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Normal neonatal TREC and KREC levels in early onset juvenile idiopathic arthritis

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ABSTRACT

Objective: Dysregulated central tolerance predisposes to autoimmune diseases. Reduced thymic output as well as compromised central B cell tolerance checkpoints have been proposed in the pathogenesis of juvenile idiopathic arthritis (JIA). The aim of this study was to investigate neonatal levels of T-cell receptor excision circles (TRECs) and kappa-deleting element excision circles (KRECs), as markers of T- and B-cell output at birth, in patients with early onset JIA.

Methods: TRECs and KRECs were quantitated by multiplex qPCR from dried blood spots (DBS), collected 2–5 days after birth, in 156 children with early onset JIA and in 312 matched controls.

Results: When analysed from neonatal dried blood spots, the median TREC level was 78 (IQR 55–113) in JIA cases and 88 (IQR 57–117) copies/well in controls. The median KREC level was 51 (IQR 35–69) and 53 (IQR 35–74) copies/well, in JIA cases and controls, respectively. Stratification by sex and age at disease onset did not reveal any difference in the levels of TRECs and KRECs.

Conclusion: T- and B-cell output at birth, as measured by TREC and KREC levels in neonatal dried blood spots, does not differ in children with early onset JIA compared to controls.

1. Introduction

Juvenile idiopathic arthritis (JIA) is the most common pediatric chronic rheumatic condition, with an annual incidence of 15/100000 children in the Nordic population [1]. The pathogenesis of JIA is complex, as genetic and immunological factors, as well as environmental triggers, contribute to disease development [2]. Clonal expansion of autoreactive T cells and genetic associations to certain MHC class II alleles indicate a critical pathogenetic role of CD4+ T cells [3]. Signs of a disturbed B-cell development and activation have also been described, particularly in children with an early onset JIA. Increased numbers of memory B cells and autoantibodies, such as anti-nuclear antibodies

Abbreviations: JIA, juvenile idiopathic arthritis; TRECs, T-cell receptor excision circles; KRECs, kappa-deleting element excision circles; ANA, anti-nuclear antibody; ACTB, beta actin.

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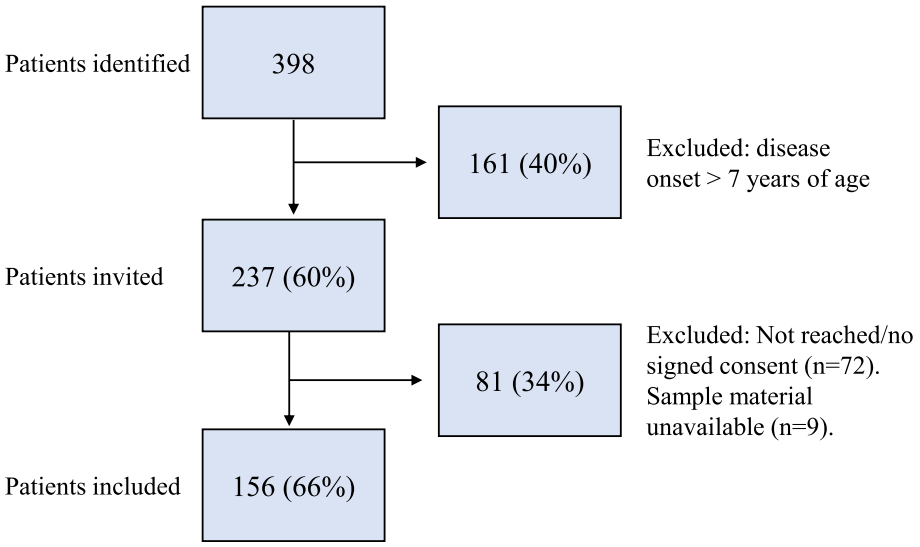

Fig. 1. Flowchart of participants.

Table 1
Clinical Characteristics of JIA patients. ANA: anti-nuclear antibody.

Clinical Characteristics		
Number of patients included		156
Girls, n (%)		116 (74%)
Debut age in months, median (range)		28 (8–80)
ICD-10 M084 (oligoarthritis), n (%)		113 (72%)
ANA positivity, n (%)		104 (67%)
Uveitis, n (%)		50 (32%)
of which ANA positive		42 (84%)
Medical treatment (anytime), n (%)	*Systemic corticosteroids*	38 (24%)
	Methotrexate	135 (87%)
	Biologic drugs	95 (61%)
	Corticosteriod injections	131 (84%)

(ANAs), are found [4,5].

Both dysregulated central tolerance and lymphopenia have been associated with an increased number of self-reactive T- and B cells in the periphery, contributing to development of autoimmune diseases [6–8]. T-cell receptor excision circles (TRECs) and Kappa-deleting excision circles (KRECs) are stable DNA episomes that form during T- and B-cell receptor rearrangements in the thymus and bone marrow. Present only in thymus derived T cells, TRECs are used in newborn screening to detect severe T-cell lymphopenia and are accepted as a measure of thymic output [9,10]. Disturbances of TREC levels have been reported in autoimmune diseases such as JIA, rheumatoid arthritis, multiple sclerosis and autoimmune thyroiditis [11–15]. However, it is not known if low T-cell levels contribute to disease development or are merely a consequence of the disease processes and treatments. Age-inappropriate T-cell senescence with low TRECs has been reported in patients with manifest JIA [12,14] while other studies demonstrate a normal thymic output [16]. KREC levels have not been investigated in JIA to our knowledge, but increased numbers of autoreactive B cells have been reported in rheumatoid arthritis [17].

Children with JIA have had symptoms for several weeks and ongoing subclinical disease processes for much longer at the time of diagnosis. At that time, it is difficult to distinguish factors promoting the development of the disease from changes caused by the disease process. In this study, we tried to circumvent this situation by comparing the neonatal levels of TRECs and KRECs in children with early onset JIA with controls using their stored dried blood spots.

2. Methods

2.1. Study population and study design

The study is a retrospective case-control study. We included 156 patients from the western part of Sweden ($n = 123$) and Iceland ($n = 33$), with onset of JIA ≤6 years of age. Patients with systemic onset JIA (sJIA) were excluded. Fig. 1 describes the enrolment of study participants. In Sweden, all children with a diagnosis of JIA, visiting a pediatric rheumatologist in Region Västra Götaland and Region Halland from January 1, 2019 to June 4, 2020, were identified. In Iceland, all children with a diagnosis of JIA attending Landspitali University Hospital from January 1, 2003, to December 31, 2020, were identified. ICD codes M080–1, M083–9, M09 or L405 were included. Children with systemic JIA were excluded. In Sweden, children born before December 1, 2005 were excluded, since their neonatal screening cards were not accessible at the time of the study. In Iceland, children born before January 1, 2003 were excluded. Medical records of the resulting 398 children were searched to confirm a diagnosis of JIA, as classified by ILAR (International League of Associations for Rheumatology) and to determine the age at disease onset. 237 children with a diagnosis of JIA ≤6 years of age were invited to participate and subsequently 156 children were included in the study. Parents answered a questionnaire providing data on pregnancy and perinatal exposures that may influence the screening assay, as well as information on heredity. Information on age at symptom onset, medication, occurrence of uveitis and ANA status was retrieved from medical records. The study was approved by the Swedish Ethical Review Authority (reference number 2019–05139) and the Icelandic National Bioethics Committee (reference number VSN-20-199) and written informed consent was obtained for all participants. The STROBE case-control reporting guidelines were used [18].

2.2. Screening assay

TREC and KREC levels were quantified in neonatal dried blood spots (DBS). The original neonatal screening cards were retrieved from storage. Each case was matched by sex, gestational age, and date of newborn screening with two randomly selected comparators from the same biobank. Thus, the screening cards of cases and controls were stored for the same time and under the same conditions. The quantitative assay has been described previously [10]. Briefly, TREC, KREC, and beta actin (ACTB) levels were analysed using a quantitative real-time polymerase chain reaction (qPCR) in triplicates from 3.2 mm punches of the original

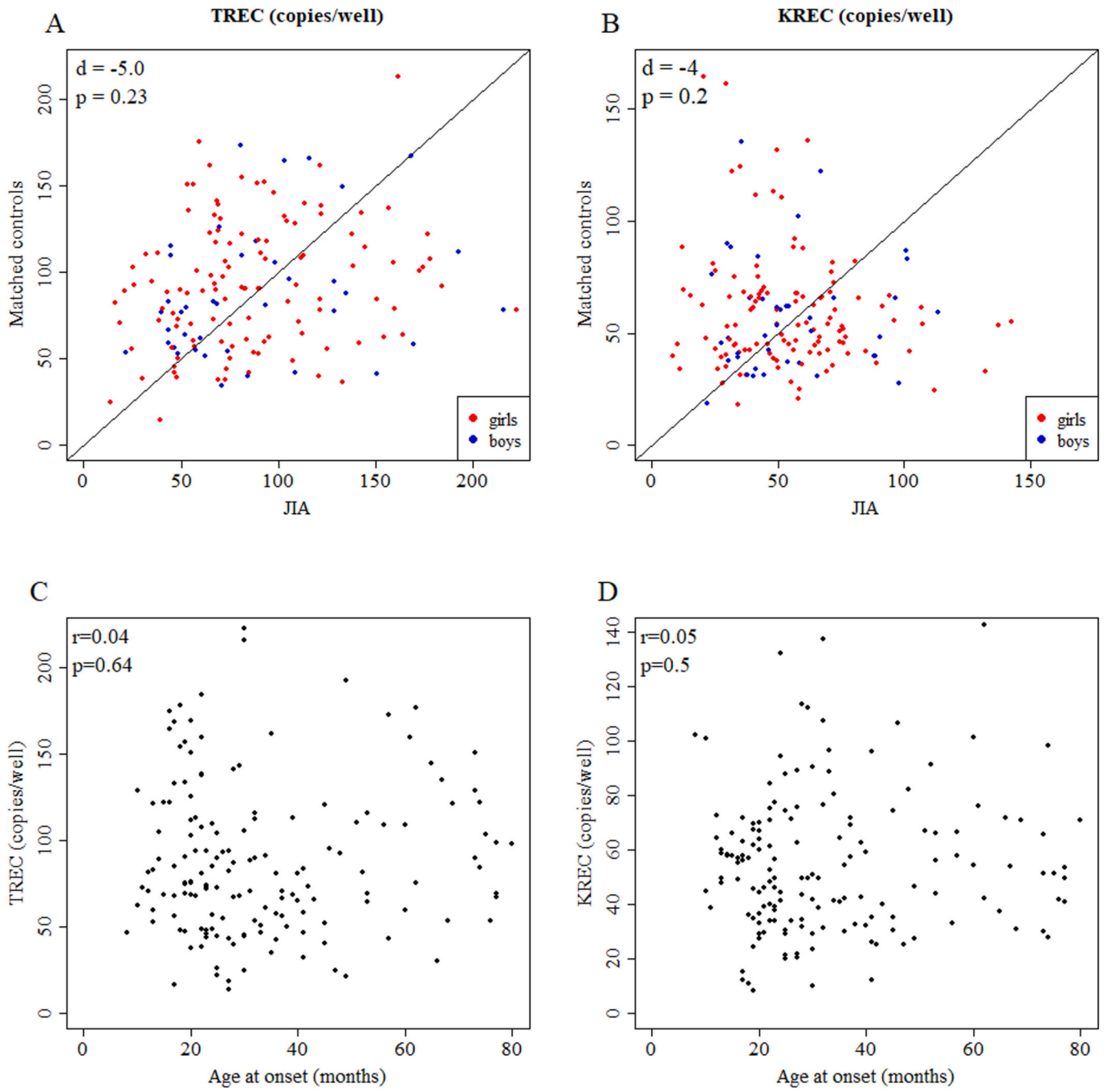

Fig. 2. *TREC (A) and KREC (B) levels in children with JIA and their matched controls. Sex stratified analysis did not reveal any difference. TRECs (C) and KRECs (D) plotted against age at onset of disease.* TREC: T cell receptor excision circles; KREC: Kappa-deleting excision circles; JIA: Juvenile idiopathic arthritis; d: Cohen's D, p: *p*-value, r: correlation coefficient r.

screening card according to the manufacturer's instructions (SPOT-it™ TK kit in 96-well format, ImmunoIVD, Nacka, Sweden). Cases and two matched controls were analysed on the same 96-well plate. On each plate, three internal DBS controls were analysed; T cell depleted, B cell depleted and both T- and B cell depleted. Quantification was carried out using 5-point standard curves (provided in the kit) ranging from 10 to 100,000 copies/well for TREC and KREC, and from 100 to 1,000,000 copies/well for ACTB. Sample quality was considered sufficient if all amplification curves showed the expected amplification profile and ACTB was ≥1000 copies/well.

2.3. Data analysis and statistics

TREC and KREC concentrations were presented as median and IQR, compared using paired *t*-test between cases and the mean of their matched controls and the relationship to age at onset was analysed with linear regression. All tests were two-tailed with significance level 0.05. R version 4.1.1 was used for all analyses.

Table 2
Neonatal TREC and KREC levels in JIA cases and controls, stratified according to sex. TREC: T cell receptor excision circles; KREC: Kappa-deleting excision circles; JIA: Juvenile idiopathic arthritis; IQR: interquartile range.

	JIA cases, median (IQR)	*Controls, median (IQR)*	*p-value*
TRECS			
All	*78 (55–113)*	*88 (57–117)*	*0.22*
Female	*79 (56–112)*	*89 (60–116)*	*0.19*
Male	*77 (51–116)*	*77 (49–121)*	*0.82*
KRECS			
All	*51 (35–69)*	*53 (35–74)*	*0.19*
Female	*52 (35–70)*	*53 (36–74)*	*0.20*
Male	*50 (37–66)*	*53 (31–70)*	*0.70*

3. Results

3.1. Clinical characteristics

Clinical characteristics are presented in Table 1. As expected, most patients were female, the most frequent disease subgroup was oligoarthritis and ANA positivity was common. Uveitis had been diagnosed in as many as 32%. Information regarding heredity, medical use and infections during pregnancy is reported in the supplementary Table S1.

3.2. Neonatal TREC and KREC levels in early onset JIA are comparable to controls

The median neonatal TREC and KREC levels in children with JIA and their matched controls did not differ (Fig. 2 A-B, Table 2). All children with JIA had levels above the referral cut-off used in the screening program for severe combined immunodeficiency in Sweden. No difference was detected in TREC and KREC levels after stratification according to sex (Fig. 2 A-B, Table 2) or age at disease onset (Fig. 2 C–D).

4. Discussion

This study found no difference in TREC and KREC levels at birth in children who went on to develop JIA before the age of seven and their matched controls. This applied to both boys and girls regardless of age at disease onset. Thus, we conclude that children with early onset JIA start out in life with an intact thymic output.

Thymic output in children with an already established JIA has been addressed by measuring TREC levels in a few studies. Prelog et al. and Horwath et al. both reported decreased TREC levels [12,14] whereas Lorenzi et al. found TREC levels to be unaffected [16]. The differences in study design makes comparisons between them difficult. Since our study addressed neonatal TREC levels it neither supports nor contradicts previous results. However, it indicates that a peripheral proliferation, homeostatic or related to the disease or its treatment, is a more likely explanation to the reported decreased TREC levels than a primary T-cell generation defect.

Disturbed B-cell development, such as increased numbers of switched memory B cells and skewed kappa:lambda light chain ratio, have been described in the pathogenesis of JIA [5,19–21]. Normal KREC levels at birth in this study suggest that this may reflect peripheral B-cell activation in manifest disease rather than a flawed central B-cell tolerance.

The main strength of this study is that it includes a large homogenous group of children with a classified JIA diagnosis, compared to controls matched for gestational age and sex, factors known to affect neonatal TREC levels. Controls were also chosen to have arrived at the laboratory on the same day as cases and were thus treated and stored under the same conditions. The controls were otherwise anonymous to us and any diseases they may have developed are unknown of. However, given that autoimmune diseases in childhood, like JIA, are relatively rare and that we chose to analyse two control samples for every JIA case, the risk that the presence of autoimmune diseases in the control group affecting the results is negligible. Analysis of TRECs and KRECs using dried blood spot samples is a very well established and validated method, used worldwide on a large scale. Measurement shortly after birth represents a stable and comparable time-point in life.

As far as we are aware of, this is the first study investigating T- and B cell generation at birth in persons who later develop an autoimmune disease. In diseases like JIA with complex etiology it is important to define intact immune functions as well as aberrations. A better understanding of disease pathogenesis, including a possible inherent congenital difference in the immune system, is important for future treatment development.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2023.109277.

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Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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