Serum perfluoroalkyl substances (PFAS) and risk of asthma and various allergies in adolescents. The Tromsø study Fit Futures in Northern Norway.

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Abstract

Background

Exposure to environmental pollutants may contribute to the development of asthma and other allergies. The aim of this study was to investigate a possible association between asthma and other allergies with exposure to perfluoroalkyl substances (PFASs) in adolescents from the Arctic region of Norway.

Methods

The Tromsø study Fit Futures 1 (TFF1) and its 3-year follow-up Fit Futures 2 study (TFF2) included 675 adolescents that completed a questionnaire about health conditions and underwent a clinical examination with blood tests and fractional nitric oxide (FeNO) measurement. Serum concentrations of 18 PFASs were measured by UHPLC-MS/MS method.

Results

Sum of all measured PFASs (ΣPFAS), total perfluorooctane sulfonate (ΣPFOS), linear PFOS (linPFOS), linear perfluorohexane sulfonate (linPFHxS) concentrations over 3rd quartiles were associated with 2-3 times higher odds of asthma in the TFF1. The positive associations between ΣPFAS, ΣPFOS, linPFOS and asthma remained statistically significant in the TFF2. ΣPFAS and linPFHxS concentrations over 3rd tertile were positively associated with the marker of eosinophilic airways inflammation FeNO>25 ppb in the TFF2 (OR 2.18 (95% CI 1.08-4.42) p=0.03 and OR 2.13 (95% CI 1.08-4.21) p=0.03, respectively). Concentrations of ΣPFOS and linPFOS over 4th quartiles were positively associated with self-reported nickel allergy (OR 2.40 (95% CI 1.19-4.82) p=0.014 and OR 2.91 (95% CI 1.43-5.93) p=0.003, respectively). Allergic rhinitis, self-reported pollen allergy, food allergy and atopic eczema were not associated with serum PFASs concentrations.

Conclusions

This study of Norwegian adolescents showed a positive association between several PFASs and asthma, as well as between PFOS and nickel allergy.

Key words: Asthma, Allergy, Adolescents, Pollutants, perfluoroalkyl substances, PFAS

Abbreviations

FeNO, fractional exhaled nitric oxide; PPB, parts per billion; PFASs, perfloroalkyl substances; PFOS, perfluorooctane sulfonate; PFHxS, perfluorohexane sulfonate.

Introduction

Prevalence of asthma remains high in children and adolescents with increasing trend in many countries in the last 30 years (1-5). In Norway, the increasing prevalence of asthma was shown in three surveys of 7-14 years old children between 1985 and 2008 (6). Another Norwegian study of adolescents reported that 11% of boys and 10.5% of girls had doctor-diagnosed asthma at the age of 13-19 years (7), which is similar to the prevalence found among adolescents from Northern Norway (8) and other high-income countries such as UK and Canada (9). The reason for the increasing asthma prevalence and the unremitting asthma in adolescence remains unknown. One of the hypotheses is that exposure to chemical substances and environmental pollutants may promote asthma and other allergies.

Perfluoroalkyl substances (PFASs) are a large group of compounds that have been widely used in the last 50 years in many consumer products such as firefighting foams, surfactants, impregnating agents for textiles, carpets, packaging paper and leather (10). The most notorious and commonly studied substances of this group are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). Both PFOS and PFOA are defined as persistent organic pollutants (POPs) and have been recently strictly regulated in the European Union (11, 12). However, despite the EU regulation PFOS and PFOA are still abundant in the environment due to their biological persistence, continuous presence in consumer products and their long-range transport by sea currents and air streams (13, 14). Moreover, other

substances from the PFASs group are not yet regulated and are widely used due to their unique water- and fat repellent and fire retardant properties. General populations are exposed to PFASs through water, food, dust from indoor environments and through direct contact with products that contain these compounds (15). Population studies from different countries have shown that more than 98% of a general population have several PFASs in their blood (16-18). In our recent publication, we showed that dietary intake of different food items is associated with serum concentrations of PFASs among adolescents in Norway (18).

Potential health effects of the long-term exposure to different PFASs are not well understood. Studies of PFASs' possible effects on allergic disease in humans are rare and with controversial results. The cord blood IgE levels were positively correlated with pre-natal PFOA and PFOS exposure in one study (19) and negatively associated with PFOA in another study (20). Some studies have reported no association between pre-natal PFOS and PFOA exposure and allergic diseases in infants (19, 20), while other studies have reported a positive association between PFASs concentrations and asthma/different allergies in older children (21-24).

The aim of the present study was to examine possible associations between 18 different PFASs in blood with asthma, various allergic diseases, IgE and fractional exhaled nitric oxide (FeNO) concentrations in a cohort study of adolescents from Northern Norway. This study is a sub-project of the Tromsø Study, Fit Futures that had the overall aim to investigate effects of lifestyle, diet and other behavioral and environmental risk factors on health of adolescents in the Arctic areas.

Ethics

The Tromsø study Fit Futures 1 (TFF1) and its follow-up Fit Futures 2 (TFF2) were approved by the Regional committee for medical and health research ethics (REK) and the Norwegian Data Protection Authority. All students and parents/guardians of students under 16 years of age gave written informed consent. REK North (2015/1384) also approved the present sub-project.

Financial support

TFF1 and TFF2 were financed by UiT The Arctic University of Norway, University Hospital of North Norway (UNN), the Northern Norway Regional Health Authority, the Troms County Council, and Odd Berg medical research fund. The Department of Laboratory Medicine, UNN, financed the PFASs analyses.

Materials and methods

Study population

All the 1117 first-year high school students from the eight high schools in the municipalities of Tromsø and Balsfjord were invited to participate in TFF1 in 2010-2011. The attendance rate was 93%. Further, all participants in the TFF1 study and all new high school students from the third-year were invited for the follow up TFF2 study in 2012-2013. Altogether 870 high school children were recruited in the TFF2 study, 78% of the TFF2 study participants attended both the TFF1 and the TFF2 studies. Overall, 675 high school students who participated in both TFF1 and TFF2 studies and provided blood samples for pollutants analyses were included in the present study. The participants completed a comprehensive questionnaire about family, living conditions, lifestyle, diet and general health and were

interviewed by study nurses about self-reported diseases and medication in both TFF1 and TFF2. Both surveys were performed at the Clinical Research Unit, University Hospital of North Norway, Tromsø.

Clinical examination

All participants underwent a clinical examination with measurement of height, weight and body mass index (BMI) in both surveys. Fractional exhaled nitric oxide (FeNO) was measured for each participant in the sitting position by the NIOX MINO® method (Aerocrine AB, Solna, Sweden) in the TFF2. A standardized blood sampling was performed for pollutant analyses in TFF1 and for total and specific IgE analyses in the TFF2. Blood samples were obtained in BD vacutainer® tubes with no additive (Becton, Dickinson and Company, New Jersey, US), serum was transferred to Supelco glass vials (Sigma-Aldrich Norway AS, Oslo, Norway) with Pasteur glass pipettes and subsequently frozen at -40°C. No significant background PFAS contamination was detected in the sampling equipment.

Laboratory analyses

The pre-analytical and analytical procedures for PFAS analyses have been previously described in detail (25). Briefly, a fully validated high-throughput sample preparation method and analysis by ultrahigh pressure liquid chromatography triple-quadrupole mass-spectrometry (UHPLC-MS/MS, Waters, Milford, MA, USA) was applied. Altogether 18 different PFAS were quantified: perfluorobutane sulfonate (PFBS), perfluoropentane sulfonate (PFPS), perfluorohexane sulfonate (PFHxS), perfluoroheptane sulfonate (PFHpS), PFOS, perfluorononane sulfonate (PFNS), perfluorodecane sulfonate (PFDS), perfluorododecane sulfonate (PFDoDS), perfluorooctane sulfonamide (PFOSA), perfluorohexanoate (PFHxA), perfluoroheptanoate (PFHpA), PFOA, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA),

perfluorododecanoate (PFDoDA), perfluorotridecanoate (PFTrDA) and perfluorotetradecanoate (PFTeDA). Linear species (lin) as well as sum of branched and linear species (Σ) were quantified for PFHxS, PFHpS, PFOS, PFNS, PFDS and PFOSA. The linear isomers were used for calculating the contribution of the branched species. For quality assurance, four blank samples, four SRM 1958 (NIST, Gaithersburg, MD, USA) samples and three bovine serum samples (Sigma Aldrich, Steinheim, Germany) were analyzed within each batch of 96 samples to control for background and carry-over effects. All the quality controls were within the acceptance limits. Analytical coefficients of variation (CVa) were < 10% for all the measured PFASs except for PFUnDA with CVa 12%.

Total IgE and allergen specific IgE (sIgE) were analysed in fresh serum samples by the ImmunoCap Phadia 2500 methods (Thermo Fisher Scientific Inc., Phadia AB, Uppsala, Sweden) at the Department of Laboratory Medicine, UNN. The following sIgE were analysed: Ip6 and IP7 screening panels for inhalant allergens (birch, timothy, mugwort pollen, Alternaria alternata, and Cladosporium herbarum; and cat, horse, dog, house dust mite, and rabbit), FX5 screening panel for food allergens (egg white, cow`s milk, cod, wheat, peanut, and soy), and shrimp IgE (F24). CVa for all tests was <10%. Specific IgE values < 0.35 kU_A/L for a screening panel were interpreted as negative for all included allergens. If screening panels had IgE ≥ 0.35 kU_A/L, then sIgE was measured to all the allergens included in the screening panel.

Definition of asthma and other allergies

The definition of asthma and allergic diseases was based on the standardized self-reported MeDALL questions (Mechanisms of the Development of Allergy, Framework Programme 7 of the European Commission) that were validated in the International study of asthma and allergies in childhood (ISAAC) (26). Norway has participated in the development and validation of the harmonized MeDALL questionnaire for children and adolescents and the standard procedure was used to translate and adopt the questionnaire for the Norwegian population (26). This study used the MeDALL question about doctor-diagnosed asthma in TFF1 and TFF2 study as the definition of *Asthma*: "Have a doctor ever said that you have asthma?"

In TFF2 we included more questions on symptoms of asthma and could therefore also define *Current asthma* with at least 2 of the following 3 criteria:

1. Self-reported doctor-diagnosed asthma
2. Any symptom of asthma in the last 12 months (chest tightness, shortness of breath, wheezing, whistling in the chest or sleep disturbed due to wheezing)
3. Use of asthma medication in the past 12 months

Clinically severe asthma was defined as self-reported severe breathing problems (asthma wheezing >12 attacks in the last 12 months or self-scoring 7 or higher at the 10-scale score of breathing problems in the last 12 months) in TFF2.

For the definitions of other allergies, we used self-reported doctor-diagnosed *Allergic rhinitis, Atopic eczema,* as well as self-reported *Pollen allergy, Nickel allergy and Food reactions* in the TFF1.

For the definition of *Current allergic rhinitis* we used the following criteria from the TFF2 questionnaire: symptoms of sneezing, a runny or blocked nose, or itchy, red and watery eyes after exposure to furred pets or pollen in the last 12 months (8).

FeNO > 25 ppb was defined as a marker of eosinophilic airway inflammation (27).

Statistical analyses

Statistical analyses were performed with IBM SPSS statistics (IBM Corp. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Chi square test was used for comparisons between girls and boys. PFASs concentrations were not normally distributed according to distribution plots, skewness estimates, QQ-plots and the Kolmogorov-Smirnov test. Therefore the non-parametric Mann-Whitney U-test was used for comparisons of PFASs concentrations between genders. Limits of detection (LODs) were set as concentrations calculated by the Targetlynx-software for each individual sample (LOD*i*) and each individual analyte with a signal to noise ratio of 3 divided by the related sample amount. Where blank contamination was detected (background contribution during sample preparation), LOD was calculated as an average of the blanks multiplied by three times of their standard deviation. If the LOD calculated from the blank contamination was higher than the LOD*i* of the sample, the LOD calculated based on the blank samples was used. Limit of quantification (LOQ) was defined as three times the LOD. To reduce possible bias of left censored data analyses we have used the actual values between LOQ and LOD. PFASs concentrations below the LOD were not quantified (in most cases there was only noise visible) and these data were replaced by LOD*i* divided by 2. Statistical analyses were performed only for PFASs with detection rate ≥70%. PFASs with detection rate <70% were included in the PFASs sum concentration (ΣPFAS).

We have further used the logistic regression analyses where we have stratified PFASs concentrations in quartiles or tertiles. This approach allows to minimize the risk of possible bias of left censored data and to evaluate the possible threshold effects.

Logistic regression analyses were used to examine the associations between PFASs, asthma and other allergic diseases. We present the results of analyses not stratified by gender because of a relatively few asthma cases (n=88) in this population based cohort study. The logistic regression was performed using both crude models and models adjusted for possible confounders (age, gender, BMI, socioeconomic variables (parent's educational level, unemployment and disability of parents, living with adoptive parents), dietary variables that were associated with serum PFASs concentrations in TFF1 (18), physical activity at leisure time, smoking and chewed tobacco use). The covariates for the regression models were selected based on the results of the previously published studies (28-40) and were reported to be associated with risk of asthma and/or with PFASs exposure, therefore being possible confounders. The ANOVA linear trend tests were performed to evaluate if there were significant linear trends. All statistical tests were two-sided. A p-value < 0.05 was considered statistically significant.

Results

Altogether 355 girls and 320 boys participated in both TFF1 and TFF2 studies. General characteristics of the study population are presented in Table 1. Prevalence of asthma in the study population was about 13% in TFF1 and 14% in TFF2. Girls reported more nickel allergy and food allergic reactions than boys. ΣPFAS serum concentration was not significantly different between genders; however, boys had higher ΣPFOS, linPFOS and linPFHxS concentrations, while girls had higher PFOA serum concentrations than boys. There

were no gender differences in prevalence of asthma, allergic rhinitis, atopic eczema and pollen allergy. There were also no significant differences in socioeconomic characteristics between boys and girls (Table S1). Further we present the results of analyses not stratified by gender.

Asthma and PFASs

There was a strong positive association of ΣPFAS with self-reported doctor-diagnosed asthma at the time of the first study TFF1 (Table 2). ΣPFAS concentrations over 25 percentiles were associated with about three times higher odds of asthma. This association remained positive after adjustment for possible confounders such as BMI, socioeconomic variables, dietary variables, physical activity at leisure time, smoking and chewed tobacco use. The association was mostly due to the positive associations between ΣPFOS, linPFOS, linPFHxS and asthma, while other PFASs had no statistically significant association with asthma when analyzed separately. PFOA concentrations over 75 percentile showed a tendency to a positive association with asthma in the TFF1 study with OR 2.18 (95% CI 1.00-4.74, p=0.050; data not shown in the Table 2). The test for linear trend was statistically significant for ΣPFAS, ΣPFOS (p=0.02) and linPFOS (p=0.04) in the TFF1.

ΣPFAS and ΣPFOS were also positively associated with both self-reported doctor-diagnosed asthma and current asthma in the follow-up TFF2 study (Table 3), but the test for linear trend was not statistically significant. ΣPFAS was positively associated with clinically severe asthma, and both ΣPFAS and linPFHxS were positively associated with the marker of eosinophilic airway inflammation FeNO >25 ppb in the TFF2 (Table 4).

Atopic eczema, Total Ig E and PFASs

Self-reported atopic eczema in the TFF1 and the TFF2 studies was not associated with the measured PFASs (ΣPFAS, ΣPFHxS, linPFHxS, PFHpS, PFHpA, ΣPFOS, linPFOS, PFOA,

PFNA, PFDA, PFUDcA; data not shown). Logistic regression analyses revealed no statistically significant association between serum concentrations of different PFASs (ΣPFAS, ΣPFHxS, linPFHxS, PFHpS, PFHpA, ΣPFOS, linPFOS, PFOA, PFNA, PFDA, PFUDcA; data not shown) and high total IgE (>114 kU/L).

Nickel allergy and PFASs

There was a positive association between both ΣPFOS and linPFOS with self-reported nickel allergy (Table 5). Other PFASs measured in the study were not associated with nickel allergy.

Food reactions and PFASs

Logistic regression analysis showed no statistically significant association of PFASs with self-reported food allergic reactions (n=117; data not shown). The screen test for food sensitization (FX5 food panel IgE ≥0.35 kU/L) had no association with ΣPFAS, ΣPFHxS, linPFHxS, ΣPFOS, linPFOS, PFNA, PFDA, PFUDcA after adjustment for age, sex, BMI, dietary variables, physical activity, socioeconomic variables and asthma or allergic rhinitis medication. Food sensitization had a statistically significant negative association with PFOA concentrations over 50th percentile (OR 0.27 (95% CI 0.12-0.65, p=0.003) and with PFHpS concentrations over 50th percentile (OR 0.24 (95% CI 0.10-0.60), p=0.002). Sensitization to other specific food antigens (soybean F14, peanut F13, egg white F1, cod F3, milk F2, IgE ≥0.35 kU/L) had no statistically significant associations with PFASs measured in this study.

Allergic rhinitis, pollen allergy and PFAS

Self-reported pollen allergy, self-reported doctor diagnosed allergic rhinitis in the TFF1 study, as well as current allergic rhinitis in the TFF2 study were not associated with any of measured PFASs concentrations (data not shown). ΣPFAS, ΣPFOS and PFNA were weakly negatively

associated with high Ip6 inhalation panel IgE concentrations (≥0.35 kU/L), the association was not linear (Table S2). Other PFASs were not associated with Ip6 inhalation panel IgE.

Discussion

Prevalence of asthma in this study was similar to other studies of adolescents in high income countries (8, 9). To evaluate the persistence of asthma and allergy status in this population we followed up the study population for three years and measured the outcome variables (asthma and other allergies) in both TFF1 and TFF2 studies. There were no substantial differences in the prevalence of asthma, allergic rhinitis and atopic eczema between the TFF1 and TFF2 studies.

In this study of Norwegian adolescents, we found several significant positive associations between PFASs exposure and different asthma outcomes (asthma in the TFF1, asthma in the TFF2, current asthma in the TFF2, FeNO>25, severe asthma). We found a significant linear trend for ΣPFAS, ΣPFOS and linPFOS concentrations and asthma in the TFF1. This shows the consistency of the association between asthma and PFASs exposure in this population. The association is less consistent in the follow-up TFF2 study, but unfortunately, we did not have measured PFASs exposure status for the TFF2. These results corroborate previous reports about associations of PFAS with asthma in other populations (23, 41, 42). The Genetics and Biomarker study for Childhood Asthma found a positive association of ΣPFHxS, PFOA, ΣPFOS, PFDA, and PFNA serum concentrations with asthma in Taiwanese children (22, 24). The NHANES study of adolescents reported a positive association of asthma with increasing PFOA and PFNA serum concentrations (23). Both the NHANES study and the Taiwanese study populations had higher ΣPFOS, ΣPFHxS , PFDA and PFNA concentrations compared with the present study. ΣPFOS concentrations were considerably higher in the NHANES study and the Taiwanese study (median 16.8 ng/mL and 28.9 ng/mL,

respectively) compared with the present study (median 6.2 ng/mL) (18, 22, 23). PFOA concentrations were 2 times higher in the NHANES study (median 4.0 ng/mL) compared with the present study (median 1.9 ng/mL) (18, 23). ΣPFHxS concentrations were also approximately 2 times higher in the Taiwanese study (median 1.3 ng/mL) and approximately 3 times higher in the NHANES study (median 2.0-2.2 ng/mL for different subgroups) compared with the present study (median 0.71 ng/ml) (18, 22, 23). Despite much lower concentrations of ΣPFOS and ΣPFHxS in our study population, we still observed the positive association with asthma as in the NHANES study and the Taiwanese study.

Asthma is a multifactorial disease with a complex pathophysiology. Both genetic and several environmental factors are involved in the development of asthma. PFASs exposure may be one of the environmental factors that can contribute to asthma development. We cannot establish the causality of the PFASs association with asthma from these data, but we consider it important to report this consistent association between different asthma variables and PFASs exposure. We did not find the same association of PFASs exposure with other allergies such as atopic eczema, allergic rhinitis, food allergy and pollen allergy. These findings are also consistent with other studies. A Japanese study found no association of food allergies, eczema and total allergies in early childhood with maternal ΣPFOS and PFOA concentrations (20). A cohort study from Greenland and Ukraine showed no consistent association between maternal PFASs concentrations and eczema in children (43). Other studies have also found no association of atopic dermatitis in children with prenatal PFASs exposure (19, 44).

In our study we found a weak negative association between ∑PFAS, ∑PFOS, PFNA and sensitization to plants and between PFOA and PFHpS concentrations over 50 percentile and food sensitization, which confirms the findings of other studies describing

immunosuppressive effects of several PFASs. The positive association of PFASs with asthma seems not to be IgE related in this population. Immunosuppressive effects of PFASs were previously described in the NHANES study that also showed decreased IgE sensitization to plants, as well as reduced mumps and rubella antibody response in children exposed to PFOS (41). Several studies showed that prenatal exposure to PFASs was associated with lower vaccine antibody response in children (45, 46). However, immunomodulating effects of different PFASs seem to be complex, as the same group of children with high PFOS concentrations in the NHANES study had significantly higher sensitization to mold (41). In a murine model of asthma (47), exposure to immunosuppressant PFOA enhanced the hypersensitivity response to ovalbumin suggesting that PFOA may augment the IgE response to some allergens (25). In mice models PFASs exposure induced mast cell-derived inflammatory reactions by histamine release and expression of pro-inflammatory cytokines (48). Another study in mice showed that PFOS exposure increased IL-4, but decreased IL-2 and interferon-gamma (IFN-γ) secretion, favoring a T-helper 2(TH2) immune response that may lead to enhancement of the humoral response and suppression of the cellular response (49). Serum PFASs were positively associated with TH2 cytokines and inversely with TH1 cytokines in Taiwanese asthmatic boys showing that PFASs may promote TH-cell dysregulation and thus contribute to the development of asthma (24). Co-occurrence of immunosuppression and inappropriate immune enhancement with increased risk of allergic sensitization was previously described for several pollutants (50).

The complexity of immunomodulating effects of PFASs may explain the inconsistency of findings in population studies of asthma and PFASs exposure. Two Norwegian cohort studies (Environment and Childhood Asthma study and The Norwegian Mother and Child Cohort Study) found no statistically significant association between asthma and prenatal exposure to PFASs in small children (44, 45). These studies measured only maternal PFASs

concentrations and had no estimation of the post-natal PFASs exposure. However, these prospective cohort studies found a positive association between maternal PFASs and respiratory tract infections in small children due to possible immunomodulating effects of PFASs. Another large 20-year prospective cohort study showed that respiratory tract infections in early childhood were strong predictors of asthma through childhood to young adulthood (51). The ISAAC study also found a strong positive association of asthma with recurrent respiratory infections (52). The PreDicta prospective cohort study reported that viral infections were the most common factor for asthma persistence from preschool to school age (53). Several meta-analysis studies confirmed a possible association between respiratory syncytial virus (RSV) and rhinovirus (RV) infections and asthma (54, 55).

Therefore, one of the possible explanations of the positive association between PFASs exposure and asthma in several populations, including the present study, may be the complex immunomodulating effects of PFASs with both inappropriate immune enhancement and immunosuppression that increase the susceptibility to recurrent respiratory infections and the risk of asthma development.

Our study also showed a positive association of ΣPFOS and linPFOS with nickel allergy, which has not been reported before. The possible mechanism of this association remains unknown and requires further investigation.

Limitations of the study

Due to the cross-sectional nature of the TFF1 study the temporal sequence of the associations and the cause-effect relationships are impossible to determine. Another limitation of our study is that the PFASs exposure at the time of the follow-up study (TFF2) was not measured. This may explain why the association between PFASs and asthma was less consistent in the

follow-up TFF2 study than in the TFF1 study, even though the half-life of many PFASs is over 3 years. The prenatal exposure to PFASs as well as exposure in early childhood and vaccine/infections status are the important parameters and possible effect-modifiers that were not available in the present study. It would have been also an advantage to investigate concentrations of other environmental pollutants in the present population and to evaluate their possible confounder effects. Due to all the limitations, the causality of positive association of PFASs exposure with asthma and nickel allergy cannot be established in this study.

However, the findings of this general population-based cohort study strongly suggest immunomodulating effects of PFASs and a possible link between PFASs exposure and asthma in adolescents, as well as between PFASs and nickel allergy. Further prospective studies with repeated measurements of environmental pollutants are needed to test this hypothesis and to evaluate the effects of pollutant mixtures on predisposition to asthma and different allergies.

Highlights

- ΣPFAS, ΣPFOS, linPFOS, linPFHxS were positively associated with asthma
- ΣPFAS and linPFHxS were positively associated with FeNO>25 ppb
- ΣPFAS was positively associated with clinically severe asthma
- ΣPFOS and linPFOS were positively associated with self-reported nickel allergy

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Table 1. Characteristics of the study population. The Tromsø study Fit Futures 1 and 2 (TFF1, TFF2).

Parameters	Girls n=355	Boys n=320	Difference** p-value
Mean age (SD), years, TFF1	16.3 (1.1)	16.3 (1.0)	NS
Mean age (SD), years, TFF2	18.4 (1.2)	18.4 (1.0)	NS
Doctor-diagnosed asthma, TFF1	12.7%	13.4%	NS
Doctor-diagnosed asthma, TFF2	13.6%	14.5%	NS
Current asthma, TFF2	10.4%	9.4%	NS
Allergic rhinitis, TFF1	8.9%	10.7%	NS
Allergic rhinitis TFF2	11.0%	11.5%	NS
Current allergic rhinitis TFF2	29.5%	25.7%	NS
Nickel allergy, TFF1	22.4%	5.2%	<0.0001
Pollen allergy,TFF1	25.1%	27.1%	NS
Atopic eczema, TFF1	16.6%	12.8%	NS
Atopic eczema, TFF2	18.2%	11.5%	0.05
Food allergic reaction, TFF2	22.3%	13.2%	0.003
ΣPFAS (IQR)*, ng/mL TFF1	10.7 (4.8)	11.2 (4.8)	NS
ΣPFOS (IQR)*, ng/mL TFF1	5.8 (2.6)	6.8 (3.1)	<0.0001
LinPFOS (IQR)*, ng/mL TFF1	3.1 (1.6)	3.5 (1.8)	<0.0001
PFOA (IQR)*, ng/mL TFF1	2.1 (1.3)	1.9 (0.7)	<0.0001
ΣPFHxS (IQR)*, ng/mL TFF1	0.82 (0.5)	0.94 (0.64)	NS
linPFHxS (IQR)*, ng/mL TFF1	0.62 (0.4)	0.76 (0.6)	<0.0001

**Chi-square test, Mann-Whitney test. *Geometric mean with interquartile range (IQR)

Table 2. Association between PFAS concentrations and self-reported doctor-diagnosed asthma in Norwegian adolescents (n=675, cases of asthma n=88). The Tromsø study Fit Futures 1 (TFF1).

PFAS	Concentration	Cases/controls	Asthma TFF1		Asthma TFF1	
	ng/mL	Number	OR (95% CI)*	p-value	OR (95% CI)**	p-value
ΣPFAS						
Quartile 1‡	2.6-8.59	10/158	1.0		1.0	
Quartile 2	8.60-10.71	28/141	3.27 (1.52-7.04)	0.002	3.38 (1.54-7.45)	0.002
Quartile 3	10.72-13.37	23/146	2.62 (1.20-5.73)	0.016	2.95 (1.32-6.61)	0.008
Quartile 4	>13.37	27/142	3.09 (1.44-6.66)	0.004	3.63 (1.64-8.06)	0.002
ΣPFOS						
Quartile 1‡	1.28-4.92	14/154	1.0		1.0	
Quartile 2	4.93-6.24	19/151	1.47 (0.70-3.05)	NS	1.54 (0.72-3.27)	NS
Quartile 3	6.25-7.84	30/139	2.55 (1.28-5.10)	0.008	2.90 (1.41 -5.99)	0.004
Quartile 4	>7.84	25/143	2.01 (0.99-4.09)	0.053	2.31 (1.10-4.86)	0.027
linPFOS						
Quartile 1‡	0.61-2.56	14/156	1.0	1.0		
Quartile 2	2.57-3.30	16/153	1.23 (0.57-2.62)	NS	1.20 (0.55-2.63)	NS
Quartile 3	3.31-4.18	30/136	2.57 (1.30-5.09)	0.007	2.94 (1.45-5.95)	0.003
Quartile 4	>4.18	28/142	2.26 (1.13-4.52)	0.021	2.55 (1.24-5.27)	0.011
linPFHxS						
Quartile 1‡	0.10-0.40	15/150	1.0	1.0		
Quartile 2	0.41-0.55	25/145	1.79 (0.90-3.56)	NS	1.80 (0.88-3.69)	NS
Quartile 3	0.56-0.90	22/151	1.51 (0.74-3.07)	NS	1.63 (0.78-3.41)	NS
Quartile 4	>0.90	26/141	1.94 (0.97-3.86)	NS	2.33 (1.13-4.81)	0.023

‡ Reference group; *Logistic regression model adjusted for sex and age; **Model adjusted for sex, age, BMI, smoking, use of chewed tobacco, physical activity, socioeconomic variables (parent's educational level, unemployment and disability of parents, living with adoptive parents) and dietary variables (fish and junk food intake). OR = odds ratio, CI = confidence interval, NS = statistically non-significant.

Table 3. Associations of serum PFAS concentrations measured in the Tromsø study Fit Futures 1 (TFF1) with asthma in the 3 years follow-up study TFF2 (self-reported asthma cases n=91, current asthma n=67).

	Case/controls	Asthma		Case/controls	Current asthma	
	Number	OR (95% CI)*	p-value	Number	OR (95% CI)*	p-value
ΣPFAS						
Quartile 1‡	14/154	1.0		10/158	1.0	
Quartile 2	31/138	2.79 (1.36-5.71)	0.005	20/149	2.45 (1.05-5.72)	0.038
Quartile 3	26/143	2.37 (1.14-4.95)	0.021	21/148	2.76 (1.18-6.44)	0.019
Quartile 4	20/149	1.83 (0.85-3.94)	NS	16/153	2.24 (0.93-5.40)	0.074
ΣPFOS						
Quartile 1‡	16/152	1.0		13/155	1.0	
Quartile 2	25/145	1.61 (0.80-3.25)	NS	15/155	1.11 (0.49-2.52)	NS
Quartile 3	31/138	2.37 (1.18-4.72)	0.015	24/145	2.22 (1.04-4.74)	0.041
Quartile 4	19/149	1.36 (0.64-2.87)	NS	15/153	1.46 (0.64-3.34)	NS
linPFOS						
Quartile 1‡	17/153	1.0		14/156	1.0	
Quartile 2	20/149	1.09 (0.53-2.26)	NS	15/154	0.98 (0.44-2.21)	NS
Quartile 3	32/134	2.52 (1.30-4.89)	0.006	21/145	1.87 (0.89-3.93)	NS
Quartile 4	22/148	1.46 (0.72-2.98)	NS	17/153	1.50 (0.68-3.28)	NS

‡Reference group; * Logistic regression model adjusted for sex, age, BMI, smoking status, use of chewed tobacco, physical activity, socioeconomic variables (parent's educational level, unemployment and disability of parents, living with adoptive parents) and dietary variables (fish, junk food intake). OR = odds ratio, CI = confidence interval, NS = statistically non-significant.

Table 4. Associations of serum PFAS concentrations in the Tromsø study Fit Futures 1 (TFF1) with markers of severe asthma in the 3 years follow-up study TFF2.

	Cases/controls	Concentration	Clinically severe asthma†	
	Number	ng/mL	OR (95% CI)*	p-value
ΣPFAS				
Quartile1‡	5/163	2.6-8.59	1.0	
Quartile 2	13/156	8.6-10.71	2.71 (0.92-8.05)	0.072
Quartile 3	15/154	10.72-13.37	3.13 (1.06-9.23)	0.039
Quartile 4	6/163	>13.37	1.35 (0.39-4.66)	NS
	Cases/control	Concentration	FeNO > 25 ppb	
	Number	ng/mL	OR (95% CI)**	p-value
ΣPFAS				
Tertile 1‡	18/191	<9.39	1.0	
Tertile 2	32/176	9.39-12.35	1.82 (0.90-3.66)	NS
Tertile 3	31/186	>12.35	2.18 (1.08-4.42)	0.030
linPFHxS				
Tertile 1‡	19/189	<0.44	1.0	
Tertile 2	24/177	0.44-0.74	1.32 (0.65-2.68)	NS
Tertile 3	38/187	>0.74	2.13 (1.08-4.21)	0.030

† Self-reported severe breathing problems (defined as asthma wheezing >12 attacks in the last 12 months or self-scoring 7 or higher at the 10-scale score of breathing problems in the last 12 months).

‡Reference group; *Adjusted for age, sex, BMI, physical activity, smoking, chewed tobacco use, socioeconomic and dietary variables (fish and junk food intake). **Adjusted for age, sex, BMI, physical activity, smoking, chewed tobacco use, socioeconomic and dietary variables (fish and junk food intake), asthma/ allergic rhinitis medication. OR = odds ratio, CI = confidence interval, NS = statistically non-significant, FeNO = Fractional exhaled nitric oxide.

Table 5. Associations of serum PFAS concentrations in the Tromsø study Fit Futures 1 (TFF1) with self-reported nickel allergy in TFF1 study (n=94).

PFAS	Cases/controls	Nickel allergy	
	Number	OR (95% CI)*	p-value
ΣPFOS			
Quartile 1‡	19/149	1.0	
Quartile 2	21/149	1.35 (0.67-2.74)	NS
Quartile 3	28/141	2.31 (1.17 -4.56)	0.016
Quartile 4	26/142	2.40 (1.19-4.82)	0.014
linPFOS			
Quartile 1‡	17/153	1.0	
Quartile 2	19/150	1.56 (0.75-3.27)	NS
Quartile 3	30/136	2.71 (1.37-5.39)	0.004
Quartile 4	28/142	2.91 (1.43-5.93)	0.003

‡ Reference group; *Logistic regression model adjusted for sex, age, BMI, physical activity, smoking, chewed tobacco use, socioeconomic and dietary variables (fish and junk food intake). OR = odds ratio, CI = confidence interval, NS = statistically non-significant

Table S1. Socio-demographic characteristics of the study population. The Tromsø study Fit Futures.

Parameters	Girls n=355	Boys n=320	Difference** p-value
Living with adoptive parents	2.3%	1.3%	
Unemployed parents*	2.0%	2.5%	
Disabled parents*	10.1%	9.1%	
Education mothers:			
Primary school (9 years)	7.2%	5.8%	
High school/ occupational high school	38.3%	38.4%	
College	22.7%	22.6%	
University	31.8%	33.2%	
Education fathers:			
Primary school (9 years)	12.4%	10.4%	
High school/ occupational high school	40.3%	39.8%	
College	19.8%	18.6%	
University	27.5%	31.2%	

*One or both parents

Table S2. Association between PFAS concentrations in the Tromsø study Fit Futures 1 (TFF1) and Ip6 inhalation Ip6 panel IgE in the follow-up study TFF2.

	Cases/controls	Ip6 IgE≥0.35kUA/L	
	Number	OR (95% CI)*	p-value
ΣPFAS	175/500		
Quartile 1‡	53/115	1.0	
Quartile 2	45/124	0.54 (0.31-0.96)	0.035
Quartile 3	39/130	0.49 (0.27-0.87)	0.015
Quartile 4	38/131	0.59 (0.33-1.05)	0.074
ΣPFOS			
Quartile 1‡	52/116	1.0	
Quartile 2	42/128	0.58 (0.33-1.02)	0.06
Quartile 3	38/131	0.42 (0.23-0.76)	0.004
Quartile 4	43/125	0.60 (0.34-1.07)	NS
PFNA			
Quartile 1‡	54/115	1.0	
Quartile 2	48/120	0.71 (0.41-1.22)	NS
Quartile 3	34/134	0.41 (0.23-0.73)	0.003
Quartile 4	39/131	0.63 (0.35-1.3)	NS

‡ Reference group; *Logistic regression model adjusted for sex, age, BMI, socioeconomic variables (parent's educational level, unemployment and disability of parents, living with only one parent, with adoptive parents), dietary variables, physical activity, smoking, use of chewed tobacco, and asthma or allergic rhinitis medication.