Short Communication

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

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ABSTRACT

Mucolipidosis 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

Mucolipidosis 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: α, β, and γ. Mutations in the gene encoding the α- and β-subunits (GNPTAB) lead to ML II alpha/beta (OMIM #252500), or to the less clinically severe...
condition, ML III alpha/beta (OMIM #252600). ML III gamma (OMIM #252605) is caused by mutations in the gene encoding the γ-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

| Enzymes Sample | Patient | References values |
|----------------|---------|------------------|
| Arylsulfatase A (EC 3.1.6.8) Plasma | + | Negative |
| α-L-Iduronidase (EC 3.2.1.76) Plasma | 176 | 32–52 nmol/h/ml |
| β-Glucuronidase (EC 3.2.1.31) Plasma | 475 | 30–300 nmol/h/ml |
| α-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 β-hexosaminidases (EC 3.2.1.52) Plasma | 12,675 | 1000–2857 nmol/h/ml |
| α-Mannosidase (EC 3.2.1.24) Fibroblasts | 16 | 60–400 nmol/h/mg |
| β-Galactosidase (EC 3.2.1.23) Fibroblasts | 132 | 394–1440 nmol/h/mg |
| β-Glucuronidase (EC 3.2.1.31) Fibroblasts | 8.6 | 62–361 nmol/h/mg |
| α-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× 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 AmpFlSTR® 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]**

![GNPTG gene sequencing of the patient, her mother and father (leukocytes). WT = wild type.](image-url)
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.

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