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A NEW MUTATION AT EXON 2 OF HPRT1 LOCUS CAUSING LESCH-NYHAN SYNDROME

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ABSTRACT

Introduction: Lesch-Nyhan síndrome (LNS) is an X-linked recessive inborn error of metabolism, due to deficiency of the enzyme Hypoxanthine-guanine-phosphoribosyl transferase (HGPRT; EC.2.4.2.8) resulting in hyperuricemia, neurological and behavioural disturbances. In the present work, we report the results of the study of a Colombian family, where LNS was previously clinically and biochemically diagnosed. **Material and Methods:** The full HPRT gene, including 9 exons and 8 introns, was amplified on eight separate DNA fragments. Both strands, forward and reverse, of the amplified DNA fragments were analyzed and the obtained sequences were compared with those deposited at National Center for Biotechnology Information. **Results and conclusions:** Sequence analysis allowed the detection of new LNS causing mutation, an adenine deletion in exon 2 of HPRT1 gene resulting in a frameshift which determines a premature stop codon. This study, besides adding a new mutation to the already large spectrum of disease causing variation at HPRT, allows therefore providing genetic counseling for the family as well as prenatal diagnosis.

Keywords: Lesch-Nyhan, HGPRT deficiency, genetic counseling, X-linked recessive, mutation

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INTRODUCTION

Lesch-Nyhan syndrome (LNS) is an X-linked recessive inborn error of metabolism, due to deficiency of the enzyme Hypoxanthine-guanine-phosphoribosyltransferase (HGPRT; EC.2.4.2.8) resulting in hyperuricemia, neurological and behavioural disturbances. In the present work, we report the results of the study of a Colombian family, where LNS was previously clinically and biochemically diagnosed. Sequence analysis allowed the detection of new LNS causing mutation, an adenine deletion in exon 2 of HPRT1 gene resulting in a frame shift which determines a premature stop codon.

The Lesch-Nyhan syndrome (LNS) is an inborn error of purine metabolism caused by a virtually complete deficiency (less than 1.5% of the normal level) of the enzyme Hypoxanthine-guanine phosphoribosy ltransferase (HGPRT) causing hyperuricemia and profound neurological disturbances (OMIM#300322). LNS is characterized by an overproduction of uric acid, neurological dysfunction, varying degrees of learning disability, and some behavioral abnormalities including self-mutilation[1]. Three main phenotypic subgroups are recognized. The mostsevere subgroup is Lesch-Nyhan disease (LND), in which full classical syndromeoccurs.

Least affected subgroup is HGPRT-related hyperuicemia (HRH), where patients exhibit overproductionof uric acid and show aresidual enzyme activity of 8%. In HRH the neurobehavioral features are absent or sufficiently mild with no clinical significance. An intermediate subgroup is HGprt-relatedneurological dysfunction (HND), where patients exhibitoverproduction of uric acid along with varying degrees of neurologicalimpairments and display 1.5–8% of residual enzyme activity[2].

The mutations are dispersed all over the gene, both in exons and intronic regions, and are very heterogeneous both in type and effects on HPRT activity[2]. However, recent studies have revealed multiple unrelated patients with similar mutations, providing an opportunity to examine genotype–phenotype correlations[3].

HPRT gene is located on X chromosome, at Xq26-q27 region, and consists of nine exons and eight introns, spanning 45 kb[4]; the mRNA transcript is 1.6 kb long, encoding a protein with 218 amino acids[1]. More than 400 different causing HPRT deficiency mutations have been described, including a total of 250 responsible for LNS. As a consequence of the sex-linked mode of transmission LNS affects almost exclusively males and an allele frequency of approximately $2x10^{-3}$ is responsible for anestimated incidence at birth of 1/380,000.

Although there are many reports on families carrying the LNS, until now a single case has been described for a Colombian family. Two affected members of this family were first clinically diagnosed in 1992[5] and later they were characterized biochemically[6]. Subsequently analysis of alleles at an STR locus in intron 3 of the HPRT gene were performed in 16 members from this family in order to establish the carrier statues of the women[7]. In this work we report the results of the sequencing study on this Colombian family with members affected by LNS, which revealed a new mutation at HPRT locus.

MATERIALS AND METHODS

The genealogy of the studied family is shown in **Figure 1.** Samples were collected after informed consent. DNA was extracted from total blood by salting-out[8]. Kinship was confirmed using apreviously described protocol for the amplification of 10 X chromosome STRs[9,10].

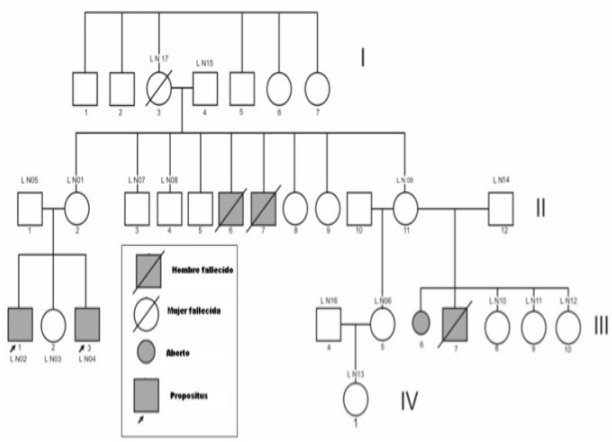

Figure 1. The genealogy of the family with Lesch-Nyhan syndrome. Greyed symbols correspond to affected individuals; a smaller circle to a newborn death; arrows point to the proposita. Note that all coded individuals, except LN17, were sequenced.

The full HPRT gene, including 9 exons and 8 introns, was amplified on eight separate DNA fragments following a previous described protocol[11]. Both strands, forward and reverse, of the amplified DNA fragments were sequenced employing Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) in an ABI PRISM 377 DNA sequencer (Applied Biosystems)[12]. Obtained sequences were compared with those deposited at NCBI[4].

RESULTS

Complete sequencing of the 9 exons and 8 introns of HPRT gene of both affected individuals, has revealed an adenine deletion at position 52 of exon 2. No other variation previously associated with the disease was found. The sequencing results of the affected male and a normal homozygote female are shown in **Fig 2** and **Fig 3** respectively.

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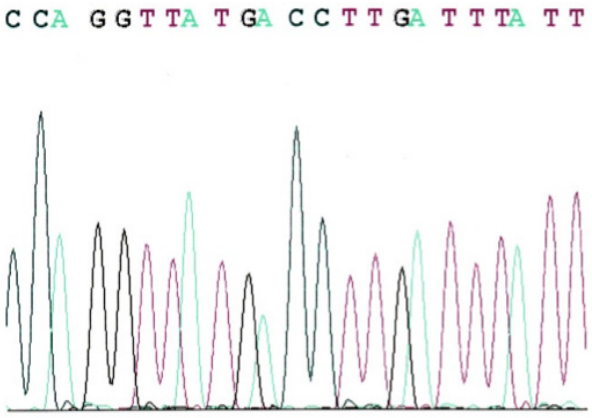

Figure 2. Sample of a woman homozygous for the normal sequence of exon 2 of HPRT1 gene.

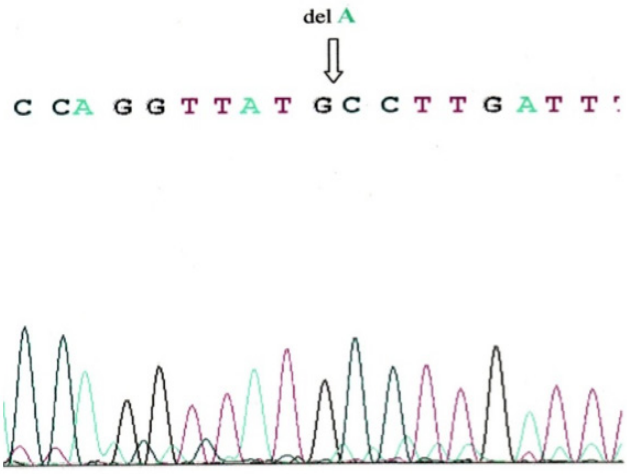

Figure 3.Sample of a male hemizygous for the sequence with the deletion of adenine at position 52 of exon 2 of HPRT1 gene.

This deletion causes a shift of the reading frame in Y17 and generates a stop codon at 41. Figure 2 shows the sequencing of an analyzed sample of a homozygous for the normal sequence of exon 2. Figure 4 shows the sequences of the normal allele and the mutated one for a carrier.

DISCUSSION AND CONCLUSIONS:

Sequencing of theen tire HPRT gene in this family members allowed the discovery of a new mutation at exon 2, an adenine deletion determining a premature codon stop at position 41 of this exon. Although other mutations at codon 2 were previously reported, none corresponds to the variant here described[13,14]. The diagnosis in patients LN02 and LN04 was confirmed and we were able to trace the transmission of the mutation in the family and namely to establish the carrier status of females (LN01, LN09, LN06, LN10, LN12).This study, besides adding a new mutation to the already large spectrum of disease causing variation at HPRT, allows therefore providing genetic counseling for the family as well as prenatal diagnosis[7].

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