ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Alu-mediated nonallelic homologous and nonhomologous recombination in the *BMPR2* gene in heritable pulmonary arterial hypertension

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Purpose: The purpose of this study was to undertake thorough genetic analysis of the bone morphogenetic protein type 2 receptor (*BMPR2*) gene in patients with pulmonary arterial hypertension.

Methods: We conducted a systematic analysis for larger gene rearrangements together with conventional mutation analysis in 152 pulmonary arterial hypertension patients including 43 patients diagnosed as having idiopathic pulmonary arterial hypertension and 10 diagnosed as having familial pulmonary arterial hypertension.

Results: Analysis of the *BMPR2* gene revealed each of the four kinds of nonsense and frameshift mutations, one missense mutation, one splice-site mutation, and two types of exonic deletion. For cases in which exons 1–3 were deleted, the 5′ and 3′ break points were located in the *Alu*Y repeat sequences in the 5′ side of the adjacent *NOP58* gene and in the *Alu*Y repeat sequences in intron 3, suggesting an *Alu*Y-mediated nonallelic homologous recombination as the mechanism responsible for the deletion. For the case in which exon 10 was deleted, nonhomologous recombination took place between the *Alu*Sx site in intron 9 and a unique sequence in intron 10.

Conclusion: Exonic deletions of *BMPR2* account for at least part of *BMPR2* mutations associated with heritable pulmonary arterial hypertension in Japan, as previously reported in other populations. One of our cases was mediated via *Alu*-mediated nonallelic homologous recombination and another was mediated via nonhomologous recombination.

Genet Med advance online publication 11 April 2013

Key Words: bone morphogenetic protein type 2 receptor; deletion break point; exonic deletion; gene mutation; pulmonary hypertension

Pulmonary arterial hypertension (PAH) is a serious disease with a poor prognosis. Possible etiological factors for PAH development include vasospasm, endothelin overactivity, intimal hyperplasia due to increased growth factor secretion, and thrombus formation in small pulmonary arteries.[1] The presence of familial disease in ~6% of PAH patients with no obvious secondary causes of PAH suggested that genetic factors play an important role in a substantial proportion of patients with PAH. Linkage analysis and positional cloning have identified mutations in the bone morphogenetic protein type 2 receptor (*BMPR2*) gene in ~60% of familial PAH (FPAH) cases and 10–40% of patients with idiopathic PAH (IPAH).[2–4] *BMPR2* mutations were also reported in FPAH patients and in 40% of the IPAH cases in a Japanese population,[5] corresponding to the clinical observation that PAH prevalence does not vary among different races.[6]

Recent studies have clarified the presence of genome-wide copy-number variations caused by genomic rearrangement such as deletion, duplication, inversion, and translocation of genetic codes spanning more than 1,000 base pairs, with genetic rearrangements of *BMPR2* in patients with FPAH resulting in deletion or duplication of one or more exons of the gene.[7,8] Another study identified exonic deletion or duplication in *BMPR2* in both FPAH and IPAH cases.[9] These findings have demonstrated that *BMPR2* mutations may account for a higher proportion of PAH patients than previously expected.

Genome-wide analysis of copy-number variations has indicated significant variation in distribution and frequency among populations with different ethnic backgrounds, although such information about the genetic rearrangements of *BMPR2* has been limited to the data obtained. Furthermore, deletion break points in exonic deletions of *BMPR2* have not been reported. We therefore undertook a thorough genetic analysis of *BMPR2* in Japanese patients with PAH and investigated the deletion break points in exonic deletions of *BMPR2*.

MATERIALS AND METHODS

Study population

The study included PAH patients and their family members in Kyorin University Hospital, Tokyo, Japan, who were enrolled

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Submitted 23 October 2012; accepted 1 March 2013; advance online publication 11 April 2013. doi:10.1038/gim.2013.41

from August 2010 to June 2012. The enrolled group comprised 43 patients with IPAH, 10 with FPAH (5 families), 20 with collagen-associated PAH, 44 with chronic thromboembolic pulmonary hypertension, 7 with Eisenmenger syndrome, 2 with pulmonary veno-occlusive disease, 2 with cor pulmonale, and 24 with other etiologies. This study was approved by the ethics committee of Kyorin University School of Medicine, and written informed consent was obtained from all patients and family members.

In this study, the definition of heritable PAH (HPAH) is as follows: HPAH includes FPAH in which at least two patients in a family have been diagnosed on clinical criteria and also includes sporadic PAH cases who carry *BMPR2* mutation.

DNA sequencing of *BMPR2* exons

DNA was isolated from patient blood samples by a previously reported method,[10] and DNA concentrations were measured using a Qubit fluorometer (Life Technologies, Carlsbad, CA). The protein-coding sequence of *BMPR2* from exon 1 to 13 was amplified by PCR using primers derived from the intron sequence (NCBI reference sequence NC_000002.11) and designed by the online Primer Blast resource (the sequences are available on request to the corresponding author). PCR was carried out using AmpliTaq Gold (Life Technologies) as follows: 1 cycle at 95 °C for 10 min; 40 cycles at 96 °C for 1 min, 56 °C for 15 s, and 72 °C for 45 s; and 1 cycle at 72 °C for 7 min. After agarose electrophoresis (3%), amplified fragments were purified by a MultiScreen Filter Plate (EMD Millipore, Billerica, MA) and then sequenced directly to detect point mutations in *BMPR2* using an Life Technologies 3130xl genetic analyzer and Big Dye Terminator Cycle Sequence Kit (ver. 3.1; Life Technologies). The protein-coding sequence of activin-like kinase receptor 1 and endoglin gene was analyzed as described above.

Detection of *BMPR2* exonic deletion and break points

BMPR2 exonic deletion was detected by multiplex ligation-dependent probe amplification using a Salsa multiplex ligation-dependent probe amplification kit (no. P093; MRC-Holland, Amsterdam, The Netherlands) and a

Table 1 Summary of *BMPR2* mutations in heritable PAH

Patient No.	Sex	Diagnosis		Exon	Mutation category	Nucleotide change	Amino acid change	Family	Reference
		Age[a]	Type[b]						
1	Female	10s	I	Exon 1	Nonsense	c.16C>T	p.Glu5X	—	This study
2	Female	40s	I	Exon 2	Nonsense	c.201insA	p.Tyr67X	—	This study
3	Female	40s	F	Exon 2	Frameshift	c.237delT	p.Leu79fsX2	Proband	This study
3-2	Female	40s		Exon 2	Frameshift	c.237delT	p.Leu79fsX2	Sib of patient 3	
3-3	Male	NS		Exon 2	Frameshift	c.237delT	p.Leu79fsX2	Father of patient 3/3-2	
4	Female	20s	F	Exon 3	Nonsense	c.255G>A	p.Trp85X	Proband	Machado et al. (Ref. 11)
4-2	Female	20s		Exon 3	Nonsense	c.255G>A	p.Trp85X	Sib of patient 4	
5	Male	30s	F	Exon 3	Nonsense	c.399insA	p.Tyr113X	Proband	This study
5-2	Female	NS		Exon 3	Nonsense	c.399insA	p.Tyr113X	Sib of patient 5	
6	Female	30s	I	Exon 4	Frameshift	c.498delT	p.Val166fsX9	Deceased	This study
7	Female	20s	I	Intron 6	Splice site	c.853-2A>G	—	—	Machado et al. (Ref. 11)
8	Female	30s	F	Exon 8	Missense	c.1016T>A	p.Val339Asp	Proband	This study
8-2	Female	10s		ND	ND	ND	ND	Daughter of patient 8	
9	Female	20s	F	Exon 12	Frameshift	c.1969insA	p.Gln657fsX17	Proband	This study
9-2	Male	20s		ND	ND	ND	ND	Sib of patient 9; deceased at 20 years	
9-3	Male	NS		Exon 12	Frameshift	c.1969insA	p.Gln657fsX17	Father of patient 9	
10	Female	30s	I	Exon 12	Frameshift	c.2128delC	p.Leu710fsX2	—	This study
11[c]	Male	20s	F	Exons 1–3	Large deletion	c.1-128k_418+7kdel	Large rearrangement	Proband	This study
11-2	Female	50s		Exons 1–3	Large deletion	c.1-128k_418+7kdel	Large rearrangement	Mother of patient 11	
12	Female	20s	I	Exon 10	Large deletion	c.1277-291_1413+4735del	Large rearrangement	—	This study and Aldred et al. (Ref. 9)

F, familial PAH; I, idiopathic PAH; ND, not determined yet; NS, no symptom; PAH, pulmonary arterial hypertension.

[a]Age at diagnosis. [b]Type of diagnosis. [c]Patient 11-3 in **Figure 1** was not included in this table because the diagnosis was not made by us.

3130xl genetic analyzer, as previously reported.[8] Multiplex ligation-dependent probe amplification data were analyzed on the basis of several control data from the manufacturer-recommended Excel-based spreadsheet and finally sorted according to exon order.

Break points in exonic deletions were examined using a combination of semiquantitative PCR, long PCR, restriction fragment analysis, and direct sequencing. Semiquantitative PCR was carried out using KOD plus polymerase (TOYOBO, Japan) under the following conditions: 1 cycle at 94 °C for 2 min and 26 cycles at 94 °C for 15 s, 56 °C for 30 s, and 68 °C for 45 s. Long PCR was carried out using PrimeSTAR GXL polymerase (TAKARA BIO, Otsu, Japan) under the following conditions: 30 cycles at 98 °C for 10 s and 66 °C for 10 min. PCR products and their restriction fragments (*Aat*II, *Bam*HI, *Hin*dIII, and *Ppu*MI) were analyzed by agarose gel electrophoresis (0.7%), followed by another cycle of PCR analysis to identify the deletion break point. The sequence reaction was carried out as described above.

RESULTS

Point mutations in *BMPR2*

Among IPAH and FPAH patients and their respective family members, each of the four kinds of nonsense and frameshift mutations, one missense mutation, one splice-site mutation, and two types of exonic deletion on *BMPR2* gene, were found. Finally, 20 individuals (12 families) including 3 individuals who do not have obvious symptoms of PAH were identified as having HPAH (Table 1). Only two kinds of point mutations were previously reported.[11] However, point mutations in *BMPR2* were not recognized in all of the cases with other etiologies, such as collagen-associated PAH, chronic thromboembolic pulmonary hypertension, Eisenmenger syndrome, and pulmonary veno-occlusive disease. No mutation was found in the activin-like kinase receptor 1 or endoglin genes (data not shown).

Exonic deletions in *BMPR2*

Figure 1 shows the multiplex ligation-dependent probe amplification analysis for exonic deletions of *BMPR2*. Two patients

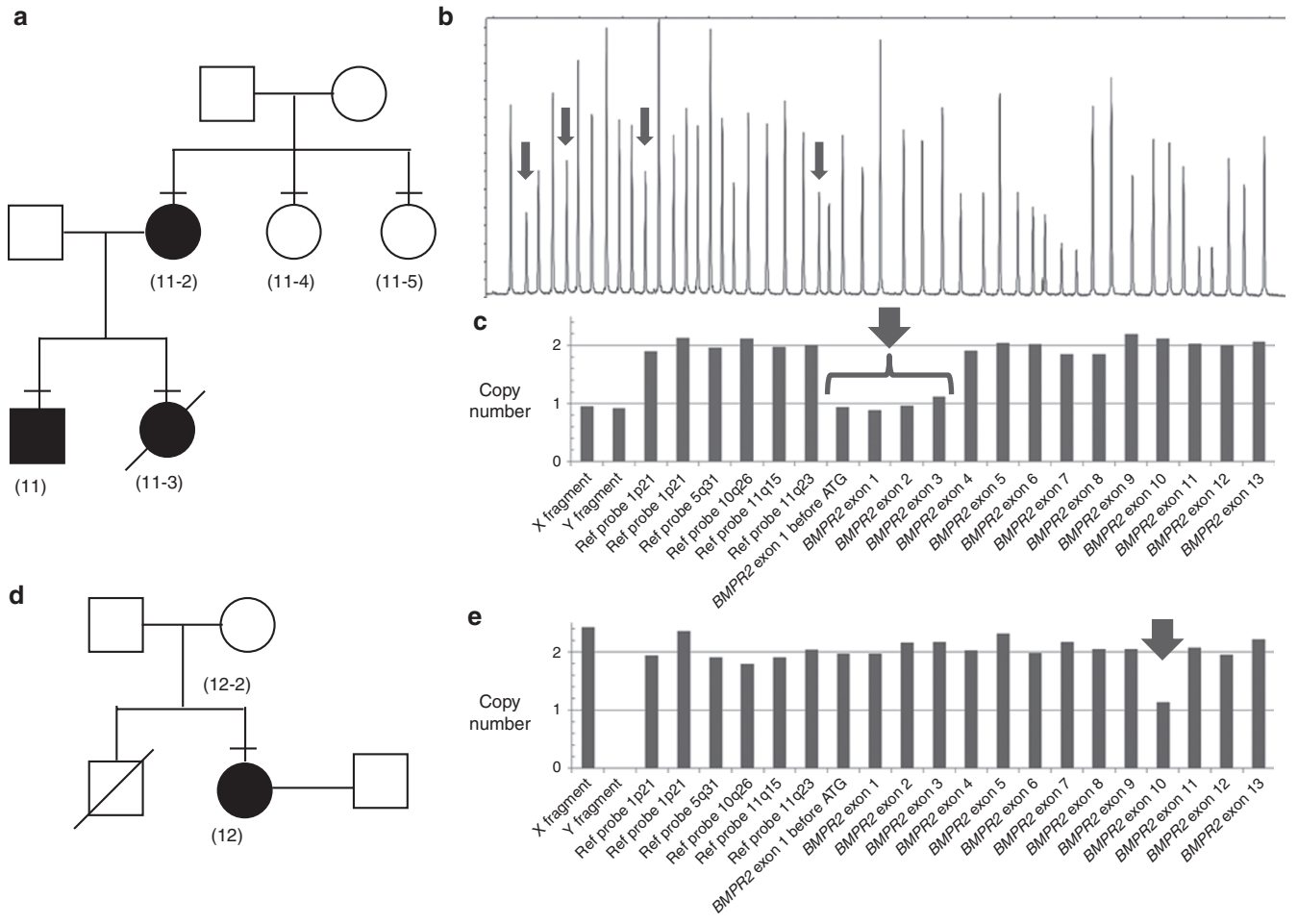

Figure 1 Multiplex ligation-dependent probe amplification (MLPA) analysis of patients 11 and 12. (**a**) Family chart of patient 11 (Nos. 11 and 11-2 in Table 1). The sister of patient 11 (No. 11-3) had been diagnosed as having pulmonary arterial hypertension, and she had already died. (**b**) Raw data of MLPA analysis on the bone morphogenetic protein type 2 receptor (*BMPR2*) gene of patient 11. Exonic deletions of exons 1–3 in *BMPR2* are shown by arrows. (**c**) The processed data of the MLPA analysis on the *BMPR2* gene of patient 11. The MLPA analysis shows an exon 1–3 deletion (arrow). (**d**) Family chart of patient 12. (**e**) The processed data of MLPA on the *BMPR2* gene of patient 12. The MLPA analysis shows the exon 10 deletion (arrow).

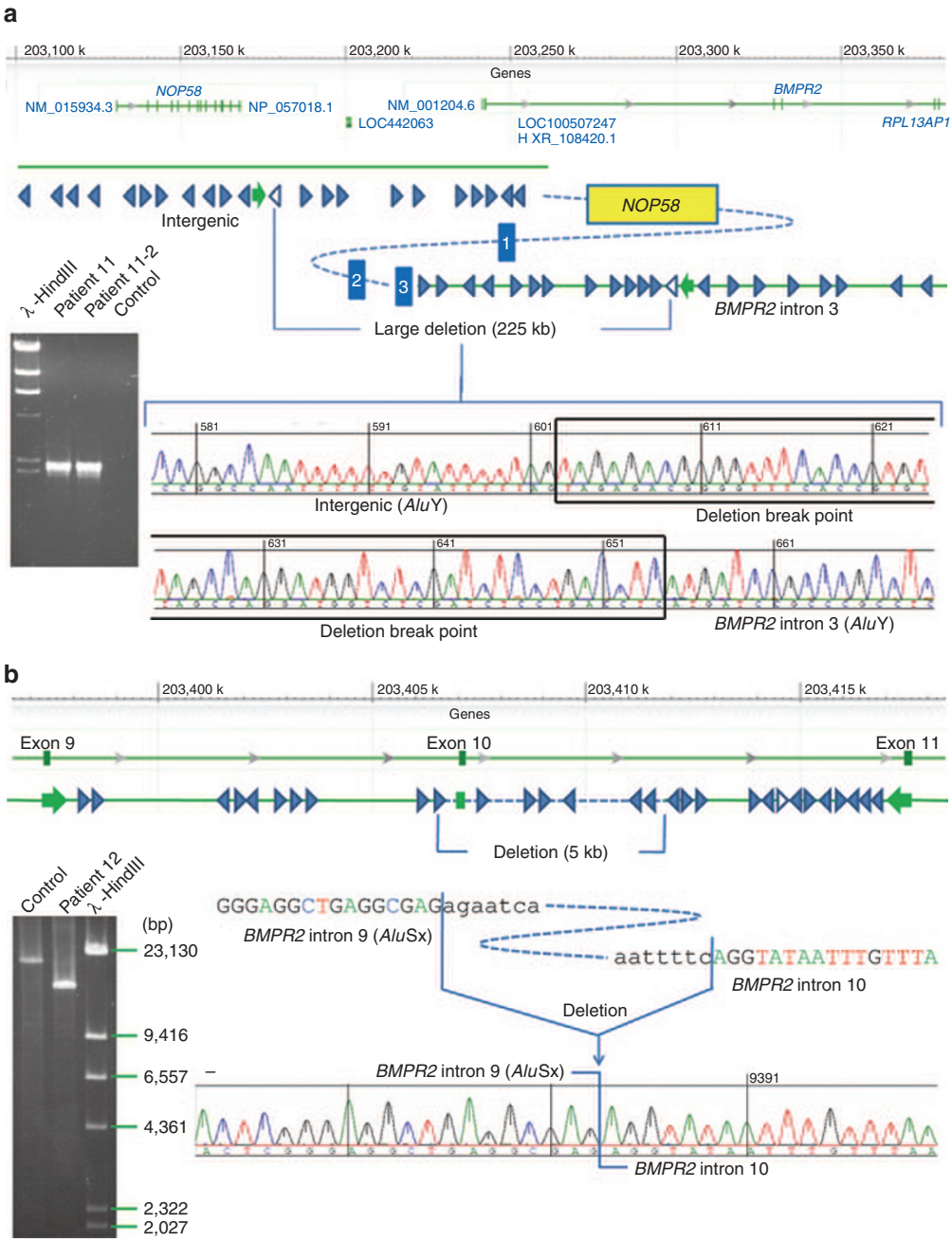

Figure 2 Deletion break point of patients 11 and 12. (**a**) Deletion break point of patient 11. Top: genomic structure of *BMPR2* gene and 5′ adjacent region. Middle: genomic map of break points. Triangles and boxes with numbers indicate *Alu* sequences and exons. Arrows indicate PCR primers to bridge the deletion. Bottom: sequence profile of break and rejoining points. Inset: gel electrophoresis of bridge clone. (**b**) Deletion break point of patient 12. Top: genomic structure of *BMPR2* gene exon 10 and neighboring region. Middle: genomic map of break points. Triangles indicate *Alu* sequences. Arrows indicate PCR primers used to bridge the deletion. Bottom: sequence profile of break and rejoining points. Inset: gel electrophoresis of bridge clone.

with FPAH (patients 11 and 11-2 in **Table 1**) showed deletion of exons 1–3, suggesting no transcript from this allele. Patient 11-3 was also diagnosed as having PAH at 17 years of age and died in another hospital. Two sibs of patient 11-2 did not have a deleted allele (data not shown).

One patient (patient 12 in **Table 1**) showed deletion of exon 10 (**Figure 1e**), and the mother of patient 12 did not have a deleted allele, suggesting that the deleted allele was transmitted from the paternal side or by *de novo* deletion. Exon 10 deletion causes loss of the C-terminal portion of the kinase domain and cytoplasmic tail from the *BMPR2* protein. Meanwhile, exonic deletions were not recognized in all of the cases with other etiologies.

Detection of deletion break points

In cases of exon 1–3 deletion (patients 11 and 11-2), semiquantitative PCR analysis revealed the lack of one copy of the 5′

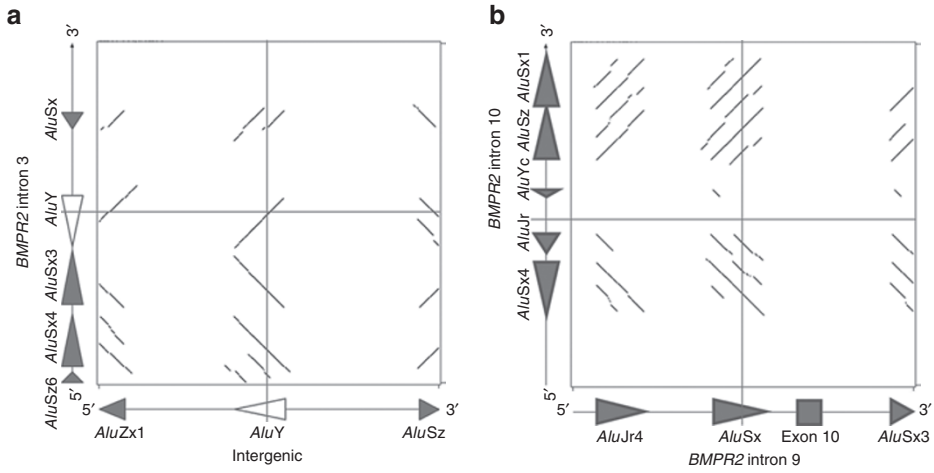

Figure 3 Dot matrix plot of break points. (**a**) Break point of patient 11. (**b**) Break point of patient 12. Triangles in *x* and *y* axes indicate *Alu* sequences in break point–neighboring regions. In cases in which DNA sequences in *x* and *y* axes are the same, dots are inserted in this plot field. Therefore, when repeated sequences such as *Alu* exist in *x* and *y* axes, lines appear because dots connect. The direction of the lines indicate direct repeat or invert repeat. Break points are located in the middle of the *x* and *y* axes. In patient 11 (**a**), the recombination point exists only on the dot line indicating direct repeat of *Alu* sequences, suggesting homologous recombination between *Alu* sequences. In patient 12 (**b**), the recombination point exists on the *Alu* sequence (*AluSx*) in intron 9, but it exists in between *Alu* sequences including some retrograded ones in intron 10, suggesting nonhomologous recombination.

adjacent *NOP58* gene but the presence of two copies of *SUMO1*, a gene located further upstream. Therefore, 5′ break points were predicted between *NOP58* and *SUMO1*. Furthermore, several forward primers in the intergenic region between *NOP58* and *SUMO1*, and several reverse primers within the 45-kb *BMPR2* intron 3 were designed, and PCRs were carried out. This strategy enabled bridging of the deletion, achieving a 2.2-kb fragment (**Figure 2a**). Sequence analysis on this fragment revealed a 225-kb deletion and the 5′ and 3′ break points, both mapping within the *AluY* repetitive sequence.

In the case of exon 10 deletion (patient 12), break points were located in intron 9 (9 kb) and intron 10 (11 kb). Long PCR between exon 9 and exon 11 obtained a 15-kb fragment, suggesting a 5-kb deletion including exon 10 (**Figure 2b**). The restriction analysis, nested PCR, and primer walking on this fragment revealed 5′–break point maps on *Alu*Sx in intron 9 and 3′–break point maps on a unique sequence within the *Alu* cluster in intron 10.

DISCUSSION

This study demonstrated that (i) *BMPR2* point mutations and exonic deletions may account for at least part of *BMPR2* mutations associated with PAH in a Japanese population and (ii) exonic deletions associated with HPAH arise through the mechanism of not only *Alu*-mediated nonallelic homologous recombination (NAHR) but also nonhomologous recombination.

One type of mutation in exon 3 recognized in HPAH sisters (patients 4 and 4-2) and one splice-site mutation (patient 7) in this study were the same as reported in Caucasian cases,[11] whereas other mutations detected in HPAH were novel. The exonic deletion of exon 10 was also reported previously,[9] although it is not clear whether their deletions are identical to those in our results, because break points were not reported in the previous study.

One patient in this study (patient 8) showed a missense mutation in exon 8 (c.1016T>A/p.Val339Asp). Her daughter (patient 8-2) had some obvious symptoms of PAH since 14 years of age and, thus, had been diagnosed as having pediatric PAH. Our preliminary investigation revealed that the frequency of c.1016T>A is <1 of 200 chromosomes in the Japanese general population (data not shown). Val339 in *BMPR2* is located in the "catalytic domain of protein kinase" and conserved across many vertebrate species. It is also conserved across the human transforming growth factor-β receptor family, although some family members such as *BMPR1A* and *BMPR1B* show replacement with the synonymous amino acid isoleucine. Such mutation of an uncharged amino acid to a negatively charged one in exon 8 is likely to impair the protein kinase activity of *BMPR2*, although we cannot rule out the possibility that an additional abnormality may account for this PAH mutation.

This study is the first to identify the exact break points of PAH exonic deletion. In one case, we demonstrated *AluY*-mediated NAHR and a large deletion (>200 kb) involving *BMPR2*-5′ exons and the 5′-neighboring gene *NOP58* (**Figure 3a**). Therefore, the *AluY*-mediated deleted/duplicated allele of *NOP58* and exons 1–3 can be expected (**Figure 4a**). It seems less possible that *NOP58*, a ribonucleoprotein homologue to yeast, is involved in the etiology of PAH because although *NOP58* is widely distributed in many animal species, there is no positive evidence of irreplaceable function. NAHR-mediated deletions, duplications, inversions, and other alterations, especially *Alu*-mediated NAHR, have been implicated not only in copy-number variations within the human genome but also in numerous human genetic disorders.[12,13] Therefore, the same type of NAHR could be responsible for other exonic deletions in PAH.

The second case of exon 10 deletion in this study, however, was not due to NAHR but instead to nonhomologous

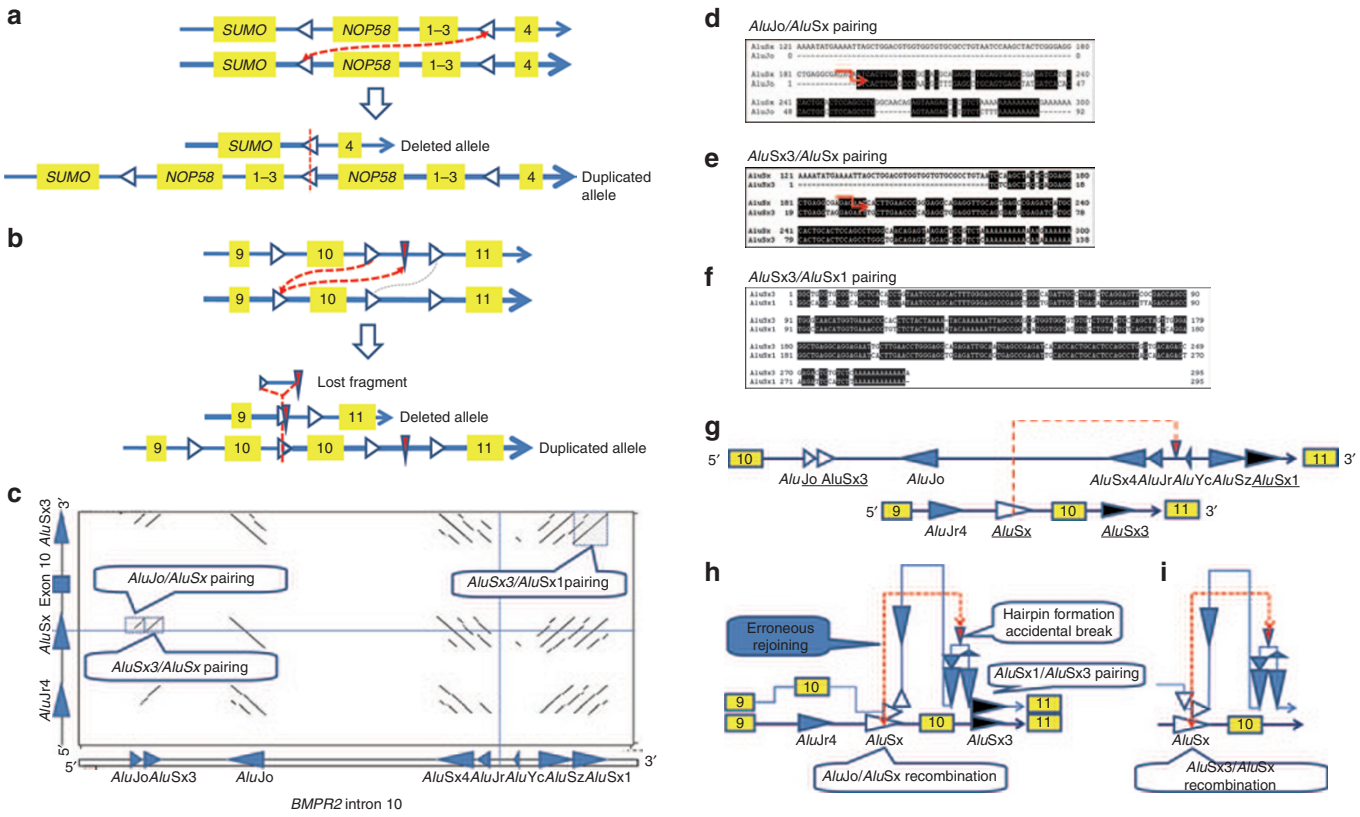

Figure 4 Potential mechanisms of *Alu*-mediated exonic deletions. (**a**) Possible *Alu*-mediated nonallelic homologous recombination in family 11. In patients 11 and 11-2, *AluY*-mediated deleted/duplicated allele of *NOP58* and exons 1–3 can be expected. (**b**) Possible *Alu*-mediated nonhomologous recombination in patient 12. Nonhomologous recombination between *Alu* sequences can induce a lost fragment and deleted/duplicated allele of exon 10. (**c**) Dot matrix plot of areas neighboring break points in patient 12. This plot shows a wider range in *x* axis than that in **Figure 3**, indicating interesting homologous regions in both 5′ and 3′ sites in intron 10. (**d,e**) Sequence alignment of two possible *Alu* pairings close to a break point line in patient 12. (**f**) Sequence alignment of possible high but nonhomologous *Alu*Sx3/*Alu*Sx1 in 3′ sites in patient 12. (**g**) *Alu* sequences in areas of exon 10 (upper) and intron 10 (lower) in patient 12. Dot plot and sequence alignment analyses of these regions allow us to raise two possible models to produce a deleted allele. (**h**) *Alu*Jo/*Alu*Sx recombination and erroneous rejoining model in patient 12. One possibility is that *Alu*Sx1/*Alu*Sx3 nonhomologous pairing (**f**) triggers hairpin loop formation and *Alu*Jo/*Alu*Sx pairing (**d**). Then, accidental breakage in the middle of the hairpin, *Alu*Jo/*Alu*Sx recombination, and erroneous rejoining can produce a deleted allele. (**i**) *Alu*Sx3/*Alu*Sx recombination and erroneous rejoining model in patient 12. Another possibility is that *Alu*Sx1/*Alu*Sx3 nonhomologous pairing (**f**) triggers hairpin loop formation and *Alu*Sx3/*Alu*Sx nonhomologous recombination (**e**).

recombination between *Alu*-rich regions (**Figure 3b**). *Alu*-mediated NAHR has been attracting attention recently and was reported in association with selected diseases.[14] *Alu* sequences are apparently involved in this recombination because the 5′-break point is in the middle of the *Alu*Sx-repetitive sequence. However, the adjacent sequence of 3′ break point in intron 10 seems to be unique, i.e., no homology to the 5′ break point or the adjacent region in intron 9 (**Figure 3b**). It is therefore possible that a two-step recombination is involved, first *Alu*–*Alu* NAHR and then possibly accidental nonhomologous recombination. Although there are many alternative models, the deleted allele is accompanied by a duplicated allele and a lost fragment. The possible ways of producing those models are simultaneous *Alu*–*Alu* pairings, hairpin loop formation and accidental break, and erroneous rejoining (**Figure 4b–i**). Our results raise the possibility that *Alu*-mediated, but not NAHR-mediated, accidental nonhomologous recombination occurs in some diseases at a certain level of frequency. Further analyses of other cases are needed to clarify the mechanism underlying this type of nonhomologous recombination.

We performed a preliminary semiquantitative reverse-transcriptase PCR to investigate the expression levels of *BMPR2* mRNA (**Supplementary Figure S1** online). As expected, the expression levels of *BMPR2* mRNA were much decreased in patients 11 and 11-2 as compared with their family members, 11-3 and 11-4, without a deletion. Of note, there was no evidence of aberrant mRNA, and the level of intact *BMPR2* mRNA was decreased in patient 12. This suggests that the transcript from the exon 10–deleted allele is depleted or instable, and it can be expected that no aberrant *BMPR2* protein is translated. Therefore, both of these exonic deletions seem to cause stoichiometric imbalance due to haploinsufficiency, indicating low probability of a dominant-negative effect on *BMPR2* function. However, these findings are based on preliminary data, and the quantification of mRNA by additional experiments such as real-time PCR is desired in the future.

Alu is a retrotransposon and the most repeated sequence in the human genome (about 10% of all the genome). The following mechanisms regarding *Alu*-mediated genetic disorders have been demonstrated: (i) deleted and duplicated allele is induced by homologous recombination between different *Alu* sequences mainly in introns;[15,16] (ii) inactivation of genes is induced by insertion of *Alu* sequences into genes, in particular, into exons;[17]and (iii) variant *Alu* sequences in introns induce exonization.[18] In particular, because *Alu* sequences share high homology with each other and their length is ~300 bases although they have grown in diversity along the course of evolution, the different *Alu* sequences are subject to homologous recombination. These findings suggest that *Alu* has an important role in genomic diversity. In this study, we demonstrated that PAH is one of the *Alu*-mediated genetic disorders and that the mechanisms could be mediated through both *Alu*-mediated NAHR and non-homologous recombination. In the near future, the further detailed studies investigating the relationship between loss of *BMPR2* function mediated by *Alu* and BMP signaling would be desirable using specimens from lung tissue of PAH patients.

In conclusion, exonic deletions of *BMPR2* found in HPAH were mediated through both *Alu*-mediated NAHR and nonhomologous recombination.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

ACKNOWLEDGMENTS

This work was supported in part by the Health and Labour Sciences Research Grant from the Ministry of Health, Labour, and Welfare of Japan; Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology; a grant from the medical department collaborative project of Kyorin University; and a fellowship from Kyorin University School of Health Sciences.

DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Rich S, Dantzker DR, Ayres SM, et al. Primary pulmonary hypertension. A national prospective study. *Ann Intern Med* 1987;107:216–223.
2. Morse JH, Jones AC, Barst RJ, Hodge SE, Wilhelmsen KC, Nygaard TG. Mapping of familial primary pulmonary hypertension locus (PPH1) to chromosome 2q31-q32. *Circulation* 1997;95:2603–2606.
3. Deng Z, Morse JH, Slager SL, et al. Familial primary pulmonary hypertension (gene PPH1) is caused by mutations in the bone morphogenetic protein receptor-II gene. *Am J Hum Genet* 2000;67:737–744.
4. Elliott CG. Genetics of pulmonary arterial hypertension: current and future implications. *Semin Respir Crit Care Med* 2005;26:365–371.
5. Morisaki H, Nakanishi N, Kyotani S, Takashima A, Tomoike H, Morisaki T. BMPR2 mutations found in Japanese patients with familial and sporadic primary pulmonary hypertension. *Hum Mutat* 2004;23:632.
6. Rich S. *Heart Disease*. WB Saunders Company: Philadelphia, 2001:1908–1935.
7. Cogan JD, Vnencak-Jones CL, Phillips JA 3rd, et al. Gross BMPR2 gene rearrangements constitute a new cause for primary pulmonary hypertension. *Genet Med* 2005;7:169–174.
8. Cogan JD, Pauciulo MW, Batchman AP, et al. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. *Am J Respir Crit Care Med* 2006;174:590–598.
9. Aldred MA, Vijayakrishnan J, James V, et al. BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. *Hum Mutat* 2006;27:212–213.
10. Thomson JR, Machado RD, Pauciulo MW, et al. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family. *J Med Genet* 2000;37:741–745.
11. Machado RD, Aldred MA, James V, et al. Mutations of the TGF-beta type II receptor BMPR2 in pulmonary arterial hypertension. *Hum Mutat* 2006;27:121–132.
12. Sasaki M, Lange J, Keeney S. Genome destabilization by homologous recombination in the germ line. *Nat Rev Mol Cell Biol* 2010;11:182–195.
13. Konkel MK, Batzer MA. A mobile threat to genome stability: the impact of non-LTR retrotransposons upon the human genome. *Semin Cancer Biol* 2010;20:211–221.
14. Zhang F, Seeman P, Liu P, et al. Mechanisms for nonrecurrent genomic rearrangements associated with CMT1A or HNPP: rare CNVs as a cause for missing heritability. *Am J Hum Genet* 2010;86:892–903.
15. Zhang G, Fukao T, Sakurai S, Yamada K, Michael Gibson K, Kondo N. Identification of Alu-mediated, large deletion-spanning exons 2-4 in a patient with mitochondrial acetoacetyl-CoA thiolase deficiency. *Mol Genet Metab* 2006;89:222–226.
16. Fukao T, Zhang G, Rolland MO, et al. Identification of an Alu-mediated tandem duplication of exons 8 and 9 in a patient with mitochondrial acetoacetyl-CoA thiolase (T2) deficiency. *Mol Genet Metab* 2007;92:375–378.
17. Apoil PA, Kuhlein E, Robert A, Rubie H, Blancher A. HIGM syndrome caused by insertion of an AluYb8 element in exon 1 of the CD40LG gene. *Immunogenetics* 2007;59:17–23.
18. Purevsuren J, Fukao T, Hasegawa Y, Fukuda S, Kobayashi H, Yamaguchi S. Study of deep intronic sequence exonization in a Japanese neonate with a mitochondrial trifunctional protein deficiency. *Mol Genet Metab* 2008;95:46–51.