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Splicing Aberration in Naevoid Basal Cell Carcinoma Syndrome

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Pre-RNA splicing is a transcriptional process whereby introns are removed from a primary transcript and exons are joined to generate a mature protein-coding mRNA transcript. The reaction is initiated by transcripts binding to spliceosomal protein complexes that recognize splice site signals at exon/intron boundaries (1). The flexibility of splice sites in individual genes facilitates complex gene regulation and protein diversity by alternative splicing (2). Alterations in splice site selection, can be affected by gene mutations and may cause splicing modifications. A new "cryptic" splice site can replace the original because of a change in spliceosome affinity (3) and has pathogenic consequences if there is an amino acid change or a protein truncation.

Naevoid basal cell carcinoma syndrome (NBCCS) is caused by germline mutations in the *patched protein homolog 1* (*PTCH1*) (4, 5). NBCCS is characterized by a predisposition to neoplasms and a range of developmental anomalies (6), including multiple basal cell carcinomas (BCCs), odontogenic keratocysts, palmoplantar pits and bifid ribs. *PTCH1* is located on chromosome 9q22.3 (7), where it encodes a receptor protein for the hedgehog signalling pathway (8) that plays an important role in developmental processes such as cell polarity and pattern formation (9).

This study tested the benefits of additional mRNA analysis in a NBCCS patient in whom a mutation was identified by genomic sequencing as splicing aberration.

CASE REPORT

A 58-year-old Japanese man with a history of congenital hydrocephaly and previous extirpation of a jaw cyst during his teenage years noticed black nodules on his face and thighs, which were excised after they were diagnosed as BCCs. He had no family history of NBCCS, but his face presented the characteristic manifestation of hypertelorism, and multiple pits were observed on his palms and soles. Pantomography identified multiple odontogenic keratocysts in his mandible, while a skull MRI indicated communicating hydrocephalus. The patient was diagnosed with NBCCS after satisfying 3 major diagnostic criteria (10), namely, the presence of multiple BCCs, palmoplantar pits and odontogenic keratocysts. For mutational analysis see http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1332 with details including references to (11) and (12).

Prior to commencing this study, written informed consent was obtained from the patient and permission was obtained from the ethical committee of Tsukuba University Hospital.

A mutation analysis of *PTCH1* was made to investigate the cause of the patient's symptoms. We extracted genomic DNA from the patient's peripheral blood and performed genomic sequencing, which revealed a mutation site where the 584th guanine (G) was replaced by adenine (A) (c.584G>A) on exon 3 (as per GenBank entry NM_000264) (Fig. 1A). This mutation was identified in a previous study (11, 13), but this point mutation suggested a missense mutation whereby the 195th amino acid arginine was replaced by lysine (p.R195K). However, the mutation could affect splicing because the mutation was located at the 3′ end of exon 3. Therefore, we established an immortalized cell line from the patient's lymphoblastoid cells to obtain mRNA, which was then subjected to RT-PCR. Electrophoresis of the product revealed an additional large band (Fig. 1C). Sequencing of the additional product indicated the activation of a cryptic 5′ splice site in intron 3 and the insertion of a 37-bp intronic sequence, which included a termination signal, resulting in premature termination of translation (Fig. 1E). This demonstrated the presence of a splicing aberration and suggested the formation of a truncated PTCH1 protein as a result of the c.584G>A mutation. Thus, we concluded that this NBCCS case was attributable to a splicing aberration.

DISCUSSION

The *PTCH1* mutation identified in the present case was located at an exon/intron boundary, where it could disrupt the splicing signal, so it was reasonable to predict a splicing aberration. However, aberrant splicing can also be caused by silent mutations in which single base substitutions do not change specific amino acids. Indeed, a mutation in *LAMB3* of a junctional epidermolysis bullosa patient initially appeared to be a silent mutation, but it actually resulted in the aberrant splicing of a cryptic splice site (14). A comparative study of mRNA and genomic DNA in neurofibromatosis patients showed that 50% of the patients had mutations resulting in splicing aberration, which was confirmed by mRNA analysis, although 37% of the patients had splice site mutations that were identified without cDNA sequencing (15). Therefore, these previous studies and the present case clearly illustrate the significance of additional cDNA sequencing after the identification of a genomic mutation. An aggregate analysis of NBCCS patients with genomic mutations revealed that two out of 28 patients (7.1%) had splice site mutations (16). However, our study indicates that the actual incidence of splicing aberrations among NBCCS patients might be higher.

In addition, we extracted a sample from the patient's peripheral blood, because peripheral blood monocytes express the PTCH1 protein. Moreover, analysis of *PTCH1* mRNA and genomic DNA can be performed using samples obtained from patients' peripheral blood. Although splicing patterns may differ depending on the tissues examined, our method was less invasive and more appropriate for multiple subjects with NBCCS compared with a procedure that requires disease tissue.

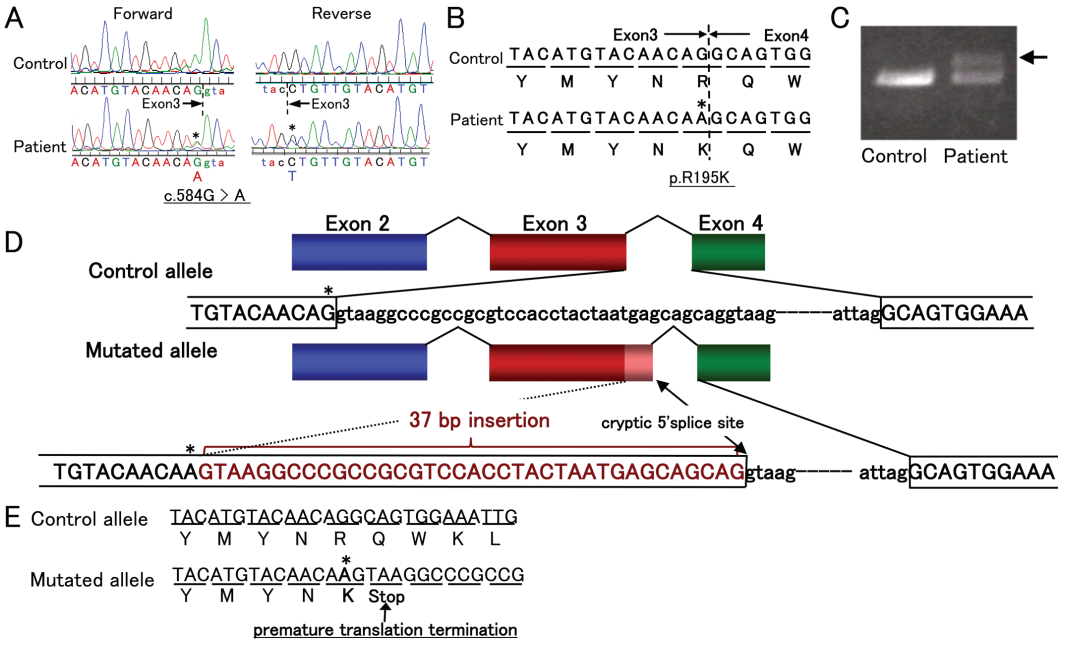

Fig. 1. The mutation identified in the patient and its effect on the splicing event. In parts (A) and (D), the exonic sequence is denoted by uppercase letters, while the intronic sequence is denoted by lowercase letters. A sample from a healthy donor was used as a control. (A) Genomic sequence of *PTCH1*. The 584th guanine (G) was replaced by adenine (A) in the patient's DNA. Note the double peaks at the last base in exon 3 (*green and red*). (B) The patient's amino acid sequence predicted from the genomic mutation c.584G>A. The 195th amino acid, arginine (R), would be substituted with lysine (K). (C) Electrophoresis of cDNA obtained from the mRNA, which was extracted from the patient's Epstein-Barr virus (EBV)-immortalized lymphoblastoid cells. Note the additional large band in the patient's lane (*arrow*). (D) Schematic representation of the splicing aberration found in the patient. A 37-bp sequence (*red letters*) was inserted following a mutation located in the 3′ end of exon 3 (*asterisks*). Because of the base substitution at the 3′ end of exon 3 (G → A), a cryptic 5′ splice site was produced in intron 3 (*double-headed arrow*), rather than the original site at the intron/exon border. This resulted in a splicing aberration on activation. (E) Results of cDNA sequencing. In addition to the amino acid alteration, a frameshift mutation due to the splicing aberration caused a termination signal (TAA) that resulted in the truncation of the PTCH1 protein.

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