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LETTER TO THE EDITOR

# Application of long-range polymerase chain reaction in the diagnosis of X-linked dystonia–parkinsonism

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Sir,

X-linked dystonia–parkinsonism (XDP, DYT3, also referred to as "Lubag") is a neurodegenerative disorder characterized by a unique combination of parkinsonism and dystonia [1]. The insertion of short interspersed nuclear element, variable number of tandem repeats, and Alu composite (SVA) retrotransposon has been identified in intron 32 of the TATA-binding protein-associated factor 1 gene (TAF1), which is mapped within the haploblock associated with XDP [2]. Several disease-specific single-nucleotide changes (DSCs) and 48-bp deletion polymorphism have also been mapped within the haploblock [3]. The DSC3, located at the TAF1/DYT3 multiple transcript system, has been investigated and shown to be associated with XDP [4]. TAF1 is a component of the transcription initiation factor TFIID which plays a central role in mediating promoter responses to various activators and repressors [5]. DSC3 containing transcripts as well as alterations of TAF1 splice variants would affect the transcription of several genes, eventually leading to neurodegeneration [2, 4]. Genetic testing for XDP has been performed using Southern analysis for SVA retrotransposon or direct polymerase chain reaction

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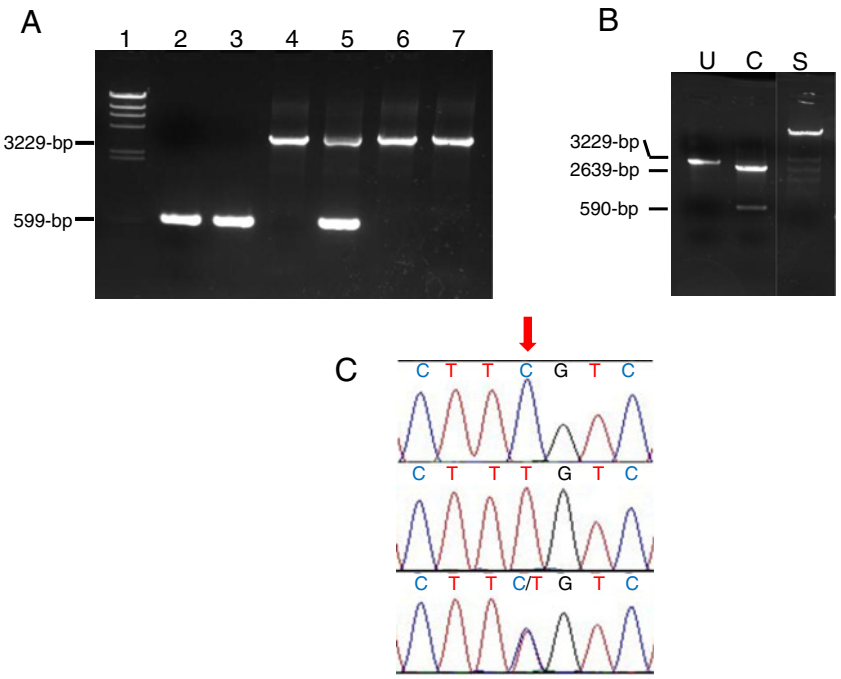


**Fig. 1** **a** Long-range polymerase chain reaction (LRP) of the SVA retrotransposon in X-linked dystonia–parkinsonism. *Lane 1*: Lambda DNA/*Hind*III marker; *Lanes 2 and 3*: normal individuals with wild allele; *Lanes 4*, *6*, *and 7*: affected individuals with SVA retrotransposon in TAF1 gene; *Lane 5*: female carrier with both wild allele and SVA retrotransposon. The length of SVA retrotransposon and normal allele is 3,229 and 599 bp, respectively. **b** LRP-RFLP analysis using the *Bam*HI. Digestion of LRP products obtained from an XDP patient shows two bands, 2,639 and 590 bp, as predicted. *C*, cut; *U*, uncut, *S*, Lambda DNA/*Hind*III marker. **c** Sequence analyses of the DSC3. Sequence fluorescent chromatograms show hemizygosity for a wild allele C in a healthy man (*upper panel*), hemizygosity for a changed allele T in an affected man (*middle panel*), and a heterozygous C/T in an obligate female carrier (*lower panel*)

(PCR) sequencing of several DSCs [2, 3]. Since 1991, 505 XDP cases have been registered with the Philippine XDP project based at the Philippine Children's Medical Center in Metro Manila and in Roxas City, Capiz [1]. Improving diagnostic methods for the detection of nucleotide changes would contribute to enhance national surveillance of XDP in the Philippines and genetic counseling to XDP families. Therefore, conventional Southern analysis or direct PCR sequencing might be replaced by an alternative method using long-range polymerase chain reaction (LRP). This article describes the LRP protocol used to accurately detect the SVA retrotransposon.

Forty-two DNA samples were used from 14 unrelated normal controls, 20 XDP patients, and 8 obligate female carriers from 16 families residing in Panay, in that had been previously diagnosed with XDP by Southern analysis [2]. In addition, three DNA samples extracted from paraffin-embedded affected brain tissue were also used. LRP was performed as described in the supplementary conditions. CR-direct sequencing of DSC3 was also performed as described elsewhere [3]. As shown in Fig. 1, DNA fragments with or without SVA retrotransposon were amplified (Fig. 1a). Restriction fragment length polymorphism analysis using the restriction enzyme *Bam*HI showed the predicted size of DNA fragments in the samples from XDP patients (Fig. 1b). The genotypes obtained by means of LRP were completely consistent with those previously determined by Southern analysis. Moreover, SVA retrotransposon is shown to be in complete linkage disequilibrium with allele T at the DSC3 (Fig. 1c). No false-positive results were obtained in controls. No amplification of the SVA retrotransposon was shown in DNA samples extracted from paraffin-embedded brain tissue, probably due to genomic DNA fragmentation. However, the direct sequencing of PCR products demonstrated the presence of the disease-associated allele T at the DSC3 in the pathologically confirmed XDP samples (data available upon request). These results prove that equivalent results can be obtained from the two methods. In conclusion, considering the multistep purification procedures in PCR-direct sequencing and laborious technique in Southern analysis, LRP-based genetic testing might be preferred for the initial surveillance on XDP.

PCR-direct sequencing of the DSCs will further confirm the results.

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**Ethical standards** The study was performed according to a protocol reviewed and approved by the Ethics Committee of the Graduate School of Medicine, University of Tokushima.

**Conflict of interest** The authors declare that they have no conflict of interest.

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