

SHORT COMMUNICATION

A novel break point of the *BMPR2* gene exonic deletion in a patient with pulmonary arterial hypertension

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The presence of genetic rearrangements of bone morphogenetic protein type 2 receptor (*BMPR2*) was identified in pulmonary arterial hypertension (PAH) patients as the deletion or duplication of one or more exons of the gene. We recently investigated the deletion break points in exonic deletions of *BMPR2* in two Japanese familial cases with PAH, and found that these were *Alu*-mediated via either non-allelic homologous recombination or non-homologous recombination. We herein report the third case of exonic deletion, which was in a 25-year-old female PAH patient with a deletion of *BMPR2* exon 3. The break point in this case was not located in an *Alu* sequence. The 5'- and 3'-break point maps between the inverted *Alu* sequences in intron 2 and in exon 3, respectively, resulted in a 759-bp deletion. This novel exonic deletion in this PAH case may be a unique and non-recurrent rearrangement, and appears to be of a different size from that in other patients.

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INTRODUCTION

Pulmonary arterial hypertension (PAH) is a frequently lethal disease with a poor prognosis. The linkage analyses and positional cloning have identified the bone morphogenetic protein type 2 receptor (*BMPR2*) as a causative gene, and it has been reported that *BMPR2* mutations account for ~60% of familial PAH cases and 10–40% of patients with idiopathic PAH.^{1–3} Recent studies have clarified the presence of genome-wide structural variations (SVs) caused by genomic rearrangements such as deletion, duplication, inversion and translocation of genetic codes spanning more than 1000 base pairs.⁴ The presence of genetic rearrangements of *BMPR2* was identified in familial PAH patients as the deletion or duplication of one or more exons of the gene.⁵ We recently undertook a thorough genetic analysis of *BMPR2* in Japanese patients with PAH and investigated the deletion break points in exonic deletions of *BMPR2* in two familial cases, and found that these were *Alu*-mediated by either non-allelic homologous recombination or non-homologous recombination.⁶ We herein describe the third case of exonic deletion, in which the *Alu* sequence does not appear to be involved in the deletion of an exon.

MATERIALS AND METHODS

Case

The case was a 25-year-old female PAH patient. No PAH history was reported in her family. She had dyspnea first at 12 years of age and dyspnea worsened

gradually. She was diagnosed to have PAH at 18 years of age and estimated pulmonary arterial pressure was 60 mm Hg by echocardiogram and New York Heart Association functional class was II at diagnosis. After diagnosis, medical therapies such as bosentan, sildenafil and beraprost were administered. After 6 years of medical therapy (the timing of genetic analysis), mean pulmonary arterial pressure was 38 mm Hg and pulmonary vascular resistance 8.1 Wood units by right-sided heart catheterization, B-type natriuretic peptide was 14.7 pg ml⁻¹ and New York Heart Association functional class was II. Figure 1 shows the chest X-ray and electrocardiogram. The genetic analysis of this patient was approved by the ethics committee of Kyorin University and written informed consent was obtained.

Genetic analysis

Genetic analyses were carried out as reported previously.⁷ In brief, DNA was isolated from the patient's blood, and the protein coding sequence of *BMPR2* was amplified by PCR. The amplified fragments were then sequenced directly using the Big Dye Terminator method. A *BMPR2* exonic deletion was detected by multiplex ligation-dependent probe amplification (MLPA) using a Salsa MLPA kit for HHT/PPH1 (Cat. No. P093; FALCO Biosystems/MRC-Holland). The break points of the exonic deletion were examined using a combination of semi-quantitative PCR, long PCR and direct sequencing.

RESULTS

Although there was no point mutation identified in the *BMPR2* gene of this patient, the MLPA analysis revealed an exon 3 deletion

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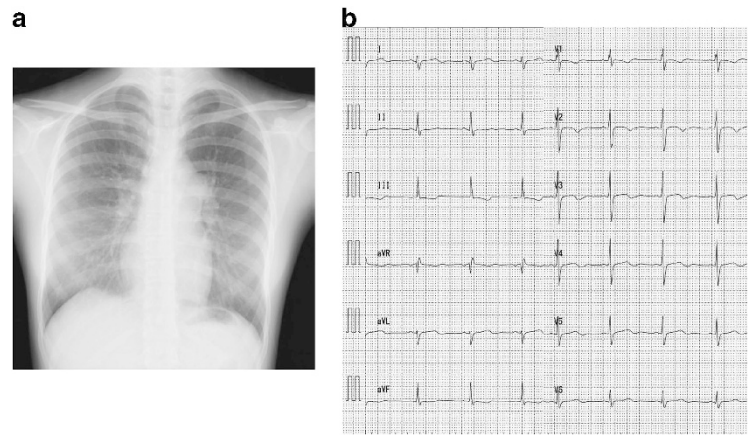


Figure 1 Chest X-ray (a) and electrocardiogram (b) of the patient.

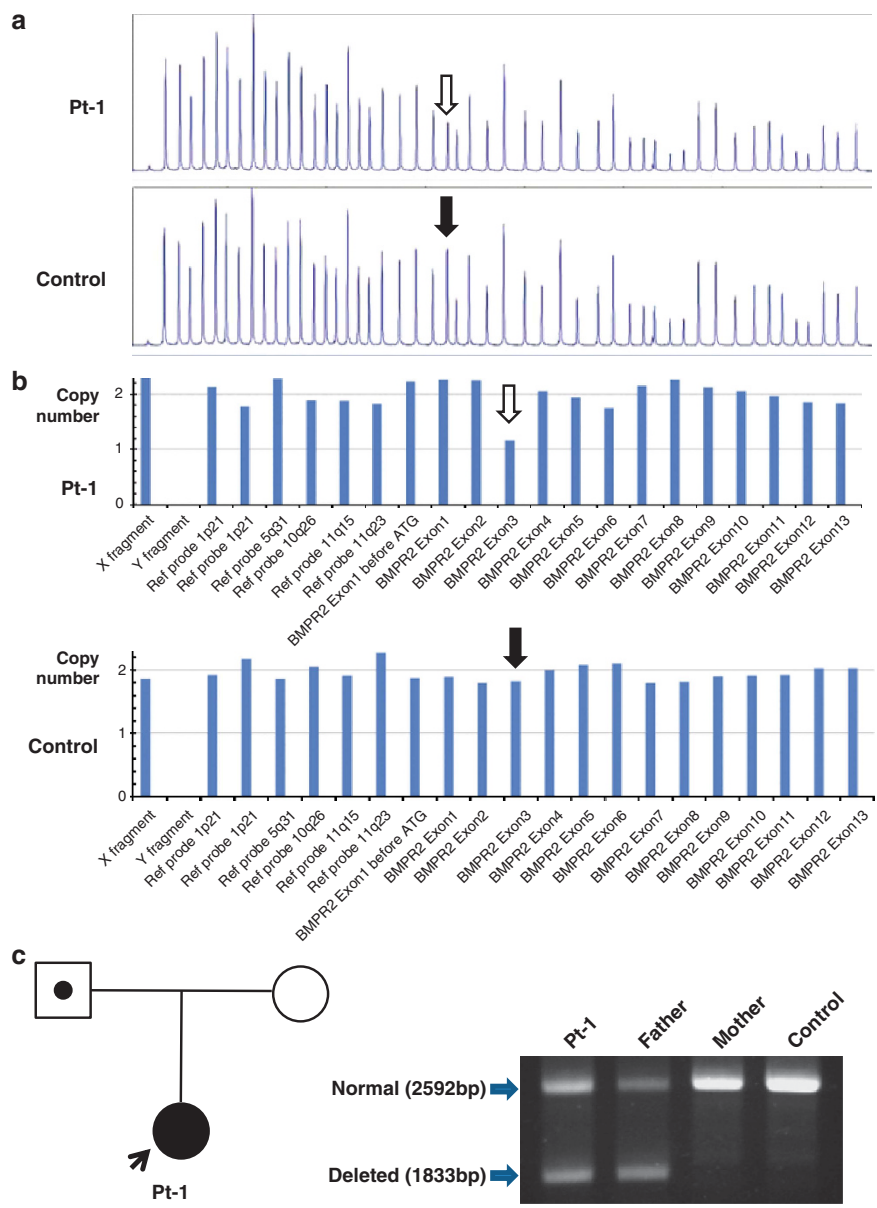


Figure 2 Deletion analysis. (a) The raw data from the MLPA analysis. (b) The processed MLPA data. Open arrows indicate the exonic deletion. Closed arrows indicate the normal diploid exon. (c) Long PCR analysis of family members.

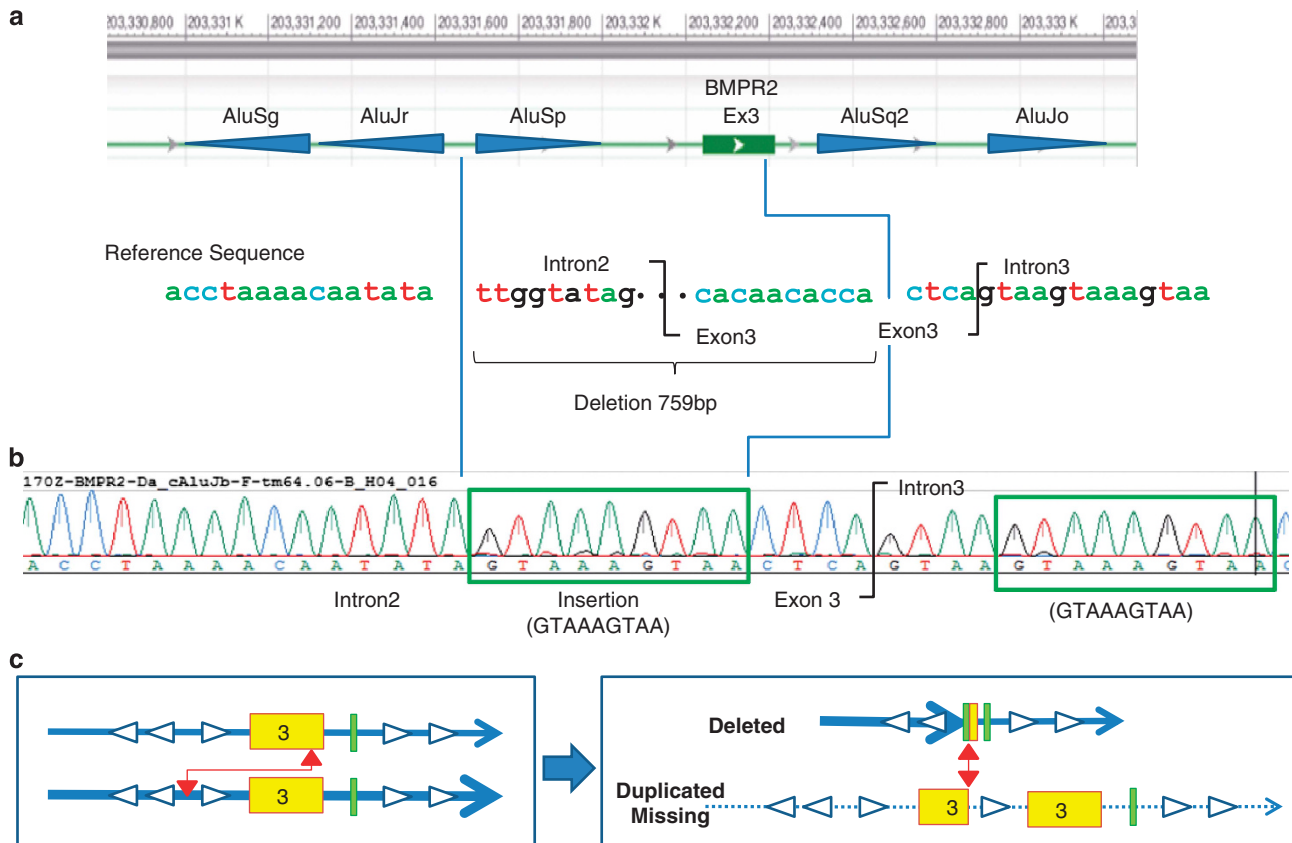


Figure 3 Deletion break point analysis. (a) A genomic map of break points. Triangles and boxes with number indicate *Alu* sequences and exons. (b) The sequence profile at the break point. (c) A schematic drawing of the recombination and rejoining.

(Figures 2a and b). The break points had to be located in intron 2 (~2.5 kb) and intron 3 (46 kb) of the *BMPR2* gene. In order to narrow down the break points in intron 3, several semi-quantitative PCRs were carried out. These analyses indicated that the 3'-break point was within a few kb downstream of exon 3. After several long PCR trials using 5'-side primers in intron 2 and 3'-side primers in intron 3, we eventually obtained a 2.6-kb normal fragment and a 1.8-kb deleted fragment (Figure 2c). The long PCR analysis also demonstrated that the patient's father, who has no symptoms of PAH, has the same deletion (Figure 2c).

These fragments were separately eluted from a gel, subjected to nested PCR and a sequence analysis. The 5'- and 3'-break point maps between the inverted *Alu* sequences in intron 2 and in exon 3, respectively, resulted in a 759-bp deletion (Figure 3a). However, a unique 9-bp sequence was inserted between the break points. The same 9 bp sequence was also located in 3'-adjacent side of exon 3. It does not appear that the rearrangement involved the *Alu* sequence, although the 5'-break point was found between the inverted *Alu* sequences (Figure 3b). Neither a variable number of tandem repeats nor transposable element insertions were involved in this rearrangement.

DISCUSSION

We previously conducted a systematic analysis for larger gene rearrangements, together with a conventional mutation analysis in 152 PAH patients, including 43 patients diagnosed to have idiopathic PAH and 10 with familial PAH, and the analysis of the *BMPR2* gene revealed four kinds of nonsense and frame-shift mutations, one

missense mutation, one splice-site mutation and two types of exonic deletions.⁶ The penetrance of heritable PAH was calculated as 22 (2/9) and 44% (4/9) for male and female subjects, respectively, from our data. Our sample numbers are small, but the estimated penetrance is consistent with the data observed in the previous report, that is, ~20%.⁸ Our previous study was the first to identify the exact break points related to PAH exonic deletions. In one case, we demonstrated an *AluY*-mediated non-allelic homologous recombination and a large deletion (>200 kb) involving *BMPR2* exons and a neighboring gene. The second case with a medium-sized deletion (5 kb) was not due to non-allelic homologous recombination, but non-homologous recombination was taking place between the *Alu*-rich regions. Here, this report revealed another patient with a deletion of *BMPR2* exon 3, the size of which was <1 kb.

Machado *et al.*⁹ and Pfarr *et al.*¹⁰ reported two and one PAH patients with the exon 3 deletions, respectively, but they did not identify the break points. Despite the presence of several highly homologous repetitive *Alu* sequences located adjacent to exon 3, the break point in this report was not located in such sequences. Therefore, this case may have unique and non-recurrent rearrangements of different sizes from those in other patients (Figure 3c).

Further break point analyses can clarify whether the deletions are mediated by repeated sequences with recombination hotspots.¹¹ Mills *et al.*¹² observed that differences in size spectra of SVs originated from the distinct mechanism of formation. There are several mechanisms that can account for a relatively small deletion: non-homologous recombination associated with the repair of DNA double-strand

breaks (that is, non-homologous endjoining) and microhomology-mediated break-induced replication.¹³ These mechanisms can describe the etiology of non-recurrent copy number variations in human diseases.¹⁴

Over the past few years, SV has been shown to be far more common in the human genome than was previously thought. Nearly 2000 SVs have been fine-mapped at the break point level, and most have been identified from healthy donors, such as in the 1000 Genomes Project. The extensive analyses of SV break points may provide an answer to whether the deletion size is correlated to the deletion mechanism, which will be helpful not only for basic science but also for the diagnosis of familial disorders.^{15–17}

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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