Wide allelic heterogeneity with predominance of large IDS gene complex rearrangements in a sample of Mexican patients with Hunter syndrome

**Short Title:** Mutational spectrum in a sample of Mexican MPSII patients

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This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cge.12738

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CONFLICT OF INTEREST STATEMENT

All contributors have reviewed the manuscript, approved the final version, consented to its submission to the journal, and agreed with the presented data analyses and conclusions. Additionally, all authors declare no conflicts of interest and affirm their independence from the research financial source, which did not influence the study design, the data collection, the data analysis, the writing of the report, or the decision to submit the article for publication.

ACKNOWLEDGEMENTS

This study was supported by research funding from the National Institute of Pediatrics ("Recursos Fiscales del Programa E022", México, Distrito Federal). We thank doctors Leticia Belmont-Martínez, Daniel Campos-Acevedo, Beatriz de la Fuente-Cortez, Jaime López-Valdez, Dolores Ruíz-Cruz, Yuritzi Santillán-Hernández, Rosalba Sevilla-Montoya, Mónica Aguinaga-Ríos and Patricia Grether-González for kindly referring patients and for their support in performing prenatal diagnosis at the National Institute of Perinatology (México, Distrito Federal). We express our gratitude to Dr. Andreas Gal who kindly provided the IDS genotype from patient IDS62.
ABSTRACT

Hunter syndrome or mucopolysaccharidosis type II (MPSII) is caused by pathogenic variants in the IDS gene. This is the first study that examines the mutational spectrum in 25 unrelated Mexican MPSII families. The responsible genotype was identified in 96% of the families (24/25) with ten novel pathogenic variants: c.133G>C, c.1003C>T, c.1025A>C, c.463_464delinsCCGTATAGCTGG, c.754_767del, c.1132_1133del, c.1463del, c.508-1G>C, c.1006+1G>T and c.(-217_103del). Extensive IDS gene deletions were identified in four patients; by DNA microarray analysis two patients showed the loss of the entire AFF2 gene, and epilepsy developed in only one of them. Wide allelic heterogeneity was noted, with large gene alterations (e.g., IDS/IDSP1 gene inversions, partial to extensive IDS deletions, and one chimeric IDS-IDSP1 allele) that occurred at higher frequencies than previously reported (36% vs. 18.9-29%). The frequency of carrier mothers (80%) is consistent with previous descriptions (>70%). Carrier assignment allowed molecular prenatal diagnoses. Notably, somatic and germline mosaicism was identified in one family, and two patients presented thrombocytopenic purpura and pancytopenia after idursulfase enzyme replacement treatment. Our findings suggest a wide allelic heterogeneity in Mexican MPSII patients; DNA microarray analysis contributes to further delineation of the resulting phenotype for IDS and neighboring loci deletions.

Keywords: AFF2 gene, DNA microarray, idiopathic thrombocytopenic purpura, idursulfase, lysosomal storage disease, Mexican population, mucopolysaccharidosis type II, prenatal diagnosis, somatic and germline mosaicism
INTRODUCTION

Hunter syndrome or mucopolysaccharidosis type II (MPSII, MIM #309900) is an X-linked recessive inherited lysosomal storage disease that is caused by a deficiency of idurionate 2-sulfatase (I2S, EC:3.1.6.13). The \textit{IDS} gene (MIM #300823) spans 24 kb in Xq28 and is organized in nine exons that code the principal I2S isoform "a" preproprotein of 550 amino acids (73-78 kDa). Upstream of the functional \textit{IDS} gene, a homologous pseudogene (\textit{IDSP1}, 1.4 kb) that contains highly \textit{IDS} homologous sequences to exons 2 and 3, intron 2 and a chimeric fragment derived of intron 3-7 lies in an inverted orientation. It is considered to be a "hot spot" for nonallelic homologous recombinations between the loci, which yields \textit{IDS}-type deletions and \textit{IDS/IDSP1} rearrangements, including \textit{IDS} inversions (1-4). These gross structural changes are present in < 20% of MPSII patients (5,6). Patients with contiguous gene deletions that include the \textit{IDS} gene frequently exhibit severe seizures, which may be attributed to the loss of the adjacent \textit{AFF2} and/or \textit{FMR1} loci (7,8).

Molecular analysis of the \textit{IDS} gene enables clinicians to confirm a diagnosis of MPSII in male patients, identify the carrier status of their female relatives, and potentially perform either prenatal or preimplantation genetic diagnosis (PGD) (9). The majority of pathogenic variants of \textit{IDS} (80-86%) are missense and nonsense mutations, small deletions, small insertions or indels (5,6,10). The high proportion of "private" pathogenic variants and the absence of a standardized consensus regarding a severity spectrum for MPSII have complicated the determination of distinct genotype-phenotype correlations (5,11). However, gross \textit{IDS} rearrangements are associated with more severe MPSII phenotypes that include significant impairments in the central nervous system (5-8).

In this study, we characterized the \textit{IDS} genotypes of twenty-five unrelated Mexican males with MPSII, assessed the carrier status of their female relatives, performed prenatal diagnosis, identified
a family with somatic and germline mosaicism, employed DNA microarray analysis in patients with complete *IDS/IDSP1* deletions to examine the breakpoints and identify loss of other loci, and sought to determine some possible genotype-phenotype correlations. We discuss two MPSII brothers that developed idiopathic thrombocytopenic purpura (ITP) and pancytopenia after idursulfase treatment.

**MATERIALS AND METHODS**

Twenty-five unrelated Mexican male patients (2-16 years old, Table 1) with deficient I2S activity (n=14), confirmed deficient I2S activity and genotype (n=4), or only clinically suspected MPSII (n=7), and their mothers (n = 24) and other affected male (n=1) and female (n=16) relatives were included. A positive family history of MPSII was documented in 7/24 patients (28%; family history was unknown for a single case). Several of the patients were referred from other medical institutions; they were classified as severe (n=20/25), mild (n=3/25), and unknown (n=2/25) MPSII phenotypes according to neurological involvement. Available clinical data of each patient are provided in Supporting Information Table S1. Three molecular prenatal diagnosis procedures were performed in two obligate unrelated carrier females (mothers of patients IDS1 and IDS11). This study was approved by the Bioethics and Research Committees of the National Institute of Pediatrics, Mexico (22/2010).

Genomic DNA samples were obtained using standard methods to process peripheral blood leukocytes and/or buccal swabs (hair roots and urinary sediment samples were also obtained from the mother of patient IDS31). Prenatal diagnosis was performed between 16 and 20 weeks of gestational age; genomic DNA was obtained from cultured (n=1) or uncultured (n=3, two from a
twin pregnancy) amniotic cells. Fetal gender assignment was performed via PCR amplification of a 270 bp SRY gene fragment (12). Following medical pregnancy termination, the mutated IDS genotype was confirmed in genomic DNA that was derived from two umbilical cord samples (from the twin pregnancy).

The IDS genotypes that were previously reported for four MPSII cases (IDS31, -51, -62 and -71, Table 1) were corroborated and the carrier status was examined in their female relatives. In the remaining twenty-one patients, we employed the following molecular approach: we tested for IDS/IDSP1 inversion using the previously reported PCR-RFLP HinfI assay (4), including as DNA sample controls: an IDS/IDSP1 inversion-affected MPSII patient, a heterozygote IDS/IDSP1 inversion female, a healthy male, and a reagent blank control. In patients who had a normal HinfI restriction pattern or failed to produce PCR-RFLP HinfI amplicons in at least two independent assays (suggesting the presence of an extensive deletion), we performed PCR amplification and direct bidirectional automated sequencing of the nine IDS exons (NG_011900.3 RefSeqGene and NM_000202.6) and their exon-intron borders (13). For the family of patient IDS5, this strategy failed to reveal a pathogenic variant in the genomic DNA. Thus, we obtained mRNA from peripheral blood leukocytes and performed RT-PCR followed by automated sequencing of four overlapping fragments that encompassed the entire coding sequence of IDS isoform "a" (NM_000202.6, primers and conditions are available upon request).

All novel missense single-nucleotide variants were assessed with respect to dbSNP (http://www.ncbi.nlm.nih.gov/snp), the NHLBI Exome Sequencing Project at the Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS/), HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), and LOVD.
(http://grenada.lumc.nl/LOVD2/MR/home.php?select_db=IDS), and the literature. Also they were searched in 133 IDS alleles from 92 healthy and ethnically matched controls. The novel missense variations were subjected to in silico analysis using the PolyPhen (http://genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant From Tolerant (SIFT; http://sift.jcvi.org/www/SIFT_enst_submit.html) online tools. All small and novel identified pathogenic variants were submitted to LOVD.

In order to exclude the possibility of amplification failure due to technical issues in patients in which a large deletion was suspected (IDS7, -28, -58 and -76) and to delimit the sizes of the IDS/IDSP1 deletions, we amplified both the trinucleotide GCC repeat-containing sequence located in the 5’ untranslated region (UTR) of the AFF2 gene (Xq28, ~335 bp; UniSTS ID: 156199, Probe GDB: 215331) as well as the CGG repeat-containing sequence in the 5’ UTR of the FMR1 gene (Xq27.3, ~150 bp; UniSTS ID: 158090, Probe GDB: 596304). The genomic DNA samples of these patients were subsequently analyzed with a CytoScan™ High Density Microarray (Affymetrix, Inc., Santa Clara, CA, USA) (7,14). The microarray experiments were performed according to the manufacturer’s standard protocol. The DNA (250 ng) was digested with NspI, PCR amplified, fragmented, and labeled. The microarrays were hybridized, washed, and scanned using a GeneChip 3000 7G scanner and the Affymetrix GeneChip Command Console software. Cell intensity files (.CEL) were generated, saved, and transported to the Chromosome Analysis Suite software (ChAS) ver. 2.0.1 (Affymetrix, Inc., Santa Clara, CA, USA). To calculate the log2 ratios, we employed a 380-sample reference dataset from ChAS, which included 284 HapMap samples and 96 healthy male and female samples. The smoothing and joining methods, which were employed to determine copy number variant segments, were run using the default settings (14).
RESULTS

Pathogenic IDS gene variations were identified in 24/25 (96%) patients (Table 1). We detected fifteen point and small frameshift pathogenic variants (60%); each was identified in only one family. Nine of the variants were novel, including c.133G>C, c.1003C>T, c.1025A>C, c.463_464delinsCCGTATAGCTGG, c.754_767del, c.1132_1133del, c.1463del, c.508-1G>C, c.1006+1G>T, in addition to the exon 1 deletion c.(-217_103del). The three novel missense variations c.133G>C or p.(Asp45His), c.1003C>T or p.(His335Tyr) and c.1025A>C or p.(His342Pro) affect the I2S mature 42 kDa chain (a.a. 34-455) and disturb highly conserved residues of the alkaline phosphatase-like superfamily (alkPPc) domain (a.a. 38-416, NCBI Conserved Domains database: http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?seqinput=NP_000193.1). The in silico analysis of these variants predicted them to be damaging and were absent in the analyzed 133 normal IDS alleles (Table S1). C>T and G>A transitions were identified in six out of ten point variants, with c.253G>A or p.(Ala85Thr), c.262C>T or p.(Arg88Cys), c.1265G>A or p.(Cys422Tyr) and c.1403G>A or p.(Arg468Gln) located within CpG dinucleotides.

We only observed two types of commonly occurring pathogenic variations: the extensive IDS/IDSP1 deletions that were delineated by the microarray analysis (n = 4 patients, 16%) and the recurring IDSP1-mediated IDS inversion (n = 3 patients, 12%, Table 1). The sizes of the extensive deletions were highly variable and ranged from 157 kb (patient IDS28) to 1.8 Mb (IDS7). The region deleted in patient IDS28, which included both IDS and IDSP1, was also deleted in the other three patients (Figure 1). Patients IDS7 and IDS76 presented the largest deletions in the DNA microarray analysis (1.8 Mb and 1.3 Mb, respectively) and included the AFF2 gene (Table 1 and Figure 1), which was consistent with our results for the absence of the PCR amplicon for Probe GDB 215331. Instead, all
patients retained the *FMRI* gene, which was according with our observation regarding the presence of the amplicon for probe GDB 596304. This finding also eliminates the possibility of an *IDS* and *IDSP1* PCR amplification failure due to an inadequate genomic DNA sample quality and/or quantity.

Unexpectedly, an abnormal restriction pattern for an *IDS/IDSP1* inversion on the PCR-RFLP *HinfI* assay and a partial deletion of *IDS* exons 4 to 7 was detected in patient IDS11, his carrier mother and her twin affected male fetuses. These findings suggested the presence of a complex rearrangement involving an *IDSP1* insertion, which was previously reported as chimeric *IDS-IDSP1* allele (2,5,15). To confirm this, a 3682 bp chimeric *IDS-IDSP1* allele-derived fragment was generated by PCR using exon 2 forward (2F) and exon 8 reverse (8R) primers (13) and further sequenced by a "primer-walking" strategy (primers and PCR conditions are available upon request). Sequencing of the 3682 bp mutation-specific 2F/8R PCR fragment from IDS11, his carrier mother and one affected twin-brother fetus confirmed the presence of the chimeric *IDS-IDSP1* allele (2,5,15), which consists of the replacement of *IDS'* exons 4 to 7 with a partial *IDSP1* insertion (nucleotide intervals g.126_1518 from NCBI Reference Sequence NG_001149.3) and 1325 bp from its flanking 3' sequence (GenBank Accession: KT724868). The nucleotide sequence at the 3'-end of exon 2, exon 3 and their respective introns is identical to *IDSP1*, including the T-to-C intronic transition at the c.418+12T position. Thus, an accurate definition of the 5' breakpoint is difficult because neither of the junction ends have a recognizable signature, as occurs with the *IDS/IDSP1* inversion (1); hence we propose the following HGVS nomenclature to describe this complex allele: NG_011900.3:g.6129_22625delinsAC244197.3:g.45710_48426.

The carrier status was assigned for 20/25 (80%) mothers, either by family history (the mother of known familial case IDS78 was not available for study) or by molecular study, in which the familial
history of MPSII was unknown (1/25), positive (6/25) or negative (12/25). The latter category includes an isolated case due to somatic and germline mosaicism (IDS31; Figure 2). Despite our inability to precisely identify the genotypic abnormality in patient IDS5, the carrier status of a splicing defect was confirmed in his obligate carrier mother by RT-PCR analysis in a mRNA blood-derived sample, which demonstrates both normal (635 bp, c.889-c.1523) and aberrant (461-bp, lacking the entire 174 bp of exon 8) IDS transcripts (data not shown).

Two obligate MPSII carrier mothers (patients IDS1 and IDS11) requested three molecular prenatal diagnostic procedures and four affected male fetuses were diagnosed. These diagnoses were based on the results of SRY amplification and identification of the chimeric IDS-IDSP1 allele (in twin fetuses) or a hemizygous IDS c.1003C>T genotype (IDS1; in the remaining two fetuses) (Table S1). Medical pregnancy termination was requested and the mutated genotype was only confirmed in umbilical cord samples from the twins.

DISCUSSION

The MPSII mutational spectrum in Latin-American populations was recently reported (6); however, that paper did not include Mexican patients. In this study, we describe the mutational IDS spectrum of a sample of unrelated Mexican MPSII families. This sample was small (n = 25) but showed remarkable allelic heterogeneity. It includes the major mutation types that were previously described in larger MPSII series (5,6,10) and nine small and "private" novel changes, one novel exon 1 deletion or c.(−217_103del), one unknown splicing defect, one chimeric IDS-IDSP1 allele (2,5) and four complete IDS/IDSP1 deletions that were delineated by microarray analysis (Table 1). The four point mutations that occur at CpG dinucleotides identified in this study (4/10; 40%) are
highly recurring C>T or G>A transitions in the MPSII cases according to LOVD and HGMD registries; thus, they are considered to be “hot spots” (6,10,15,16). This proportion of C>T or G>A transitions at CpG dinucleotides is consistent with 47% of the previously reported pathogenic variants at IDS (16). Of the five small frame shift variants that were observed, only c.596_599delAACA was recurring; this variant has been described in other populations (15,17,18), which may reflect the tendency for slipped mispairing during DNA replication, involving two flanking direct repeats (15).

To define the precise splicing defect in IDS5, introns 7 and 8 should be further examined, although it predicts an in-frame deletion of 58 residues or p.(Trp337_Gly394del), which are caused by the aberrant skipping of exon 8. It precludes the generation of an identifiable amount of a normal IDS transcript in the mRNA blood-derived sample of the affected male, at least by our employed RT-PCR, gel electrophoresis and automated sequencing assays. This defect may disturb the Arylsulfatase A superfamily and related enzymes domain (AslA, a.a. 33-544, NCBI Conserved Domains database: http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?seqinput=NP_000193.1). This abnormal transcript is quite different from the normal I2S isoform “b” precursor (NM_006123.4, NP_006114.1), that contains a distinct C-terminus due to the employment of an alternate 3' terminal exon and lacks all the exon 9-derived sequence. These findings correlate with the very low documented I2S activity (1.2 nmol/hr/mL, normal range: 12-23 nmol/hr/mL), diagnosis of MPSII at early age (2 yr, 8 mo), the presence of visceromegalies, dysostosis multiplex, valve cardiac involvement, severe psychomotor delay and the early age of death of two maternal uncles (at 18 and 7 years old) who were referred with a phenotype highly suggestive of a lysosomal storage disease.
The utilized molecular strategy successfully determined the responsible MPSII genotypes in 96% of our patients. This proportion is comparable to the ones obtained using similar strategies in larger populations of Caucasians (100% of 155 patients) (5), Latin Americans (100% of 103 patients) (6) and Koreans (98.5%, 69/70 patients) (18). The large gene alterations, such as IDSPI-mediated IDS gene inversions (n=3), large (n=4) and partial (n=1) IDS deletions and the chimeric IDS-IDSPI allele (n=1), were more frequent in our sample (9/25, 36%) compared with previous reports in Caucasian (27/155, 17.4%) (5), Latin American (20/103, 19.4%) (6), Chinese (11/38, 29%) (17) and Korean (14/74, 18.9%) (18) patients. The chimeric IDS-IDSPI allele, in which IDS’ exons 4 to 7 are replaced with an IDSPI pseudogene sequence (2), was previously reported as an uncommon rearrangement in 1/155 (5) and 1/18 (15) of the studied MPSII patients. The insertion of the IDSPI pseudogene in the deleted IDS functional gene produces a rearrangement that is identical to the one observed in the recurrent IDS/IDSPI inversion. Thus, with the 1st set of primers (4), it generates a 1310 bp PCR recombinant-derived amplicon with four HinfI restriction sites, which produce 554, 399, 271, 71 and 15 bp fragments. These primers also amplify the adjacent non-inverted or normal IDSPI locus of 1315 bp with five HinfI restriction sites, which produce 451, 399, 271, 108, 71 and 15 bp fragments. Due to the presence of two amplifiable IDSPI elements in the same chimeric IDS-IDSPI allele of the affected MPSII males or their carrier females relatives, the 554 and 451 bp restriction HinfI fragments are generated, which produces an abnormal restriction pattern that is identical to the observed for a IDS/IDSPI inversion genotype in carrier females. This finding was also reported by Lualdi et al. in one male patient (4), which led the authors to suggest a possible IDSPI duplication; however, additional analysis to explain this phenomenon was not provided. So, we suggest that the real frequency of this complex allele may be underestimated because the PCR
amplification of the nine IDS exons reveals the deletion of exons 4 through 7 but, unlike the PCR-RFLP HinfI assay, does not reflect this complex rearrangement. Thus, when deletions that affect these exons are identified by PCR (9,18), Southern blotting (19) or RT-PCR amplification of the IDS cDNA (15) this complex rearrangement should be ruled out by using another assay. An example is illustrated by Korean patients KH-14, KH-15 and KH-16, who were initially classified as having partial IDS gene deletions (exons 6-8, 4 or 5-6, respectively) according to Southern blot analyses (19) but were subsequently subjected to the PCR-RFLP HinfI assay (4) and reclassified as having an IDS-IDSP1 recombination allele (18).

Studies of large series have estimated that complete IDS deletions are responsible for 3-4.5% of MPSII cases and have indicated that are usually associated with severe phenotypes (5,6,20). MPSII patients with IDS deletions that encompass both FMR1 and AFF2 exhibit significant neurodevelopmental delay and/or mental retardation (7,21), hydrocephalus (7), severe hypotonia (22) and seizures (7,8). The contiguous deletions of IDS-IDSP1-AFF2 identified in patients IDS7 and IDS76 (Table 1 and Figure 1) are similar to the contiguous deletions that have been previously described (22). With the exception of hypotonia, the phenotype of patient IDS7 is consistent with the reported severe clinical picture (22), leading to an early diagnosis of MPSII when he was 1 year old. Enzyme replacement therapy (ERT) with idursulfase was initiated at 23 months of age. At the age of 4 years and 5 months, he presented a stroke-like event with right-side hemiparesis; at this time, brain magnetic resonance imaging and computed tomography scanning revealed communicating high-pressure hydrocephalus that was managed with ventriculoperitoneal shunting. Despite the procedure, he presented gradual loss of bilateral vision, hearing, locomotion, and verbal communication, as well as the establishment of seizures (maximum of fifteen episodes
per day). Conversely, patient IDS76, who also had the \textit{IDS-IDSP1-AFF2} deletion, was referred for molecular diagnosis at ten years of age with severe intellectual disability but without hydrocephaly or seizures, indicating that the contiguous deletions of \textit{AFF2} or \textit{FMR1} do not assure the presence of seizures in MPSII (21-23). Honda et al. (22) also reported a patient with a contiguous deletion of \textit{IDS-IDSP1-AFF2}, who did not have seizures or hydrocephalous at the age of 23 months. Nevertheless, as illustrated by our patient IDS7, a strict clinical follow-up complemented by brain imaging should be offered for the surveillance of epilepsy or hydrocephalous in individuals who are known to have large contiguous deletions.

The 20\% \textit{de novo} mutation rate that we observed is similar to the rates that were previously described in larger series (15.6-24\%) (5,6,10). The identification of somatic and germline mosaicism in 1 of the 20 MPSII carrier mothers suggests that this condition should be considered in genetic counseling. Somatic and germline mosaicism has only been reported in another two MPSII families (2/155, 1.29\%) (5); however, a “pure” germline mosaic has not been reported yet. We identified the pathogenic variant \texttt{c.463_464delinsCCGTATAGCTGG} in only the genomic DNA obtained from urinary sediment from the mother of patient IDS31 (Figure 2); although, very low levels of somatic mosaicism in DNA samples from peripheral blood leukocytes, buccal cells and hair roots cannot be ruled out because the automated sequencing assays that we employed was not quantitative. In fact, the real prevalence of somatic and/or germline mosaicism in single MPSII cases due to \textit{de novo} mutations may be underestimated given that the mutated allele is rarely quantified in genomic DNA from different maternal tissue samples, nor are molecular studies currently performed in other family members. Thus, we also emphasize that the molecular study
should be extended to all at-risk family members and prenatal diagnosis should be offered (5), even when a normal IDS genotype is documented in the mother of a unique MPSII case.

As a rule, heterozygous MPSII females are assumed to be asymptomatic even though they may show a significantly lower number of median plasma and leukocyte I2S activities than non-carrier females, but overlap between these values has been noted (24). A complete clinical MPSII picture in females is rare, with approximately fifteen cases reported to date, including one of Mexican-descent (25). The major implicated mechanism are skewed X-inactivation in heterozygous females and translocations or large deletions of the X chromosome (24,25). None of the twenty-four mothers and sixteen female relatives in this study was referred to have symptomatology of MPSII.

The three prenatal diagnoses proceedings done in this study show that this strategy should be considered as a real preventive strategy for Mexican MPSII families. This is relevant because experience in prenatal diagnosis in Latin America is limited; only one MPSII affected fetus of Latin American-descent and prenatally diagnosed by DNA and I2S activity analyses has been reported (26). In addition, the characterization of responsible MPSII genotype in couples at-risk opens the possibility to offer PGD (9); even though, this method is not available in our country.

Although a correlation between ERT and adverse effects with IDS genotype has not been reported, the affected twin brothers of IDS33 (with an IDS/IDSP1 inversion genotype) developed ITP and pancytopenia after the discontinuation of ERT (0.5 mg/kg/week). Idursulfase was initiated when they were 7 years old; however, the first twin developed rash, urticaria and glottis edema after six sessions, which caused the discontinuation of ERT. Sixteen months after ERT withdrawal, he presented severe gingival hemorrhage and a platelet count of 2x10⁹/L. Based on complete blood
count and bone marrow biopsy, a diagnosis of ITP was established. Oral methylprednisolone and intravenous immunoglobulin were started; however, the response was unsatisfactory. A splenectomy was performed at 8 years and 6 months, improving the platelet count ($37 \times 10^9/L$). After one year, the patient developed a respiratory tract infection and upper gastrointestinal bleeding, which caused his demise at 9 years and 6 months of age. The second affected twin received 25 eventless idursulfase infusion sessions but his parents decided to suspend ERT when he reached 9.5 years old due to clinical progression of the disease and increased frequency of respiratory infections. Four months after the suspension of ERT, the patient developed severe bronchopneumonia, generalized edema and disseminated petechiae. His blood counts revealed leukopenia ($0.5 \times 10^9/L$) and thrombocytopenia ($25 \times 10^9/L$), which required platelet transfusion and treatment with methylprednisolone. However, response after 24 hours in his platelet count was discrete ($42 \times 10^9/L$). A bone marrow biopsy revealed hypocellularity, diminished megakaryocytes and erythroid lineages, suggesting ITP. At the age of 9 years and 9 months, the patient died as a consequence of septic shock, bronchopneumonia and pancytopenia. Conversely, IDS33 (the twins’ younger brother) has undergone a minimum of seven years of uninterrupted ERT and has never presented any clinical or laboratory sign suggestive of ITP or any other idursulfase-related adverse effect.

Another MPSII patient has been reported to develop ITP related to idursulfase after an eight-month treatment course. The 20-year-old Caucasian adequately responded to intravenous immunoglobulin treatment, and ERT was considered to be the principal etiological factor for his development of ITP (27). However, no ITP or other serious hematological adverse effect were reported in a recent multicenter, open-label safety study of idursulfase in children with MPSII; only
one out of twenty-eight treated patients developed eosinophilia (28). Alternative explanations exist for the hematological abnormalities observed in our patients, such as viral or bacterial infections; however, thrombocytopenia has also been described following galsulfase treatment for MPS VI (29). Thus, the clinical manifestations of our patients may have originated by a probable idiosyncratic and late-onset adverse reaction to ERT.

In summary, our findings support a wide allelic heterogeneity in Mexican MPSII patients, in which large gene alterations occur at higher frequencies than the previously reported ones in other populations (36% vs. 18.9-29%). The microarray analysis allows further delineation of the resulting phenotype for extensive IDS and neighboring loci deletions because it advocates that loss of IDS and AFF2 is not a full-penetrant genotype for seizure development. Additionally, identification of somatic and germline mosaicism in our relatively small MPSII study sample (1/25 families, 4%) emphasizes the need to consider this phenomenon in genetic counseling. The development of ITP and pancytopenia in two MPSII brothers after idursulfase ERT warrants attention.
REFERENCES


Figure 1. Details of the Xq28 deletions found in four patients using Affymetrix CytoScan™ High Density microarray analysis. Dots represent the weighted intensity signals of patients compared to control DNA samples, as expressed in log₂ ratios on a scale from −1.5 to 1.5 (y-axis). The bars indicate deletions in the Xq28 region (0 copies). Horizontal segmented lines represent the genes in the Xq28 region. The x-axis represents an ideogram of the X chromosome with respect to the genome coordinates (hg19) expressed in kilobases (kb). The shaded vertical rectangle indicates the minimal common region (MCR) among these four patients. Description of deletion coordinates:

**Patient IDS28:** Complete *IDS/IDSP1* deletion (157 kb). *AFF2* and *FMR1* preserved. arr Xq28(148,467,083-148,624,248)x0 mat.

**Patient IDS58:** Complete *IDS/IDSP1* deletion (562 kb). *AFF2* and *FMR1* preserved. arr Xq28(148,176,414-148,738,845)x0 mat.

**Patient IDS76:** Complete *IDS/IDSP1* deletion (1.39 Mb). *AFF2* deletion. *FMR1* preserved. arr Xq28(147,340,291-148,731,894)x0.

**Patient IDS7:** Complete deletion (1.83 Mb). *AFF2* deletion. *FMR1* preserved. arr Xq28(147,392,284-149,225,394)x0.
Figure 2. Partial electropherograms of exon 4 of *IDS* in relatives of patient IDS31 (II-3) and a healthy male control (CTL) for identification of the novel frameshift variant c.463_464delinsCCGTATAGCTGG or p.(Phe155Profs*12). Note in II-3 that the inserted sequence is identical to that in c.448-c.459 positions. Reverse strands were analyzed to overcome sequence reading difficulties due to the presence of a homopolymeric thymine tract at 3’-end of intron 3. II-1 and II-2 were determined to be heterozygous carriers for c.463_464delinsCCGTATAGCTGG (arrows indicate the indel start point). Unexpectedly, the mutated allele was undetectable in three (peripheral blood leukocytes, buccal cells and hair roots) out of four genomic DNA samples that were derived from different somatic tissues from I-1. Additionally, I-1 was confirmed as the mother of the three siblings by DNA profiling with the 13 CODIS STR markers (data not shown). Thus, we assume that the pathogenic indel variant is present both in the somatic (urinary tract) as germline cells of I-1.
Figure 1 Alcántara-Ortigoza et al. R1.tif
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Title:
Wide allelic heterogeneity with predominance of large IDS gene complex rearrangements in a sample of Mexican patients with Hunter syndrome

Date:
2016-05-01

Citation:

Persistent Link:
http://hdl.handle.net/11343/290930