



Short Communication

# *A de novo* or germline mutation in a family with Mucopolidiosis III gamma: Implications for molecular diagnosis and genetic counseling



Renata Voltolini Velho<sup>a,b</sup>, Taciane Alegra<sup>b</sup>, Fernanda Sperb<sup>a,d</sup>,  
Nataniel Floriano Ludwig<sup>a</sup>, Maria Luiza Saraiva-Pereira<sup>b,f</sup>, Ursula Matte<sup>a,b</sup>,  
Ida V.D. Schwartz<sup>b,c,d,e,f,\*</sup>

<sup>a</sup> Gene Therapy Center, Experimental Research Center, Hospital de Clínicas de Porto Alegre, RS, Brazil

<sup>b</sup> Genetics and Molecular Biology Postgraduate Program, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

<sup>c</sup> Department of Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

<sup>d</sup> Postgraduation Program in Medical Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

<sup>e</sup> Brain Laboratory, Experimental Research Center, Hospital de Clínicas de Porto Alegre, RS, Brazil

<sup>f</sup> Medical Genetics Service, Hospital de Clínicas de Porto Alegre, RS, Brazil

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## ABSTRACT

Mucopolidiosis III (ML III) gamma is a very rare autosomal-recessive disorder characterized by the abnormal trafficking and subcellular localization of lysosomal enzymes due to mutations in the *GNPTG* gene. The present study consists of a report of a Brazilian compound heterozygote patient with ML III gamma resulting from one mutant paternal allele and one allele that had most likely undergone a *de novo* or maternal germline mutation. This is the first report of a *de novo* mutation in ML III gamma. This finding has significant implications for genetic counseling.

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## 1. Introduction

Mucopolidiosis II (ML II disease, inclusion cell disease or I-cell disease) and III (ML III, pseudo-Hurler polydystrophy) are autosomal recessive disorders caused by defects in the GlcNAc-1-phosphotransferase (EC 2.7.8.17) complex, which is composed by three subunits:  $\alpha$ ,  $\beta$ , and  $\gamma$ . Mutations in the gene encoding the  $\alpha$ - and  $\beta$ -subunits (*GNPTAB*) lead to ML II alpha/beta (OMIM #252500), or to the less clinically severe

\* Corresponding author at: Serviço de Genética Médica, Rua Ramiro Barcelos, 2350, Santa Cecília, 90035-903 Porto Alegre, RS, Brazil.  
E-mail address: [ischwartz@hcpa.ufrgs.br](mailto:ischwartz@hcpa.ufrgs.br) (I.V.D. Schwartz)

condition, ML III alpha/beta (OMIM #252600). ML III gamma (OMIM #252605) is caused by mutations in the gene encoding the  $\gamma$ -subunit of GlcNAc-1-phosphotransferase [1,2], and is thought to be the mildest form of the disease. Very few cases of ML III gamma are reported in the literature, maybe because the disease is underdiagnosed due to its relatively mild and unspecific clinical findings, which is suggested by a recent report of ML III gamma patients diagnosed through next generation sequencing [3]. To date, approximately 28 mutations have been reported in the *GNPTG* gene. A large number of these mutations are unique or rare [4].

*De novo* mutations are not rare events and the perception they are potentially important in genetic diseases, even in autosomal recessive conditions, have major implications for genetic counseling [4,5]. The present study consists of a report of a Brazilian compound heterozygote patient with ML III gamma resulting from the inheritance of one mutant paternal allele and one maternal allele that had most likely undergone a *de novo* or germline mutation.

## 2. Materials and methods

### 2.1. Case report

The proband, a female born at term to young (maternal and paternal age at conception was 27 years old) and non-consanguineous parents, has been described previously [6]. She was referred for clinical genetic evaluation due to large joint contractures. On physical examination, the patient presented contractures and restrictions of movement, especially in the hands, feet and shoulders, and heart systolic murmur, audible mainly at the left sternal border. The two-dimensional color Doppler echocardiography revealed the presence of mild thickening of aortic valve leaflets with mild regurgitation. All other clinical parameters were within normal limits for the patient's age. The exam was performed through the subcostal window, as thoracic deformity prevented the use of standard echocardiographic measures of pulmonary artery systolic pressure. Electroneuromyography of the upper limb was normal, and showed no electrophysiological evidence of peripheral neuropathy. The somatosensory evoked potential of the upper and lower limbs was also normal. The patient was diagnosed as having ML III when she was 8 years old (Table 1), and is currently stable. At the time of the study, she was 16 years old and attended regular school. Previous *GNPTAB* sequencing showed no alterations.

### 2.2. *GNPTG* analysis and maternity testing

Genomic DNA was extracted from leukocytes (patient and both parents), buccal cells (patient and mother) and fibroblasts (patient) after informed consent was given.

*GNPTG* was amplified in five fragments containing exons 1 to 2, 3, 4 to 7, 8 to 9 and 10 to 11 as described by Persichetti et al. [7] with modifications. The fragment which comprises exons 4 to 7, where the mutation c.244\_247dupGAGT is located, was also amplified using a second pair of primers [8]. Samples were submitted to DNA sequencing, performed on an ABI PRISM 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA). All samples were amplified and sequenced twice. The sequence of the *GNPTG* gene used as a template was GenBank accession no. NG\_016985.1.

**Table 1**  
Biochemical characterization of a patient with Mucopolipidosis III gamma.

Enzymes	Sample	Patient	References values
Arylsulfatase A (EC 3.1.6.8)	Plasma	+	Negative
$\alpha$ -L-Iduronidase (EC 3.2.1.76)	Plasma	176	32–52 nmol/h/ml
$\beta$ -Glucuronidase (EC 3.2.1.31)	Plasma	475	30–300 nmol/h/ml
$\alpha$ -Mannosidase (EC 3.2.1.24)	Plasma	1,548	17–56 nmol/h/ml
Iduronate-sulfatase (EC 3.1.6.12)	Plasma	1894	122–463 nmol/h/ml
Total $\beta$ -hexosaminidases (EC 3.2.1.52)	Plasma	12,675	1000–2857 nmol/h/ml
$\alpha$ -Mannosidase (EC 3.2.1.24)	Fibroblasts	16	60–400 nmol/h/mg
$\beta$ -Galactosidase (EC 3.2.1.23)	Fibroblasts	132	394–1440 nmol/h/mg
$\beta$ -Glucuronidase (EC 3.2.1.31)	Fibroblasts	8.6	62–361 nmol/h/mg
$\alpha$ -Fucosidase (EC 3.2.1.51)	Fibroblasts	2.1	46–221 nmol/h/mg

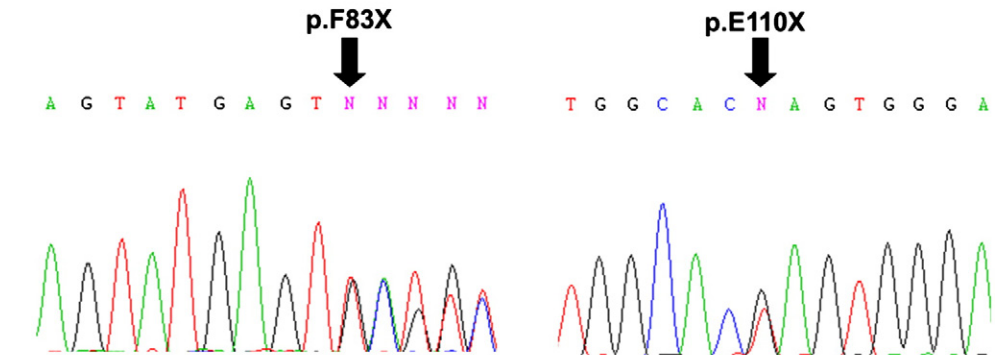
Total RNA extraction was performed on a whole blood sample of the patient and her relatives, as well as three controls using the Paxgene blood RNA Kit (Qiagen, Germany), and conversion to cDNA was made using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's instructions. The *GNPTG* mRNA levels were determined by qRT-PCR using  $2 \times$  SYBR Green PCR Master Mix (Applied Biosystem) with the Mx3000P (Stratagene, Amsterdam, NL). *GAPDH* was chosen as housekeeping gene. Primers and conditions were performed as described by Ho et al. [9] with modifications. The relative quantification of the RNA was normalized to the level of *GAPDH* mRNA in the same cDNA using the comparative CT method ( $2^{-\Delta\Delta CT}$ ).

For maternity testing, DNA from each sample was analyzed by PCR multiplex reaction using the AmpFISTR® Identifier® PCR Amplification kit (Applied Biosystems®), which enables the analysis of the 15 STR markers (STRs) using fluorescent primers, according to the manufacturer's instructions. PCR products were then resolved by capillary electrophoresis in an ABI3130xl genetic analyzer (Applied Biosystems®) using GeneScan™ 500 LIZ® as an internal marker and alleles were identified through GeneMapper® Software® v1.2 (Applied Biosystems®).

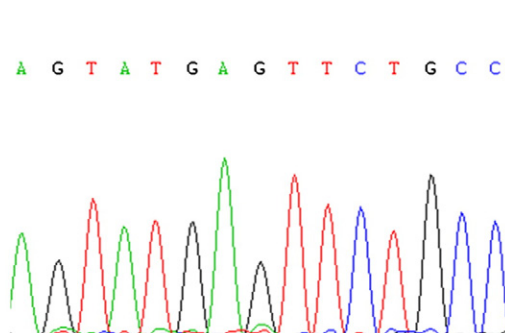
### 2.3. Statistical analysis

Values obtained for the relative quantification of *GNPTG* mRNA in patient, parents and control samples were compared using Student's test (IBM SPSS Statistics version 20). P values lower than 0.05 were considered statistically significant.

#### Patient: p.[F83X];[E110X]



#### Mother: WT/WT



#### Father: WT/ p.E110X

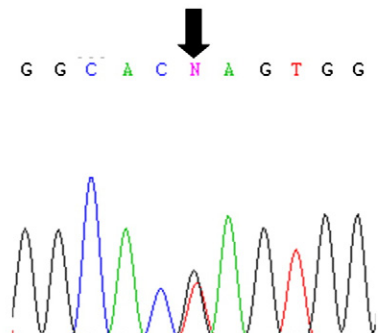


Fig. 1. *GNPTG* gene sequencing of the patient, her mother and father (leukocytes). WT = wild type.

### 3. Results

The genotype of the proband was c.[244\_247dupGAGT];[− 112C > G; 328G > T] (or p.[F83X];[E110X]), as determined by leukocyte DNA sequencing. To confirm this result, parents were also analyzed. Paternal allele sequencing identified a heterozygous p.E110X mutation, which had been previously found by our group in two other unrelated Brazilian ML III patients always *in cis* with the − 112C > G mutation; sequencing of 100 control alleles did not show the presence of both mutations (data not shown). Surprisingly, no mutation was detected in maternal leukocytes (Fig. 1), even using different pairs of primers. Based on these findings, buccal cells were collected from both individuals, and fibroblasts were obtained from the patient in order to investigate the possibility of mosaicism for the c.244\_247dupGAGT mutation in the patient and her mother.

Quantitative RT-PCR revealed that the amount of *GNPTG* mRNA found in blood samples of the patient, her father and her mother was approximately 1.6%, 54% and 88.5% of the levels found in controls (Fig. 2).

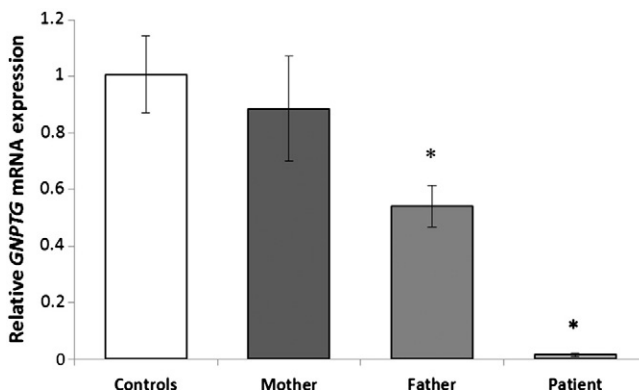
The probability of maternity was determined as 99.9999% by assessment of 15 different DNA markers in the patient and her parents.

### 4. Discussion

On the basis of the present results, we propose that the new mutation c.244\_247dupGAGT is attributable to a *de novo* event which occurred in only one ovum, or to germline mosaicism in the mothers' ova. Although there is the possibility of mosaicism in the patient, this is an unlikely alternative, since more than one tissue was analyzed in the present study. Unfortunately, the possibility of germline mutation or germline mosaicism could only be confirmed in this case through ovarian biopsy.

We also consider very unlikely the possibility of preferential amplification of the normal allele (e.g., allele dropout of the mutant allele) in the mother due to the presence, *in cis*, of any genetic variation in the annealing region of one of the primers used to amplify exons 4 to 7 of *GNPTAB*, since we use two different pairs of primers to amplify this fragment, both showing the same results. Besides that, RNA studies showed a decreased *GNPTG* mRNA in patient and her father, but not in her mother, supporting the hypothesis of a *de novo* or germline mutation.

On average, 74 *de novo* single-nucleotide variants (SNVs) and three novel indels are believed to occur in an individual's genome per generation. The rates at which these phenomena occur are strongly influenced by factors such as parental sex and age, and DNA sequences located next to the mutation [5,9,10]. In the present case, maternal and paternal age at conception was not advanced, but just upstream to c.244\_247dupGAGT mutation, an indel (TMP\_ESP\_16\_1411876 DELETION) has already been described [11], a finding which suggests this region of the *GNPTG* is prone to mutations (e.g., a hot spot site).



**Fig. 2.** Quantitative mRNA studies. The relative mRNA level of *GNPTG* was determined in a blood sample of the patient and her relatives, as well three controls, by real-time PCR and normalized to *GAPDH* mRNA expression. The final values are the mean values of three real-time PCRs made from two RNA preparations for each individual, and expressed as the fold change ± SD. The values found in father and patient were found to differ from those found in controls. \* $p < 0.001$  Student's test.

As expected, *de novo* germline mutations have been described more frequently in dominant disorders such as achondroplasia, Apert syndrome and multiple endocrine neoplasia [12] than in recessive disorders. Studies of autosomal recessive disorders have also detected *de novo* germline mutations in one patient with Ataxia-telangiectasia and in two patients with Gaucher disease [13]. From a genetic counseling perspective, the recurrence risk for an autosomal recessive disorder changes from 25% – if both parents are carriers of a pathogenic mutation – to a negligible value if only one parent is a carrier and there is no germline mosaicism in the other parent. There are few studies estimating the rates of germline mosaicism in recessive disorders, but for X-linked recessive disorders such as Duchenne muscular dystrophy and hemophilia A, maternal germline mosaicism is believed to occur in up to 5% [14] and 11% of mothers [15], respectively.

This is the first report of a *de novo* mutation in ML III gamma, and suggests that this kind of event probably occurs more often than currently recognized in recessive disorders. The present findings have major implications for genetic counseling, and strongly recommend that the carrier status of non-consanguineous parents of a child with-recessive disorders should always be confirmed through DNA analysis.

### Conflict of interest

The authors declare no conflict of interest.

### Acknowledgments

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