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No evidence of intrauterine sensitization against inhalant allergens

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48 **Summary:**

49 Specific IgE against inhalant allergens in cord blood was a very rare finding when the risk of
50 transfer from the mother was minimized; hence, findings of such IgE should be interpreted
51 with caution.

52 **Keywords:** Cord blood; Infant; Intrauterine; Atopy, IgE

53 **Abbreviations:** CB – Cord Blood; MB – Maternal Blood; IgE – immunoglobulin E

54

To the Editor:

Allergen specific IgE can be detected in cord blood (CB), but the origin and relevance of this finding is controversial. We previously reported that specific IgE in CB against inhalant allergens mainly seemed to be the result of transfer of maternal IgE to the fetus¹, while others suggested that such IgE is of fetal origin²⁻⁶, thus representing intrauterine sensitization. This distinction is important: intrauterine sensitization could be targeted by prenatal intervention and would suggest CB specific IgE as a relevant disease marker in the newborn, while maternally transferred IgE, either as a sampling artifact or due to transplacental transfer, would have no independent value as disease marker and no long term consequences for allergy in the offspring.

Here, we aimed to test the hypothesis that specific IgE against inhalant allergens in CB is of maternal origin. We hypothesized that applying strict sampling procedures and including unselected women with low levels of IgE would result in limited, if any, specific IgE in CB.

Women with singleton pregnancies were invited to participate in the study, as previously described⁷. The investigation conformed to the Declaration of Helsinki and was approved by the Scientific Ethics Committee and the Danish Data Surveillance Authority.

Blood was taken from the umbilical vein by needle puncture performed by a single investigator. Samples were available for 260 maternal blood (MB)-CB pairs. IgE antibody levels were determined using the ImmunoCAP assay (Phadia AB, Uppsala, Sweden). MB and CB were analyzed for levels of specific IgE against a mixture of common ImmunoCap inhalant and food allergens (lower detection limit 0.1 kUa/L) and total IgE (lower detection limit 0.1 kU/L using a combination of the standard and low level total IgE assays). IgA in CB samples were analyzed by ELISA (detection limit: 0.1 µg/L). Further information on methodology can be found in the online repository.

An elevated IgA level in CB ($>50 \mu\text{g/L}$) was considered indicative of contamination with MB^{1,8}. This was found in only 3 (1%) of the CB samples compared to 21 samples (9%) in our previous study⁸. High levels of specific IgE in MB (sum of specific IgE $>100 \text{ kUa/L}$) was found in 4 samples (2%) compared to 12 samples (5%) in our previous study¹ (**Table I**). Correspondingly, specific IgE against inhalant allergens ($\geq 0.1 \text{ kUa/L}$) was found in only 2 CB samples ($<1\%$) compared to 22 (9%) in our previous study¹ (**Table I and Figure 1**). In **Figure 1** the relationship between specific IgE against inhalant allergens in CB and MB is depicted. We have included prediction lines for the level of specific IgE in CB in relation to the level in MB stratified by level of CB IgA based upon results from our previous study¹. As predicted, specific IgE against inhalant allergens in CB was only present when either the level of CB IgA was very high or the corresponding maternal sample had highly elevated levels of specific IgE. For both CB samples with specific IgE against inhalant allergens, corresponding IgE against the same allergen was found in the maternal sample (**Figure E1**). Specific IgE against food allergens (milk) was found in 3 CB samples. For these, there were no sign of transfer from the mother since the corresponding specific IgE was not detectable in MB.

Levels of total IgE in CB and MB correlated significantly ($p < 0.0001$) (**Figure E2**). Total IgE was detectable in 100% of CB samples ($N = 31$) with maternal levels $>125 \text{ kU/L}$ compared to only 66% of samples with maternal levels $<60 \text{ kU/L}$. Further results can be found in the online repository.

In accordance with our hypothesis, we found a very low frequency of specific IgE against inhalant allergens in CB after applying strict sampling procedures and predominately including women with low levels of specific IgE. Based upon the current study and our previous studies^{1,8}, we hypothesize two different mechanisms of transfer of IgE from MB to CB. First, there seems to be an IgA-associated mechanism. IgA does not pass the placental barrier, and a high level of IgA in CB is expected to reflect contamination with MB. Our current results support such (preventable) mechanism taking place during CB sampling,

since strict sampling procedures performed by a single researcher, rather than sampling done by many different midwives, resulted in just 1% of the samples with IgA levels in the range interpreted as contamination ($>50 \mu\text{g/L}$), compared to 9% in our previous study⁸. Second, we suggest a mechanism of transfer of IgE from the mother to the fetus that is not IgA-associated and not related to CB sampling procedures. This is based on our previous findings that very high maternal levels of IgE (specific or total) always resulted in detectable IgE in CB independent of CB IgA^{1,8} and such mechanism is further supported by the current study where we similarly observed that total IgE in CB always was detectable when the mother had very high levels of total IgE ($>125 \text{ kU/L}$). Combining the results from our 2 studies, we find that IgE in cord blood was detectable in all of 84 samples with a corresponding maternal level $>145 \text{ kU/L}$. Such consistent relationship seems more compatible with maternofetal transfer than a biological mechanism of fetal IgE production. The possibility of such mechanism is further supported by a recent study demonstrating that transplacental transport of IgE can occur with IgE bound to an IgG-anti-IgE immune-complex⁹. Our results contrast other studies claiming that CB IgE is of fetal origin²⁻⁶. Pfefferle et al.⁶ found detectable specific IgE against inhalant allergens in 11% of CB samples without correlation to presence of such IgE in MB. Limitations to that study include: IgA was only measured in a subset of samples, and a much higher level of IgA was used as indicator of contamination. Another possible explanation for the discrepancy between studies is the use of different methods for IgE measurement (ImmunoCAP, Thermofisher vs. Allergy Screen, Mediwiss Analytic). We found 3 CB samples with elevated specific IgE against milk and no sign of transfer from the mother. This is in contrast to our previous study where no such IgE was found in CB¹. CB IgE against milk could be due to cross-reactivity with fetal proteins, but we cannot rule out the possibility of (rare) fetal synthesis of IgE against food allergens.

CB IgE is currently used as a surrogate marker of later sensitization and it is therefore crucial to be able to discriminate between IgE transferred from the mother and synthesis by the fetus. Our data strongly suggest that specific IgE against inhalant allergens in CB is of maternal origin. Findings of such IgE in previous and future studies should therefore be interpreted with caution.

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187 **Table I:** IgA and specific IgE in CB and MB in the present study compared to a previous study*.

	Present study	Previous study*
	N = 260 (%)	N = 243 (%)
IgA in CB (>50 µg/L)	3 (1)	21 (9)
Maternal specific IgE >100 kUa/L	4 (2)	12 (5)
Specific IgE in CB, inhalant allergens (kUa/L)	2 (1)	22 (9)
Specific IgE in CB, food allergens (kUa/L)	3 (1)	1 (1)

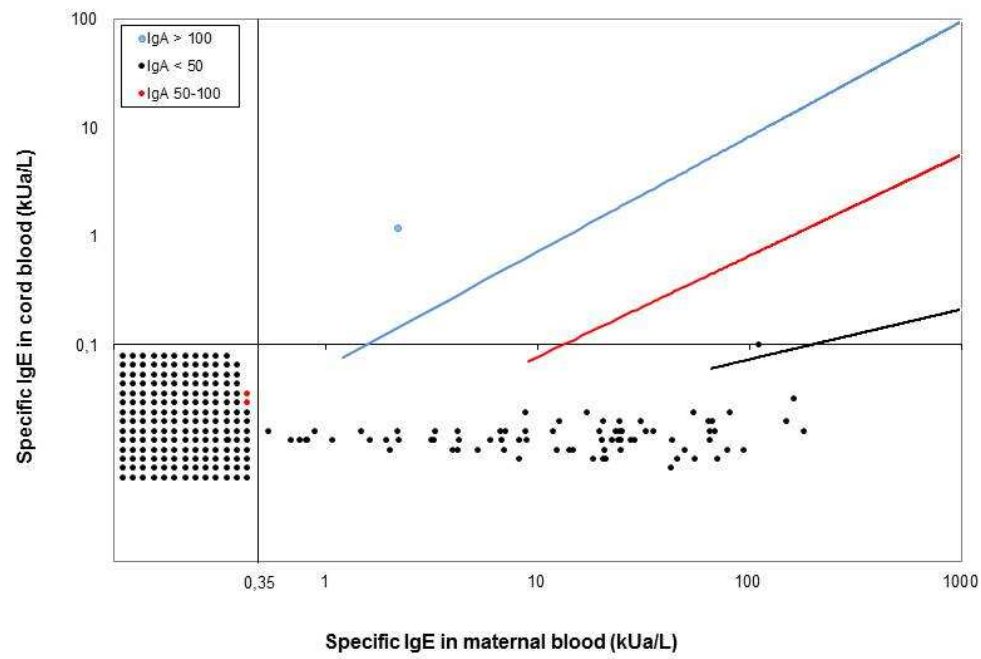
188 *Bønnelykke K, Phipps CB, Bisgaard H. Sensitization does not develop in utero. J Allergy Clin
 189 Immunol 2008;121:646–51

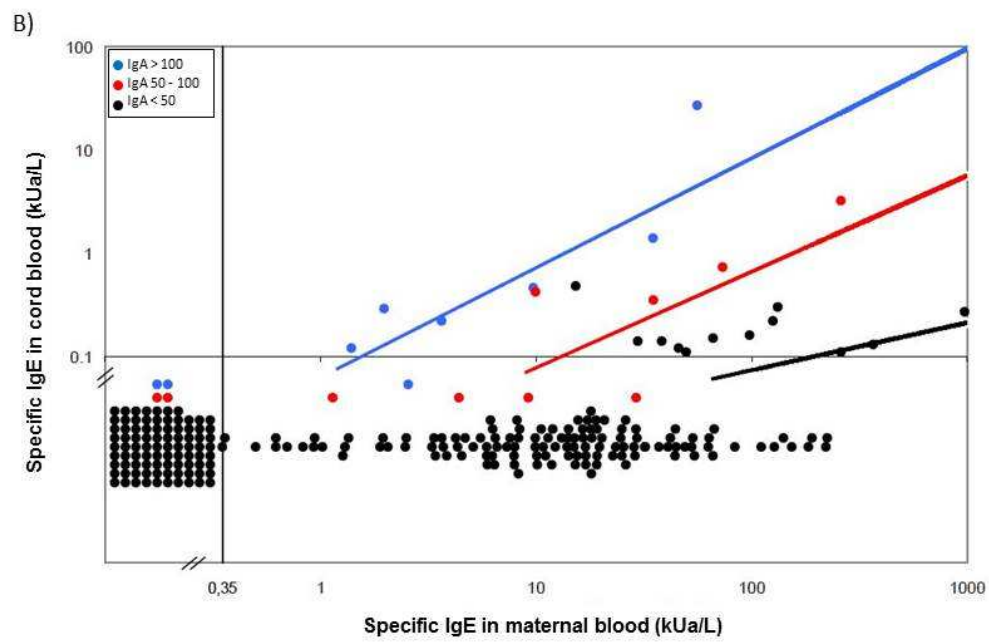
190 **Figure legend**

191 **Figure 1:** Specific IgE in CB (sum of single inhalant allergens) against specific IgE in MB (sum of
192 inhalant allergens). The regression lines are shown for each level of IgA and stratified from our
193 previous work*. **A)** Present study and **B)** Previous study for comparison*.

194

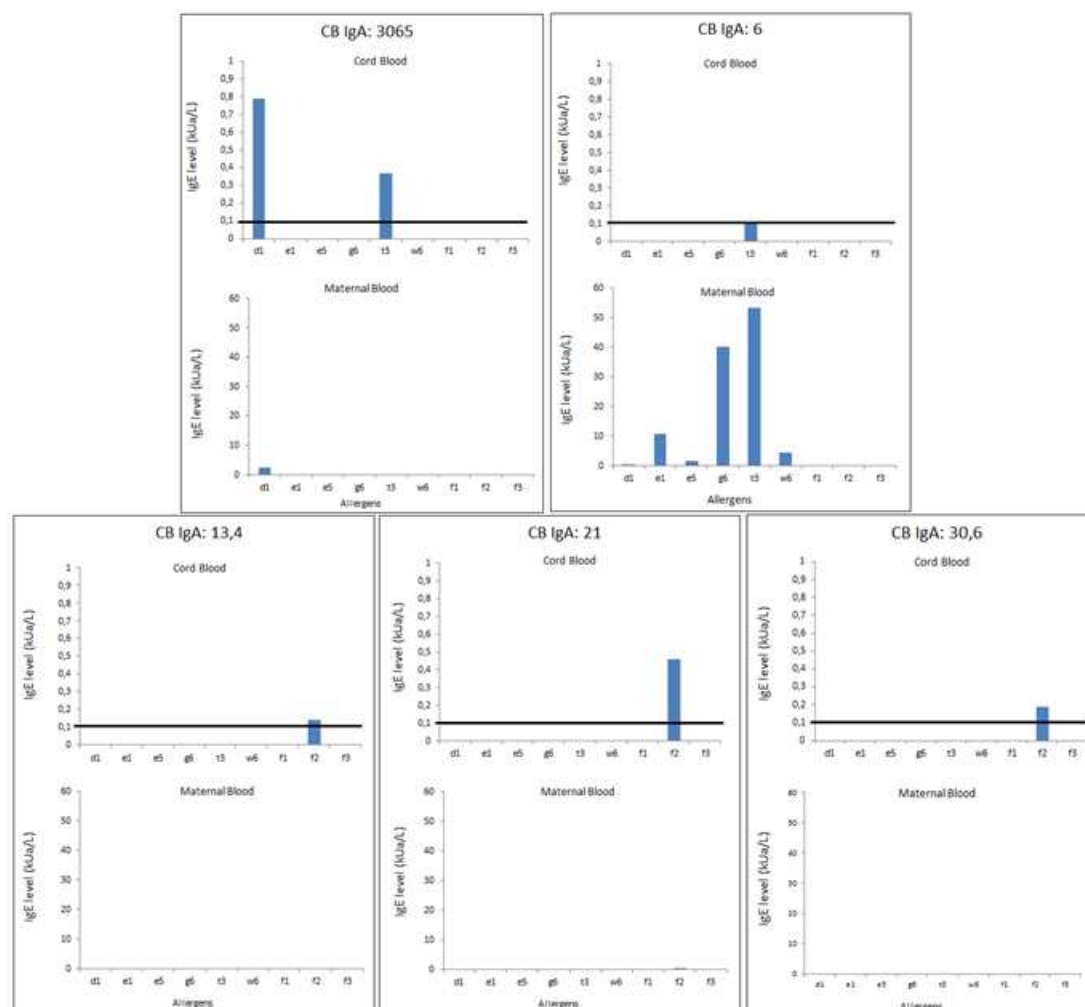
A)





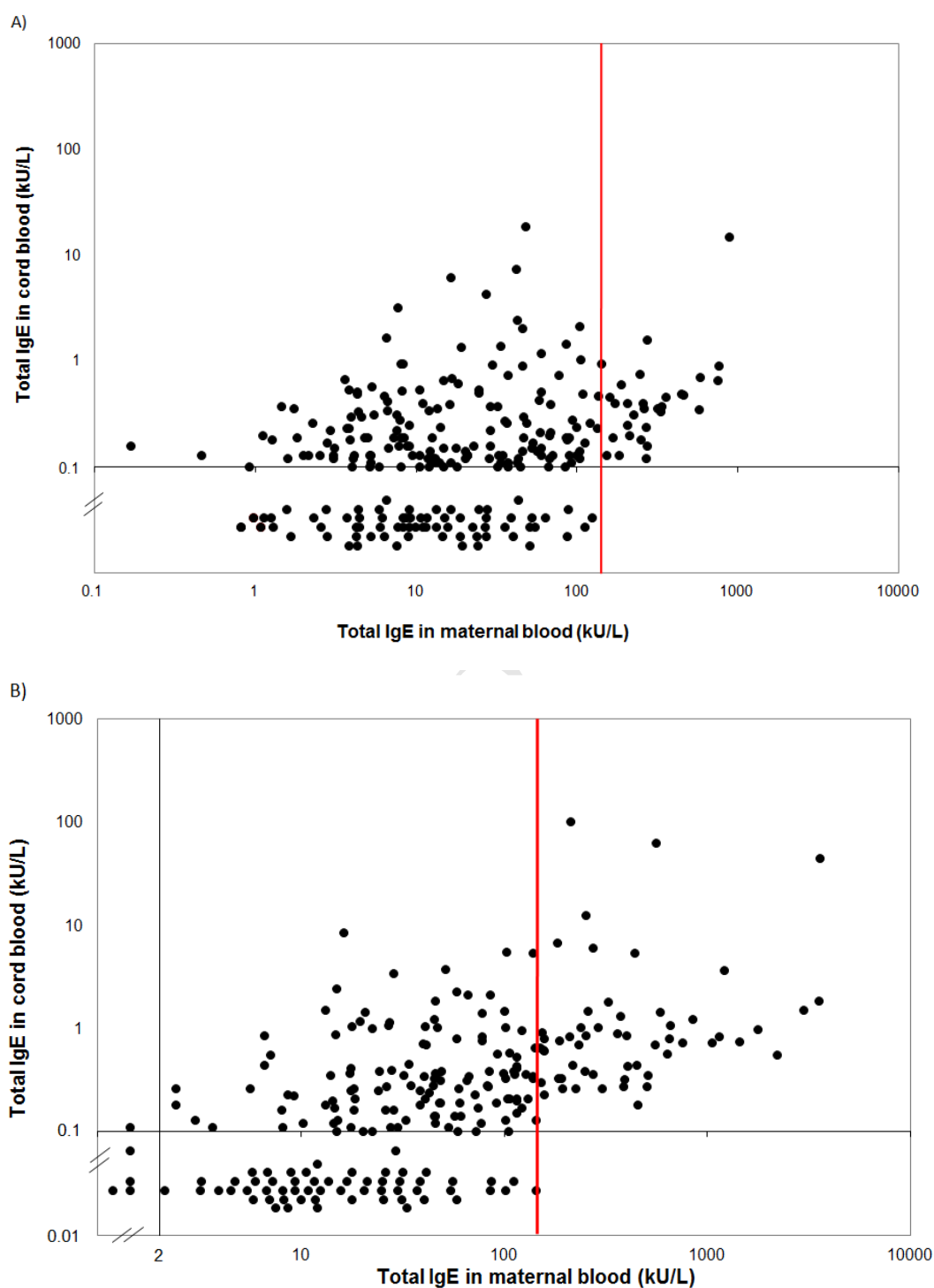
*Bønnelykke K, Phipp CB, Bisgaard H. Sensitization does not develop in utero. *J Allergy Clin Immunol* 2008;121:646–51.

- 1 **Figure E1:** Patterns of specific IgE against inhalant allergens in individual mother–cord blood pairs.
- 2 The *black line* represents the detection limit of specific IgE in cord blood (0.1 kUa/L).



- 3
- 4 Allergens: d1: *D. pteronyssinus*, e1: Cat epithelium and dander, e5: dog dander, g6: timothy grass, t3: common
- 5 silver birch, w6: mugwort, f1: Egg white, f2: Milk, f3Fish (cod)
- 6 CB: Cord blood.
- 7

Figure E2: Total IgE in cord blood against total IgE in maternal blood. **A)** Present study, **B)** Previous study for comparison*. The red line illustrates that, in both studies, all samples with maternal IgE above 145 IU/mL resulted in detectable levels in cord blood. Values are log transformed.



* Bønnelykke K, Pipper CB, Bisgaard H. Transfer of maternal IgE can be a common cause of increased IgE levels in cord blood. *J Allergy Clin Immunol* 2010;126:657–63.

Online supplement:

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METHODS

Study participants:

A total of 756 randomly selected women with singleton pregnancies admitted to the Department of Obstetrics and Gynecology, were invited to participate in the study (June 2003 to April 2004) as previously described¹. Women with diabetes mellitus, multiple gestations, a previous pregnancy with stillbirth, intrauterine growth restriction, or congenital malformations were not invited to participate. At the antenatal visit at ~12 weeks of gestation, 557 of the 756 women gave informed and written consent. The investigation conformed to the principles outlined in the Declaration of Helsinki and was approved by the Scientific Ethics Committee (H-1-2009-106) and the Danish Data Surveillance Authority (2015-41-3879).

Of the 557 women 116 did not meet the inclusion criteria, and 175 women gave birth when the investigator (MRA) was unavailable; leaving 266 women for inclusion. Six blood samples were not obtained by needle puncture from the fetal umbilical vein immediately after placental delivery, leaving 260 women and their newborns in the main study.

Sampling of cord blood and maternal blood:

Maternal blood (MB) samples were taken at either ~12, ~20, or ~39 weeks of gestation¹. To avoid contamination, sampling of cord blood (CB) was done by one well-trained person; blood was taken from the umbilical vein by needle puncture.

Determination of serum total IgE, specific IgE, and total IgA:

IgE antibody levels were determined using the ImmunoCAP assay (Phadia AB, Uppsala, Sweden)². CB was analyzed for levels of total IgE and specific IgE against a mixture of common inhalant and food allergens (Phadiatop Infant)³.

Samples positive for Phadiatop Infant (≥ 0.1 kUa/L) were further analyzed for specific IgE against relevant single allergens from this panel (*Dermatophagoides pteronyssinus*, cat dander, dog dander, birch, timothy, mugwort, peanut, cow's milk, and hen's egg). The detection limit for total and specific IgE was 0.1 kUa/L. Specific IgE results in CB were double-tested. MB was analyzed similarly for levels of total IgE and specific IgE against the same inhalant and food allergens after screening with Phadiatop and fx5. A screening value ≥ 0.35 kUa/L was considered positive. Specific IgE against single allergens in MB was analyzed irrespective of the screening result if such IgE was found in the corresponding CB sample. The lower detection limit was 0.1 kUa/L for allergen-specific IgE and 0.1 kU/L for total IgE (by using a combination of the standard and low level total IgE assays with lower detection limits of 2.0 and 0.1 kU/L, respectively).

CB samples were analyzed for total IgA by using a sensitive ELISA assay designed to measure low levels of IgA (detection limit, 0.1 µg/L; analyzed by *Phadia AB*). A CB level of IgA >50 µg/L was considered indicative of transfer of MB to CB based upon results from our previous studies using the same IgA methodology^{5,6}. In these previous studies it was seen that for cord blood samples with IgA levels > 50 µg/L there were a clear association between cord blood IgA and IgE, which was not seen at lower levels of IgA. This was further supported by a shift upwards in the cord blood vs maternal IgE regression lines for IgA levels greater than 50 µg/L.

Statistical analysis:

We modeled the underlying association between specific IgE and total IgE in CB and MB by linear regression and by assuming an underlying normal distribution. By extracting detection limit for IgA calculated in a previous study we analyzed for the association between specific IgE in CB and MB for each level of CB IgA. Maximum likelihood estimates with asymptotic 95% Wald Confidence Intervals (CI) were calculated, and likelihood ratio tests for hypotheses were performed. All values were transformed on a logarithmic scale.

Analyses were carried out using SAS version 9.3 (SAS Institute, Cary, NC).

RESULTS

The median level (range) of IgA in CB was 10.6 (0-3065) µg/L.

Total IgE in CB was detectable in 73% (N = 190) of the samples. Any specific IgE in MB was found in 80 samples (31%).

Specific IgE against inhalant allergens (≥0.1kUa/L) was found in 2 CB samples (<1%). One sample had detectable IgE against two inhalant allergens (common silver birch and *D. pteronyssinus*) one sample had detectable levels against one inhalant allergen (*D. pteronyssinus*). Two CB samples with an intermediary level of IgA (between 50-100 µg/L) indicative of some degree of contamination, had

non-detectable specific IgE in CB, which is in accordance with the prediction lines, since the corresponding maternal sample was negative for specific IgE (**Figure 1**). The comparison of single specific IgE against inhalant allergens in CB and MB is shown in Supplementary **Figure E1**, for the samples with detectable specific IgE in CB. The two samples with IgE against inhalant allergens in CB corresponded to a positive detection in the maternal sample.

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