Complete deficiency of the activity of hypoxanthine phosphoribosyltransferase (HPRT) leads to the classic phenotype of Lesch-Nyhan syndrome (Lesch, 1964; Nyhan, 2005). All patients with HPRT deficiency have hyperuricemia and hyperuricosuria and are at risk for gout, hyperuricemia and gouty arthritis, renal disease, and tophaceous deposits.

Those with the Lesch-Nyhan phenotype also have an impressive neurologic disorder and unusual self-mutilating behavior; this is the most common clinical presentation of HPRT deficiency and is probably due to major dysregulation of the HPRT gene (OMIM 310000) such as deletions, insertions, and stop codons, but it is also the most common consequence of genetic variants of the HPRT gene (Page et al., 2000). Missense mutations that are more conservative tend to lead to variant enzymes with varying amounts of partial activity and 2 that phenotype correlates moderately well with amounts of enzyme activity based on assay in an intact cell system. (Page, 1981) Those with the greatest amounts of activity display hyperuricemia and gout or urinary tract stone disease without neurologic or behavioral abnormality. (Kogut et al., 1980). An intermediate group we have called “neurologic variants” appear neurologically identical to patients with classic Lesch-Nyhan, but intelligence is normal or near normal, and there are no abnormalities of behavior. (Bakay et al., 1979)

The purpose of this study is offering an original contribution on molecular diagnosis of 39 Italian Lesch-Nyhan patients. Special attention is given to the methods required to confirm the diagnosis. Using DNA sequence analysis it is possible to detect mutations in the HPRT1 gene in the majority of males who display the full Lesch-Nyhan syndrome phenotype (Sinnah et al. 2000, Jinnah et al. 2004). On the other hand, considering that heterozygous female carriers of Lesch-Nyhan syndrome may display a milder phenotype, initial diagnosis may be confirmed only by the presence of a carrier DNA sequence analysis. For this purpose, we present our methods for testing the potential healthy carrier females. Our work shows how RNA study combined with qPCR (Lapres et al. 2006) is needed for a correct molecular diagnosis of LNS in female carriers and in some cases also in affected males.

**RESULTS**

In our cohort of 39 Italian Lesch-Nyhan patients we found 9 different missense mutations in 10 patients (25.6%), 2 different nonsense mutations in 3 patients (7.7%), 1 small deletion (2.6%), 3 different small deletions or insertions in 4 patients (10.3%), 1 small insertion-deletion (2.6%), 2 different splice site mutations in 9 patients (23.1%), and 6 different gross deletions in 9 patients (23.1%) and 1 exclusion of exons 4 and 5 at BNA level in 2 patients (5.1%). Mutations were spread along the entire gene region.

The current molecular diagnosis required the use of three different methods: 37 (95%) of the affected males males carried a single HPRT deficiency mutation. However 2 cases (patient 18 and patient 37) DNA sequencing failed to identify the disease causing mutation and only mRNA levels studies were able to show a transcript missing exons 4 and 5. Additional studies of the intronic regions in these patients are currently underway in order to establish the origin of this “alternative splicing” pattern.

Note: In the case of female carriers the number of patients we could test that 72% of the carrier females were diagnosed by genomic DNA sequencing, 5% were identified only by BNA level studies and 23% were identified by qPCR. The mRNA level testing for LNS is used in patients as confirmation of the clinical and biochemical diagnosis and is of primary importance in carrier detection and prenatal diagnosis.

**CONCLUSIONS**

DNA analysis

Each of the 9 exons of the HPRT1 gene was amplified by PCR, using specific oligonucleotide primers. PCR was performed in a 25 uL reaction mixture containing 100 ng of genomic DNA, 10 pmol of each primer, 10 mM Tris-HCl (pH 8.3), variable MgCl₂ concentration, depending on the exon amplified, 50 mM KCl, 1.5 mM MgCl₂, and 0.5 mM dNTP. The reaction mixture was amplified in an automatic thermal cycle (PCR TC-512, TIB Molbiol). The PCR products were purified with the GFX PCR DNA and Gel Band Purification Kit (Amersham-Pharmacia-Biotech) and directly sequenced with the same primers as used for PCR, using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) on an automatic ABI PRISM 3130. Sequencing products were analyzed by gel electrophoresis and confirmed using an automated DNA sequencer (Applied Biosystems, Foster City, CA, USA).

RNA Studies

First-strand DNA was synthesized using 1 μg of total RNA (Diaseq-treated) in a 20 μl reverse transcriptase reaction mixture.

1-2 micrograms of total RNA were incubated with 2 units of amplification-grade ribonuclease (Biolase free) DNase I ( Gibco BRL) in buffer (final concentration: 20 mM Tris pH 8.3, 50 mM KCl, 2.5 mM MgCl₂) at 20 °C for 15 min at room temperature. DNase I was inactivated by the addition of 2 ml of 25 mM EDTA and heated to 65 °C for 10 min. The mixture was then used in a control reaction and in the sample for complete DNA digestion. In case a band of containing genomic DNA was recovered, digestion with DNase was repeated. RNA treated by DNase was used in a final volume of 60 μl of reaction mixture (100 ng of plasmid+3 μl of cDNA). Reaction conditions were: 40 cycles of 94 °C for 1 min, annealing temperature for each qRT, reverse transcriptase reaction before having the following composition: 50 mM Tris-HCl pH 8.3, 15 mM KCl, 2.5 mM MgCl₂, 0.25 μM of each primer and 0.8 μM of random hexamers pretreated by 10 min at 90 °C and cooled on ice for 5 min before added to reaction.

The mixture described was added of 200 μl of MuLV Reverse Transcriptase (Perkin Elmer) and incubated at 28 °C for 15 min, at 45 °C for 60 min and finally heat inactivated at 70°C for 10 min. DNA was purified with the QIAGEN PCR DNA and Gel Band Purification Kit (Amersham-Pharmacia-Biotech). Aliquots of 2 μl of DNA were directly analyzed by 2% agarose gel electrophoresis.

Real Time PCR for HPRT deletions detection

Real Time PCR analysis was performed in a 96-well optical reaction plate using an ABI Prism 7000 sequence detector (Applied Biosystems). Oligonucleotide primers were designed with Primer Express. Amplification of FAMC gene was used as reference. Reaction were performed in a final volume of 25 μl containing 2× SYBR Green PCR Master Mix (Applied Biosystems, USA), 500 nM of each primer, and 5 to 40 ng genomic DNA, according to serial dilution protocol.

**BIBLIOGRAPHY**


