "a lot" and 93 "a great many" perceived symptoms.

Venous blood was collected in EDTA tubes for analysis of high-sensitivity C-reactive protein (HsCRP). In addition, venous blood was collected in 5 ml SST tubes for analysis of eosinophilic cationic protein (ECP) and myeloperoxidase (MPO) in serum. Nitric oxide (NO) in exhaled breath (FENO) was analysed by NIOX MINO (Aerocrine, Stockholm, Sweden). This instrument has a filter that removes ambient NO. Lung function was measured by dynamic spirometry (SPIRA), including forced expiratory flow for one second ($FEV_1$) (SPIRA). All the tests were carried out in a standardised way with the same well-calibrated spirometer, by a trained nurse. The measurements were taken three times on each subject each time, and the highest values were noted. A test was considered adequate when the deviation between the two best tests was <5 %. The results were expressed as a percentage of normal values based on standardisation to age, sex, height, smoking habits and body mass using a local reference material from Uppsala (Hedenström 1986).

### Home environment

The general questionnaire included one question about ETS in the current home, graded at four levels: never, 1–3 times per month, 1–3 times per week, and daily exposure (coded 0–3) (Wang et al. 2014). One yes/no question asked about window pane condensation in winter, an indicator of high humidity and poor ventilation flow (Wang et al. 2014). Four yes/no questions asked about any signs of dampness or indoor moulds during the last 12 months (water leakage, floor dampness, visible moulds and mould odour) (Wang et al. 2014; Norbäck et al. 1999). Finally one yes/no question asked about any signs of water leakage or moulds at home during the last 5 years (Wang et al. 2014). Finally, one question on any dampness or mould was created by combining window pane condensation and the questions on dampness and moulds the last 12 months and the last 5 years.

### Building inspection and indoor climate in the day care centres

Details on construction, building materials, construction year and signs of dampness or mould growth were noted. Temperature (°C), relative humidity (RH, %) and concentration of $CO_2$ (ppm) were measured continuously for 3 days in three rooms in each day care centre with a Q-TrakTM IAQ monitor (TSI Incorporated, St. Paul, Minnesota, USA), by logging average values over 1 min. The instruments were regularly calibrated. Average of daytime temperature, RH and $CO_2$ was calculated for the period when the day care centres were occupied by children and staff. Moreover, the level of nitrogen dioxide ($NO_2$), a proxy variable for traffic exhaust, was measured outside each day care centre for 7 days by diffusion sampling (Mi et al. 2006). The samplers were obtained from IVL Swedish Environmental Research Institute Ltd (Gothenburg, Sweden) and analysed by an accredited laboratory (IVL).

### Dust sampling

Settled dust was collected by a 1200-W vacuum cleaner provided with a special dust collector (ALK Abello, Copenhagen, Denmark) equipped with a Millipore filter (pore size 6 μm). The filter is made of cellulose acetate, and according to the manufacturer, it retains 74 % of particles 0.3–0.5 μm, 81 % of particles 0.5–1.0 μm, 95 % of particles 1–10 μm and about 100 % of larger particles (>10 μm). Vacuum cleaning was performed for 4 min per sample, 2 min on the floor and 2 min on other surfaces (desks, chairs) as in previous studies (Kim et al. 2005; Norbäck et al. 2014). Dust was collected from three rooms in each day care centre the same day as the baseline clinical investigation. Each classroom was divided into two parts: one near the corridor and the one near the windows, and two samples were collected. Dust was sieved through 0.3-mm mesh screen to obtain the fine dust, weighted, and used for analysis of fungal DNA. For fungal DNA analysis, sieved dust from the two filters was combined to one sample. The sieved dust samples were stored in a low-temperature freezer (−80 °C).

### Analysis of fungal DNA

The method for analysis of fungal DNA in dust samples has been previously described (Cai et al. 2011b; Norbäck and Cai 2011). Briefly fungal DNA was extracted from 10 mg of sieved dust, and five multiplex reactions were performed in five separate tubes targeting the DNA of the following species: total fungi, *Aspergillus* spp. and *Penicillium* spp. (*Asp/Pen*), *Aspergillus versicolor (A. versicolor)*,

*Stachybotrys chartarum* (*S. Chartarum)* and *Streptomyces* spp. The reaction targeting *A. versicolor* simultaneously amplified an internal positive control that was used to detect PCR inhibition. The oligonucleotides used for amplification and detection were designed using the design software Primer Express 2.0 (Applied Biosystems, Foster City, CA USA). Amplification and detection were performed on a 7300 Real-time PCR Instrument (Applied Biosystems, Foster City, CA USA) using the Taqman® Universal Master Mix (Applied Biosystems, Foster City, CA USA). The fungal DNA level was expressed as cell equivalents (CE), assuming one sequence per cell (Cai et al. 2009). The final result was presented as CE/g dust. Since previous epidemiological studies (Kim et al. 2005; Smedje and Norbäck 2001; Zhao et al. 2008) have shown that the amount of a biological compound (allergens, microbial markers) per sample has a stronger association with respiratory symptoms than concentration per gram dust, this measure was chosen as the exposure variable in this study. The amount of a particular sequence (CE) per sample was calculated by multiplying CE/g dust with the weight of the sieved fine dust in the sample.

### Statistical methods

Crude data analysis was performed by Mann–Whitney *U* test (comparing two groups) or by Kendal Tau beta rank correlation test. Associations between fungal DNA levels and health parameters were examined by linear mixed models controlling for age, BMI, current smoking, atopy, ETS at home and dampness/mould at home. Biomarkers and dyspnoea ratings, but not lung function, were log-transformed, and each of the three individual health data measurements was entered in the models (repeated measurement). The variance component on centre level was very small, and thus, we analysed associations by one-level models, only. Initially, we analysed models including only the potential confounders (age, BMI, smoking, atopy, ETS at home and dampness/mould at home). As the next step, we analysed associations between fungal DNA and amount of fungal DNA adjusting for the six confounders, keeping one type of fungal DNA exposure in the model. The arithmetic mean of the measurements in each room was addressed to each day care centre. Coefficients (Beta) with 95 % confidence intervals (95 % CI) were calculated. Then, antilog data ($e^x$) for adjusted beta values with 95 % CI were calculated, given the multiplicative associations with confounders and exposure variables. Statistics were performed with the STATA statistical package using two-tailed tests at a 5 % significance level.

## Results

Baseline data were measured at the beginning of the study. Mean age was 46 years (SD = 10), mean height was 165 cm (SD = 6), mean weight was 70 kg (SD = 13) and mean BMI was 25.5 kg/m$^2$ (SD = 4.1). Fourteen per cent were obese (BMI > 30 kg/m$^2$), 31 % were overweighed (BMI 25–30 kg/m$^2$) and 53 % had a normal BMI (20–25 kg/m$^2$). All participants were females with only few smokers ($n = 4$). In total, 16 % had any type of pollen allergy (11 % birch, 8 % timothy and 10 % mug worth), 11 % had cat allergy, 6 % dog allergy, 2 % horse allergy, 2 % house dust mite allergy (*D. pteronyssinus* and *D. farinae*) and none had mould allergy (*Cladosporium* or *Alternaria*). Totally, 15 % reported any ETS at home, mostly a few times per month (8 %), 2 % reported ETS a few times per week and 5 % reported daily exposure to ETS at home. Due to small numbers in each category, ETS was treated as a yes/no variable in the further analysis. Totally, 16 % reported window pane condensation in winter, 10 % any sign of dampness or mould the last 12 months and 15 % any sign of dampness the last 5 years. Totally, 29 % had either window pane condensation or dampness or mould the last 12 months or the last 5 years (any dampness or mould at home). Most of the participants (95 %) were preschool teachers or assistant preschool teachers. Two were kitchen staff, and one was a cleaner. An occupational exposure assessment was performed for these three subjects. All day care centres received precooked food from a central kitchen located in another place, and the kitchen staff only served food. Most day care centres had outsourced the cleaning to a private company not included in this study. The cleaner who was still employed at the day care centre used mostly dry methods (electrostatic mops) sometimes combined with wet moping with water with some detergents. Thus, it was concluded that these three subjects had no significant occupation exposure, and it was decided to include them in the further analysis. Descriptive baseline data are given in Table 1.

The average lung function (FEV1) was normal (104 %) using a local reference material including adjustment for age, gender and smoking habits (Hedenström 1986). The average rating of dyspnoea was relatively low. The geometric mean value for FeNO was 15.3 ppm, and 16 % of the subjects had an average FeNO value exceeding the cut-off value of 25 ppb suggested by American Thoracic Society (ATS) (Dweik et al. 2011). There were no significant correlations between any of the health variables, except between ECP and MPO (Kendal Tau beta = 0.50; $p < 0.001$). Descriptive data for the health parameters included in the analysis are given in Table 2.

**Table 1** Demographic and home environment data for the participants ($N = 62$)

| Type of parameter | % |
|---|---|
| Female gender | 100 |
| Current smoker | 6 |
| Current cold (last week) | 5 |
| Atopy[a] | 18 |
| Doctors' diagnosed asthma | 13 |
| Other chronic disease | 8 |
| Environmental tobacco smoke (ETS) at home | 15 |
| Window pane condensation at home in winter | 16 |
| Signs of dampness/mould at home last 12 months | 10 |
| Signs of dampness/mould at home last 5 years | 15 |
| Any signs of dampness/mould at home[b] | 29 |

[a] At least one positive skin prick test to common allergens (pollen, furry pets, mould or house dust mites)

[b] Window pane condensation in winter, signs of dampness/mould last 12 months or signs of dampness/mould last 5 years

**Table 2** Health variables investigated in the study ($N = 62$)

| Health variable | Mean (SD) |
|---|---|
| $FEV_1$ (L/s) (% predicted) | 104 % (14) |
| | GM (GSD) |
| Dyspnoea[a] | 7.4 % (3.74) |
| FeNO | 15.3 ppb (1.78) |
| HsCRP | 147 mg/L (1.78) |
| Serum MPO | 12.5 mg/L (1.83) |
| Serum ECP | 1.19 mg/L (2.76) |

*GM* geometric mean, *GSD* geometric standard deviation

[a] Reports on difficulty breathing on a 0–100 % rating scale

Totally, eight subjects (13 %) had ever had asthma diagnosed by a doctor: five of them had no asthma attacks the last 12 months and did not use any asthma medication, while three had current asthma (asthma attacks last 12 months or current asthma medication). Five subjects reported other chronic diseases than asthma diagnosed by a doctor; three had thyroid disorders, one had gluten intolerance (coeliac disease) and one had ulcerous colitis. None of these five subjects had asthma. Mean lung function, dyspnoea ratings, FeNO, MPO, ECP and HrCRP were compared between those with and without asthma diagnose. FeNO was higher among asthmatics (GM = 14.6 ppb among non-asthmatics and 20.5 ppb among asthmatics, $p = 0.035$ by Mann–Whitney $U$ test). The highest FeNO levels were found among those with current asthma. For other health variables, there were no significant differences between asthmatics and non-asthmatics. Moreover, there were no significant differences for any of the investigated health variables when comparing those with other chronic disease ($N = 5$) and those without any chronic diseases ($N = 49$) (neither asthma nor other chronic diseases).

All of the day care centre buildings were one-floor buildings built on a concrete slab (no basement) constructed from 1971 to 1991. Four had a brick facade, and one had a wooden facade. Four buildings were heated by waterborne radiators, and one had floor heating. All had a mechanical ventilation system, three building had displacement ventilation and the other two had mixing supply–exhaust ventilation. Three had polyvinyl chloride (PVC) floor coating, and two had linoleum floors. None of the rooms had visible signs of dampness, visible indoor mould or mould odour. One room had odour other than mould odour. Two of the buildings, constructed in 1971 and 1975, had a history of dampness problems that were renovated about 5 years before our investigation. Mean daytime indoor $CO_2$ was 434 ppm (range 381–505), mean daytime temperature was 22.6 °C (range 21.5–25.0) and mean daytime relative air humidity (RH) was 40 % (range 28–51). Mean outdoor level (1 week average) of $NO_2$ was 5 $\mu g/m^3$ (range 3–9).

By using the quantitative PCR method, total fungal DNA, *Aspergillus/Penicillium* DNA, *Aspergillus versiscolor* DNA and *Steptomyces* sp. DNA were detected in all dust samples, but concentrations differed by one order of magnitude. *Stachybotrys chartarum* DNA was detected in eight of the 15 dust samples but at very low concentrations. The arithmetic mean value was 5 CE/g dust in those eight samples where this sequence was detected (range 1–15 CE/g). There were no significant correlations between the concentrations of different types of fungal DNA (specific sequences), except for a positive correlation between *Aspergillus versiscolor* DNA and *Stachybotrys chartarum* DNA (Kendal Tau beta 0.48, $p = 0.02$). Moreover, there were no significant correlations between the concentration of total Fungal DNA and the concentration of any of the four specific DNA sequences. Because of very low concentrations of *Stachybotrys chartarum* DNA, this sequence was not included in the further health analysis. The concentration of total fungal DNA was lower in the two buildings that had been renovated because of previous dampness problems (GM = 1.35; GSD 1.59) as compared to the other three buildings (GM = 2.94; GSD 1.52) ($p = 0.01$). Surface contamination of fungal DNA depends on the concentration in the dust but is also influenced by the amount of dust on the surfaces. The mean amount of fine dust was 275 mg/sample (range 97–495). As a proxy variable for surface contamination, the amount of fungal DNA was calculated by multiplying the dust concentration of fungal DNA with the amount of fine dust and was expressed as

**Table 3** Concentration and amount of fungal DNA in settled dust ($N = 15$)

| DNA sequence | Concentration (CE/g dust) | | Amount (CE/sample) | |
|---|---|---|---|---|
| | GM (GSD) | Min–max | GM (GSD) | Min–max |
| Total fungal DNA | $2.16 \times 10^5$ (1.78) | $0.78–5.95 \times 10^5$ | $5.48 \times 10^4$ (1.99) | $1.7–14.8 \times 10^4$ |
| *Asp/Pen* DNA | $2.31 \times 10^3$ (1.69) | $0.98–5.32 \times 10^3$ | $5.88 \times 10^2$ (1.93) | $2.0–18.7 \times 10^2$ |
| *A. versicolor* DNA | 17.4 (4.65) | 1–210 | 4.4 (3.98) | 1–64 |
| *Streptomyces* DNA | 14.1 (2.59) | 3–60 | 3.6 (2.98) | 1–22 |

Dust was sampled in three randomly selected rooms in each of the five each day care centres

*CE* cell equivalents, *GM* geometric mean, *GSD* geometric standard deviation

CE per sample. Data on the concentration and amount of fungal DNA are given in Table 3.

Initially, we analysed health associations in regression models including six potential confounders (age, BMI, smoking, atopy, any ETS at home and any dampness/mould at home). Lung function ($FEV_1$) was 10.4 % lower among those with any ETS at home (95 % CI −19.7 to −1.1 %) ($p = 0.03$). The antilog data for adjusted beta values with 95 % CI were calculated to express associations on a multiplicative scale. Subjects with a positive skin prick test (atopy) had 2.66 times higher rating of dyspnoea (95 % CI 1.19–5.92) ($p = 0.02$). Older subjects had higher levels of HrCRP ($p = 0.01$), with a 1.34 times higher value (95 % CI 1.07–1.69) per 10 years of age. Moreover, BMI was positively associated with HrCRP ($p < 0.001$) with 1.10 times higher values (95 % CI 1.04–1.16) for a change in BMI by one unit. MPO was 0.71 times lower (95 % CI 0.51–0.97) among subjects living in homes with any sign of dampness or mould ($p = 0.03$). Moreover, subjects with atopy had 1.54 times higher (95 % CI 1.07–2.24) levels of ECP ($p = 0.02$). FeNO was not significantly associated with any of the potential confounders.

As a next step, we analysed associations between each of the exposure variables (amount of the four fungal DNA sequences) and each of the health variables, adjusting for age, BMI, smoking, atopy, ETS at home and any dampness/mould at home. Beta coefficients on the log scale with a 95 % CI are presented in Table 4. Taking the antilog data ($e^x$) for adjusted beta values, a $10^4$ CE/sample increase in total fungal DNA was associated with a 1.05 times (95 % CI 1.02–1.09; $p = 0.004$) increase in FeNO ($p = 0.004$) and a 1.07 times (95 % CI 1.01–1.14) times increase in HsCRP ($p = 0.03$). An increase in *Asp/Pen* DNA by 100 CE/sample was associated with a 1.14 times (95 % CI 1.03–1.26) increase in dyspnoea scale ratings ($p = 0.01$) and a 1.06 times (95 % CI 1.01–1.10) increase in FeNO ($p = 0.01$). There was a borderline association between *Streptomyces* DNA and FeNO ($p = 0.07$). No associations with any of the four fungal DNA sequences were found for $FEV_1$, MPO or ECP.

Finally, we analysed associations between health and fungal DNA after excluding subjects with obesity (BMI > 30) ($N = 53$). Beta coefficients on the log scale with a 95 % CI are presented in Table 4. All associations found in the total material remained significant, and moreover, the positive association between *Streptomyces* DNA and FeNO was statistically significant. Taking the antilog data ($e^x$) for adjusted beta values, a $10^4$ CE/sample increase in total fungal DNA was associated with a 1.06 times (95 % CI 1.02–1.10) increase in FeNO ($p = 0.004$) and a 1.07 times (95 % CI 1.01–1.15) times increase in HsCRP ($p = 0.03$). An increase in *Asp/Pen* DNA by 100 CE/sample was associated with a 1.13 times (95 % CI 1.00–1.26) increase in dyspnoea scale ratings ($p = 0.04$) and a 1.07 times (95 % CI 1.02–1.12) increase in FeNO ($p = 0.005$). A one CE/sample increase in *Streptomyces* DNA was associated with a 1.02 time increase (95 % CI 1.00–1.04) of FeNO ($p = 0.03$). No associations with any of the four fungal DNA sequences were found for $FEV_1$, MPO or ECP.

## Discussion

We found significant associations between levels of fungal DNA in dust samples from day care centres in mid-Sweden and dyspnoea, fractional exhaled nitrogen oxide and C-reactive protein. Positive associations were found for total fungal DNA as well as for *Aspergillus/Penicillum* DNA and *Streptomyces* DNA. Moreover, BMI was associated with higher levels of C-reactive protein.

Epidemiological studies can be influenced by selection bias and information bias. The day care centres were selected randomly within public day care centres within on city in mid-Sweden, and the participation rate was high (90 %). The majority of the participants (95 %) were preschool teachers. Two kitchen staff and one cleaner were not excluded since a detailed assessment of their exposure conditions did not reveal any significant occupational exposure. The number of subjects was relatively small, but each person participated in the clinical investigation three times, with a 2-week interval, which increased the precision of the medical data

**Table 4** Associations between fungal DNA in vacuumed dust and health variables in the total material ($N = 62$) and among non-obese subjects (BM < 30) ($N = 53$)

| Type of fungal DNA | Subjects | Log Dyspnoea Beta (95 %CI) | Log FeNO Beta (95 %CI) | Log HsCRP Beta (95 %CI) | Log Serum MPO Beta (95 %CI) | Log Serum ECP Beta (95 %CI) |
|---|---|---|---|---|---|---|
| Total fungal DNA | All | 0.077 (−0.011 to 0.165) | 0.052 (0.017–0.088)** | 0.068 (0.006–0.131)* | −0.028 (−0.069 to 0.013) | −0.015 (−0.056 to 0.026) |
| Total fungal DNA | BMI < 30 | 0.087 (−0.009 to 0.183) | 0.055 (0.018–0.092)** | 0.072 (0.007–0.137)* | −0.026 (−0.070 to 0.019) | −0.005 (−0.051 to 0.040) |
| *Asp/Pen* DNA | All | 0.127 (0.026–0.229)* | 0.054 (0.011–0.097)* | 0.069 (−0.006 to 0.144) | −0.016 (−0.065 to 0.033) | 0.005 (−0.043 to 0.054) |
| *Asp/Pen* DNA | BMI < 30 | 0.119 (0.004–0.232)* | 0.065 (0.020–0.111)** | 0.067 (−0.013 to 0.146) | −0.023 (−0.077 to 0.031) | 0.006 (−0.048 to 0.060) |
| *A. versicolor* DNA | All | 0.002 (−0.038 to 0.041) | 0.005 (−0.011 to 0.022) | 0.019 (−0.009 to 0.047) | −0.016 (−0.034 to 0.002) | −0.013 (−0.031 to 0.005) |
| *A. versicolor* DNA | BMI < 30 | 0.017 (−0.025 to 0.059) | 0.003 (−0.015 to 0.020) | 0.022 (−0.006 to 0.050) | −0.009 (−0.028 to 0.010) | −0.005 (−0.024 to 0.014) |
| *Streptomyces* DNA | All | 0.026 (−0.019 to 0.070) | 0.017 (−0.001 to 0.035) | 0.010 (−0.023 to 0.042) | 0.003 (−0.017 to 0.024) | 0.007 (−0.014 to 0.028) |
| *Streptomyces* DNA | BMI < 30 | 0.011 (−0.041 to 0.063) | 0.023 (0.003–0.044)* | 0.002 (−0.033 to 0.038) | −0.005 (−0.029 to 0.019) | 0.002 (−0.022 to 0.026) |

Adjusted for age, BMI, current smoking, atopy, ETS at home and dampness/mould at home in linear mixed models

Coefficient calculated for $10^4$ CE increase in total fungal DNA per sample

Coefficient calculated for 100 CE increase in *Asp/Pen* DNA per sample

Coefficient calculated for 1 CE increase in *A. versicolor* DNA per sample

Coefficient calculated for 1 CE increase in *Streptomyces* DNA per sample

Coefficients with 95 % CI are expressed for natural logarithm transformed data

*BMI* body mass index

* $p < 0.05$; ** $p < 0.01$

in the study. The participants or the medical staff had no information about the indoor climate measurements, or the dust analysis, when the study was performed. Reports on dyspnoea are subjective symptoms that could be influenced by attitudes among the participants, but clinical data are not affected by recall bias. Moreover, none of the buildings had any visible signs of dampness or moulds. Thus, we do not think our study was seriously biased by selection or information bias. However, the cross-sectional design of the study limits the possibility to draw conclusions on causality.

The study population consisted of 100 % females, very few were smokers, the prevalence of IgE-mediated allergies was relatively low (18 %) and on average they had normal lung function. Totally, 13 % had doctors' diagnosed asthma, somewhat higher than the 11.5 % of doctors' diagnosed asthma found in a large national questionnaire survey in Sweden (Wang et al. 2014). Moreover, fourteen per cent were obese (BMI > 30 kg/m$^2$) and 31 % were overweighed (BMI 25–30 kg/m$^2$). We found a strong association between BMI and C-reactive protein, which is in agreement with population-based studies from different parts of the world such as Northern Europe (Thjodleifsson et al. 2008), Switzerland (Marques-Vidal et al. 2012) and China (Zhao et al. 2010). This confirms the rising evidence linking obesity to inflammation.

All five buildings were well ventilated, with low $CO_2$ levels (381–505 ppm), and the general impression when visiting the buildings was that the buildings had a good indoor environment. The $NO_2$ levels outside the buildings were low (3–9 μg/m$^3$) well below the current health-based standard of WHO. The WHO air quality guideline for $NO_2$ is 40 mg/m$^3$ (annual mean) and 200 mg/m$^3$ for 1-h mean (WHO 2005). The $CO_2$ measurements showed that the ventilation flow was well above the current ventilation standard, suggesting that the CO2 levels should be below 1000 ppm (ASHRAE 1999). However, all buildings were built on a concrete slab. Concrete slabs with thermal insulation between the concrete slab and the building were commonly used in from the 1980s to the 1990s in Sweden. This construction is classified as a risk construction (Cai et al. 2011a) and is not allowed nowadays in newly constructed buildings in Sweden. Risk constructions have been demonstrated to have elevated levels of total fungal DNA (Cai et al. 2011a). Two of the buildings had a history of dampness problems, but these building had been renovated about 5 years before our investigation due to the dampness problems. These buildings had on average two times lower levels of total fungal DNA than the other buildings, which suggest that the renovations had been successful. One limitation of the study is that we did not measure formaldehyde or other volatile organic compounds (VOC) in the day care centres. One old study from Denmark found high levels of formaldehyde (430 μg/m$^3$) in mobile day care centre with urea formaldehyde particle board, while levels were much lower (80 μg/m$^3$) in normal day care centre buildings (Olsen and Døssing 1982). A more recent study from Canada reported that the mean formaldehyde level in day care centres was 23 μg/m$^3$ and that all buildings were below the Canada's Residential IAQ Guideline of 50 μg/m$^3$ (St-Jean et al. 2012). We have performed an indoor environment study measuring formaldehyde by diffusion sampling in 52 rooms in 26 day care centres in one municipality in west Sweden (Mölndal). The mean level of formaldehyde (1 week average) was 5 μg/m$^3$ (range 2–20 μg/m$^3$) (Edoffson et al. 2002). Since none of the five day care centres in our study were new or newly redecorated, it is most likely that the levels of indoor formaldehyde were low. Moreover, there is no reason to believe that formaldehyde or other VOC in the indoor air would be a confounder in our study.

The study analysed self-reported data on ETS and dampness/mould at home. Subjects exposed to ETS at home had lower $FEV_1$. This is in agreement with a large cross-sectional study (NHANESIII) which found lower $FEV_1$ in non-smoking women with the highest levels of serum cotinine (Eisner 2000). One intervention study found that establishing of smoke-free bars and taverns in California increased the $FEV_1$ in bartenders (Eisner et al. 1998), and a similar intervention study in Norway found evidence of larger cross-shift decrease in lung function among restaurant workers before compared with after the implementation of ban of smoking in bars and restaurants (Skogstad et al. 2006). Surprisingly, in our study, we found a negative (protective) association between self-reported dampness/mould at home and serum MPO, an indicator of neutrophilic inflammation. We have no clear explanation to this finding which could be a chance finding. We found no previous studies on associations between serum MPO and home dampness, but one previous home environment study did not find any association between MPO in nasal lavage and self-reported dampness at home (Bakke et al. 2007).

Fungal DNA levels in the day care centres were associated with higher-level dyspnoea, FeNO and C-reactive protein among staff in day care centres. FeNO is an indicator of Th2-driven (allergic) inflammation in the lower airways (Alving and Malinovschi 2010), while C-reactive protein is a marker of systemic inflammation. Associations were found for total fungal DNA as well as for *Aspergillus/Penicillum* DNA and *Streptomyces* DNA. *Streptomyces* sp. is a gram-positive bacterium that is found indoors in moisture-damaged building materials and has been shown to be a potent inducer of airway inflammation (Jussila et al. 2003). Previous studies have found associations between respiratory symptoms and SBS symptoms among staff in day care centres with dampness and mould growth (Ruotsalainen et al. 1995; Li et al. 1997a, b). Moreover, one of the studies from Taiwan reported positive associations between levels

of *Aspergillus* species in air and SBS symptoms in day care centre staff (Li et al. 1997b). In a recent review on associations between specific mould species measured indoors and asthma, it was concluded that *Aspergillus* and *Penicillium* species were consistently associated with development of asthma as well as exacerbation of asthma symptoms (Sharpe et al. 2015).

We found no previous epidemiological studies investigating associations between fungal DNA levels in day care centres and respiratory health, but we have previously published two studies on associations between fungal DNA in schools and asthma and respiratory symptoms among school children. One study from Malaysia found positive associations between doctor's diagnosed asthma and levels of *Streptomyces* DNA sampled on petri dishes (Cai et al. 2011b). Another multicentre school study from Europe found positive associations between wheeze and cough and levels of *Streptomyces* DNA (Simoni et al. 2011).

In conclusion, fungal surface contamination in day care centres, measured as fungal DNA in settled dust, was associated with FeNO and C-reactive protein, two biomarkers of airway inflammation. In addition, we found associations between fungal DNA levels and ratings of dyspnoea. The effects of these clinical endpoints were relatively small and further larger, and longitudinal studies are needed to evaluate the clinical significance if these findings. The study supports the view that exposure to ETS can impair the lung function and further work is needed to reduce ETS exposure in homes. Finally, the association found between BMI and C-reactive protein supports the rising evidence linking obesity to inflammation.

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**Compliance with ethical standards**

**Conflict of interest** The authors have no conflicting financial interest.

**Ethics** The researchers informed all invited staff at meetings and collected participants' signed consent forms, followed later by investigations. All medical investigations have been performed in compliance with the relevant laws and institutional guidelines for this kind of studies in April 2005. The study was performed on May 2005. WHO regulation of clinical studies came in 2006. Informed consent was obtained from all the participants. The protocol of the study was approved by the Regional Ethical Committee of Uppsala University (2005/65).

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