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A variety of *Alu*-mediated copy number variations can underlie IL-12R β 1 deficiency

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Compliance with Ethical Standards

Research involving human participants. Informed consent for participation in this study was obtained in accordance with local regulations, with approval from the IRB. The experiments described here were performed in France, in accordance with local regulations, and with the approval of the IRB of Necker Hospital for Sick Children, France.

Informed consent. Written informed consent was obtained from the guardians of the patients.

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Abstract

Purpose—Inborn errors of IFN- γ immunity underlie Mendelian susceptibility to mycobacterial disease (MSMD). Autosomal recessive complete IL-12R β 1 deficiency is the most frequent genetic etiology of MSMD. Only two of the 84 known mutations are copy number variations (CNVs), identified in two of the 213 IL-12R β 1-deficient patients and two of the 164 kindreds reported. These two CNVs are large deletions found in the heterozygous or homozygous state. We searched for novel families with IL-12R β 1 deficiency due to CNVs.

Methods—We studied six MSMD patients from five unrelated kindreds displaying adverse reactions to BCG vaccination. Three of the patients also presented systemic salmonellosis, two had mucocutaneous candidiasis, and one had disseminated histoplasmosis. We searched for CNVs and other variation by *IL12RB1*-targeted next-generation sequencing (NGS).

Results—We identified six new IL-12R β 1-deficient patients with a complete loss of IL-12R β 1 expression on PHA-activated T cells and/or EBV-transformed B cells. The cells of these patients did not respond to IL-12 and IL-23. Five different CNVs encompassing *IL12RB1* (four deletions and one duplication) were identified in these patients by NGS coverage analysis, in either the homozygous state ($n=1$) or in *trans* ($n=4$) with a single nucleotide variation ($n=3$) or a small indel ($n=1$). Seven of the nine mutations are novel. Interestingly, four of the five CNVs were predicted to be driven by nearby *Alu* elements, as well as the two previously reported large deletions. The *IL12RB1* locus is actually enriched in *Alu* elements (44.7%), when compared with the rest of the genome (10.5%).

Conclusion—The *IL12RB1* locus is *Alu*-enriched and therefore prone to rearrangements at various positions. CNVs should be considered in the genetic diagnosis of IL-12R β 1 deficiency.

Keywords

IL-12R β 1; mycobacterium; histoplasma; salmonella; copy number variation; *Alu* elements

INTRODUCTION

Complete IL-12R β 1 deficiency (MIM:614891) is the most frequent genetic etiology of Mendelian susceptibility to mycobacterial disease (MSMD) [1–19]. The human *IL12RB1* gene is located on chromosome 19p13 and has 17 exons. *IL12RB1* encodes the β 1 chain common to the receptors for IL-12 and IL-23 [20, 21]. To date, 213 patients from 164 kindreds worldwide have been reported to have complete IL-12R β 1 deficiency [1–19] and many more have probably been diagnosed. Mycobacterial disease is the most frequent infection and has been reported in 80% of the patients. It is highly heterogeneous in terms of its clinical manifestations, severity, and outcome [1, 22, 23]. About half the IL-12R β 1-deficient patients also present infectious diseases caused by *Salmonella* species. About a third also display mucocutaneous candidiasis due to an impairment of IL-17 immunity [1, 22–24]. A sizeable minority of patients (about 20%) remain asymptomatic in adulthood; IL-12R β 1 deficiency thus displays incomplete clinical penetrance, even at an advanced age [1].

All reported *IL12RB1* mutations functionally assessed to date cause a complete loss of the induction of IFN- γ production by T and NK lymphocytes in response to IL-12 and IL-23 [1, 8, 21]. Eighty-four mutations have been reported to date, including missense mutations ($n=24$), nonsense mutations ($n=23$), mutations of canonical splice sites ($n=13$), small out-of-frame insertions, deletions, duplications, or indels ($n=19$), and small in-frame insertions, deletions, duplications, or indels ($n=3$). These small mutations invariably cause a complete lack of IL-12R β 1 expression. Some of these mutations are recurrent: a founder effect, dating back 475 years, has been characterized for the c.1623_1624delinsTT mutation, which has been reported in 18 kindreds from eight countries, all of European descent [15, 22, 25–27] whereas a hotspot is suspected for the c.1791+2T>G mutation [21], which has been reported in 18 kindreds from 11 countries on four continents (Africa, America, Asia, and Europe) [2, 3, 8, 16, 22, 28–34].

Only two copy number variations (CNVs; *i.e.* deletion or duplication of more than 200 base pairs) [35] have been reported, both of them prior to the advent of next-generation sequencing (NGS). These two CNVs, described in two families, are large deletions [31, 36]. A homozygous 12,167-nucleotide deletion encompassing exons 8 to 13 (c. 700+362_1619-944del; 8-13) has been reported in a consanguineous Arab family living in Israel [36, 37]. This in-frame deletion leads to the residual expression of a non-functional IL-12R β 1 protein on the cell surface in homozygous individuals [36]. The second large deletion was described in a French woman compound heterozygous for the frameshift c. 1745_1746insCA indel and for a 3,011 bp deletion (c.1483+182_1619-1073del; 13) encompassing exon 13 [31]. This patient has no IL-12R β 1 expression. Both these large deletions were predicted to result from the proximity of short repetitive *Alu* elements. The breakpoints of the 8-13 deletion lie within *AluSx1* and *AluJr* elements and the breakpoints of the 13 deletion lie within *AluSx* and *AluJr* elements. These two CNVs are so far private [38]. In this study, we took advantage of *IL12RB1*-targeted NGS to identify six new patients from five unrelated families with IL-12R β 1 deficiency due to CNVs.

MATERIALS AND METHODS

Cellular immunology

Patients were referred to the laboratory of Human Genetics of Infectious Diseases (HGID) for cellular and genetic diagnosis. Informed consent forms were signed by patients and/or their parents, as requested and approved by the institutional review board. Whole-blood activation was performed as previously described [39, 40]. IFN- γ secretion was assessed with phytohemagglutinin (PHA)-IL-2 activated T cells. Briefly, in 96-well plates containing RPMI medium supplemented with 10% fetal bovine serum, PHA-IL-2 activated T cells were either left unstimulated or were stimulated with PHA, PHA+IL-12, PHA+IL-23 or PMA-ionomycin (see supplemental information).

Molecular genetics

Genomic DNA was extracted from whole blood or from PHA-activated T cells with the iPrep PureLink gDNA Blood kit and iPrep instruments from Thermo Fisher Scientific. Genomic DNA (1 to 3 μ g) from each individual was mechanically fragmented to a median size of 200 bp with a Covaris apparatus. Double-stranded fragmented DNA (100 ng) was end-repaired, and adaptors containing a specific eight-base barcode were ligated to the repaired ends (one specific barcode per patient). DNA fragments were amplified by PCR to obtain final precapture barcoded libraries that were pooled at equimolar concentrations. Capture was performed with SureSelect reagents (Agilent), 750 ng of the pool of precapture libraries and home-made biotinylated probes. The single-stranded biotinylated DNA probes were designed to cover a 150 kb chromosomal region including the *IL12RB1* gene on chromosome 19 (Chr19:18,152,973-18,303,007) according to the reference human genome sequence (GRCh37/hg19). During capture, barcoded library molecules complementary to the biotinylated beads were retained on streptavidin-coated magnetic beads and amplified by PCR to generate a final pool of postcapture libraries covering the targeted region. A pool of 12 libraries (nine DNA samples from kindreds and three from healthy controls) covering a 150 kb region including the entire *IL12RB1* gene was sequenced in two different runs on an

Illumina HiSeq2500 (paired-end sequencing 130×130 bases, high-throughput mode, 13 samples on half a FlowCell lane). Sequences were aligned with the hg19 reference human genome with Burrows-Wheeler Aligner version 0.6.2.13 [41]. CNVs were analyzed as previously described [42, 43]. The Sanger sequencing of *IL12RB1* exons and their flanking regions was performed as previously described [36]. Targeted sequencing of CNVs was performed by amplifying genomic DNA with the Expand Long Template PCR System (Roche) and subjecting it to Sanger sequencing (See Table S1 for conditions). RNA was extracted from EBV-B cells with the RNeasy Mini Kit (Qiagen). RNA was reverse transcribed to generate cDNA with the SuperScript II Reverse Transcriptase (Thermo Fisher scientific). Full-length cDNA was amplified by CloneAmp HiFi PCR (CloneTech) with the primers indicated in Table S2, cloned with the Zero Blunt PCR Cloning kit (Invitrogen), and used to transform *E. coli* (One Shot TOP10, Invitrogen). Single colonies of *E. coli* were then amplified and sequenced with the primers listed in Table S2.

Bioinformatic analyses

The breakpoint regions were analyzed for the presence of repetitive elements, non-B DNA conformations and sequence motifs, to evaluate the involvement of genomic architecture in the origin of the CNVs. The presence of known repetitive elements intersecting with the breakpoints was investigated with the RepeatMasker (<http://www.repeatmasker.org/>) track in the UCSC genome browser (<https://genome.ucsc.edu/>) [44]. If both breakpoints overlapped with repetitive elements from the same class, BLAST was used to calculate the percentage sequence identity between the elements (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The Non-B DNA Motif Search Tool (<https://nonb-abcc.ncicrf.gov/apps/nBMST/default/>) was then used to search for DNA sequences adopting non-B DNA conformations, such as direct, inverted and mirror repeats forming slipped hairpin, cruciform and triplex structures, respectively; GT-repeats forming left-handed Z-DNA; and G-quadruplex-forming repeats. Non-B DNA conformations were recorded only if both counterparts flanked the breakpoint. Finally, the presence of previously described structural variation-associated sequence motifs [45] was analyzed with Fuzznuc [46], as these motifs may lead to genomic stability, facilitating copy number variation.

RESULTS

Impairment of IFN- γ production and abolition of IL-12R β 1 expression in six MSMD patients

We investigated six patients with MSMD from five unrelated kindreds from Iran (Kindreds A and E), Mexico (Kindred B), Ukraine (Kindred C), and Greece (Kindred D) (Figure 1A; see Supplemental information for detailed case reports). All patients presented a clinical spectrum of infections (BCG-itis or BCG-osis, with or without invasive salmonellosis or mucocutaneous candidiasis) suggestive of an inborn error of IFN- γ immunity, and of IL-12/IL-23 immunity in particular. Two of the patients (P2 and P3) also presented recurrent invasive salmonellosis and a third (P1) had gastroenteritis caused by *Salmonella*. In P2, salmonellosis was associated with leukocytoclastic vasculitis (Figure 1B), a feature previously reported in eight other patients with IL-12R β 1 deficiency and *Salmonella* infections [3, 4, 9, 14, 18, 22, 47–49]. Three patients (P1, P2, and P6) experienced fungal disease, mostly mucocutaneous infection with *Candida* spp. P2 also suffered from

disseminated histoplasmosis. No IFN- γ induction in response to IL-12 in P1, P2, P3 and P4 was detected in whole-blood and no IFN- γ secretion was detected in response to IL-12 and IL-23 in PHA-activated T cells from P3 and P6 (Supplemental Figure 1B and 1C). PHA-activated T cells from a healthy control (C+) expressed IL-12R β 1, whereas PHA-activated T cells from P1, P2, P3, P4 and P6 had no detectable IL-12R β 1 expression, yielding results similar to those for the negative control (IL12-R β 1^{-/-}) (Supplemental Figure 2A). The cells from P1, P2, P3, P4, and P6 tested displayed no IL-12R β 1 expression on the cell surface and did not respond to IL-12 (Supplemental Figures 1 and 2 and Supplemental information).

NGS-based detection of inherited IL-12R β 1 deficiency

We thus investigated *IL12RB1* as a probable candidate gene underlying the clinical and cellular phenotypes of the five kindreds. We performed high-throughput sequencing for the entire *IL12RB1* locus of the patients, their parents (when possible), and from healthy wild-type controls. We searched for rare single-nucleotide variants (SNVs) or indels in the exons and their flanking splice sites, and compared the quality of coverage in terms of read numbers. No SNV or indel was identified in kindred A. However, there was no coverage of the sequence between the 5' region and intron 7 for P1, and coverage of this region in both parents of P1 was also half that in a healthy control, suggesting the presence of a large homozygous deletion in P1 that was inherited from the two heterozygous parents (Figure 2A). In P2, from kindred B, we identified a heterozygous nonsense mutation in exon 13 (c.1561C>T; p.R521*). This nonsense mutation has already been reported to be responsible for IL-12R β 1 deficiency [22]. Coverage from intron 7 to intron 8 in P2 was also half that in a healthy control, consistent with a heterozygous deletion in P2 encompassing exon 8 (Figure 2A). In kindred C, we identified a new heterozygous indel in exon 8 that was predicted to cause a frameshift (c.747_752delinsAAAAT; p.Q250Kfs*7). Coverage from intron 11 to intron 12 for P3 was also half that for a healthy control, suggesting a heterozygous deletion encompassing exon 12 (Figure 2A). In kindred D, we identified a splice-site mutation in P4 affecting the donor flanking splice site of exon 15 (c.1791+2T>G), which seemed to be homozygous. This mutation is the most frequently reported mutation of *IL12RB1* [2, 3, 8, 15, 16, 22, 28–34]. However, coverage between intron 14 and the 3' region in P4 was also half that for a healthy control, suggesting a heterozygous deletion encompassing exons 15 to 17 and overlapping the c.1791+2T>G mutation (Figure 2A). In kindred E, we identified a previously undescribed missense mutation in exon 7 (c.635G>A; p.R212Q) in P6. This mutation is a rare and found only as a heterozygous variant in one of the 246,185 chromosomes in the gnomAD database. It was predicted to be damaging by the PolyPhen-2 (0.944) and CADD (23.4) pathogenicity prediction scores. Moreover, coverage in P6 and his father (I.2) was one third higher than that for a healthy control, for the region between introns 9 and 12, suggesting heterozygous duplication encompassing exons 10, 11, and 12 (Figure 2A). The CNVs in kindreds A, B, C, D and E are novel and do not match with the ones previously reported [31, 36].

Sanger sequencing-based diagnosis of autosomal recessive IL-12R β 1 deficiency

For confirmation of these findings, we performed targeted amplification and Sanger sequencing for the *IL12RB1* locus. In kindred A, Sanger sequencing identified a homozygous 13,336 bp deletion extending from the 5' region to intron 7 (1-7) in P1

(Supplemental Figure 3A). In kindred B, we confirmed the presence of the heterozygous c.1561C>T (p.R521*) mutation in P2 (Supplemental Figure 3B) and also identified a heterozygous 991 bp deletion encompassing exon 8 (–8) (Supplemental Figure 3A). In kindred C, Sanger sequencing for P3 confirmed the presence of a heterozygous indel c.747_752delinsAAAT (Supplemental Figure 3B) and identified a heterozygous 1,919 bp deletion encompassing exon 12 (–12) (Supplemental Figure 3A). In kindred D, Sanger sequencing identified a heterozygous 4,872 bp deletion extending from intron 14 to the 3' region (–15-17) in P4 (Supplemental Figure 3A) and confirmed the presence of the c.1792+2T>G mutation, which appeared to be hemizygous due to the overlapping deletion on the other allele (Supplemental Figure 3B). In kindred E, Sanger sequencing confirmed the presence of the heterozygous p.R212Q mutation in P5 and P6 (Supplemental Figure 3B) and identified a 5,418 bp duplication extending from intron 9 to intron 12 (dup10-12) (Supplemental Figure 3A). We also investigated familial segregation when the necessary material was available. In kindred A, the sibling of P1 (II.2) was not available for testing. In kindreds B, D, and E, parental segregation analyses indicated that P2, P4, P5, and P6 were compound heterozygous for their *IL12RB1* mutations (Figure 1A). The healthy siblings of P2 and P4 were either single-heterozygous for the mutant alleles or homozygous for the wild-type allele (Figure 1A). In kindred C, the heterozygous c.747_752delinsAAAT mutation of P3 was found to have been inherited from the mother (Figure 1A). Cloning and sequencing of the full-length *IL12RB1* cDNA from P3 identified the c.747_752delinsAAAT mutation on one allele and a skipping of exon 12 on the other, indicating that P3 was compound heterozygous for the c.747_752delinsAAAT and –12 mutations (data not shown). However, the father of P3 was not available for testing, making it impossible to determine whether the –12 deletion was inherited from the father or was *de novo*. We thus identified homozygous *IL12RB1* mutations in P1 (–1-7/–1-7) and compound heterozygous mutations in P2 (p.R521*/–8), P3 (c.747_752delinsAAAAT/–12), P4 (c.1791+2T>G/–15-17), and P5 and P6 (p.R212Q/dup10-12) (Figure 1A and Figure 2B).

Analysis of *Alu* elements at the *IL12RB1* locus

We investigated whether the breakpoint regions of the five CNVs identified in the five kindreds were enriched in structural variation–associated sequence motifs or sequences adopting non-B DNA conformations or whether the breakpoints were located within repeated elements, as the presence of such elements may lead to genomic instability and, consequently, to CNVs [50, 51]. Such genomic architectural features were observed in all breakpoint regions (data not shown) but enrichment was observed only for *Alu* elements. This term refers to the segment of sequences that can be cleaved by the *Arthrobacter luteus* restriction enzyme AluI. *Alu* elements are 280 bp sequences that are extensively repeated in the human genome [52]. The different human *Alu* subfamilies display more than 75% sequence identity. We used RepeatMasker [53] to determine the percentage of the genomic nucleotide sequence of the *IL12RB1* locus consisting of *Alu* elements. We arbitrarily defined the *IL12RB1* locus as extending from the *IL12RB1* start codon to its stop codon plus or minus 10 kilobases, as previously described [50]. Using this method, we found that 44.7% of the *IL12RB1* locus consisted of *Alu* elements. These *Alu* elements are located in the non-coding regions of the *IL12RB1* locus: the 5' region, 3' region, and intronic regions. For comparison, the mean *Alu* element content of chromosome 19 is 25.8% [54] and that of

the human genome is 10.5% [53]. The *IL12RB1* locus can therefore be considered to be enriched in *Alu* elements. We then used the same approach to determine the percent *Alu* content of the other MSMD genes. We found that *IL12RB1* was the MSMD gene displaying the strongest enrichment in *Alu* after *TYK2*, which consists of 54.6% *Alu* elements and is also located on chromosome 19 (Table 1). Alignments of the breakpoints with University of California, Santa Cruz (UCSC) Genome Browser Data [44] showed that the 1-7, 12, 15-17, and dup10-12 CNVs were located within eight different *Alu* elements: *AluSg4* in the 5' region and *AluY* in intron 7 for the 1-7 deletion; *AluSx1* in intron 11 and *AluSx* in intron 12 for the 12 deletion; two different *AluSz* elements located in intron 14 and in the 3' region for the 15-17 deletion; and the *AluSx* element in intron 9 and the *AluSx1* element in intron 12 for the 10-12 duplication (Figure 3A and 3B). The breakpoints of the 1-7, 12, 15-17, and dup10-12 CNVs involved two different *Alu* elements presenting flanking sequence similarity (Figure 3B). The 1-7, 12, 15-17, and dup10-12 CNVs displayed a 12 bp, 14 bp, 26 bp, and 17 bp junctional microhomology between their breakpoints, respectively (Figure 3B). The 8 deletion breakpoint is flanked by an *AluY* element in intron 8 but is neither flanked by nor located within a repetitive element in intron 7 (Figure 3A and 3B). Moreover, BLAST found no significant similarity between the sequences of introns 7 and 8 (Figure 3B) [55]. However, the two breakpoints of the 8 deletion display microhomology over two base pairs. None of the five CNVs identified here was present in the Database of Genomic Variants [38], so indicating that they are so far private to the affected families.

DISCUSSION

We report here six IL-12R β 1-deficient patients from five unrelated kindreds originating from Greece, Iran (two kindreds), Mexico, and Ukraine. Since its first description in 1998 [56, 57], IL-12R β 1 deficiency has been reported in various countries, including Iran [8, 11, 21, 22], Mexico [21, 34, 58], and Ukraine [22]. This report is the first to describe IL-12R β 1 deficiency in a Greek patient. The six patients described here have infectious diseases that are typical of patients with IL-12R β 1 or IL-12p40 deficiency [1, 22, 59]. They present complete IL-12R β 1 deficiency due to biallelic mutations. Seven new *IL12RB1* mutations were identified: four large deletions (1-7, 8, 12, and 15-17), one large duplication (dup10-12), one indel (c.747_752delinsAAAAT), and one missense mutation (p.R212Q). The other two mutations (p.R521* and c.1791+2T>G) identified here have been described before [2, 3, 8, 15, 16, 22, 28–34]. In the five kindreds, IL-12R β 1 deficiency was caused by a CNV (deletion or duplication) in the homozygous state or in compound heterozygosity with a nonsense mutation, missense mutation, small indel, or splice-site mutation. The five CNVs (1-7, 8, 12, 15-17, and dup10-12) reported here expand the spectrum of known genetic causes of IL-12R β 1 deficiency, as only two CNVs (13, 8-13) underlying this deficiency had previously been reported [31, 36]. We report the first large-scale deletion of *IL12RB1* (dup10-12). CNVs are not easy to detect by routine Sanger sequencing [42, 50, 60, 61]. In this study, a simple comparison of the read-depth data obtained for targeted high-throughput sequencing unmasked five CNVs that were then delimited more precisely by Sanger sequencing. The biallelic mutations reported here lead to a complete absence of IL-12R β 1 expression on PHA-activated T cells and EBV-B cells.

An analysis of the breakpoints of the five CNVs described for the first time here (1-7, 8, 12, 15-17, and dup10-12) and of the two previously described CNVs [31, 36] (13, 8-13) showed that six of these CNVs (1-7, 12, 15-17, dup10-12 and 13, 8-13) involved two different *Alu* elements, suggesting that *Alu-Alu*-mediated non-allelic homologous recombination (NAHR) may have been responsible for the creation of these CNVs. The non-coding regions of the *IL12RB1* locus are rich in *Alu* elements, suggesting that this locus is probably prone to *Alu*-mediated recombination (Figure 3). *Alu*-mediated deletion is a common mechanism underlying the formation of CNVs and has been reported for other genes, including the causal genes of other primary immunodeficiencies (PID) [50, 62]. However, the level of sequence identity is probably too low to provide the minimal efficient processing segments (MEPS) required for NAHR [35]. Nevertheless, microhomology was detected at the junctions of six CNVs (1-7, 8, 12, 15-17, dup10-12 and 8-13), suggestive of a replication-based mechanism (RBM) or end-joining mechanism, such as non-homologous end-joining (NHEJ) or the alternative microhomology mediated end-joining (MMEJ) pathway (Table 2). The difference between these two pathways is that the presence of 1–4 bp of microhomology is optional in NHEJ, whereas at least 5 bp of microhomology is required for MMEJ [63, 64]. NHEJ might also explain the perfect junction observed in 13, although this deletion may also have been caused by NAHR, as mentioned above. Overall, the *IL12RB1* locus is enriched in *Alu* elements, which makes it prone to CNVs, including non-recurrent CNVs. With the mutations that we describe here, the proportion of CNVs among mutation responsible for IL-12R β 1 deficiency is 7% (7 CNVs out of 91 total mutations). This proportion is in the same range than previously described for other Mendelian diseases, including PID (affecting probably 10% of all genetic PID diagnosed), caused by *Alu*-enriched genes [16, 50]. In summary, heterozygous and homozygous CNVs at the *IL12RB1* locus should be considered during the genetic diagnosis of MSMD patients. The finding that other MSMD genes, such as *TYK2* and *IFNGR2*, are also enriched in *Alu* elements suggests that more CNVs remain to be described in patients with MSMD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Pedigree and clinical features of IL-12R β 1 deficiency

A. Pedigrees of the five unrelated kindreds. Each generation is designated by a Roman numeral, and each individual by an Arabic numeral. Individuals whose genetic status could not be determined are denoted “E?”. The index case is indicated by an arrow.

B. Vasculitis in the arm (left panel) and leg (middle panel), and cervical adenitis secondary to *Salmonella* infection (right panel, indicated by an arrow) in P2.

Figure 2. Biallelic mutations of *IL12RB1* in the six patients

A. Data for sequencing coverage of the *IL12RB1* locus for the five kindreds and for three different healthy controls (C1, C2, and C3). The seventeen exons of *IL12RB1* gene are depicted in gray. Deletions are indicated in red and duplications in brown.

B. Overview of the reported *IL12RB1* mutations, including those described here for the first time (indicated in green).

Figure 3. *Alu* elements are involved in *IL12RB1* copy number variations (adapted from [44])

A. Overview of the *IL12RB1* locus located on chromosome 19p13.11 (g.

18,160,418-18,207,633 comprising 10 kb upstream and downstream from the start and stop codons). *Alu* elements are represented as gray rectangles. Large deletions are indicated in red and large duplications in blue.

B. Junctional and flanking microhomology of *IL12RB1* CNVs reported to date (including those reported here). Breakpoint sequences of the *IL12RB1* CNVs in kindreds A (1-7), B (8), C (12), D (15-17), E (dup10-12), and in the previously described large deletions of *IL12RB1* (13 and 7-13) [31, 36]. *Alu* repeats shown in italics. Homologous sequences are underlined.

Table 1***Alu* content of MSMD genes**

For each gene, the percentage corresponding to *Alu*, long interspersed nuclear elements (LINEs), and long terminal repeat (LTRs) was determined with RepeatMasker for the genomic sequence extending from the start to the stop codon plus or minus 10 kilobases.

Gene	Chromosome location	Size (kb)	% <i>Alu</i>	% LINEs	% LTR elements
<i>TYK2</i>	19	47.6	54.55 %	4.29 %	0.57 %
<i>IL12RB1</i>	19	47.2	44.73 %	8.81 %	3.14 %
<i>IFNGR2</i>	21	53.4	38.34 %	3.75 %	6.09 %
<i>ISG15</i>	1	20.9	24.07 %	6.20 %	9.54 %
<i>IKBKKG</i>	X	32.5	15.82%	13.38%	14.78%
<i>IFNGRI</i>	6	41.3	8.57 %	4.03 %	23.42 %
<i>IRF8</i>	16	38.3	5.75 %	0.54 %	6.19 %
<i>IL12B</i>	5	30	5.24 %	9.87 %	8.05 %
<i>STAT1</i>	2	59.3	4.96 %	8.56 %	3.93 %
<i>CYBB</i>	X	50.8	3.15 %	13.84 %	8.05 %

Table 2
Overview of *IL12RB1* copy number variations (CNV) reported in previous studies and in this report

, with their probable underlying mechanisms. na = not applicable; NAHR = non-allelic homologous recombination; MMEJ = microhomology-mediated end joining; RBM = replication-based mechanism; NHEJ = non-homologous end joining.

Kindred	Type of CNV	Size	Micro- homology (bp)	Repetitive elements	Sequence identity between repetitive elements	Potential molecular mechanism
A	1-7	13,336	12	5': <i>AluSg4</i> Intron 7: <i>AluY</i>	88%	NAHR/MMEJ/RBM
B	8	991	2	Intron 7: none Intron 8: <i>AluY</i>	na	NHEJ/RBM
C	12	1,919	14	Intron 11: <i>AluSx1</i> Intron 12: <i>AluSx</i>	87%	NAHR/MMEJ/RBM
D	15-17	4,872	26	Intron 14: <i>AluSz</i> 3': <i>AluSz</i>	83%	NAHR/MMEJ/RBM
E	dup10-12	5,418	17	Intron 9: <i>AluSx</i> Intron 12: <i>AluSx1</i>	89%	NAHR/RBM
[31, 36] ref.	13	3,011	0	Intron 12: <i>AluSx</i> Intron 13: <i>AluIr</i>	77%	NAHR/NHEJ
[31, 36] ref.	8-13	12,167	1	Intron 8: <i>AluSx1</i> Intron 13: <i>AluIr</i>	79%	NAHR/NHEJ/RBM