

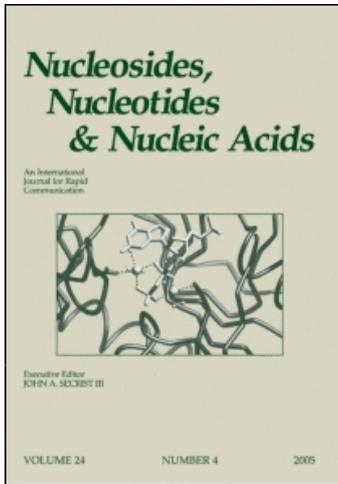
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## METHYLATION STATUS OF HPRT1 PROMOTER IN HPRT DEFICIENCY WITH NORMAL CODING REGION

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□ *Lesch-Nyhan syndrome is an X-linked recessive inborn error of metabolism due to a complete deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity (OMIM 300322). Partial deficiency of HPRT (OMIM 300323) is characterized by the effects of excess uric acid synthesis and a continuum spectrum of neurological manifestations, without the manifestations of full-blown Lesch-Nyhan syndrome. Both diseases have been associated with mutations in the HPRT1 gene. We have described one Lesch-Nyhan patient and four partial HPRT deficient patients with a normal HPRT1 coding region. These patients showed markedly decreased HPRT mRNA expression, but no mutation in their genomic regulatory sequences from HPRT1 gene. In this study, we analyzed the promoter region methylation status of the HPRT1 gene in these five HPRT deficient patients. Methods: DNA was bisulphite modified and a 620 bp fragment including 320 bp 5' to start codon was amplified and sequenced. The methylation status of 35 CpG island 5' to start codon and 28 CpG island 3' to start codon were investigated in male controls, female controls, patients, and the patient's mothers. Primer pairs were designed for methylated-specific and unmethylated-specific amplification and PCR was performed employing DNA bisulphite treated as template. Results: No alterations in the methylation pattern of the HPRT1 promoter were found in the five HPRT deficient patients. Conclusions: The promoter region methylation status of these five HPRT deficient patients was similar to that of normal subjects. Thus, some other genetic alteration must explain a reduced enzyme activity with a normal gene coding region.*

**Keywords** Lesch-Nyhan syndrome; HPRT; molecular diagnosis; methylation

### INTRODUCTION

Lesch-Nyhan syndrome is an X-linked recessive inborn error of metabolism due to a virtually complete lack of hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity (OMIM 300322).<sup>[1]</sup> Partial deficiency of HPRT (OMIM 300323) is characterized by the effects of excess uric acid

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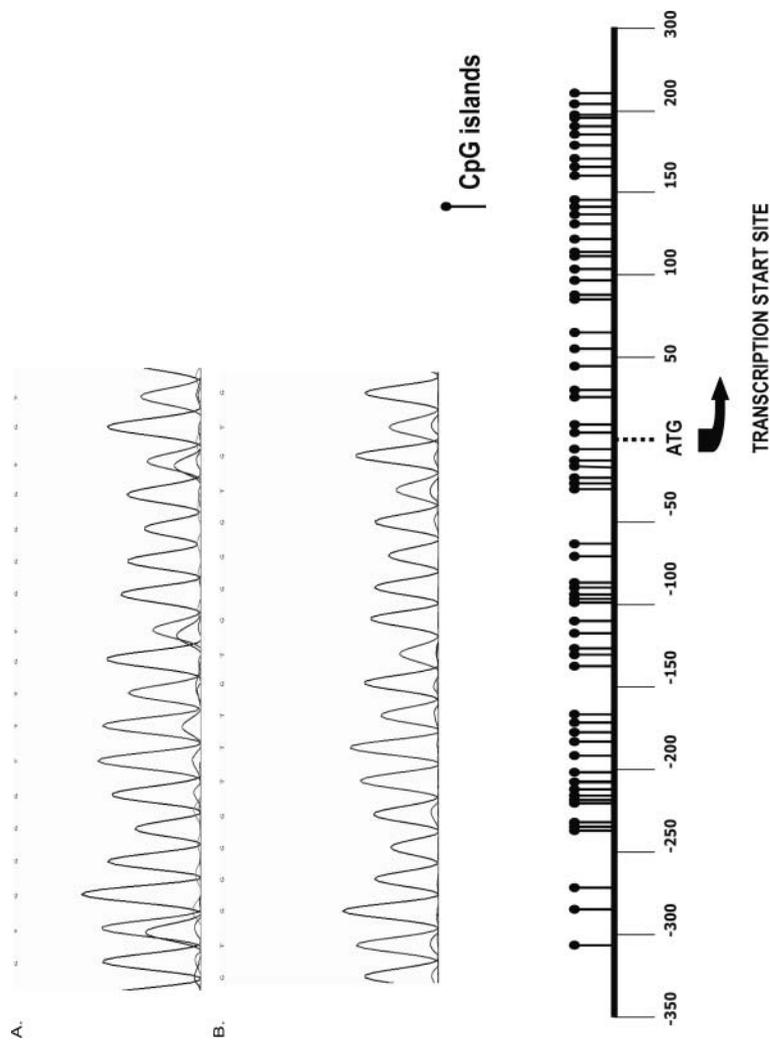
synthesis and a continuum spectrum of neurological manifestations, without the manifestations of the full-blown Lesch-Nyhan syndrome.<sup>[2,3]</sup> Both diseases have been associated with mutations in the HPRT1 gene, located in the long arm of the X chromosome at Xq26. To date, more than 300 different mutations have been described associated to HPRT deficiency. They are heterogeneous in type and localization within the gene.<sup>[4]</sup> In 2005, Dawson et al.<sup>[5]</sup> described, for the first time, an individual with gout in whom HPRT deficiency appeared to be due to a defect in gene regulation. After this first report, we described for the first time one Lesch-Nyhan patient and four partial HPRT deficient patients with a normal HPRT coding region.<sup>[6]</sup> These patients showed markedly decreased HPRT mRNA expression, but no mutation in their genomic regulatory sequences of the HPRT1 gene. Gene expression can be modulated by several factors including the methylation status of the promoter.<sup>[7]</sup> The aim of this study is to analyze the promoter region methylation status of the HPRT1 gene in these five HPRT deficient patients. We intended to assess whether an alteration of the methylation status of the promoter could account for the decreased HPRT mRNA expression.

## PATIENTS AND METHODS

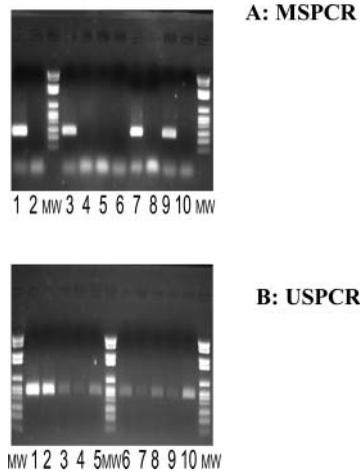
RNA-free genomic DNA samples were isolated from whole blood of one Lesch Nyhan patient, four partial HPRT deficient patients, the patient's mothers, and 6 control subjects (3 males and 3 females) using the QIAamp RNA Blood Mini Kit (QIAGEN GmbH, D-40724, Hilden, Germany). Informed consent was obtained from the parents and/or the patients and from control subjects. DNA (0.5  $\mu$ g) was bisulphite modified by means of the EZ DNA Methylation-Gold (Zymo Research Co., Orange, CA, USA). A 620 bp fragment including 320 bp 5' to start codon was amplified employing GoTaq Hot Start Polymerase and DNA bisulphite treated as template. The amplified fragments were automated sequenced employing BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Carlsbad, CA, USA) in an ABI PRISM 377 DNA Sequencer (Applied Biosystems). The methylation status of 35 CpG island 5' to start codon and 28 CpG island 3' to start codon were investigated in male controls, female controls, patients, and patients' mothers. Primer pairs were designed for methylated-specific (MSPCR) and unmethylated-specific (USPCR) amplification and polymerase chain reaction (PCR) was performed employing GoTaq Hot Start Polymerase (Promega Biotech Ibérica, Madrid, Spain) and DNA bisulphite treated as template. The amplified fragments were visualized by agarose gel electrophoresis.

## RESULTS

Female controls CpG island sequences were heterozygous showing both cytosine and thymine peaks due to random chromosome X inactivation (Figure 1A). However, all CpG island analyzed were unmethylated in male



**FIGURE 1** HPRT1 promoter scheme showing the 35 CpG island 5' to start codon and the 28 CpG island 3' to start codon analyzed by automated sequencing. A): A female chromatogram showing both cytosine and thymine peaks due to random chromosome X inactivation. B): A male chromatogram showing the complete bisulphite modification.



**FIGURE 2** A) Agarose gel showing methylated-specific PCR fragments: 1-female control, 2-male control, MW-molecular weight marker, 3-patient's mother, 4, 5, 6-partial HPRT deficiency brothers, 7-patient's mother, 8-partial HPRT deficiency patient, 9-patient's mother, 10-Lesch-Nyhan patient. B) Agarose gel showing unmethylated-specific PCR fragments: MW-molecular weight marker, 1-female control, 2-male control, 3, 4, 5-patient's mothers, 6, 7, 8, 9-partial HPRT deficiency patients, 10-Lesch-Nyhan patient.

controls and only thymine peaks were detected in the chromatogram due to the complete bisulphite modification (Figure 1B). CpG island of the patients were also complete unmethylated while patient's mothers showed both cytosine and thymine peaks. MSPCR showed amplification of the methylated fragment only in female controls and the patient's mothers (Figure 2A). However, USPCR showed amplification of the unmethylated fragment in both patients, female and male controls and the patient's mothers (Figure 2B).

## DISCUSSION

In this article, we presented 5 HPRT deficient patients in whom the cause of the diminished enzymatic activity is a decrease HPRT mRNA expression of unknown cause. We have discarded any mutation in coding regions, in splice regions of the HPRT1 gene (100 bp upstream and downstream the exon-intron junction) and in a total of 1670 bp fragments corresponding to the HPRT1 promoter. We also analyzed sequences of intron 1 and intron 2 linked to HPRT gene regulation in ES cells (980 bp 3' to exon 1 corresponding to part of intron 1, and the total sequence of intron 2) and no alteration was found. Thus, in the present study we hypothesized that an alteration in the methylation status of HPRT1 promoter could be the cause of the decreased expression of HPRT1 gene in these patients. We have found that the promoter region methylation status of these five HPRT deficient patients was similar to that of normal subjects.

In mammalian genome there is a vast extent of non-protein coding intronic and intergenic sequences. It has been postulated that these sequences may contain elaborate regulatory information. On the other hand, a great majority of the mammalian genome is transcribed to produce non-coding RNAs. To date, few mutations in non-coding RNAs have been described affecting phenotype as compared to those in protein-coding sequences. However, further studies will clarify the effect of these non-coding RNAs in gene regulation and disease. Epigenetic mechanisms regulate chromosomal organization and gene expression and include several levels of organization, all of which contribute to the activity of gene expression. Beside DNA methylation, other mechanisms of epigenetic changes in mammals are the modifications of histone tails which result in altered chromatin structure. Thus, some other genetic alteration, as mutations in unknown HPRT1 gene regulatory regions or epigenetic alterations could be the cause of decreased HPRT mRNA expression in complete or partial HPRT deficient patients.

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