HPRT$_{\text{Yale}}$ proposed as a pathogenic variant for Lesch-Nyhan syndrome: a case report

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ABSTRACT. Lesch-Nyhan syndrome (LNS) is an X-linked recessive disorder caused by a deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT), an enzyme encoded by the $\text{HPRT1}$ gene. The classic disease phenotype described by Lesch and Nyhan in 1964 includes hyperuricemia, mental retardation, severe motor deficiency, and recurring self-mutilation. Here, we report the case of a family with 4 affected males and several female obligate carriers. In 1989, Fujimori et al. reported on a patient diagnosed with LNS who had an HPRT variant thereafter codenamed HPRT$_{\text{Yale}}$. The same patient was studied by Wilson et al. in 1986, who found no detectable HPRT enzymatic activity, even though normal HPRT mRNA and protein levels were observed. Disease severity is closely related to residual enzymatic activity, which fits the phenotype presented for
this previously reported case, as well as for the patients we report on herein. As it has been reported in only one patient, this mutation is still considered a variant of unknown significance. The HPRT<sub>Yale</sub> mutation is a G>C transversion that leads to a different amino acid with different biochemical properties at position 71, potentially causing the major lack of function. To evaluate the impact of this variant, we used the PolyPhen-2 software, which classified it as possibly damaging. Furthermore, the frequency of this mutant allele is likely extremely rare, since it has only been reported on twice, and a population frequency is not yet available. In conclusion, we propose that the HPRT<sub>Yale</sub> variant is pathogenic, and should be included on lab reports hereafter.

**Key words:** Lesch-Nyhan syndrome; X-linked recessive; HPRT1; Somatic mutation

**INTRODUCTION**

Lesch-Nyhan syndrome [LNS; Online Mendelian Inheritance in Man (OMIM, 2015) ID #300322] is an X-linked recessive disorder caused by deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT; ExPASy - ENZYME Enzyme Commission No. 2.4.2.8) (SIB, 2015), which is responsible for purine metabolism (Ragazzini et al., 2014). HPRT uses hypoxanthine and guanine as substrates to synthesize inosine monophosphate and guanosine monophosphate, respectively. When this enzyme is non-functional, substrates accumulate and are converted to uric acid by the action of xanthine-oxidase (Sapag et al., 2013). HPRT is encoded by the HPRT1 gene, which contains 9 exons and is located on chromosome Xq26. Currently, there are 615 null mutations known that are located at multiple positions within the HPRT1 gene (Jinnah et al., 2000; Torres et al., 2000; Fu et al., 2014). The classic clinical phenotype of LNS was first described by Michael Lesch and William Nyhan in 1964, and is characterized by hyperuricemia, intellectual disability, severe motor deficiency, and recurring self-mutilation (Lesch and Nyhan, 1964). Complete deficiency of HPRT function and hematological dysfunction are also found in this disease (Torres et al., 2014). Patients with a lesser degree of the disease and that have no self-destructive behavior are classified as having Lesch-Nyhan variant (Torres et al., 2012). Symptoms of LNS may vary greatly according to the level of residual HPRT activity, which can be determined by the specific mutation found (Fu et al., 2014). LNS diagnosis is based on clinical symptoms and biochemical results (hyperuricemia and uricosuria), together with neurological evaluations of mental function, molecular testing for pathogenic mutations, and enzymatic analysis for HPRT function (Chandekar et al., 2015). Treatment for LNS is symptom based, whereas little to nothing is currently done to correct the underlying cause of the disease (Torres and Puig, 2007).

**MATERIAL AND METHODS**

The current study is a case report of a family with 4 male individuals affected with LNS and several female obligate carriers.
RESULTS

Case report

A couple with 2 male sons with spastic cerebral paralysis searched for genetic counseling. The familial history did not reveal consanguineous relatedness between husband and wife, but the wife had 2 deceased brothers with cerebral paralysis and other features similar to her sons. See Figure 1 for the family heredogram.

Figure 1. Family heredogram. The arrow points to the consulting proband’s mother. Affected individuals are hash-colored and carriers have an inner dot. The c.211G>C (HPRT<sub>Yale</sub>) mutation is shown in text wherever it was found by sequencing.

Karyotype, metabolic tests, and computerized tomography were performed prior to molecular genetic analysis, but nothing new was revealed. The affected children also presented with urine crystals, debilitating scoliosis, self-mutilation, mental retardation, and choreoathetosis. Furthermore, the deceased brothers (II-2 and II-3) exhibited self-mutilating behavior, which is a classic characteristic of LNS.

Owing to the X-linked recessive inheritance of LNS, we concluded that the oldest known carrier was the grandmother (I-1), who had 6 children including 3 girls and 3 boys, of which 2 boys were affected and died at approximate ages of 14 and 16 years. Individual II-1 had 3 affected boys, of which the oldest (III-1) died at age 16. Individual II-5 had 2 girls. Individual II-6 had 1 girl and 1 unaffected son.

In 1998, linkage analysis was performed for all live affected family members, as well as for putative carriers. The affected X-chromosome was found on male affected individuals II-2 and II-3, and on female carriers II-1 and II-5. In 2015, DNA from affected males and female carriers was sequenced, and the results showed that the HPRT1 variant of unknown significance c.211G>C (p.G71R) based on GenBank accession No. NM_000194.2 (also known as HPRT<sub>Yale</sub>) was present in all affected and carrier individuals (NCBI, 2015). DNA from unaffected and non-carrier individuals was also tested, and was found to not have this variant. Taken together, these results strongly suggest that HPRT<sub>Yale</sub> is a pathogenic variant that causes LNS.
DISCUSSION

LNS is an X-linked recessive disorder caused by a variety of heterogeneous mutations in the \textit{HPRT1} gene. There are currently 615 known mutations, including duplications, deletions, translocations, insertions, nonsense, and missense mutations, as well as splice site mutations. Of these, single base substitutions are relatively common, yielding a single amino acid change in most cases (Fu et al., 2014).

In the current report, the family history and clinical data motivated the molecular analysis of affected and carrier individuals, as well as of unaffected family members, which were used as controls to identify the pathogenic mutation. As seen in Figure 1, gene sequencing revealed presence of the point mutation c.211G>C in individuals II-1 and II-5 (carriers), as well as in III-2 and III-3 (affected). Individuals III-4, III-5, and III-7 were also tested, but results showed that they had the wild type gene sequence. This variant results in a glycine to arginine substitution at residue 71 (p.G71R) of the HPRT enzyme, due to a codon change of GGC to CGC.

After a careful literature search, we found only one other description of this mutation in a Lesch-Nyhan family. Specifically, Fujimori et al. (1989) previously reported on a patient identified as K.T., who was diagnosed with LNS and who had this mutation, and the authors codenamed this variant HPRT\textsubscript{Yale}. The same patient was also studied by Wilson et al. (1986), who found no detectable enzymatic activity (<0.7% compared to control), even though normal mRNA and protein levels (92% of normal control) were observed.

Single base substitutions are the most common type of mutation found in classic and attenuated LNS cases (Fu et al., 2014). Nonetheless, disease severity is closely related to residual enzymatic activity after the resultant amino acid change caused by the pathogenic mutation (Jinnah et al., 2000). Particularly, disease severity is inversely proportional to residual enzymatic activity (Nguyen et al., 2012).

Although the c.211G>C mutation has been previously reported in a LNS patient, it is still regarded as a VUS and its pathogenicity has not yet been established. However, in all affected individuals studied herein where this mutation was found, classic LNS was clinically diagnosed, and the mutation was found in all obligate carriers. This mutation is a G>C transversion that leads to a different amino acid with different biochemical properties at position 71 in HPRT. Specifically, a glycine, which is a small and neutral amino acid, is replaced by an arginine, which is basic and hydrophilic. This non-conservative substitution may be related to the loss of enzymatic function observed by Wilson et al. (1986) in patient K.T., which leads us to believe that the same is happening in our affected patients.

Mutations that cause a loss of enzymatic activity may be located far from the catalytic site, but still interfere with activity due to conformational changes, protein stability, and subunit interactions (Duan et al., 2004; Fu and Jinnah, 2012; Fu et al., 2014). According to de Gemmis et al. (2010), exon 3 mutations account for 25.7% of all \textit{HPRT1} gene mutations, suggesting that this exon is a mutation hotspot. Furthermore, according to Fu et al. (2014), the interval between amino acids 65-74 is one of 5-10 amino acid interval hotspots. The LKGG residues at amino acids 68-71 are conserved in proteins of the phosphoribosyltransferase family and participate in the phosphoribosylpyrophosphorylation reaction. Mutations in this region have been reported in 22 disease cases, of which 18 were LNS (Fu et al., 2014). Of 6 cases involving changes to amino acid 71, 3 were in regards to LNS, and described the HPRT\textsubscript{Yale} mutation at position 211 (Fu and Jinnah, 2012).
To further evaluate the impact of this variant, we used the PolyPhen-2 software, which classified this mutation as possibly damaging with a score of 0.890 with a sensitivity of 0.70 and specificity of 0.89 (Adzhubei et al., 2010). Furthermore, we believe that the frequency of this mutant allele is extremely rare, since it has been reported only twice in decades, and no mutation database reports a population frequency for this variant.

In conclusion, we reported on a family with several cases of classic LNS and obligate female carriers with the HPRT<sub>Yale</sub> mutation. A similar case with this mutation was previously reported in the literature, and together with a bioinformatics analysis of HPRT<sub>Yale</sub> enzymatic function, point to its pathogenicity. Due to these observations, we propose that this variant is pathogenic, and suggest that it should be included on lab reports for LNS patients in the future.

Conflicts of interest

The authors declare no conflict of interest.

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We would like to thank family members for all the help we received in putting this information together with all details. We are certain that their help will enable other families to have a more conclusive diagnosis for LNS.

REFERENCES


