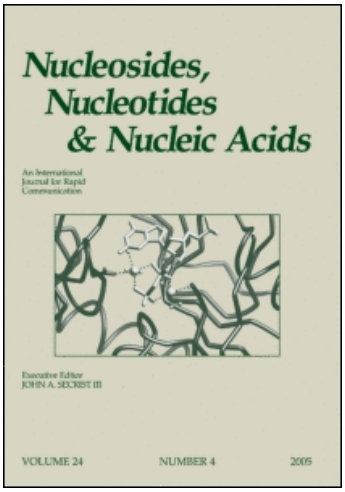

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Disruption of the Hypoxanthine-Guanine Phosphoribosyl-Transferase Gene Caused by a Translocation in a Patient with Lesch-Nyhan Syndrome

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ABSTRACT

In this study, we have identified a novel mechanism of mutation involving translocation between the *HPRT1* loci and other loci on the X chromosome. In HRT-25's cDNA obtained from a patient with Lesch-Nyhan syndrome, the upstream region of exon 3 was amplified, but the full-length region was not amplified. The use of 3′ rapid amplification of cDNA ends polymerase chain reaction (3′RACE-PCR) for HRT-25 revealed part of intron 3 and an unknown sequence which have not identified the *HPRT1* gene starting at the 3′ end of exon 3. We analyzed *HPRT1* genomic DNA in order to confirm the mutation with the unknown sequence in the genomic DNA. Unknown sequence compared through BLAST analysis of human genome (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/) showed that at least 0.5 to 0.6-Mb telomeric to

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HPRT1 on chromosome Xq where located near LOC340581. This study provides the molecular basis for the involvement of genomic instability in germ cells.

Key Words: Lesch-Nyhan syndrome; *HPRT1*; Mutation; Translocation.

INTRODUCTION

In humans, HPRT deficiency is associated with Lesch-Nyhan syndrome (LNS), which is characterized by hyperuricemia, mental retardation, choreoathetosis, and compulsive self-mutilation. The gene encoding HPRT (*HPRT1*) is located in region q26 of the X chromosome, and the entire nucleotide sequence has been determined.[1] Of the 271 mutations reported to date, thirty four mutations show macro-deletions with one or several exons deleted in *HPRT1* locus, and four mutations with macro-duplications.[2] In these large genomic rearrangements, breakpoint junctions have been defined for only ten mutations. In this report, we have identified a novel mechanism for disruption of the *HPRT1* gene leading to LNS in which a translocation occurs between intron 3 in the *HPRT1* gene and another gene.

MATERIALS AND METHODS

A lymphoblast cell line (HRT-25) was established from a Lesch-Nyhan patient, using Epstein-Barr virus. The HPRT activity in HRT-25 was less than 0.7% of the normal

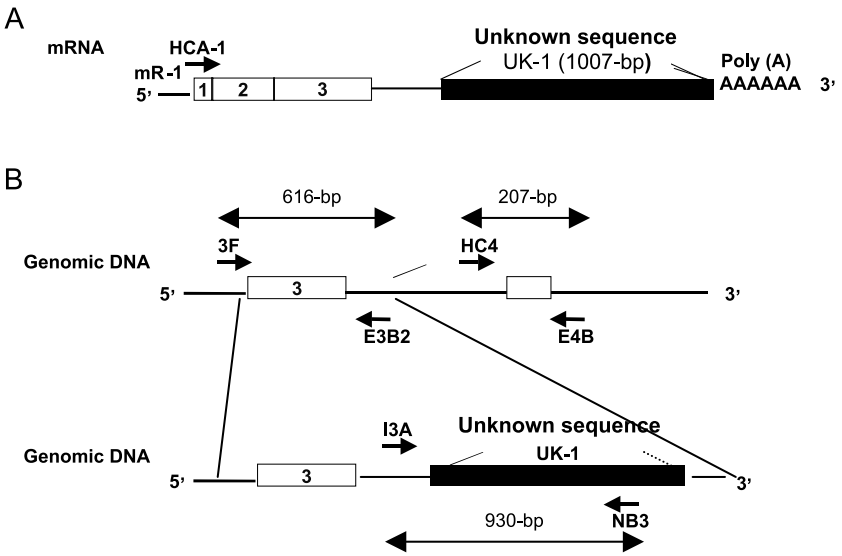

Figure 1. Structure of *HPRT1* mutation with unknown sequence in HRT-25. A. Use of the 3′ RACE-PCR method on mRNA from HRT-25 reveals a 1007-bp fragment of unknown sequence starting from the 3′ end of exon 3 in the region downstream from exon3. B. Amplification of genomic DNA from HRT-25 by using primer pair I3A/NB3 shows the same unknown sequence found in the mRNA also in the genomic DNA.

activities. The *HPRT1* cDNA analysis was performed as previously described,[3] and one additional antisense primer, MCR5 (5′-CAGAGGGCTACAATGTGATGGC-3′), was designed to anneal to exon 3. For the 3′-RACE PCR, we have used the following: polyA primer: [5′-AACTGGAAGAATTCGCGGCCGCAGGAA-T(18)-3′], an anchor primer: (5′-CTG GTT CGG CCC AGA CTC GAG TCG ACA TCG-3′), and the HCA1 primer: (5′-GAATTCCTCCTCCTGAGCAGTCAG-3′). The amplified products were sequenced by the use of a Big Dye Terminator Cycle Sequencing kit on an ABI PRISM 3700 DNA sequencer (PE Biosystems). Multiplex PCR of genomic DNA was done according to method by Gibbs et al.,[4] with the modification by Yamada et al.[5] To confirm alteration of exon 3 and 4, we performed exon-specific PCRs by using other primers, E3B2 (5′-TCATACCAAACGTCTCTAAGTTTC-3′), HCA4 (5′-TGACCAGTCAACAGGGA-CAT-3′) and E4B (5′AATTATGAAACATGAGGGCAAAGG-3′). Three primers, I3A (5′-TCGGGAAACCTGCGTTTC-3′), NB1 (5′-AGACAGAGTCTCACTCTGTCAC-3′) and NB3 (5′-TCAGTTAAGGCAGGAACAGGCCA-3′) designed for the amplification of specific translocation breakpoints are given in Fig. 1. Analysis of the amplified genomic DNA products was performed by the same method as used for cDNA analysis.

RESULTS AND DISCUSSION

Since it was indicated that the HRT-25's cDNA lacked the region downstream from exon 4, HRT-25's cDNA was not amplified by the use of MCF1/MCR1 primer pair for the *HPRT1* coding region, but rather it was amplified with MCF1/MCR5 one for the part of the *HPRT1*. It was indicating that the HRT-25's cDNA lacked the region downstream from exon 4. 3′ RACE-PCR for delineation of the 3′ end of HRT-25 *HPRT1* cDNA, revealed a part of intron 3 sequence and an unknown sequence (UK-1) of 1007-bp in the region downstream from exon 3 (Fig. 1A). In order to localize the unknown sequence which was detected in cDNA of HRT-25, I3A in intron3 as sense primer, NB1 and NB3 in unknown sequence as antisense primer (Fig. 1B) were used to screen the intron3 region in genomic DNA of HRT-25. Amplification of genomic DNA from HRT-25 with primer pair I3A/NB3 showed the same unknown sequence as mRNA also in genomic DNA (Fig. 1B). We have searched the *HPRT1* mutation in his mother's cell as same as HRT′25 cells. The HPRT activity in the mother had the normal activities and she was not found to have any mutations in her cDNA and genomic DNA in the *HPRT1*. It was suggested that this case is de novo mutation. Unknown sequence compared through BLAST analysis of human genome (NCBI; http://www.ncbi.nlm.nih.gov/BLAST/) showed that at least 0.5 to 0.6-Mb telomeric to *HPRT1* on chromosome Xq where located near LOC340581. The patient was studied for specific repeat sequence in the region near the breakpoint, but none was detected. Further investigation is required to define if this translocation mutations within the X-chromosome are responsible for the diseases.

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