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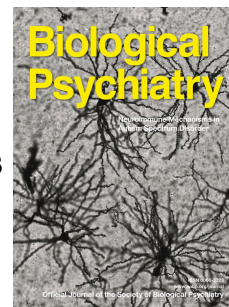
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ABSTRACT

Background: Recent research suggests that immune dysregulation in pregnancy could be a risk factor for anxiety/depression symptoms in offspring. Whereas animal studies have demonstrated the importance of the link between perinatal cytokines and abnormal behaviors in offspring, human epidemiological studies in this area remain limited. The objectives of the study were to describe the network of cord serum cytokines at birth and test whether they are associated with subsequent anxiety/depression symptom trajectories in offspring.

Methods: We used data and biological samples from 871 mother-child pairs followed up from pregnancy to 8 years of age and participating to the French mother-child cohort EDEN. Cord serum cytokines were measured at birth. Children's symptoms of anxiety/depression were assessed with the emotional difficulties subscore of the Strength and Difficulties Questionnaire at ages 3, 5 and 8 years, from which trajectories of anxiety/depression symptoms were derived.

Results: Results showed a significant association between cord serum interleukin (IL)-7 at birth and the trajectory of children's anxiety/depression symptoms between ages 3 to 8 years (adjusted Odds Ratio (aOR)=0.73, 95%CI=(0.57, 0.93). The associations considered relevant confounders, including prenatal maternal depressive symptoms.

Conclusions: Early immune changes may contribute to subsequent anxiety/depression symptoms in childhood. Beyond the understanding of mechanisms underlying the occurrence of emotional difficulties in children, our findings open avenues for future research in human and animals.

INTRODUCTION

The fetal origin of neurological and mental illness has long been known through the severe impact of in utero exposure to infectious teratogenic agents on the developing fetal brain (e.g. Toxoplasma, Rubella, Cytomegalovirus, Herpes, Influenza)(1,2). The risk these infections carry for direct fetal cerebral damage, leading to cognitive, socio-emotional, motor and sensory deficits, makes them the cornerstone of the primary obstetric focus on infection prevention. Recently, less severe common infections and immune system dysfunctions during gestation have also been shown to have an adverse impact on fetal brain development in the long term, indirectly through immune activation and irrespective of etiology(3).

A compelling body of animal and human research connects infectious and non-infectious inflammation processes in pregnancy (e.g. maternal history of autoimmune disorder, atopia, obesity, psychosocial stress, depression) to a range of adverse neuropsychiatric outcomes across offspring's life course(4,5). In humans, beyond schizophrenia and autism spectrum disorders, exposure to maternal infection in pregnancy has been linked to the excess risk of the most common psychiatric conditions, namely depression and anxiety(6–9). The strongest evidence regarding the contribution of in utero immune function to later risk of anxiety/depression-like symptoms comes from preclinical research. Animal models (i.e. rodents and primates) showed that patterns of immune activation/dysfunction in pregnancy, irrespective of their timing, predict offspring behavioral and cerebral anomalies compatible with anxiety/depression(10–14). These latter anomalies are varied, encompassing neuroinflammation with activation of microglia and astrocytes, oligodendrocyte dysfunction, neuronal loss, dysgenesis of serotonergic neurons, and dysregulation of the hypothalamic-pituitary-adrenal-axis(3,15,16). Interestingly, the role of the placental-fetal immune response may be key. In normal gestation, the placental-fetal unit is physiologically under the influence of the immune system and any disruption may have pathological consequences(17). In addition, immune dysregulation in pregnancy modulates placental serotonin secretion, which in turn modifies serotonin concentrations in the fetal brain(3,4,15). Placental-fetal blood (as assessed in the umbilical cord) hence represents a relevant biological material to investigate immune biomarkers at birth. Among these biomarkers, cytokines may be crucial because their balance regulates not only the immune system but also plays a critical role with regard to brain function, neurodevelopment, neurogenesis and neurotransmission, with cytokine receptors being expressed in microglia, astrocytes and neurons(18). In particular, Interferon (IFN)- γ , IL-1 β , IL-6, IL-8, IL-10, IL-17A, and Tumor Necrosis Factor (TNF)- α are thought to play a role in the association between immunity and mental/physical health. According to the neuroimmune network hypothesis (19), early-life

adversities could be bidirectionally related to later behavior, brain structures and functions through immune dysregulation processes.

Current knowledge regarding the interaction between cytokines at birth and behavior later in life is limited. First, while there is lack of human data, extrapolations from animal models to humans regarding mental health can be speculative. Second, epidemiological data have hardly considered the relationship between perinatal immune biomarkers as assessed in the cord serum and anxiety/depression in offspring, despite its pathophysiological pertinence. Third, by focusing on individual cytokines, studies have disregarded cytokine networks, thereby overlooking potentially important information regarding their interrelationships. Fourth, no study has explored the emergence of anxiety/depression symptoms and their trajectory in childhood in relation to *in utero* immune function, yet emotional disturbances may begin early on, especially in the event of antenatal exposures. Overcoming these shortcomings is critical to understanding the mechanisms associating perinatal immunity and subsequent anxiety/depression in offspring.

Using longitudinal data from a birth cohort study, we aimed: (1) to describe the network of cord serum cytokines and its links with the trajectories of anxiety/depression symptoms in offspring from age 3 to 8 years; (2) to test whether cord serum cytokines are associated with subsequent trajectories of anxiety/depression symptoms in offspring.

METHODS

Study design and participants

The study sample is nested within the EDEN cohort which is a mother-child cohort study investigating pre-/postnatal determinants of child development and health(20). Pregnant women (N=2002) were recruited before 24 weeks of amenorrhea in the maternity clinics of Nancy and Poitiers (France, 2003-2005). We then included 1907 newborns. Exclusion criteria were multiple pregnancy, pre-pregnancy diabetes, illiteracy, and planning to move outside the region within three years. At birth, cord serum samples were collected for 1427 mother-child pairs. Data collection was conducted in multiple waves (pregnancy, at the child's birth, 4/8/12/24 months, 1/2/3/5/8 years). Participating mother-child pairs at ages 1, 2, 3, 5 and 8 years of the child were respectively 1717, 1611, 1527, 1255 and 883. Data were collected from medical records (pregnancy, birth) by trained interviewers (pregnancy, birth, 1/2/3/5/8 years) and by mothers' and fathers' self-reports (all waves). Informed written consent from the parents was obtained at enrollment. The EDEN cohort was approved by the Bicêtre Hospital ethics committee and the Commission Nationale Informatique et Libertés. The initial EDEN sample was similar to a nationally representative French sample of pregnant women, except for its higher educational attainment(20,21). The current study sample

comprised the subset of 871 participants for whom both cord blood serum and anxiety/depression trajectories were available. This subset differed from the initial sample in comprising fewer families in which the mother had a low educational level and low income, was younger, did not live with the child's father, smoked during pregnancy, had more depressive symptoms in pregnancy, and whose child had a lower birth weight. However, the significant effect sizes were small to very small (**Table S1**).

Measures

Outcome variable: children's anxiety/depression trajectories from 3 to 8 years

Children's symptoms of anxiety/depression were assessed through the Strengths and Difficulties Questionnaire (SDQ) completed by mothers when children were aged 3, 5 and 8 years(22). This validated tool includes an emotional symptoms subscale comprising the following five items: "often unhappy, depressed or tearful", "nervous or clingy in new situations, easily loses confidence", "many fears, easily scared", "often complains of headaches, stomach-aches or sickness", "many worries or often seems worried". All items refer to the past six months or the current school year and are scored 0 (never), 1 (sometimes true) or 2 (certainly true). At each time-point, a quantitative score (range 0-10) was obtained by summing the items. We conducted latent class mixed-effect models for nonlinear outcomes to model anxiety/depression symptoms trajectories at 3, 5 and 8 years. Changes in anxiety/depression symptoms scores were modeled using quadratic splines with five internal knots (at quantiles of time). The statistical model included an intercept that represented the anxiety/depression symptoms score at baseline, the quadratic mean trajectory for the latent process and correlated random intercept and slope to account for intra-individual variability (the random quadratic slope was removed as the model did not converge). The SDQ data were available as follows: N=797 at 3 years, N=871 at 5 years, N=869 at 8 years. We identified five groups of symptoms (**Figure S1**). For further analyses, symptoms of anxiety/depression were dichotomized (high symptoms vs other groups, n=135 (15.50%) and 736 (84.50%), respectively). The other groups were merged because they likely corresponded to clinically non-problematic groups.

Main exposure: cord serum cytokines

Cord serum concentrations of cytokines were determined for the 871 individuals of the study sample. Concentrations of Chemokine (C-C motif) ligand (CCL) 2, CCL3, CCL4, CCL11, CCL13, CCL17, CCL26, C-X-C motif chemokine 10 (CXCL10), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interferon (IFN)- γ , Interleukin (IL)-1 α , IL-1 β , IL-2, IL-4, IL-5, IL-6,

IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17A, Tumor Necrosis Factor (TNF)- α , and TNF- β were initially measured. More details are available in **eSupplement 1**.

Covariates

Parental characteristics at baseline included maternal and paternal education (less than 12 years, between 12 and 14 years, and more than 14 years), maternal age at inclusion (years), maternal pre-pregnancy body mass index (BMI) (low –less than 19kg/m²-, normal – between 19 and 25kg/m²-, and high –more than 25kg/m²), parental separation during pregnancy (biological father not living with the mother and child (yes/no)), and family income at birth (mean of the eight class numbers, (1):<450€/month, (2):450-800€/month, (3):800-1500€/month, (4):1500-2300€/month, (5):2300-3000€/month, (6):3000-3800€/month, (7):3800-4500€/month, (8):>4500€/month). *Pregnancy characteristics* included maternal alcohol intake (>2 glasses per week/0-2 glasses per week), maternal daily smoking throughout pregnancy (mean number of cigarettes), maternal daily caffeine intake (0 / 0-100mg / 100-200mg / 200mg+), multiparity (firstborn/other), maternal depression (depressive symptoms measured with the Center for Epidemiologic Studies Depression Scale Revised, CES-D (mean score)(24)), diagnosed gestational diabetes (yes/no), maternal urinary/cervical/vaginal infection during pregnancy (yes/no), and maternal diet during pregnancy: healthy pattern (i.e. high in nutrient-dense food, lowest quartile vs. others) and western pattern (i.e. high in fats and sugar, highest quartile vs. others)(25). *Children's characteristics at birth* included sex (male/female), birth weight (kg), gestational age (weeks of amenorrhea), delivery mode (vaginal, forceps/ventosa, C-section), and birth trimester.

Data Analyses

Network of cord serum cytokines and anxiety/depression in offspring

Skewed distributions of standardized cytokines were normalized using the nonparanormal transformation(26). Based on partial correlations, we estimated the Mixed Graphical Models (MGMs)(27) (mgm:Estimating time-varying mixed graphical models in high-dimensional data. arXiv preprint <http://arxiv.org/abs/1510.06871v2>) for the cytokines and trajectories of anxiety/depression symptoms (R software package, mgm). We used LASSO regularization regression to identify the most relevant edges among the large number of pairwise associations. The penalty parameter of the LASSO was selected using the Extended Bayesian Information Criterion (EBIC) for which the tuning parameter gamma was set to 0. We then used the Fruchterman-

Reingold algorithm to determine the layout of the graph by positioning the strongly connected nodes on the center of the network and weakly connected nodes on the periphery (R package, qgraph). This provided a node distribution layout where edges represent conditional dependences relations between nodes (controlling for the effects of all network variables). Accuracy and stability were assessed by 100 non-parametric bootstraps of the edge weights(28) and displaying the proportion of non-zero estimates (R package, bootnet). We used the node strength centrality index to assess the strength of internode connections.

Longitudinal associations between cord serum cytokines and anxiety/depression in offspring

First, covariates with missing data were imputed based on exposure (cytokines), outcome, and all other covariates to generate 40 datasets using the Fully Conditional Specification method (mice R packages). Second, a non-parametric bootstrap with replacement was conducted, involving 100 resamplings with the full sample size for each of the 40 multiple imputed datasets. Third, we used an Elastic Net regularized logistic regression model to conduct a variable selection procedure enabling the most relevant predictors for the final multivariate model to be determined. This procedure makes it possible to account for multicollinearity between variables, and deals well with low events per variable in the minority class and reduces over-fitting. In the Elastic Net procedure, we randomly partitioned the data into a training sample (75%) and a holdout sample (25%). In the training sample, we chose the optimal tuning hyperparameters (alpha and lambda) for the Elastic Net regression via 10-fold cross-validation, to maximize the Area Under the Receiving Operator Curve (Caret and Glmnet R packages)(29). The holdout sample was used to test the predictive performance of the model by estimating the AUROC. As the Elastic Net is not a deterministic method, the optimization step was repeated three times, leading to 12,000 models. To select the subset of variables associated with the outcome, we computed the Variable Inclusion Probability (VIP, i.e. percentage of runs in which the associated coefficient was different from zero) out of the 12,000 models. The VIP can be interpreted as the posterior probability of including a variable in the model. We set a stringent VIP threshold at 80% to select variables stably associated with trajectories of anxiety/depression symptoms. Fourth, we tested associations between cytokines and anxiety/depression trajectories (high trajectory vs. others) adjusting only for covariates selected by the Elastic Net (multivariable logistic analyses), therefore limiting over-adjustment. To account for multiple testing, we applied a Bonferroni correction. We tested pairwise interactions between all the variables kept in the model, notably the interactions between maternal depressive symptoms and the cytokines retained in the final model after the variable selection procedure. Finally, we conducted a sensitivity analysis to test the robustness of our findings by adjusting additionally on sex and on

conditions known to involve low-grade chronic inflammation or to influence cytokine profiles (prenatal BMI, gestational diabetes, nutrition profiles, infections, alcohol/tobacco consumption, social factors, delivery mode) in order to limit the risk of residual confusion. Here again we tested pairwise interactions between all the variables kept in the model and the cytokines, looking for moderating effects of the third variables on the cytokines.

Role of the funding source

The EDEN study sponsors were not involved in the study design, data collection or data analyses.

RESULTS

Descriptives

The study sample included 871 children (462 (53%) males, 409 (47%) females). **Table 1** shows sample characteristics by childhood anxiety/depression trajectory. **Table 2** displays the standardized cytokine levels by childhood anxiety/depression trajectory. **Figure S2** shows the correlations between all cytokine pairs.

Network of cord serum cytokines and anxiety/depression in offspring

The network of the 19 cord serum cytokines and anxiety/depression trajectories is displayed in **Figure 1**. Non-parametric bootstraps on edge weight are shown in **Figure S3**. The node strength centrality indices for the 20 variables of the network are shown in **Figure S4**. Coherently, cytokines formed communities in the network structure. IL-6, TNF- α , and CXCL10 were the most central cytokines of the network. The most influential cytokines in the network were IL-6, TNF- α , CXCL10, CCL11, IL-8, IL-1 β , and IL-17A. Notably, IL-7 was the only cytokine directly connected (negatively) with the anxiety/depression trajectory, with a regularized edge weight equal to -0.046. IL-7 was also negatively related to IL-1 β and CXCL10 (regularized edge weights respectively equal to -0.052 and -0.035) and positively related to CCL17, IL-15, IL-17A, CCL4, and IL-8 (regularized edge weights respectively equal to 0.250, 0.146, 0.080, 0.067, and 0.044).

Longitudinal multivariate associations between cord serum cytokines and anxiety/depression in offspring

Given the large number of covariates potentially impacting cytokine levels or anxiety/depression trajectories, we first used an Elastic net penalized regression to eliminate covariates with a

negligible impact on anxiety/depression predictions. The predictive performance of the model by estimating the AUROC was 0.70. Multiparity, birth trimester, maternal depressive symptoms, IL-7 and IL-10 were selected as main predictors of anxiety/depression trajectories (Table S3). Table 3 shows adjusted associations between selected cord serum cytokines and childhood anxiety/depression trajectories adjusting for the above selected variables. IL-7, second birth trimester and multiparity were negatively associated with the outcome (respectively adjusted odds ratio (aOR)=0.73, 95%CI=(0.57, 0.93); aOR=0.53, 95%CI = (0.29, 0.98), aOR=0.50, 95%CI=(0.32, 0.77)). Prenatal maternal depressive symptoms were significantly associated with childhood anxiety/depression trajectories (aOR=1.06, 95%CI = (1.03, 1.09)). There was no statistically significant interaction between variables included in the model, notably the interaction terms maternal depressive symptoms * IL-7 (p=0.88) and maternal depressive symptoms * IL-10 (p=0.39), which suggests that maternal depression does not play any moderating role. The sensitivity analysis additionally adjusting for sex, prenatal BMI, gestational diabetes, nutrition profiles, infections, alcohol/tobacco consumptions, social factors, and delivery mode showed the same pattern of results (**Table S4**). None of the interaction terms tested in the sensitivity analysis was significant (all p non-significant), which suggests that prenatal and social variables do not play any moderating role.

DISCUSSION

Principal findings of the study

To our knowledge, this birth cohort study is the first to test whether cord serum cytokines are associated with the emergence of anxiety/depression symptoms in early childhood. We found that no birth cytokine other than IL-7 was directly related to anxiety/depression. Only higher IL-7 levels in the cord serum at birth were associated with a lower likelihood of symptoms of anxiety/depression between ages of 3 and 8 years in offspring, and vice-versa. This relationship was not confounded or moderated by a wide range of other factors (including prenatal BMI, gestational diabetes, nutrition profiles, infections, alcohol/tobacco consumption, social factors, and delivery mode), as shown by the multivariate models. The association between IL-7 and anxiety/depression was consistent while accounting for 18 other cytokines, as revealed by the network analysis. Interestingly, IL-7 was related to four of the seven most influential cytokines in the network (i.e. CXCL10, IL-1 β , IL-8, and IL-17A). Although caution is needed and no firm conclusion can be drawn from our findings, it might be that the influence of IL-7 on anxiety/depression is due to complex interactions with other cytokines of the network, since cytokines act in multiple and

interactive cascades. Our findings are new since a) previous knowledge regarding relations between cytokines and behavior was mostly based on studies tapping a limited range of cytokines, and b) human studies to date measured cytokines in maternal serum, which poorly reflects the placental-fetal immune compartment. Notably, IL-7 has previously been cross-sectionally associated with depression in adolescent/adult/geriatric populations, although the direction of this link remains controversial(30–33). Moreover, we have previously shown in the EDEN cohort that IL-7 measured in serum at age 5 was positively associated cross-sectionally with anxiety/depression symptoms at 5 years(34). This reverse pattern of association between IL-7 and anxiety/depression symptoms measured at different waves (i.e. birth and age 5 years) suggests the role of IL-7 in susceptibility to anxiety/depression and calls for this research path to be extended. Variations in IL-7 levels could correspond to different functional patterns of immunity depending on the timing due to the environmental milieu in the brain and/or the periphery, but also to life experience, which could shape the brain and psychosocial factors that impact development in children.

Interpretation

The finding that IL-7 was the only cytokine significantly and directly associated with children's anxiety/depression was somewhat unexpected. Particularly, the absence of a significant link between IFN- γ , IL-1 β , IL-6, IL-8, IL-10, IL-17A, TNF- α and anxiety/depression was surprising, given the rich literature on their potential role in prenatal immune activation and/or in the pathophysiology of depression. It is challenging to interpret these negative findings since this study is unique in its design and methodology. Indeed, most prior research relied on animal models when exploring prenatal immune activation or considered cytokines in serum/blood samples and not in cord blood in human data, which make comparisons tricky. However, the fact that IL-7 was itself related to IL-1 β , IL-8 and IL-17A in the network is noteworthy and might suggest indirect effects through complex interactions. This interpretation remains speculative and requires further research. Considering IL-7 specifically, it is an essential cytokine for the homeostasis of the immune system, particularly for B and T cell development and maturation, such as CD4⁺/CD8⁺/Th17 lymphocytes(31,35,36). Importantly, the pathophysiology of depression and anxiety have been related to impaired T cell function, notably Th17(37–40). Hence, reduced circulating levels of IL-7 at birth may contribute to T cell defects acquired during early immune programming at birth, thereby triggering the susceptibility towards anxiety/depression, with lasting effects beyond the perinatal window of early brain development.

IL-7 and its receptors are expressed early in the developing brain(41), suggesting that it could play a role in neural system development and function from the early stages onwards, with

sustainable influences after initial programming of brain regions that govern emotional, cognitive and self-reflective functions. Indeed, the unbalanced prefrontal cortical inhibition of the limbic brain regions (amygdala, insula, hippocampus and anterior cingulate cortex) observed in patients with anxiety/depression could result from early neurodevelopmental defects(42). Several mechanisms could underlie the link between IL-7 and neurodevelopment. First, IL-7 is expressed in embryonic hippocampal progenitor cells in rodents and induces a shift of differentiation toward the glial lineage of human embryonic neural progenitors(41,43). This possibly involves the significant early contribution of IL-7 to formation of the neurocircuitry possibly involved in vulnerability to anxiety/depression. Second, IL-7 likely prevents neuroinflammation in response to non-specific stimuli(44). Although speculative, increased IL-7 could confer lasting protection against neuroinflammation during neurodevelopment. Alternatively, and finally, the association we found could be non-causal and merely correlative, due to other unmeasured confounders.

Strengths and weaknesses

The strengths of the present study include the novelty of the finding that lower IL-7 at birth is associated with a higher risk of anxiety/depression symptoms later. It also has methodological strengths: the measurement of cord serum cytokine biomarkers in a large population-based sample, the use of a widely validated instrument to assess anxiety/depression symptoms longitudinally, the use of state-of-the-art modeling strategies (mixed-effect modeling approach for anxiety/depression trajectories, network analysis, Elastic Net) and the robustness of the results in sensitivity analyses. However, the findings should be considered and interpreted in light of the study's limitations. First, the selection of a more socially advantaged sample and the self-reported nature of some covariates as well as symptoms of anxiety/depression possibly introduced a selection bias and measurement errors. This may have led to conservative findings by lowering the strength of the associations between IL-7 and anxiety/depression. Second, it is important to note that the measure of anxiety and depression using the SDQ in early childhood may not be the most sensitive, although it is certainly appropriate for children over 6. This could lead to underestimating associations between risk factors and internalizing problems. Third, measurement error regarding cytokines is possible. Cord blood is susceptible to clot easily, which can interfere with downstream assay quality. In addition, cytokine levels can spike during labor/delivery, which was not adjusted for in the analyses. A potential measurement error could attenuate the associations between cytokines and anxiety/depression and thus contribute to their non-significance. Fourth, we did not explore central cytokines directly. It is hence not clear how levels of cord serum cytokines reflect the actual levels in the neonatal brain. To our knowledge, whether IL-7 crosses the blood-brain barrier remains unknown, even though

peripheral immune patterns very likely influence central immune patterns(11). First, the period reflected by the cord serum measurement is uncertain. Assessment at birth could reflect either long- or very short-term cytokine patterns. Here again this could lead to underestimated associations and limit the extrapolation of these findings in terms of a developmental window. Sixth, we lacked detailed information on other factors such as maternal medications or physiological stress at birth and could not include these variables in the models. Finally, other residual confounders (e.g. unmeasured genetic/epigenetic/environmental factors) may also hamper the study's interpretation.

Unanswered questions and future research

Importantly, practical implications of the findings lie ahead. The association between IL-7 cord serum levels and anxiety/depression symptoms in offspring appears robust. However, it cannot be used as yet for predictive purposes in clinical or public health practice. Rather, these preliminary findings pave the way for conducting further etiological studies in humans and potentially experimenting new treatments in the field of preventive psychiatry, firstly in animals. The modulation of IL-7 and its downstream IL-7R signaling pathway could represent a new therapeutic target to prevent anxiety/depression symptoms before their emergence. Both molecules targeting the IL-7/IL-7R signaling pathway and larger immuno-modulating drugs could be experimented. IL-7-based molecules (e.g.recombinant Human IL-7, Cytheris cyt107) exist and have been tested in fields such as HIV+ infection and cancer. From a mechanistic perspective, it is relevant to understand whether and how the characteristics of immune dysregulation (such as timing, intensity, patterns of immune response, accumulation of inflammation challenges) relate differentially to the emergence of anxiety/depressive symptoms. It is also important to consider the notion of vulnerability to immune dysregulation. In other terms, whether certain factors (e.g.genetic/epigenetic background, microbiome and other environmental factors) confer a particular liability towards anxiety/depression as result of immune dysregulation remains to be confirmed. In addition, it would be appropriate in the future to use detailed information on physiological stress related to birth to test whether the change in the levels of IL-7 could reflect an altered response specifically related to birth stress, or a more long-lasting pattern of altered levels of IL-7. Finally, how early life and chronic stresses (e.g.low socioeconomic status, maltreatment) magnify the crosstalk between central/peripheral inflammation and the neural system deserves further research in the future(19).

Overall, we found evidence that IL-7 in the cord serum is associated with the risk of anxiety/depression symptoms in offspring at ages 3 to 8 years. Despite the modest magnitude of the association, the results point to the role of the immune system as a pathway in the fetal origins of

mental disorders, at least for a subpart of the anxiety/depression spectrum. Ultimately, the quest for early primary prevention of anxiety/depression linked to immune dysregulation, although still far off in humans, is now clearly on the agenda of animal research.

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Journal Pre-proof

Figure 1. Network displaying relationship between 19 standardized cord serum cytokines and anxiety/depression (A/D) symptoms trajectories measured at ages 3, 5 and 8 years, n=871

Green edges indicate positive relationship and pink negative relationships. The absolute value of associated edge-parameter is proportional to the width and saturation of the edges.

Journal Pre-proof

Table 1. Sample sociodemographic characteristics by childhood anxiety/depression trajectory, EDEN, n=871

Characteristics	N	Reference group n=736	High anxiety/ depression trajectory n=135	Effect-size ^a
Male, No. (%)	871	397 (53.94)	65 (48.15)	-0.06
Prenatal and biological factors				
Gestational diabetes, No. (%)	871	55 (7.47)	8 (5.93)	-0.02
Maternal pre-pregnancy BMI (kg.m2), No. (%)	871			-0.07
Low pre-pregnancy BMI		90 (12.23)	12 (8.89)	
Normal pre-pregnancy BMI		456 (61.96)	93 (68.89)	
High pre-pregnancy BMI		190 (25.82)	30 (22.22)	
Urinary/Cervical/Vaginal infection during pregnancy, No. (%)	870	246 (33.42)	45 (33.33)	-0.001
Multiparity, No. (%)	871			0.18 ^b
1st child		318 (43.21)	83 (61.48)	
2nd child or more		418 (56.79)	52 (38.52)	
Caffeine consumption (mg/day), No. (%)	858			0.003
0		65 (8.83)	12 (8.89)	
0 to <100		354 (48.10)	64 (47.41)	
100 to <200		208 (28.26)	44 (32.59)	
>=200		97 (13.18)	14 (10.37)	
Alcohol consumption during pregnancy, No. (%)	871			0.04
No or ≤ 2 glasses/week		683 (92.80)	120 (88.89)	
Yes (> 2 glasses/week)		53 (7.20)	15 (11.11)	
Smoking during pregnancy (cigarette(s)/day), mean (SD)	859	0.99 (2.67)	0.96 (2.58)	0.02
Maternal diet during pregnancy	871			
Lowest quartile of healthy pattern		252 (34.24)	49 (36.30)	0.02
Highest quartile of Western pattern		151 (20.52)	27 (20.0)	-0.005
Perinatal factors				
Birth weight (kg), mean (SD)	871	3.32 (0.48)	3.30 (0.53)	-0.04
Gestational age (week of amenorrhea), mean (SD)	871	39.29 (1.55)	39.11 (1.76)	-0.01
Delivery mode, No. (%)	870			0.001
Vaginal		565 (76.77)	104 (77.04)	
Forceps / Ventosa		78 (10.60)	14 (10.37)	
C-section		92 (12.50)	17 (12.59)	
Birth trimester, No. (%)	871			0.01
1st trimester		179 (24.32)	41 (30.37)	
2nd trimester		224 (30.43)	27 (20.0)	
3rd trimester		186 (25.27)	33 (24.44)	
4th trimester		147 (19.97)	34 (25.19)	
Social factors				

Journal Pre-proof				
Maternal age at birth, mean (SD)	871	30.28 (4.75)	29.41 (4.09)	-0.19
No intact family at birth, No. (%)	841	21 (2.85)	6 (4.44)	0.02
Maternal education, No. (%)	869			-0.08
Less than 12 years		155 (21.06)	29 (21.48)	
Between 12 and 14 years		293 (39.81)	63 (46.67)	
More than 14 years		286 (38.86)	43 (31.85)	
Paternal education, No. (%)	807			0.01
Less than 12 years		215 (29.21)	36 (26.67)	
Between 12 and 14 years		276 (37.50)	52 (38.52)	
More than 14 years		190 (25.82)	38 (28.15)	
Family income at birth, mean (SD)	870	4.98 (1.27)	4.82 (1.28)	-0.13
Maternal prenatal psychopathology				
Depressive symptoms, No. (%)	867	10.25 (7.28)	13.89 (8.87)	0.48 ^b

^a Calculated as Cohen's d effect size or success rate difference (very small: <0.20; small: 0.20-0.50; medium: 0.50-0.80; large: 0.80-1.20; very large: 1.20-2.0; huge: > 2.0). p values are based on unpaired t test or Mann-Whitney test for continuous variables and chi2 test for categorical variables.

^b p < 0.01

^c p < 0.05

Table 2. Sample standardized cord serum cytokine characteristics by childhood anxiety/depression trajectories, EDEN, n=871.

Cytokines (pico/ml)	Reference group n=736 (84.50%)		High anxiety/depression trajectory n=135 (15.50%)		Effect size ^a
	Mean	Standard deviation	Mean	Standard deviation	
CCL2	330.51	263.23	329.37	154.05	-0.01
CCL3	36.45	173.79	22.32	13.85	-0.09
CCL4	266.90	137.35	258.00	102.81	-0.07
CCL11	224.93	93.75	212.73	91.96	-0.13
CCL17	1481.86	1066.65	1435.02	1226.92	-0.04
CCL26	13.08	15.45	13.14	20.21	0.01
CXCL10	203.98	464.10	201.62	324.84	-0.01
IFN- γ	2.81	3.43	2.58	2.13	-0.07
IL-1b	1.15	5.63	0.51	0.70	-0.12
IL-6	27.04	191.63	12.32	52.43	-0.08
IL-7	9.45	3.95	8.26	2.92	-0.31 ^b
IL-8	65.31	358.19	22.87	50.46	-0.13
IL-10	1.05	2.78	1.87	9.57	0.18
IL-12p40	656.94	251.26	653.82	242.71	-0.01
IL-15	2.90	7.83	2.23	1.06	-0.10
IL16	456.94	605.51	402.68	378.10	-0.09
IL-17A	9.74	6.65	8.85	5.96	-0.14
TNF- α	3.64	2.67	3.31	1.06	0.13
TNF- β	0.81	0.57	0.70	0.40	-0.21

^a Calculated as Cohen's d effect size or success rate difference (very small: <0.20; small: 0.20-0.50; medium: 0.50-0.80; large: 0.80-1.20; very large 1.20-2.0; huge: > 2.0). p-values are based on unpaired t test or Mann-Whitney test with Bonferroni's multiple testing correction.

^b p < 0.01

Table 3. Adjusted associations between childhood anxiety/depression trajectories and standardized cord serum cytokines, EDEN, n=871

	OR (97.5% CI)^a
Prenatal and biological factors	
Multiparity (2nd child or more vs. 1st child)	0.50 (0.32, 0.77)
Birth trimester	
1st trimester	1 (REF)
2nd trimester	0.53 (0.29, 0.98)
3rd trimester	0.87 (0.49, 1.55)
4th trimester	1.01 (0.56, 1.80)
Maternal prenatal psychopathology	
Depressive symptoms	1.06 (1.03, 1.09)
Cord serum cytokines	
IL-7	0.73 (0.57, 0.93)
IL-10	1.13 (0.95, 1.34)

^a Odds ratios and their confidence intervals (97.5% confidence from Bonferroni correction)

