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ORIGINAL RESEARCH

Neonatal Levels of T-cell Receptor Excision Circles (TREC) in Patients with 22q11.2 Deletion Syndrome and Later Disease Features

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Abstract
Purpose Newborns with severe T-cell lymphopenia, including those with 22q11.2 deletion syndrome (DS), have low numbers of T-cell receptor excision circles (TRECs). The aim of this study was to determine a possible correlation between neonatal TRECs in 22q11.2DS and the development of different phenotypes to elucidate the prognostic value of TREC in this disease.
Methods In this national survey including 46 patients with 22q11.2DS born after 2005, TREC levels were determined using stored newborn screening blood spots on filter cards. Patients were grouped into quartiles according to their TREC values, except the two infants with thymus aplasia.
Results The two patients with thymic aplasia had no detectable TREC. The rest had no severe clinical immunodeficiency. There was a significant correlation between low TRECs and the proportion of patients with $CD3^{+}CD4^{+}$T-cells below the 5th percentile of healthy infants (p=0.027) as well as the proportion with an abnormal thymus feature either no thymus or remnant thymus as observed during heart surgery (p=0.022). Significantly lower TRECs (p=0.019) were found in patients with cardiac defects compared to no such defects. Patients within the lowest quartile of TREC values (<71 TRECs/μL, n=11) had more frequent severe cardiac defects than the other quartiles (p=0.010). Eight of these patients in the lowest quartile needed an operation/intervention within two weeks after birth or died because of a cardiac defect.
Conclusion The low TREC values not only correlate with decreased T-cell immunity, but also with the occurrence of heart defects in the patients.

Keywords T-cell receptor excision circles · 22q11.2 deletion syndrome · cardiac defect · thymus · newborn screening

Abbreviations

CDC	Centre of disease control
CGH array	Comparative genomic hybridization array
CMV	Cytomegalovirus
COMT	Catechol-O-methyltransferase

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DBS	Dried blood spot
DS	Deletion syndrome
FISH	fluorescent in situ hybridization
SCID	Severe combined immunodeficiency
S1	DNA Purification solution
S2	Elution solution
TBX1	T-Box 1
TCR	T-cell receptor
TREC	T-cell receptor excision circles
MAPCA	major aortopulmonary collateral arteries
VSD	Ventricle septum defect

Introduction

The 22q11.2 deletion syndrome (DS) has an estimated incidence of 1:4000 live births. It is characterized by a deletion of 1.5-3 Mb inside the 22q11.2 chromosome band, where more than 40 genes are located [1–5]. The main characteristics of the syndrome are conotruncal cardiac defects, thymic hypoplasia/aplasia, velopharyngeal insufficiency, hypoparathyroidism, dysmorphic face and immunodeficiency [3, 5, 6]. The transcription factor T-Box 1 (TBX1) has been considered to be important for the phenotype [7–12]. However, recent studies have described patients with typical disease features such as cardiac defects and atypical deletions that do not include TBX1 [13, 14]. Thymic hypoplasia occurs probably in more than 80 % of the patients, and this results in varying degrees of T-cell deficiency [6]. However, less than 1 % of the patients have thymic aplasia with absent peripheral T-cells and severe immunodeficiency [5, 15].

T-cell receptor excision circles (TRECs) are episomal, circular DNA fragments generated during the sequential rearrangement of variable V, D, and J segments of T-cell receptor (TCR) genes [16, 17]. These DNA circles do not replicate and therefore are present in a higher number in T-cells that have recently emigrated from the thymus. Over 70 % of rearranged α/β T-cell receptors form a circular DNA TREC from the excised TCRδ gene that lies in the TCRα genetic locus [16].

T-cell lymphopenia correlates with low or absent TRECs. Two recent studies describe low levels of neonatal TRECs in 22q11.2 DS patients with low numbers of T lymphocytes, while absent TRECs were seen in patients with thymic aplasia [18–20]. The purpose of this study was to determine neonatal TREC levels on stored blood filter cards of Norwegian patients later diagnosed with 22q11.2 DS and study the prognostic value on disease features such as heart defects, thymus size, lymphocyte subpopulations and neonatal hypocalcemia. This retrospective information may be valuable for a future neonatal screening program primarily intended to detect newborns with severe combined immunodeficiency and not 22q11.2 DS.

Material and Methods

Patients and Samples

In this national survey we included 46 of the 55 22q11.2 DS patients born between 2005 and 2014. The patients were recruited through the four genetic institutions in Norway. The study was approved by the Regional Committee for Research Ethics (2012/806b) and the Data Protection Officer of the hospital. Letters were sent to all 55 parents, and 46 (84 %) consented to participate. The rest did not respond to the invitation. Sixteen patients were diagnosed by fluorescent in situ hybridization (FISH), 24 by multiplex ligation-dependent probe amplification (MLPA), and six by comparative genomic hybridization array (CGH array). Stored filter cards from these children and from 10 random controls that were sampled in 2012, were retrieved from the National newborn screening diagnostic biobank (Oslo University Hospital, Norway). They were stored in a frozen condition at temperature between −20 and −25 °C. Previously published studies show that there is no significant difference in TREC values of new and old filtercards [20, 21].

Chart reviews of the patients were conducted by the first author. Clinical data were collected, including the occurrence of cardiac defects, treatment with heart operation/intervention, the presence of cleft palate variants and velopharyngeal insufficiency in addition to their serum calcium levels. The used definition for hypocalcemia was ionized calcium <1.0 mmol/L in the neonates (<1 week old), and <1.15 mmol/L in the infants (>1 week old). The total number of $CD3^+$ T-cells, $CD3^+CD4^+$ T-cells, $CD3^+CD8^+$ T-cells were retrieved from the medical records as the result of the first flowcytometry that was performed in the children age ranging 1 to 65 months.

Extraction of DNA

DNA was isolated from a 3.2 mm punch from dried blood spot (DBS) of stored filter cards using a modification of a protocol previously published [22]. Briefly, each DBS punch was washed twice with 150 μL of DNA Purification Solution (S1) followed by 150 μL DNA Elution solution (S2) (both Qiagen) for 10 min each at 300 rpm in a thermo shaker (TS-100 Biosan) at ambient temperature. The washing steps were followed by DNA elution in 100 μL S2 at 99.5 °C with continuous shaking in a thermo shaker for 30 min at 300 rpm.

TREC qRT-PCR

The qRT-PCR reactions were performed on an ABI 7300 (Applied Biosystems). The TREC and

β-actin were run as singleplex in a final volume of 25 μL containing 12.5 μL Taqman Universal master mix (Applied

Biosystems) and primers. The following primers were used: TREC Forward

5′- CAC ATC CCT TTC AAC CAT GCT-3′ 0.625 μL (20 μM), TREC Reverse 5′- GCC AGC TGC AGG GTT TAG G-3′ 0.625 μL (20 μM), TREC Probe: 5′- FAM-ACA CCT CTG GTT TTT GTA AAG GTG CCC ACT-3′-TAMRA 0.25 μL (15 μM), ACTB primers: β-actin Forward Primer
5′ ATT TCC CTC TCA GGC ATG GA-3′ 0.63 μL (10 μM), β-actin Reverse Primer 5′- CGT CAC ACT TCA TGA TGG AGT TG-3′ 0.63 μL (10 μM) each, β-actin Probe: 5′- FAM-GTG GCA TCC ACG AAA CTA-3′-TAMRA 0.25 μL (15 μM) and BSA 1 μL (10 mg/ml). DNA concentration in each sample for the TREC assay was 10 μL, and 5 μL for the β-actin assay. For the β-actin assay 5.62 μL H_2O (Nuclease free water, Ambion) was added. The 96-well plate reactions were started with an initial cycle at 50 °C for 2 min, a heating cycle at 95 °C for 10 min, followed by 45 °C cycles of 30 s at 95 °C and 60 s at 60 °C.

The TREC plasmid, generated by Douek (7), was provided by the Medical College of Wisconsin and used to construct a standard curve. We measured the concentration of TREC plasmid with Nanodrop (Spectrophotometer ND-1000), and then an 8 point standard curve was established after 2-fold serial dilutions in dilution solution (Qiagen Generation solution 2 containing 100 ng/μL tRNA). All the analyzed qRT-PCR assays fulfilled the quality requirements of similar slopes and with R^2 values>0.99. β-actin was used as a housekeeping gene to assure an adequate DNA extraction for PCR. Quality control was done with cards provided by Centre of Disease Control (CDC).

The TREC value per μL was calculated assuming that a 3.2 mm punch contains~3 μL of whole blood.

Flow Cytometric Enumeration of T-cells

Determination of absolute counts of T-cells ($CD3^+$ T-cells) and T-cell subpopulations ($CD3^+CD4^+$,and $CD3^+CD8^+$) was done using Truecount Tubes and Multitest mAb CD3, FITC/CD8, PE/CD45, PerCP/CD4 APC (BD Bioscience) according to the manufacturer's instructions. The samples were analyzed on a FacsCalibur (BD) or Canto II flowcytometer (BD) and data analysis was performed with either Cell Quest or Canto Software (BD).

Statistical Analysis

The statistical analysis was performed using PASW Statistics 18 (SPSS Inc, USA).

The Mann–Whitney test was applied to compare TREC values in patients with 22q11.2 DS with controls. The Chi-square test was used to determine the correlation between proportions of patients in different groups (TREC quartiles) and heart operation/intervention, and the same was done for thymus size. The binomial test was used to calculate p-values for $CD3^+$ T-cells, $CD3^+CD4^+$ T-cells, and $CD3^+CD8^+$ T-cells compared with the 5th percentile of age related reference values previously published [23]. Crosstabs and Chi-square Linear by linear association were performed to study a possible association between TREC quartiles and the number of patients below the 5th percentile of the reference values. A significance level of 5 % was used.

Results

Neonatal TREC Levels in 22q11.2 Deletion Syndrome

Two patients with 22q11.2 DS had thymus aplasia with no detectable TRECs but normal β-actin values (>10 000 copies/μL). For the rest of the patients (n=44), the median number of TRECs was 135 (range: 8–973 TRECs/ μL) while for the controls (n=10), median TREC number was 882 (range: 487–1737 TRECs/ μL). The patients displayed a broad range in their TREC values, but only two had a value overlapping with those of the controls.

For further evaluation we grouped the patients, except the two with thymus aplasia, into quartiles according to their TREC values: TREC quartile 1 (8–70 TRECs/ μL; n=11 patients), TREC quartile 2 (71–130 TRECs/ μL; n=11 patients), TREC quartile 3 (131–180 TRECs/ μL; n=11 patients), TREC quartile 4 (181–973 TRECs/ μL; n=11 patients).

Six of the 44 patients were born prematurely with a gestational age between 30 and 36 weeks. Two of these patients were in TREC quartile 1, two in TREC quartile 2, one in TREC quartile 3, and one in TREC quartile 4. The median TREC value of the premature patients was 100 (range: 52–226 TRECs/ μL). The two with thymus aplasia were term born.

Neonatal TREC Quartiles and Thymus Size

Information about the thymus of the patients with 22q11.2 DS was obtained from the heart surgery report in 19 of 27 operated patients (Table 1). The number of patients with no visible thymus was increasing with decreasing TREC levels. There was a significant correlation between TREC quartiles and an abnormal thymus feature when comparing TREC quartile 1 with TREC quartile 3 or 4 (p=0.022).

Table 1 The clinical characteristics of the patients in different TREC quartiles and the correlation with thymus size, deletion size, hypocalcemia, cleft palate and polyhydramnion

CHARACTERISTIC	Total Number	TREC QUARTILE 1	TREC QUARTILE 2	TREC QUARTILE 3	TREC QUARTILE 4
Thymus size	19/27[a]				
No visible thymus	9	5	2	1	1
Hypoplastic thymus	2	1	1	0	0
Normal sized thymus	8	0	3	2	3
No information available	8	3	2	3	0
Deletion size	30/44				
3 MB deletion	29	11	5	6	7
Other deletion	1	0	1[b]	0	0
Hypocalcemia	11/44	4	4	3	0
Cleft palate	10/44	0	2	4	4
Polyhydramnion	10/44	2	2	2	4

[a] Total number of patients who were heart operated

[b] This patient had a deletion affecting both classical 22q11.2 deletion area and parts of CAT-eye area and another deletion on the short arm of chromosome three

Neonatal TREC Quartiles and Lymphocyte Subpopulations

Flow cytometry data of $CD3^+$ T-cells, $CD3^+CD4^+$ T-cells, $CD3^+CD8^+$T-cells, $CD19^+$B-cells and $CD16^+/CD56^+/CD3^-$NK- cells were obtained in 36 of the patients at an age that ranged from 1 to 65 months (Supplementary Table). The T-cell numbers of the patients with 22q11.2 DS, except the two with thymus aplasia and almost no T-cells, were compared to 5th percentile of age related reference values [23]. For $CD3^+$T-cells, 13 of 34 patients ($p<0.001$) had values below the 5th percentile. For $CD3^+CD4^+$T-cells, 15 of 34 patients ($p<0.001$); and for $CD3^+CD8^+$ T-cells, 10 of 34 patients ($p<0.001$) had values below the 5th percentile.

For patients with TREC values in quartile 1, five out of nine (56 %) had $CD3^+$ T-cells below the 5th percentile, while this was found in only one of eight patients (12.5 %) in those with TREC quartile 4 (trend not significant, Table 2). However, for $CD3^+CD4^+$ T-cells six of nine (67 %) patients with TREC in quartile 1 had values below the 5th percentile, while in TREC quartile 4, only one of eight had such low values (12.5 %, Table 2, $p=0.027$). For $CD3^+CD8^+$T-cells, the same trend was observed, although not significant (Table 2). (T-cell subpopulations in different TREC levels in all patients are found in Supplementary Figure) No significant correlations were found between B-cell and NK-cell numbers or immunoglobulin values (Supplementary table) and the TREC levels. Vaccine responses were not studied.

The two patients with thymic aplasia died of opportunistic infections at the age of 4 and 6 months. For the other patients a history of infections was reported in 31 of the 44 patients (Table 3). Recurrent middle ear infection occurred in 11 patients, upper airway infection in 16, respiratory syncytial virus infection in 5, pneumonia in 14, gastrointestinal infection in 1 and urinary tract infection in 2. Twentythree of the patients were hospitalized and 20 received antibiotics. Two patients were not hospitalized but treated with antibiotics. No correlation was found between TREC levels, infections, hospitalization or use of antibiotics.

Table 2 The distribution of T-cell subpopulations in the patients in different TREC quartiles

Flowcytometry	Total	No of patients <5th percentile[a]
$CD3^+$T-cells: 0.074[b]	34	
TREC 1	9	5 (56 %)
TREC 2	8	3 (37.5 %)
TREC 3	9	3 (33.3 %)
TREC 4	8	1 (12.5 %)
$CD3^+CD4^+$T-cells : 0.027[b]	34	
TREC 1	9	6 (67 %)
TREC 2	8	3 (37.5 %)
TREC 3	9	3 (33.3 %)
TREC 4	8	1 (12.5 %)
$CD3^+CD8^+$T-cells: 0.118[b]	34	
TREC 1	9	4 (44 %)
TREC 2	8	3 (37.5 %)
TREC 3	9	2 (22.2 %)
TREC 4	8	1 (12.5 %)

[a] 5th percentile of reference values for lymphocyte subpopulations (15)

[b] The correlation between number of patients below the 5th percentile of age matched lymphocyte subpopulation and TREC quartiles (linear by linear association)

Table 3 The infectious complications in patients in different TREC quartiles

TREC QUARTILE 1	TREC QUARTILE 2	TREC QUARTILE 3	TREC QUARTILE 4
(P13) NI	**(P35) Pneumonia, UAI**	(P34) *RSV*	**(P04) UAI, EI**
(P26) UAI, Pneumonia	(P16) UAI	**(P28) Sepsis,Pneumonia, UAI, EI**	P11) NI
(P23) UAI	(P15) NI	(P38) UAI	**(P30) Pneumonia**
(P45) NI	(P03) NI	**(P33) GII, UAI**	**(P18) Pneumonia, EI**
(P36) Pneumonia	(P14) *RSV*	(P21) NI	(P43) NI
(P37) UAI	**(P17) Pneumonia, RSV**	(P12) NI	(P47) NI
(P20) *RSV*	(P32) NI	(P06) UAI, EI	(P19) NI
(P10) Pneumonia	**(P31) Pneumonia**	(P02) Pneumonia, EI [a]	**(P29) RSV, UTI, EI**
(P27) Pneumonia	**(P22) UAI**	**(P41) UAI, UTI**	**(P07) Sepsis, UAI, EI**
(P46) NI	(P25) UAI	(P44) NI	**(P05) Pneumonia, UAI, EI**
(P42) Pneumonia, EI	**(P39) UAI**	(P09) EI [a]	**(P01) Pneumonia, EI**

EI Middle ear infection, *GII* Gastrointestinal infection, *NI* No infections reported, *RSV* Respiratory syncytial virus infection, *UAI* Upper airway infection, *UTI* Urinary tract infection

Italic indicate hospitalization, [a] indicate use of antibiotics and bold indicate hospitalization and use of antibiotics. Patient identification is indicated in brackets

Neonatal TREC Quartiles and Cardiac Defects

Conotruncal heart defects were common in the 22q11.2 DS patients (Table 4), and many (35 %) had a heart operation/intervention during the first two weeks of life or died because of cardiac defects. In one of the two patients with thymus aplasia an interrupted aortic arch and an aorta stenosis were present. This infant had an early heart operation. The other patient had no heart defect. Significantly lower TRECs (p =0.019) were found in patients with cardiac defects compared to those with no such defects. In patients who had an operation/intervention within two weeks after birth or died of cardiac disease, the correlation was even higher (p=0.014). Consequently, there was a significantly higher frequency of operation/intervention or death in TREC quartile 1 compared to TREC quartiles 2, 3 or 4 (p=0.010) (Figure 1). In TREC quartile 1, nine patients (82 %) underwent heart operation/intervention or/and died of the cardiac defect, including six within two weeks after birth and two who died because of the cardiac defect. One of these patients, who died, had pulmonary atresia and major aortopulmonary collateral arteries (MAPCA). This patient was operated, but died of the cardiac defect 2 years old. The other patient had pulmonary atresia, MAPCA and ventricle septum defect (VSD). She died one month after birth before she had had any repair of the heart defect. For TREC quartile 2, a total of eight patients (73 %) underwent heart operation/intervention, including one within two weeks after birth and one who died 6 months old due to his cardiac defect

Table 4 The cardiovascular anomalies in patients in different TREC quartiles

TREC QUARTILE 1	TREC QUARTILE 2	TREC QUARTILE 3	TREC QUARTILE 4
(P13) TOF	**(P35) IAA, VSD**	**(P34) IAA, VSD,ASD,BAV**	**(P04) IAA, VSD, BAV**
(P26) IAA, ASD, DCVSD	**(P16)** TOF	**(P28)** ***PA, VSD, MAPCA***	**(P11)** TOF
(P23) IAA, VSD	**(P15)** TOF	**(P38)** TA, VSD	**(P30)** PA VSD MAPCA
(P45) IAA, VSD	**(P03)** VSD, ASD	**(P33)** TOF	**(P18)** ASD
(P36) PA, VSD	**(P14)** TOF	**(P21)** VSD, ASD	**(P43)*PA, OA,VSD, MAPCA***
(P37) TOF	**(P17)** APV, OA, VSD	**(P12)** NO: VSD, ASD	**(P47)** NO
(P20) VSD	**(P32)** TOF, VSD	**(P06)** NO	**(P19)** NO
(P10) TOF	**(P31)** ***HRHS, PS, VSD***	**(P02)** NO	**(P29)** NO
(P27)*OA, PA, VSD, MAPCA*	**(P22)** NO	**(P41)** NO	**(P07)** NO
(P46) ***N0: PA, VSD, MAPCA***	**(P25)** NO	**(P44)** NO	**(P05)** NO
(P42) NO	**(P39)** NO	**(P09)** NO	**(P01)** NO

AS Aorta stenosis, *ASD*: atrial septum defect, *APV* absent pulmonary valve, *BAV* bicuspid aorta valve, *DCVSD* double comitted ventricle septum defect, *HRHS* hypoplastic right heart syndrome, *IAA* interrupted aortic arch, *MAPCA* major aortopulmonary collateral arteries, *OA* overriding aorta, *PA* pulmonal atresi, *TOF* triade of Fallot, *VSD* ventricular septum defect, *NO* not operated. Bold indicate an operation/intervention within 2 weeks after birth. Italic and bold indicate that the patient died because of the cardiac disease. Patient identification is indicated in brackets

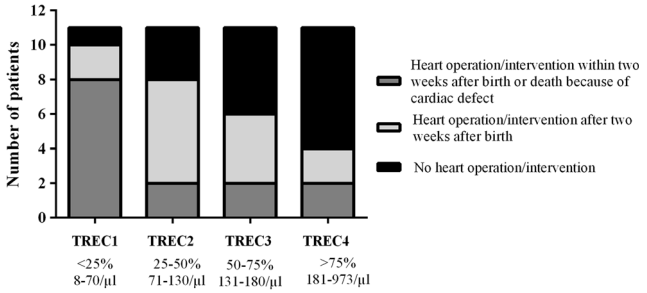

Fig. 1 Number of patients in different TREC quartiles and heart defects. There is a significant (p=0.010) correlation between TREC quartile 1 and TREC quartile 2, 3 or 4 for having heart operation within two weeks or death because of cardiac disease

(hypoplastic right heart syndrome, pulmonary stenosis and ventricle septum defect). For TREC quartile 3, a total of six patients (55 %) underwent heart operation/intervention, including one within two weeks after birth and one who died because of the defect (pulmonary atresia ventricular septum defect and MAPCA) at the age of 2.5 years. For TREC quartile 4, a total of five patients (45 %) underwent heart operation/intervention, including one within two weeks after birth and one infant died 1.5 years old because of the defect (pulmonary atresia, overriding aorta, VSD and MAPCA).

Neonatal TREC Quartiles and Chromosomal Deletion Size

The size of the deletion is known in 30 patients since 24 were diagnosed with MLPA and six by CGH array. In the 29 patients with the classical 3 MB deletion no correlation with the TREC level was found. One patient had a deletion o 3.3 Mb affecting both TBX1 and catechol-O-methyltransferase (COMT). It included the classical 22q11.2 DS area and parts of the area that is duplicated in Cat Eye syndrome (which is the neighbor area on chromosome 22). This patient had an additional 7.4 Mb deletion of the short arm of chromosome three. The patient was in TREC level 2 and had a heart operation after two weeks of age.

Neonatal TREC Quartiles and Other Disease Features

Hypocalcemia was detected in 13 patients with 22q11.2 DS. One patient with thymus aplasia had hypocalcemia within the first month of life. In the other twelve patients, calcium was measured within the first week, and for the remaining one within the first month. There was no correlation between TREC quartiles and hypocalcemia, cleft palate variants or polyhydramnion.

Discussion

In this population based study, we found that newborns who later were diagnosed with 22q11.2 DS, had significantly lower TREC levels compared to randomly selected control neonates when testing stored screening filter cards from the newborn screening program. This difference in TREC has been reported previously for older age groups when comparing patients with 22q11.2 DS and age matched controls [24–26]. Our results indicate that the thymus hypoplasia/aplasia found in our patients is congenital and not acquired after birth. We also found correlations between newborn TREC levels and the clinical characteristics of these patients.

In patients with 22q11.2 DS, thymic aplasia but particularly hypoplasia is often encountered [27, 28], and may correlate with T-cell lymphopenia. The spectrum of immunodeficiency in these patients ranges from a total lack of T-cells and thymic aplasia to normal T-cell numbers [5, 29–31]. In our patient group a normal, visible thymus as observed during heart operation, was predominantly found in infants in the higher TREC quartiles. There was a significant correlation (p= 0.022) between low TREC quartile and an abnormal thymus feature (no or remnant thymus). We also found a significant correlation between TREC quartiles and the proportion of patients with low number of $CD3^+CD4^+$T-cells even if they were not analysed at the same time. There was a trend in the same direction for $CD3^+$T-cells and $CD3^+CD8^+$T-cells.

Our study included two infants with no detectable TREC and thymic aplasia. Both these infants died of systemic Cytomegalovirus (CMV) infection at 4 and 6 months of age, respectively. For the rest of the patients including those children with low TRECs and low T-cell counts, we received no reports of opportunistic infections. This is in agreement with several other studies that have shown only minor clinical immunodeficiency for most 22q11.2 DS patients [5, 30, 32]. However, one study [33] showed an increase in non-cardiac mortality for children with 22q11.2 DS and T-cell counts less than the 10th percentile for their age group controls, including four of 11 children who died of infections or lymphoproliferative disease. We did not find any correlation between TREC levels and infections or hospitalization. In patients with 22q11.2 DS the special pharyngeal anatomy may contribute to the often recurrent middle ear infections.

Cardiac defects are the major cause of death in patients with 22q11.2 DS [34]. In our cohort five patients died of a cardiac defect. We found a significant correlation between cardiac defects and low TREC quartiles. A surprising finding was that the most severe cardiac defects that needed acute operation or intervention, occurred in patients in the lowest TREC quartile (<71 TRECs/ µL). There is no clear explanation for this. The common deleted region of chromosome 22q11.2 contains more than 40 genes including the gene for T-Box 1 (TBX1), which is considered essential for the phenotypic features of the

syndrome [7, 8]. Haploinsufficiency for TBX1 in mice leads to a compromised development of the thymus and the parathyroid glands [11, 12]. TBX1 is also expressed in the secondary heart field, which gives rise to the cardiac outflow tract and right ventricle, interventricular septation, and conal alignment [9–12]. Our main hypothesis is that embryological factors are involved both in the heart defects and thymus aplasia/hypoplasia since they are developed from the same pharyngeal arches [35, 36]. Stress has been associated with a smaller thymus in neonates [37], and this may have an additive effect in sick infants with severe heart defects.

Our study includes patients up to seven years of age. Some clinical features like learning disabilities and psychiatric illnesses, are observed in older patients with 22q11.2 DS, and are therefore not studied in our report.

Conclusions

In this study we found that low TREC values correlated with $CD3^+CD4^+$T-cells and with a lack of thymus/remnant thymus. More surprising is that we also demonstrated a correlation between low TREC values and conotruncal cardiac defects. Thus, newborn TREC values can be used as a prognostic marker for these disease features in patients with 22q11.2 DS.

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Conflicts of Interest All the authors confirm that no conflict of interest exists.

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