IMPACT OF PFAS EXPOSURE ON HUMAN IMMUNITY

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PFASs: FOREVER CHEMICALS

Per- and polyfluoroalkyl substances (PFASs), from which PFOA and PFOS are the most abundant and studied, are widely used as **surfactants**. They are sometimes called forever chemicals as they are **very persistent** in the environment. PFASs have been found in the water, soil, sediment, biota and also in humans. They are transported to humans mainly through **contaminated food and water.** PFASs enter the environment from manufacture sites as well as by using product containing PFASs as firefighting foam, non-stick cookware, food packaging, waterproof textiles (1).

There have been reported **adverse health outcomes** linked to PFAS exposure. Epidemiological studies reported association of PFAS exposure with cancer, liver damage, cardiovascular problems, developmental adverse effects, and importantly **immune disruption** (2).

Immune disrupting effects associated with PFAS exposure have been described among children, adolescents, and adults (2). Strong evidence is reported for association of PFAS exposure with **suppressed antibody responses** after vaccination (3–6) and increased **risk of infectious diseases** (7). Further, **asthma** (5,8,9), **allergies** (8,10) and **autoimmune diseases** (11,12) associated with PFAS exposure have been described. In addition, an immune disorder can affect the axis of the neuroendocrine complex, thus the immunotoxicity of PFASs may cause deregulation of other organs and/or tissues (13).

What is the mechanism of immunomodulation induced by PFAS exposure?

A: It is still unknown and probably very complex. Our research revealed involvement of plasma B cells in the immunomodulation processes associated with PFAS exposure.

CELSPAC:Young Adults POPULATION STUDY

CELSPAC pro zdravou budoucnost

This work employs data from participants of CELSPAC: YA study (Central European Longitudinal Studies of Parents and Children: Young Adults) based in the **Brno region**. Participants underwent examinations including **blood collection**, measurement of height and weight, and filling **questionnaires** reporting characteristics such as health condition, diet, lifestyle and environment.

The population used for analysis consists of a comparable number of men and women, a total of **309 participants.** The majority of the population was investigated around 27 years old and generally of normal weight (BMI 18.5 – 24.9 kg/m2), non-smokers, with university education. The most prevalent immune-mediated disease was **pollen, dust, and mite allergy** (41%), followed by atopic eczema (15%), asthma (14%) and drug allergy (14%).

PFAS concentration in participants' serum were measured by high pressure liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS). PFOA and PFOS reached higher concentrations than other PFASs with a median of 1.11 ng/mL and 1.93 ng/mL respectively (Figure 1).

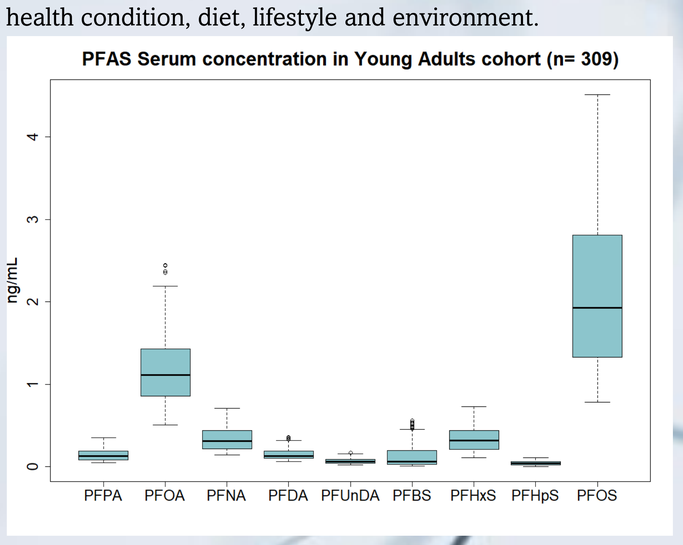
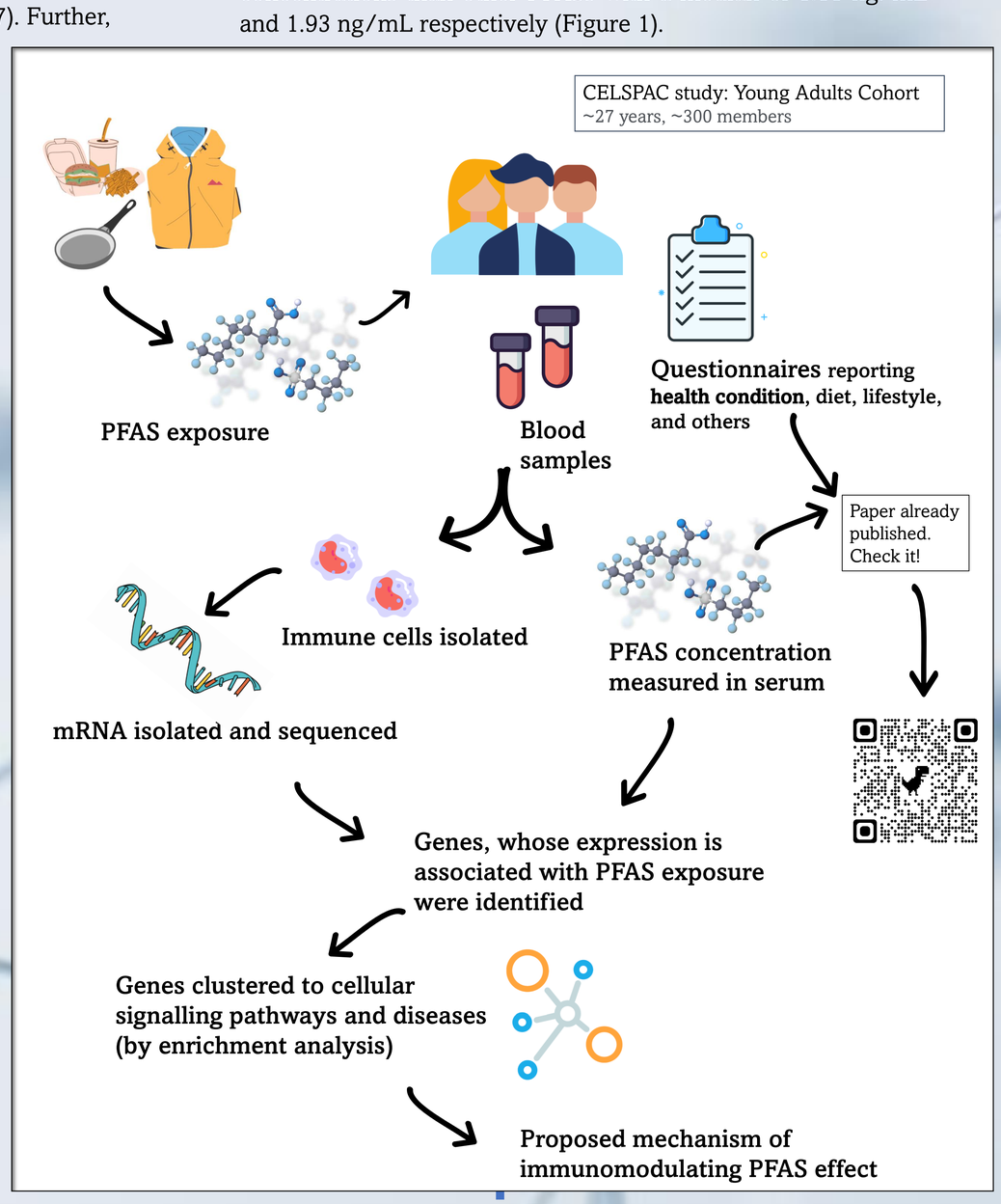


Figure 1: PFAS serum concentrations in CELSPAC: Young Adults cohort (n = 309). Perfluoropentanoate (PFPA), perfluorooctanoate (PFOA), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorobutane sulfonate (PFBS), pefluorohexane sulfonate (PFHxS), pefluoroheptane sulfonate (PFHpS), perfluorooctane sulfonate (PFOS).



Peripheral mononuclear cells were isolated from blood of participants and RNA was extracted from samples. The **quality of RNA** was checked (RNA integrity number (RIN) >7, A260/A230>1.8; A260/A280>1.8). Only high quality RNA was sequenced (QuantSeq library, Ilumina NovaSeq sequencing) to obtain ~10-15 mil. reads per sample.

- Genes, whose expression was associated with **PFAS exposure** were identified.
  - Genes, whose expression was associated **with at least 4 PFASs** were chosen, analysis was adjusted for relevant confounders.
  - These genes were employed for Gene Set Enrichment Analysis (GSEA) and Sub-Network Enrichment Analysis (SNEA) by Pathway Studio.
  - Enrichment analyses revealed involvement of **B cell signalling**, specifically mechanisms involved in the production of **antibody-secreting plasma B cells** (Figure 3). These processes probably play a key role in PFAS-induced immunomodulation.
- Further, gene expression associated with each individual PFAS for the whole population and also separately for the **men and women** was identified and these genes were clustered to the pathways by enrichment analysis (data not shown).

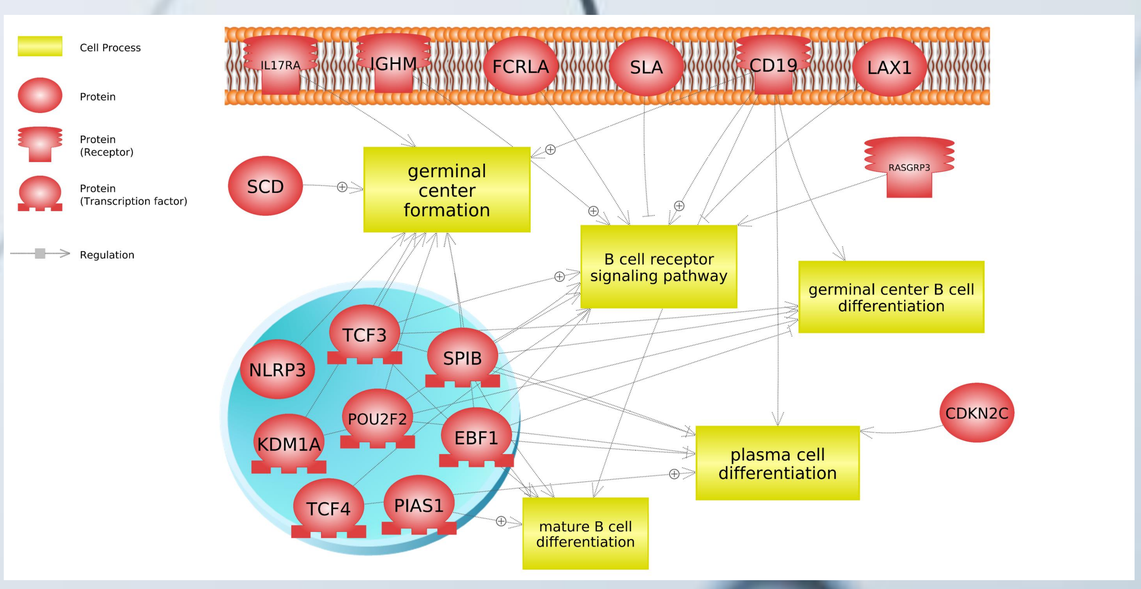


Figure 3: Cell processes enriched by set of genes associated with at least 4 PFASs by Sub-Network Enrichment Analysis.

Does PFAS exposure have an impact on the prevalence of immune-mediated diseases?

A: Yes, we observed a negative association between PFAS exposure and immune-mediated diseases in our population.

We employed **logistic regression** to reveal impact of individual PFASs and **Bayesian kernel machine regression** (BKMR) to reveal impact of PFASs as a mixture

- When assessing individual PFAS exposure, we observed **negative association** between PFAS exposure and **eczemas** and **inhalant allergy** (Table 1).

	PFOA	PFOS
Atopic eczema	**0.58 (0.37-0.90)**	**0.56 (0.32-0.95)**
Contact dermatitis	**0.37 (0.16-0.85)**	**0.33 (0.11-0.94)**
	PFUnDA	
Pollen, dust, and mite allergy	**0.62 (0.43-0.89)**	

Table 1: The association between individual PFASs and immune-mediated diseases; results are expressed **as OR (95% CI) per IQR increase**. The model was adjusted for sex, age, BMI, smoking, education, and family history. OR = odds ratio, CI = confidence interval, IQR = interquartile range

- A **negative trend** was also observed in output of BKMR model assessing effect of 9 PFASs mixture.
- Interestingly, we observed **sex-specific difference** between men and women in the association of PFAS exposure and atopic eczema, based on both OR (data not shown) and BKMR results (Figure 2).

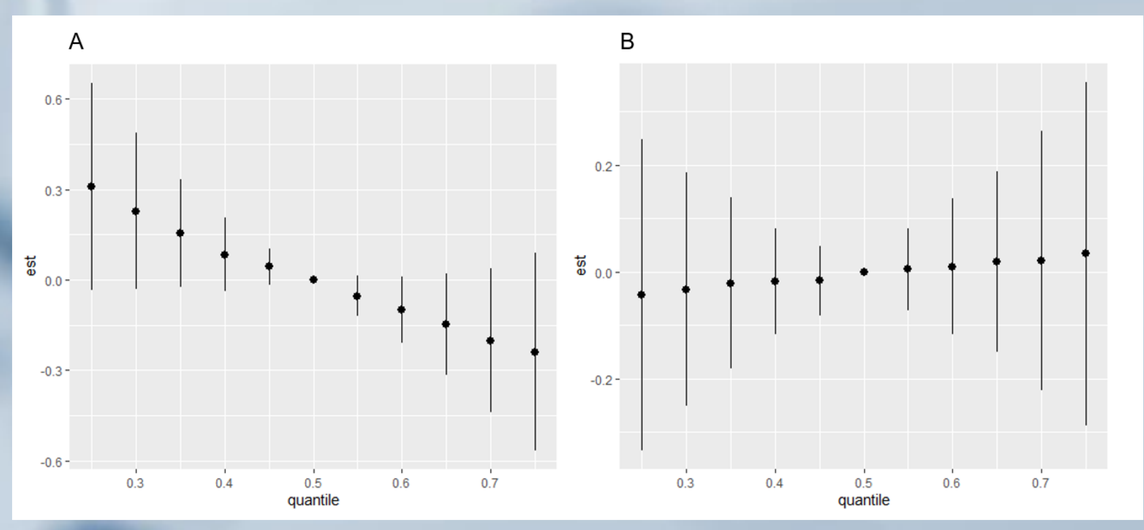


Figure 2: The overall effect of the PFAS mixture on atopic eczema separately in women (A) and men (B) depicted in quantile graphs by BKMR model. The quantile graph shows the estimated change in effect when all the exposures at a particular percentile are compared to when all the exposures are at their 50th percentile. The estimate is accompanied by 95% CI.

TAKE HOME MESSAGE

- We performed genome-wide sequencing of blood immune cells of Czech adults
- 167 genes were associated with multiple PFAS exposure (associated with at least 4 PFAS)
- Signalling pathways responsible for maturing of B plasma cells were identified by enrichment analyses based on genes associated with multiple PFAS exposure
- PFAS exposure is negatively associated with prevalence of immune mediated diseases in Czech adults
- Sex-specific difference were observed in the association of PFAS exposure and atopic eczema

The CELSPAC: YA study was approved by the ELSPAC Ethics Committee (Ref. No: ELSPAC/EK/2/2019, dated March 13, 2019).
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References: 1. Silva AOD, Armitage JM, Bruton TA, Dassuncao C, Heiger-Bernays W, Hu XC, et al. PFAS Exposure Pathways for Humans and Wildlife: A Synthesis of Current Knowledge and Key Gaps in Understanding. Environ Toxicol Chem. 2021;40(3):631–57. , 2. Agency for Toxic Substances and Disease Registry (ATSDR) (2021). Toxicological profile for Perfluoroalkyls. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service., 3. Kielsen K, Shamim Z, Ryder LP, Nielsen F, Grandjean P, Budtz-Jørgensen E, et al. Antibody response to booster vaccination with tetanus and diphtheria in adults exposed to perfluorinated alkylates. J Immunotoxicol. 2016;13(2):270–3. , 4. Looker C, Luster MI, Calafat AM, Johnson VJ, Burleson GR, Burleson FG, et al. Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. Toxicol Sci Off J Soc Toxicol. březen 2014;138(1):76–88. , 5. Rappazzo KM, Coffman E, Hines EP. Exposure to Perfluorinated Alkyl Substances and Health Outcomes in Children: A Systematic Review of the Epidemiologic Literature. Int J Environ Res Public Health. 27. červen 2017;14(7). , 6. Timmermann CAG, Jensen KJ, Nielsen F, Budtz-Jørgensen E, van der Klis F, Benn CS, et al. Serum Perfluoroalkyl Substances, Vaccine Responses, and Morbidity in a Cohort of Guinea-Bissau Children. Environ Health Perspect [Internet]. 10. srpen 2020 [citován 16. červen 2021];128(8). Dostupné z: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7416537/, 7. Dalsager L, Christensen N, Halekoh U, Timmermann CAG, Nielsen F, Kyhl HB, et al. Exposure to perfluoroalkyl substances during fetal life and hospitalization for infectious disease in childhood: A study among 1,503 children from the Odense Child Cohort. Environ Int. 1. duben 2021;149:106395. , 8. Averina M, Brox J, Huber S, Furberg AS, Sørensen M. Serum perfluoroalkyl substances (PFAS) and risk of asthma and various allergies in adolescents. The Tromsø study Fit Futures in Northern Norway. Environ Res. 1. únor 2019;169:114–21. , 9. Dong GH, Tung KY, Tsai CH, Liu MM, Wang D, Liu W, et al. Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case-control study of Taiwanese children. Environ Health Perspect. duben 2013;121(4):507–13. , 10. Buser MC, Scinicariello F. Perfluoroalkyl substances and food allergies in adolescents. Environ Int. 1. březen 2016;88:74–9. , 11.Steenland K, Zhao L, Winquist A. A cohort incidence study of workers exposed to perfluorooctanoic acid (PFOA). Occup Environ Med. 1. květen 2015;72(5):373–80. , 12.Steenland Kyle, Zhao Liping, Winquist Andrea, Parks Christine. Ulcerative Colitis and Perfluorooctanoic Acid (PFOA) in a Highly Exposed Population of Community Residents and Workers in the Mid-Ohio Valley. Environ Health Perspect. 1. srpen 2013;121(8):900–5. , 13. Neagu M, Constantin C, Bardi G, Duraes L. Adverse outcome pathway in immunotoxicity of perfluoroalkyls. Curr Opin Toxicol. 1. březen 2021;25:23–9.