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BRIEF COMMUNICATION

Timo M. Breit · Ingrid L. M. Wolvers-Tettero
Ad J. J. C. Bogers · Ronald R. de Krijger
Juriy W. Wladimiroff · Jacques J. M. van Dongen

Rearrangements of the human *TCRD*-deleting elements

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There are two types of T-cell receptors (TCR) present on human peripheral blood (PB) T lymphocytes: TCR-αβ and TCR-γδ (Davis and Bjorkman 1988). Although little is known about the mechanisms that commit a T cell to the αβ- or γδ lineage, it is generally assumed that the *TCRD* gene plays a pivotal role in the divergence of the two lineages (De Villartay et al. 1988; Hockett et al. 1988, 1989; Van Dongen et al. 1990). This assumption is based on two features of the *TCRD* gene. Firstly, the *TCRD* gene is located in the middle of the *TCRA* gene and is therefore deleted during Vα-Jα rearrangement, which in principle excludes co-expression of *TCRD* and *TCRA* chains (Hockett et al. 1988; Isobe et al. 1988). Secondly, in normal polyclonal thymocytes a predominant rearrangement is observed, which represents the rearranged *δREC* and *ΨJα* gene segments (De Villartay et al. 1987, 1988; Hockett et al. 1989). These two gene segments flank the major part of the *TCRD* gene and are called *TCRD* deleting elements, because their non-productive rearrangement deletes the intermediate germline and/or rearranged *TCRD* gene sequences (De Villartay et al. 1988). Therefore, a model is postulated in which a germline or a rearranged *TCRD* gene is deleted by the *δREC-ΨJα* rearrangement, which in turn can be replaced by a *Vα-Jα* gene rearrangement (Fig. 1; Van Dongen et al. 1990).

T. M. Breit · I. L. M. Wolvers-Tettero · J. J. M. van Dongen (✉)
Department of Immunology, University Hospital Dijkzigt/Erasmus University, P. O. Box 1738, 3000 DR Rotterdam, The Netherlands

A. J. J. C. Bogers
Department of Thoracic Surgery, University Hospital Dijkzigt/Erasmus University, Rotterdam, The Netherlands

R. R. de Krijger
Department of Pediatrics, Sophia Children's Hospital/Erasmus University, Rotterdam, The Netherlands

J. W. Wladimiroff
Department of Gynaecology/Obstetrics, Division of Prenatal Diagnosis, University Hospital Dijkzigt/Erasmus University, Rotterdam, The Netherlands

Because new genetic nomenclature concerning *TCR* gene segments has recently been introduced, we propose renaming the formerly designated *δREC* and *ΨJα* gene segments according to this new nomenclature as defined by the WHO-IUIS Nomenclature Subcommittee on TCR Designation (WHO Bulletin 1993). The *δREC* gene segment could be renamed *TCRDREC1*, homologous to the *δREC1* to *δREC3* gene segments defined in the mouse (Takeshita et al. 1989). The *ΨJα* gene segment could be renamed *TCRAJ61P*, as it is the most upstream-located gene segment of the 61 human *Jα* gene segments (Koop et al. 1994). We designate the *δREC-ΨJα* rearrangement *TCRDREC1AJ61P*. Hereafter, we will use the WHO nomenclature for all *TCR* genes and rearrangements, with the abbreviations *DREC1* (δREC), *AJ61P* (ΨJα), and *DREC1.AJ61P* (δREC-ΨJα).

Although the *DREC1.AJ61P* rearrangement by its result and prominent occurrence in the thymus seems to play a distinct role in the divergence of the human TCR-αβ and TCR-γδ T-cell lineages, limited information is available in the literature concerning this rearrangement. The first *DREC1.AJ61P* rearrangements were analyzed only by identification of circular excision products with a *AJ61P.DREC1* signal joint (De Villartay et al. 1988). The subsequent determination of the *DREC1.AJ61P* junctional region of a T-cell line (DU.528) showed not only N-region nucleotide insertion and deletion of nucleotides by trimming of the flanking sequences comparable to a normal rearrangement, but revealed also evidence for *TCRDD* (*Dδ*)-gene-derived junctional region nucleotides (Begley et al. 1989). Other rearrangements involving one of the two *TCRD* (TCR-δ)-deleting elements have also been observed, such as *DREC1.J1* (δREC-Jδ1), *DREC1.AJI* (δREC-JαI), and *DREC1.AJII* (δREC-JαII) in T-cell acute lymphoblastic leukemia (T-ALL; Breit et al. 1991a, b; Hara et al. 1991), as well as *DD3.AJ61P* (Dδ3-ΨJα) and *AV3S1.J61P* (Vα3.1-ΨJα) in normal thymocytes (De Villartay et al. 1988).

A total of 150 *DREC1.AJ61P* junctional regions were analyzed to determine their precise sequence and to investigate whether *TCRDD* gene segments can occur in

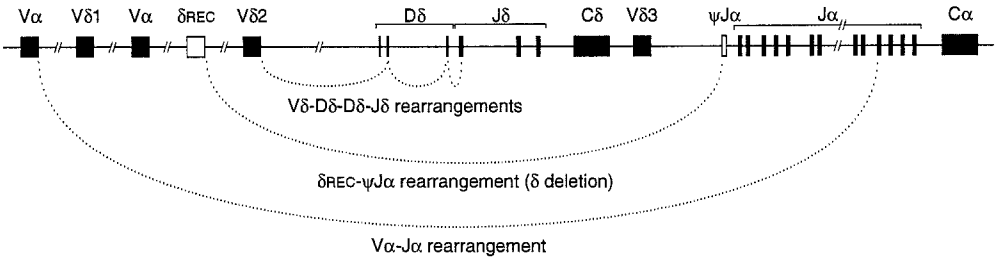

Fig. 1 Schematic representation of the human *TCRAD* (TCR-α/δ) locus. Indicated are the various gene segments including the *TCRD*-deleting elements: *DREC1* (δREC) and *AJ61P* (ΨJα). The *dotted lines* indicate the possible consecutive gene rearrangements: *TCRDV.D.DJ* (Vδ-Dδ-Dδ-Jδ), *DREC1.AJ61P* (δREC-ΨJα), and *TCRAV.J* (Vα-Jα)

these junctional regions and if so, in what frequency they are present. To investigate at the same time potential differences in the *DREC1.AJ61P* rearrangement during human ontogeny, we studied 15 thymus and blood cell samples from fetuses, neonates, and adults.

The 15 cell samples consisted of five fetal thymi (12, 15, 16, 17, and 18 weeks of gestation), five postnatal thymi (3 days, 1 month, 1 year, 5 years, and 15 years), one fetal cord blood sample (18 weeks of gestation), one neonatal cord blood sample, and three adult PB samples (16 years, 27 years, and 28 years). DNA was extracted from the obtained cell samples as described (Van Dongen and Wolvers-Tettero 1991). Fifteen μg of each DNA sample was digested with *Eco* RI, *Hin* dIII, and/or *Bgl* II and analyzed by Southern blot analysis, using the *DREC1* probe (TCRDRE) and *AJ61P* probe (TCRAPJ; Breit et al. 1993). Of each DNA sample, 0.25–0.5 μg was amplified in a normal 100 μl polymerase chain reaction (PCR) reaction mix, using the

Fig. 2A, B Southern blot analysis of the *DREC1.AJ61P* rearrangement in various human cell samples. Lane 1, control DNA (cell line HELA); lane 2, fetal thymus 15 weeks; lane 3, fetal thymus 18 weeks; lane 4, neonatal thymus 3 days; lane 5, infant thymus 15 years; lane 6, neonatal cord blood; lane 7, infant PB 16 years; lane 8, adult PB 28 years. **A** Hybridization of *Hin* dIII digests with the TCRDRE (*DREC1*) probe. **B** Rehybridization with the TCRAPJ (*AJ61P*) probe. The band representing the preferential *DREC1.AJ61P* (δREC-ΨJα) rearrangement is indicated. All other rearranged bands represent other preferential rearrangements to either the *DREC1* or *AJ61P* gene segment. (*G* indicates the germline band)

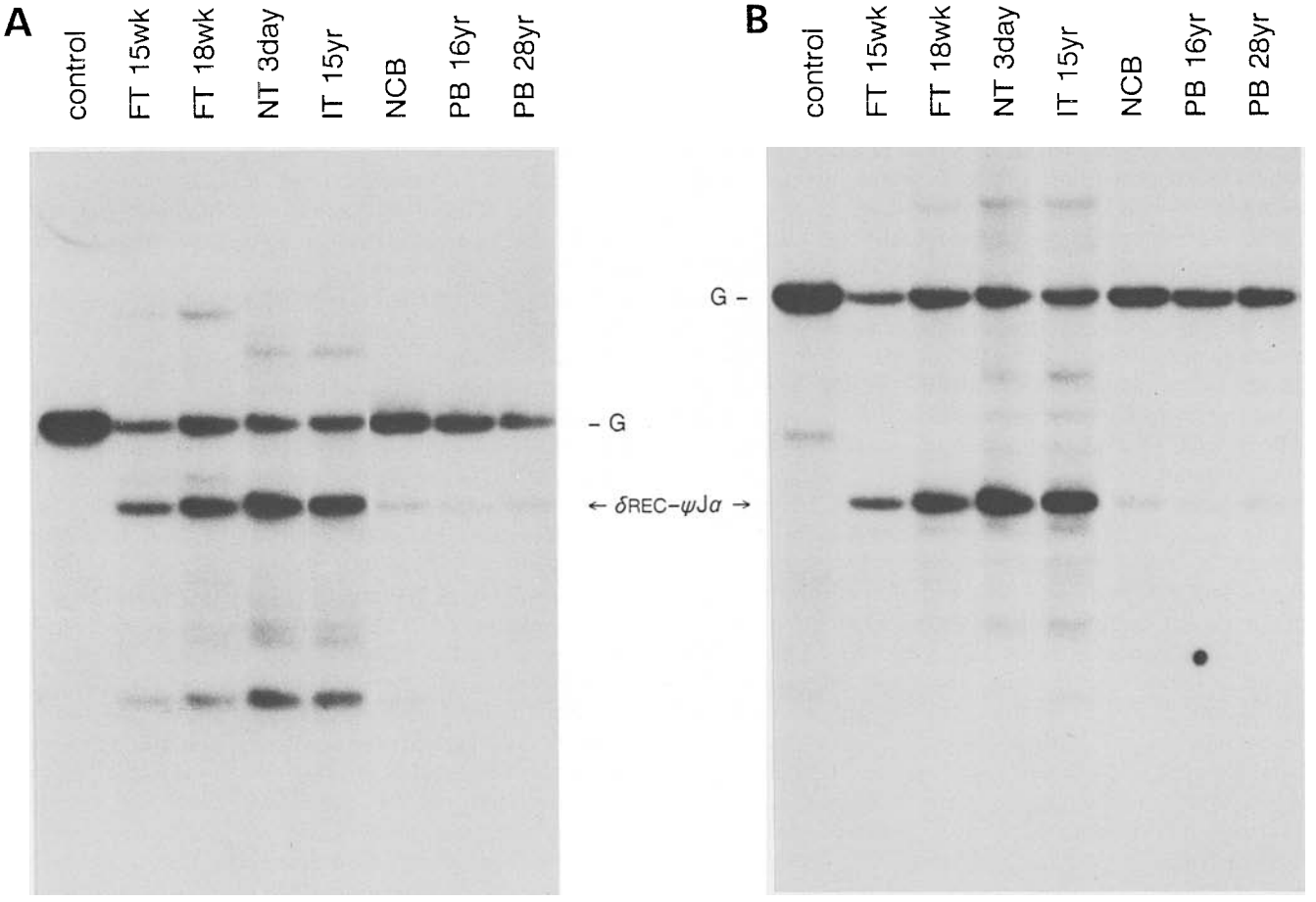

Table 1 Junctional region diversity of human *DREC1.AJ61P* rearrangements, as determined by analyzing at least ten junctional regions per cell samples

	No. of inserted nucleotides				No. of deleted nucleotides		
	N-region	*TCRDD*	P-region	Total	*DREC1*	*AJ61P*	Total
Fetal thymus							
12 weeks	1.3	0.3	0.1	1.7	4.8	4.7	9.5
15 weeks	1.7	0.8	0.2	2.7	1.8	4.3	6.1
16 weeks	1.6	0	0.4	2.0	1.9	2.9	4.8
17 weeks	1.7	1.7	0.3	3.7	3.7	2.1	4.8
18 weeks	2.3	1.0	0.3	3.6	2.0	2.4	4.4
Postnatal thymus							
3 days	3.7	2.2	0.6	6.5	3.3	8.4	11.7
1 month	3.9	1.8	0.6	6.3	3.4	4.5	7.9
1 year	2.6	1.4	0.2	4.2	3.8	3.9	7.7
5 years	2.8	1.9	0.6	5.3	1.2	4.4	5.6
15 years	3.0	1.8	0	4.8	4.6	5.9	10.5
Fetal cord blood							
18 weeks	2.5	1.7	0.4	4.6	3.7	5.1	8.8
Neonatal cord blood							
NCB5	1.6	1.4	0.1	3.1	2.8	4.6	7.4
Peripheral blood							
16 years	5.4	2.0	0.7	8.1	4.0	4.6	8.6
27 years	3.2	0.3	0.4	3.9	3.9	4.7	8.6
28 years	5.3	2.3	0.1	7.7	4.2	5.1	9.3

oligonucleotide primers δREC-5′E (ctaagaatTCGATC-CTCAAGGGTCGAGACTGTC) and ΨJα-3′ H (cctg-aagcTTAAGGCACATTAGAATCTCTCACTG) as described (Breit et al. 1993b). The obtained polyclonal PCR products of ~500 base pairs (bp) were digested with *Eco* RI and *Hin* dIII and cloned in the pUC19 vector. Ten single bacteria colonies of each sample were randomly picked and sequenced with the universal pUC reverse sequencing primer as described (Sambrook et al. 1989).

Southern blot analysis of the various cell samples confirmed the prominent presence of the *DREC1.AJ61P* rearrangement in all thymic cell samples, but in PB mononuclear cells this rearrangement was hardly visible (5% detection limit; Fig. 2). The latter observation is probably caused by the predominant biallelic *TCRAV.J* (Vα-Jα) gene rearrangements in peripheral TCR-αβ+ T-lymphocytes, which have deleted the preexisting *DREC1.AJ61P* rearrangements.

The sequences of the *DREC1.AJ61P* junctional regions in the various cell samples are presented in Figure 3. Although there are some differences, in all cell samples N-region and P-region nucleotide insertion occurred in addition to deletion of nucleotides from the flanking sequences. The characteristics of the *DREC1.AJ61P* junctional regions in Table 1 show that the total nucleotide insertion in fetal thymocytes (1.7–3.7) is on average lower than in postnatal cell samples (4.2–6.5). Especially in 12 weeks thymocytes, N-region nucleotide insertion was very low, probably due to low expression of the enzyme terminal deoxynucleotidyl transferase (Campana et al. 1989), which mediates the random N-region nucleotide insertion. Nucleotide deletion was also more extensive in postnatal cell samples, suggesting that rearrangements in early fetal thymocytes are performed by an "immature" recombinase complex, which is less capable of nucleotide deletion. Furthermore, in almost all cell samples, nucleotide deletion by trimming of the *DREC1* gene segment was less extensive than trimming of the *AJ61P* gene segment, indicating that the activity of the recombinase enzyme complex has a direction, which may be related to the size of the spacers in the recombination signal sequences (Breit et al. 1993a).

Usually, *TCRDD* nucleotides are identified in *TCRD* junctional regions based on the guideline that at least one-third of the *TCRDD* gene segment has to be present with a minimum of three consecutive nucleotides. By using this guideline, we could identify putative *TCRDD* gene-derived nucleotides in 36% of the *DREC1.AJ61P* junctional regions. However, there was no complete *TCRDD* gene segment present in any junctional region, whereas in normal *TCRDV.J* (Vδ-Jδ) junctional regions complete *TCRDD2* (Dδ2) and *TCRDD3* (Dδ3) gene segments frequently occur (Breit et al. 1991b; Panchamoorthy et al. 1991). Moreover, applying the same guideline to published *TCRAV.J* or even *TCRGV.J* (Vγ-Jγ) junctional regions, revealed comparable frequencies of putative *TCRDD* gene-derived nucleotides, although these junctional regions should not contain *TCRDD* gene segments (Breit et al. 1991b; Porcelli et al. 1993). In fact, the *DREC1.AJ61P* junctional regions are highly homologous to *TCRAV.J* and *TCRGV.J* junctional regions (with only one N-region) and are essentially smaller than *TCRDV.J* junctional regions (with primarily two or three N-regions). We therefore conclude that most putative *TCRDD* gene-derived nucleotides observed in the *DREC1.AJ61P* junctional regions probably represent N-region nucleotides, which is in line with the finding that putative *TCRDD* gene-derived nu-

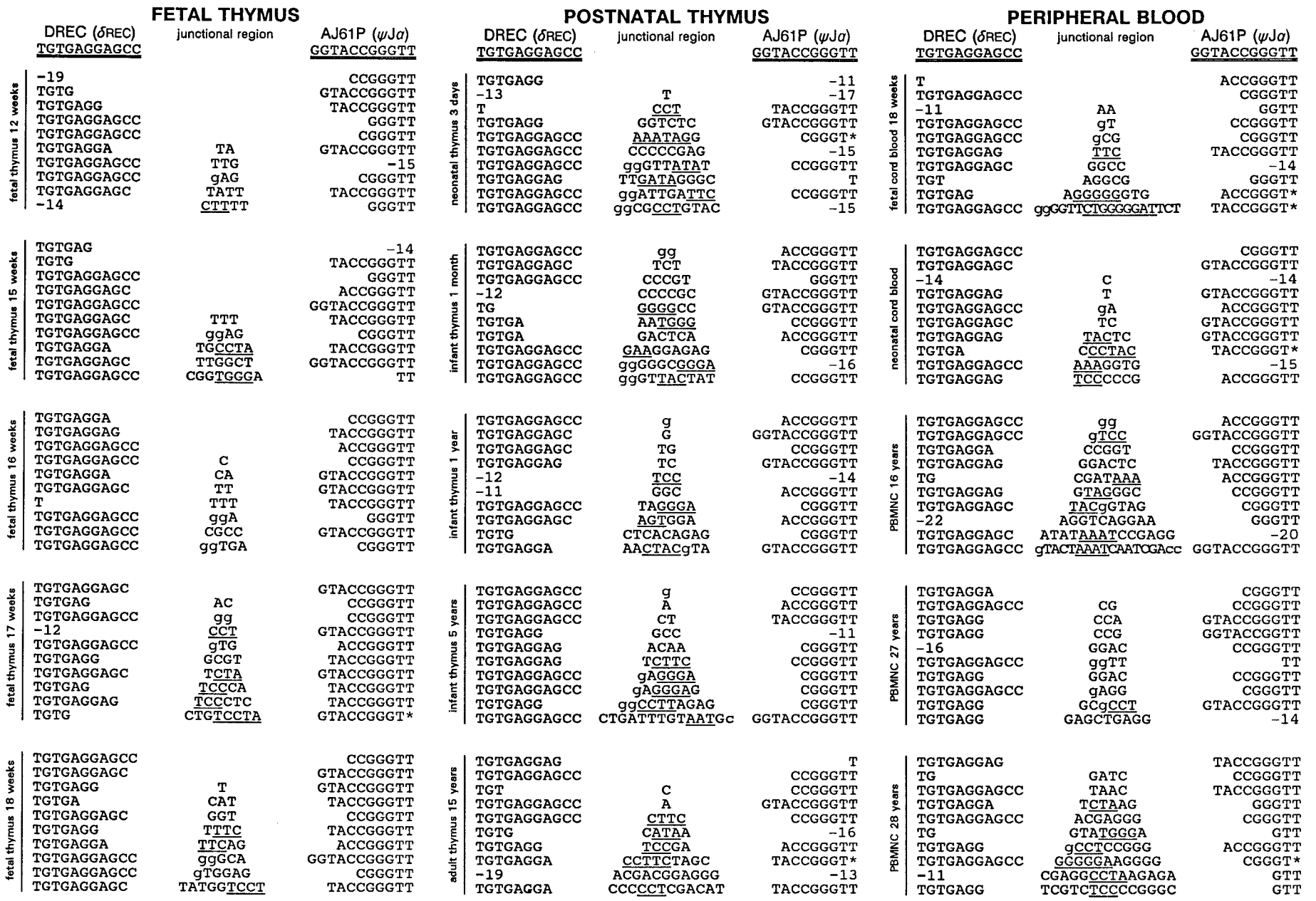

Fig. 3 (For legend see p. 74)

Fig. 3 Junctional region sequences of *DREC1.AJ61P* rearrangements in various human cell samples. Sequences of the *DREC1.AJ61P* junctional regions are aligned with the known (*double underlined*) *DREC1* (δREC) and *AJ61P* (ΨJα) germline sequences. *Single underlined* sequences represent putative *TCRDD* gene-derived nucleotides. *Lower-case* characters represent P-region nucleotides and all other junctional region nucleotides represent N-region nucleotides. (* Indicates junctional regions (n = 7) with a long string of (≥5) *TCRDD* gene-derived nucleotides)

cleotides are virtually absent, if the number of N-region nucleotides is low (12-week-old fetal thymocytes).

Nevertheless, in some *DREC1.AJ61P* junctional regions (7/150) we discovered longer strings (≥5) of putative *TCRDD* gene nucleotides (Fig. 3), suggesting that in these particular instances *TCRDD* gene-derived nucleotides are indeed present. All three *TCRDD* gene segments were present at least once, indicating that the *DREC1* and *AJ61P* gene segments are able to rearrange to each *TCRDD* gene segment.

The finding that *DREC1.AJ61P* and *TCRAV.J* junctional regions rarely include a *TCRDD* gene segment may be due to the order of rearrangements on one allele: firstly a *TCRDD.J* (Dδ-Jδ) or *TCRDV2/3.D.J* (Vδ2/3-Dδ-Jδ) rearrangement, followed by the *TCRD* gene-deleting *DREC1.AJ61P* rearrangement, and finally a *TCRAV.J* rearrangement (Fig. 1). In principle, *TCRDD* gene segments can only be involved in *DREC1.AJ61P* or *TCRAV.J* rearrangements, if germline *TCRDD* gene segments are available at the time of rearrangement, i.e., germline *TCRD* genes or incompletely rearranged *TCRD* genes. Apparently this does not occur frequently, or there are other (yet unknown) restrictions excluding *TCRDD* gene segments from these rearrangements. Overall, the *DREC1.AJ61P* rearrangement appears to be just a *TCRAV.J*-like rearrangement, committing the thymocyte to the TCR-αβ lineage.

It can be concluded that the predominant *TCRD* gene-deleting *DREC1.AJ61P* rearrangement is present in human thymocytes throughout ontogeny. The size of the *DREC1.AJ61P* junctional regions increases during thymic ontogeny, but no further ontogenic differences were observed. The *TCRD* gene-deleting elements can potentially rearrange to *TCRDD* gene segments, but our extensive sequencing analyses of 150 *DREC1.AJ61P* junctional regions revealed that they rarely contain *TCRDD* gene-derived nucleotides.

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