Mucopolysaccharidosis type II: Identification of 30 novel mutations among Latin American patients

A.C. Brusius-Facchin,1,I.V.D. Schwartz,1,C. Zimmer,2,M.G. Ribeiro,3,A.X. Acosta,4,D. Horovitz,5,I.L. Monillé,6,M.I.B. Fontes,6,A. Fett-Conte,6,R.P. Oliveira Sobrinho,1,A.R. Duarte,3,R. Boy,4,P. Mabe,4,M. Ascarra,10,M. de Michelena,10,K.L. Tylee,6,G.T.N. Besley,5,M.C.V. Garreton,11,R. Giugliani,1,a,b,c,S. Leistner-Segal,1,a,b,c,⁎

1 Post Graduation Program in Medical Sciences, UFRGS, Porto Alegre, RS, Brazil
2 Department of Genetics, UFRGS, Porto Alegre, Brazil
3 Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Brazil
4 Clinical Genetics Service, IPPMG, UFRJ, Rio de Janeiro, Brazil
5 Department of Pediatrics, UFBA, Salvador, Brazil
6 Instituto Fernandes Figueira, FIOCRUZ, Rio de Janeiro, Brazil
7 Department of Pediatrics, UNICISAL, Maceió, Brazil
8 Department of Molecular Biology, FAMERP, São José do Rio Preto, Brazil
9 Department of Medical Genetics, UNICAMP, Campinas, Brazil
10 Clinical Genetics Service, IPPMG, Porto Alegre, Brazil
11 Medical Genetics Service, IMIP, Recife, Brazil
12 Mother and Child Department, UERJ, Rio de Janeiro, Brazil
13 Genetics and Metabolic Diseases Unit, INTA, University of Chile, Chile
14 Department of Genetics, ILCS-UNA, Asunción, Paraguay
15 Universidad Peruana Cayetano Heredia, Lima, Peru
16 Willink Biochemical Genetics Unit, Royal Manchester Children’s Hospital, Manchester, UK
17 Unidad de Genética Clínica, Hospital Roberto del Río, Santiago, Chile

⁎ Corresponding author at: Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, 90035-903 Porto Alegre, RS, Brazil. Fax: +55 51 33598010.
E-mail address: segalh@hcpa.ufrgs.br (S. Leistner-Segal).
1 These authors contributed equally to the manuscript.

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A B S T R A C T
In this study, 103 unrelated South-American patients with mucopolysaccharidosis type II (MPS II) were investigated aiming at the identification of iduronate-2-sulfatase (IDS) disease causing mutations and the possibility of some insights on the genotype–phenotype correlation The strategy used for genotyping involved the identification of the previously reported inversion/disruption of the IDS gene by PCR and screening for other mutations by PCR/SSCP. The exons with altered mobility on SSCP were sequenced, as well as all the exons of patients with no SSCP alteration. By using this strategy, we were able to find the pathogenic mutation in all patients. Alterations such as inversion/disruption and partial/total deletions of the IDS gene were found in 20/103 (19%) patients.
Small insertions/deletions/indels (<22 bp) and point mutations were identified in 83/103 (88%) patients, including 30 novel mutations; except for a higher frequency of small duplications in relation to small deletions, the frequencies of major and minor alterations found in our sample are in accordance with those described in the literature.

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1. Introduction

Mucopolysaccharidosis type II (MPS II, McKusick 309900) is an X-linked recessively inherited lysosomal storage disorder (LSD), resulting from deficiency of iduronate-2-sulfatase activity (IDS, EC 3.1.6.13). IDS is involved in the degradation of glycosaminoglycans (GAGs) dermatan sulfate and heparan sulfate (DS–HS). Failure to hydrolyze the terminal iduronate-2-sulfate esters in these GAGs results in progressive accumulation of undegraded substrates within the lysosomes and in the clinical manifestations associated to MPS II [1].

The IDS gene is located on the Xq27/28 boundary of the long arm of the X-chromosome, comprising 9 exons spanning approximately 24 kb [2]. A pseudogene (IDS2), containing sequences homologous to exon II, intron 2, exon III and a chimerical intron 3–intron 7, is located approximately 20 kb far, telomeric to the active IDS gene, which makes this region prone to the occurrence of recombination events. Approximately 10–20% of MPS II patients present large
gene alterations, including rearrangements and total IDS gene deletions, while 80–90% of them present small gene alterations [3,4]. According to the Human Gene Mutation Database [5], 479 different mutations in the IDS gene have been described until July 2013, most of which being private point mutations (52%) or small deletions (17%).

MPS II shows not only wide allelic heterogeneity, but also wide phenotypical heterogeneity. Patients are usually classified as having the severe, intermediate, or attenuated forms, depending mainly on the degree of mental retardation present [1,4,6]. In general, it seems that patients with the more severe forms are diagnosed earlier, have mental retardation that is evident by the age of 4–6 years, and are dead by the age of 10–15 years, while patients with the attenuated forms present normal intelligence and have a longer life expectancy [7,1,4]. However, as there is no standardized scoring index of severity for MPS II, this classification is not always reliable, especially in the case of patients diagnosed during childhood [4]. Consequently, studies of the genotyp–phenotype correlation are subject to various biases. As simple Mendelian disorders are in fact complex traits [8], it is likely that genotyp–phenotype correlation does not exist for several IDS gene mutations [4].

In the present study, we have investigated 103 unrelated South-American MPS II patients in order to characterize their genotypes.

2. Material and methods

The Molecular Genetics Laboratory of the Medical Genetics Service of Hospital de Clínicas de Porto Alegre, Brazil, is a reference center for MPS diagnosis and receives samples from all over Brazil and from many Latin American countries. The indication for molecular analysis was based on clinical criteria, abnormal urinary GAG excretion and definitive IDS activity in dried blood, plasma, leucocytes or fibroblasts. In all cases, the possibility of multiple sulfatase deficiency was excluded by the measurement of the activity of another sulfatase. Regarding the origin of the 103 patients, 91/103 were from Brazil, 4/103 were from Chile, 2/103 were from Peru, 1/103 was from Paraguay, 2/103 were from Argentina, 1/103 was from Cuba and 2/103 were from Bolivia. DNA samples from 66 mothers and 22 affected brothers/cousins of the probands were also analyzed. This study was approved by the Institutional Review Board and by the National Research Ethics Committee of Chile, 2/103 were from Peru, 1/103 was from Paraguay, 2/103 were from Argentina, 1/103 was from Cuba and 2/103 were from Bolivia.

Genomic DNA of patients/relatives was extracted from blood following the salt precipitation method [9] and preserved in a Tris–EDTA 0.1 M solution (Tris–HCl (10 mM, pH 7.5) and EDTA (1 mM, pH 8.0)).

Samples were first analyzed through PCR/RFLP for the presence of the inversion/disruption of the IDS gene caused by intrachromosomal recombination between intron 7 of the gene and its homologous region on the pseudogene, according Lualdi et al. [10]. Samples with no evidence of the IDS–IDS2 recombination were analyzed by PCR/SSCP throughout the 9 exons and intron–exon boundaries of the IDS gene.

The PCR amplifications were performed using 5 μL of buffer concentrated 10× (200 mM Tris HCl (pH 8.0); 500 mM KCl), 0.2 mM dNTPs, 1.5–3.0 mM MgCl2, 0–6% of DMSO (Sigma), 1 U of Taq DNA Polymerase (Invitrogen), and 20 pm of each primer in a final reaction volume of 50 μL. PCR conditions were as follows: 94 °C for 5 min; 34 cycles of 94 °C for 40 s, 50.5 °C–61 °C for 40 s (based on each exon) and 72 °C for 40 s; and a final extension time of 72 °C for 10 min. The primer sequences used for amplification of all the IDS exons and for the identification of the inversion/disruption events have been previously described [11–13]. Exon III was amplified using nested-PCR (amplification with primers 2A and 3B followed by amplification with primers 3A and 3B) [12]. This extra step prevents the amplification of the pseudogene sequence. Two negative PCRs for one determined exon of the IDS gene were considered as indicative of deletion of this exon. In the case of total deletions, DNA integrity was also tested by simultaneous amplification of the α1-antitrypsin gene.

SSCP was used to screen samples, using mutation detection enhancement gels (BMA) ranging from 0.7 to 1×. The electrophoresis was carried out at 160 V for 16–24 h at room temperature.

The samples showing mobility shifts on SSCP were sequenced on ABI Prism™ 3500 Genetic Analyzer (Applied Biosystem). Mutations were confirmed on a second PCR product by the sequencing of both strands using the same methodology. In the case of novel missense mutations, 100 control alleles were analyzed in order to confirm the pathogenicity of the identified mutation, and bioinformatics analyses were performed using the Polyphen software. The samples of patients that did not show any alteration on SSCP were submitted to sequencing of the 9 IDS exons including exon/intron boundaries, according to the laboratory protocol.

After the identification of mutation in the DNA sample from the index case, family members were tested by restriction endonuclease analysis, PCR (mothers of patients presenting the inversion/disruption), SSCP or sequencing.

3. Results

3.1. Detection of large gene alterations (n: 20/103) (Table 1)

The recombination between the IDS gene and pseudogene (IDS2), which causes a disruption of the IDS gene and an inversion of the intervening region, was found in 13/103 (12%) patients (patients H1–H13). Four patients (H14–H17) appear to have partial deletion of the IDS gene, after exon by exon amplification. Other three patients (H18–H20) showed a total deletion of the IDS gene and we performed whole-genome array–Comparative Genomic Hybridization (Array-CGH) to delineate the deletion breakpoints and to characterize the deletion extension; the deletion showed in the 3 patients extended from the proximal IDS region towards contiguous genes. The results were described previously by our group [14]. All of these patients have neurological impairment.

3.2. Identification of small gene alterations (n: 83/103) (Tables 2 and 3)

Amplification, SSCP analysis, and sequencing of exons 1 to IX of the IDS gene allowed for the identification of a small alteration (<22 bp) in 83/103 patients. The success rate found for SSCP screening was 72%. All the missense point mutations were considered to be causative for the disease since the mutation was not found in 100 alleles of unaffected and unrelated controls and bioinformatic analyses, performed by Polyphen v.2, predicted that these mutations are probably damaging (score >2), it is with high confidence supposed to affect protein function/structure or possibly damaging (score 1, 5–20) it is supposed to affect protein function or structure (Table 1). The multiple alignment in different species showed that mutations involve a highly conserved gene sequence (Table 2).

We have found 57 different small gene mutations in 83 patients. Data on the novel (n: 30) and recurrent mutations (n: 27) found in our sample are shown in Tables 1 and 2. Only 8 mutations were found in at least two unrelated patients: p.R88C, p.Y103X, p.W109X, p.S333L, p.R443X, p.P467L and p.R468W. Intronic mutations were
found in 3/100 (3%) patients, all of which involving the consensus splice donor or acceptor site.

Regarding the type of the 57 different small alterations found, 2/57 (3.5%) were deletions, 2/57 (3.5%) were in indels, 4/57 (7.0%) were duplications, and 49/57 (85%) were point mutations. Among point mutations, 7/49 (10.6%) were splice site, 25/49 (50%) were nonsense, and 21/49 (42.9%) were sense mutations.

In 23/103 (22%) patients, the mutation was located in exon IX. Considering only the patients that presented exonic point mutations (n: 75/83), exon IX was the exon with the highest frequency of mutations found (21/75 patients), followed by exons II, VIII, and VII (17/75, 13/75, 12/75 patients each) and exons I, 8/75 patients (Tables 2 and 3). Point mutations seemed to be rare in exons V (17/75, 13/75, 12/75 patients each) and exons II, 8/75 patients (n: 75/83), exon IX was the exon with the highest frequency of point mutations (n: 21 patients). It has been reported that, while the distribution of small rearrangements in the IDS gene seems random, point mutations tend to be more frequent in exons II, VIII, and IX [32].

### 3.3. Family studies

The analysis performed in 63 available mothers of MPS II patients showed that 48/63 (76%) were carriers. Seven of 10 mothers of patients presenting the inversion/disruption were carriers. Also, all brothers/cousins with MPS II that were studied (n: 22) presented the same mutation identified in the proband.

### 4. Discussion

This is the largest sample of Latin American MPS II patients reported to date. Our findings regarding the genotype analysis are, in general, in agreement with the literature. We have found great allelic heterogeneity among our patients (57 different mutations in 103 patients), and 30 novel mutations. Only 8 point mutations were recurrent in two or more unrelated patients (p.R88C, p.Y103X, p.W109X, p.S333L, c.1122 C>T, p.R443X, p.P467L, and p.R468W); of these, only p.P467L did not involve CpG sites.

Discordant with the literature is the relatively higher number of small duplications/insertions in relation to the small deletions that we found (7.0% versus 3.5%). According to the literature, the frequency for small duplications/insertions ranges from 1.5% to 6% and from 10 to 20% for small deletions [24,30,31].

As expected, the exon with the highest frequency of point mutations was exon IX (n: 21 patients). It has been reported that, while the distribution of small rearrangements in the IDS gene seems random, point mutations tend to be more frequent in exons II, VIII, and IX [32]. In our sample, the number of patients showing point mutations in exons I, IV, and V was almost the same. We found a high frequency of the mutation c.1122C>T, in codon 374. This is a silent mutation that creates an
alternative splice site with loss of 20 IDS amino acids and that corre-
sponds to around 45% of the mutations in exon VIII [32]. This was
expected, given the ethnic background of our samples and the fact
that this is the most frequent mutation of the IDS gene found in Spain
and Portugal, where more than 25 patients have been reported. All of
these patients have an attenuated phenotype, suggesting that the very
low percentage of normal transcript was sufficient to avoid a severe
phenotype [21,25,30,40]. On its turn, the high frequency of point muta-
tions in exon IX may be explained by the existence of mutational
hotspots (codon 468, for example) and by the size of this exon (473 bp, ap-
proximately twice the size of the remaining exons).

Regarding the pathogenicity of the novel missense mutations de-
scribed, it is important to point out that all occur in codons that are con-
served among the human and the murine IDS [15] and that they were
not found in 100 control alleles. Of the 63 mothers tested for mutation
analysis, 19 are obligatory carriers, based on family pedigree. Taking
into account only the mothers that are not mandatory carriers 44/63,
we identified 46% of carriers and 24% as non-carriers, which is in ac-
cordance with the rate of spontaneous mutation proposed by Haldane.
Assuming no selection among carriers and non-carriers, and the fact that
MPS II is X-linked recessive, it is expected that approximately 1/3 of
the cases are secondary to new mutations [50]. In the work of Chase
et al. [51], 23% of mothers of patients with MPS II were identified as
non-carriers, similar to the expected value (approximately 33%); how-
ever, Machill et al. [52], Rathmann et al. [32], Froissart et al. [16]
and Bellows and Thompson [53] found a ratio of carrier mothers (approximately 90%) higher than expected (66% approximately).

Although there are no uniform criteria to classify the clinical severi-
ty of patients with MPS II, most studies that analyze the correlation be-
between genotype and phenotype in this disease classify patients into the severe (presence of severe or moderate mental retardation), intermediate (ab-
sence or presence of mild mental retardation and presence of severe so-
matic disease) or the attenuated form (absence of mental retardation
and presence of attenuated somatic disease). However, these studies usu-
ally do not provide a more detailed clinical description of the patients.

For instance, IDS gene total deletions and the p.S333L mutation are
always reported as being associated with the severe phenotype [4,24,30,31,32], while the c.1122C>T mutation (codon 374) is always re-
ported as being associated with normal intelligence [30]; two of our pa-
tients present a severe phenotype. Patients with, p.A85T, p.R443X,
p.R468W and p.R468Q mutations, are reported as having the severe or
the attenuated form of MPS II. Therefore, some few mutations present
the severe form (total deletions, p.S333L), other few mutations present correlation with the severe form (c.1122C>T), while a greater number of mutations are correlated both with the severe
is important to point out that these generalizations can only be made in
relation to the small number of recurrent mutations, since most muta-
tions in the IDS gene are private or have been described in a limited
number of patients.

The three patients that present a frameshift mutation, clearly appear
to have a severe phenotype.

According to our findings, point mutations (including mutations in
splice sites) seem to be associated with any of the clinical phenotypes.
It is interesting to point out that, different from what was expected,
the clinical phenotypes associated with the 7 nonsense mutations found range from the attenuated to the severe form of the disease. The mutations p.Y54X and p.Y536X are clearly associ-
ated with the severe and the attenuated phenotype, respectively. Mu-
tation p.Y536X is the nearest to the carboxyl terminus of the IDS
protein described to date. It may be compared with the recurrent
mutation p.Q531X, which has already been described in two patients
with the attenuated form of MPS II [16,40]; both mutations lead to
the synthesis of a protein missing the C-terminal amino aciIDS re-
moved during IDS protein maturation and should not prevent the
IDS processing to the lysosome [4]. The phenotypes found in the
patients that present the following recurrent mutations (p.R88X,
p.Q389X, p.R443X, and p.L482X), are in agreement with those re-
ported in the literature: The p.R88X phenotype could be explained
by the proximity of the mutation to the initiation codon, a context in
which nonsense-mediated decay could be circumvented [10], and by
the presence of the low-fidelity stop codon, which may result in
limited natural read through possibilities [30]. In patients carrying
the mutation p.R443X a complete spectrum of phenotypes from atten-
uated to severe has been described suggesting that the natural
read-through could vary between individuals and/or that other fac-
tors could influence the phenotype [30]. It seems that the severity
of nonsense mutations, in the case of the IDS gene, shows a relation
with its location within the gene, since mutations located in the
amino and carboxy terminal ends tend to be associated with attenuated
phenotypes. Interestingly, one of the novel point mutations described in

Table 3
Recurrent IDS gene point mutations found in this work and worldwide patients described in the literature.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Mutation</th>
<th>Origin</th>
<th>Clinical phenotype (literature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H52 a, b, c</td>
<td>R8X</td>
<td>Brazil</td>
<td>Intermediate: 1 patient [3]; attenuated: 2 patients [18,30]</td>
</tr>
<tr>
<td>H53</td>
<td>Y54X</td>
<td>Brazil</td>
<td>Severe: 1 patient [33]</td>
</tr>
<tr>
<td>H54</td>
<td>A77D</td>
<td>Brazil</td>
<td>Attenuated [34]</td>
</tr>
<tr>
<td>H55</td>
<td>N63D</td>
<td>Brazil</td>
<td>Mild: 1 patient [12]</td>
</tr>
<tr>
<td>H56</td>
<td>A85T</td>
<td>Brazil</td>
<td>Severe: 1 patient [26]; intermediate: 2 patients [16,24,25,32,35,36]</td>
</tr>
<tr>
<td>H57</td>
<td>P86L</td>
<td>Brazil</td>
<td>[37]</td>
</tr>
<tr>
<td>H58</td>
<td>S87N</td>
<td>Brazil</td>
<td>Attenuated: 1 patient [37]</td>
</tr>
<tr>
<td>H59,60,61</td>
<td>R88C</td>
<td>Brazil</td>
<td>Severe: 3 patients [32]</td>
</tr>
<tr>
<td>H62</td>
<td>R88H</td>
<td>Brazil</td>
<td>Severe: 3 patients [32,33]</td>
</tr>
<tr>
<td>H63,64</td>
<td>Y103X</td>
<td>Brazil</td>
<td>Intermediate [38]</td>
</tr>
<tr>
<td>H65</td>
<td>TI30I</td>
<td>Brazil</td>
<td>Mild: 1 patient [33]</td>
</tr>
<tr>
<td>H66</td>
<td>P231L</td>
<td>Brazil</td>
<td>Mild: 1 patient [25]; severe: 1 patient [31]</td>
</tr>
<tr>
<td>H67</td>
<td>c.708G &gt; A</td>
<td>Brazil</td>
<td>Severe: 1 patient [28]; intermediate: 1 patient [32]</td>
</tr>
<tr>
<td>H68</td>
<td>c.709-2A &gt; G</td>
<td>Paraguay</td>
<td>(no clinical description)</td>
</tr>
<tr>
<td>H69</td>
<td>S305P</td>
<td>Brazil</td>
<td>Attenuated: 1 patient [39]</td>
</tr>
<tr>
<td>H70,H71,H72,H73</td>
<td>S333L</td>
<td>Brazil</td>
<td>Severe: 5 patients [16,18,26,31,33,40,41]</td>
</tr>
<tr>
<td>H74</td>
<td>S333L</td>
<td>Chile</td>
<td>Same as above</td>
</tr>
<tr>
<td>H75</td>
<td>H335R</td>
<td>Brazil</td>
<td>Intermediate: 2 patients [16,31]</td>
</tr>
<tr>
<td>H76</td>
<td>G336V</td>
<td>Brazil</td>
<td>Severe [42]</td>
</tr>
<tr>
<td>H77,H78,H79,H80</td>
<td>c.1122C &gt; T</td>
<td>Brazil</td>
<td>Intermediate: 1 patient [3]; attenuated: 21 patients [11,12,18,21,31,32,37,43,44]</td>
</tr>
<tr>
<td>H78</td>
<td>c.1122C &gt; T</td>
<td>Bolivia</td>
<td>Same as above</td>
</tr>
<tr>
<td>H81</td>
<td>c.1122C &gt; T</td>
<td>Brazil</td>
<td>Severe: 1 patient [18]; attenuated: 2 patients [25,45,47]</td>
</tr>
<tr>
<td>H87,H88,H89</td>
<td>R443X</td>
<td>Brazil</td>
<td>Intermediate: 1 patient [25]; attenuated: 5 patients [16,32,38,40]; attenuated: 6 patients [3,25,30,31]</td>
</tr>
<tr>
<td>H89</td>
<td>R443X</td>
<td>Bolivia</td>
<td>Same as above</td>
</tr>
<tr>
<td>H90,H91</td>
<td>R468W</td>
<td>Brazil</td>
<td>Severe: 10 patients [12,24,31,21,15,37,44,45]; attenuated: 2 patients [45,47]</td>
</tr>
<tr>
<td>H92</td>
<td>R467L</td>
<td>Chile</td>
<td>Same as above</td>
</tr>
<tr>
<td>H94 a, b, H95</td>
<td>R467L</td>
<td>Brazil</td>
<td>Same as above</td>
</tr>
<tr>
<td>H96,H97,H98,H99</td>
<td>R468W</td>
<td>Brazil</td>
<td>Severe: 10 patients [12,24,31,21,15,37,44,45]; attenuated: 2 patients [45,47]</td>
</tr>
<tr>
<td>H100</td>
<td>R468W</td>
<td>Argentina</td>
<td>Same as above</td>
</tr>
<tr>
<td>H101</td>
<td>R468Q</td>
<td>Argentina</td>
<td>Severe: 3 patients [3,12,16,18,24,26,32,38,40,48,49]; intermediate: 1 patient [12]</td>
</tr>
<tr>
<td>H102</td>
<td>D476G</td>
<td>Brazil</td>
<td>[45]</td>
</tr>
<tr>
<td>H103 a, b, c</td>
<td>L482X</td>
<td>Brazil</td>
<td>Intermediate: 2 patients [26,30]</td>
</tr>
</tbody>
</table>

MID: delay in motor development; IDD: delay in language development; MR: mental retardation; BP: behavioral problems; NR: neurological regression; NA: not available; see specific reference for the clarification of the criteria used for the classification of clinical severity in each study.

a Patients described with the same number and different letters (a, b, c) are relatives.
b cDNA numbering follows [29], beginning with the A of the initiation codon.
c Cases when author has not characterized the clinical phenotype or when the clinical phenotype was unknown have not been included in this column.
the carboxy terminal end (p.V503D) is also associated with the attenuated-
phenotype. As to the mutations that are being described in this study for the sec-
ond time in the literature (p.S87N, c.709-2A>G, p.C422Y), there is total disor-
dance as to the phenotype associated to p.S87N: the Brazilian pa-
tient clearly presents the severe form of MPS II, while the other patient
described [37] presents the attenuated form. In our sample, the pheno-
type associated with c.709-2A>G is the severe one; however, Lissen
et al. [23] did not describe the phenotype of their patient. There is some
agreement as to the phenotype associated with p.C422Y: the pa-
tient being described herein presents an extremely attenuated form
of MPS II, while Gort et al. [25] classified their patient as having the in-
termediate form. Moreover, we are describing two brothers with the
p.708G>A which apparently have no neurological involvement. Interest-
ingly, this mutation was described in one patient with the intermediate
form [32] as well as in other patient with the severe form of MPS II [18].

The clinical phenotypes associated with the other recurrent mutations

We believe that the methodology employed in this study is appro-
priate for both the diagnosis and the identification of mutations in pa-
tients with MPS II and possible carriers. The correlation between
pheno-
type and genotype for MPS II exists for a small subset of muta-
tions, with most mutations having no clear correlation to the pheno-
type. The correlation between the mutations described [32] and the severe form of MPS II has been reported previously. The p.N211K mutation, for example, has been described in patients with
the severe form of MPS II [18].

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