

## SHORT COMMUNICATION

# Identical deletion at 14q13.3 including *PAX9* and *NKX2-1* in siblings from mosaicism of unaffected parent

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**By screening patients with undiagnosed multiple congenital anomalies and intellectual disability using array-comparative genomic hybridization, we identified an 884 kb heterozygous microdeletion at 14q13.3 in two siblings presenting with oligodontia, hypothyroidism and persistent pulmonary hypertension of the newborn, resulting from their parental gonosomal mosaicism. Among the six genes included in the deletion, haploinsufficiency of *PAX9* and *NKX2-1* was probably associated with their phenotypes. These results highlighted a possibility of recurrence of pathogenic copy-number variants associated with parental mosaicism, which requires careful genetic counseling.**

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## INTRODUCTION

In the last decade, several types of genomic microarrays have been applied for screening of undiagnosed congenital disorders,<sup>1–3</sup> allowing the identification of a number of pathogenic DNA copy-number variants (CNVs).<sup>4,5</sup> We have also screened patients with undiagnosed multiple congenital anomalies and intellectual disability using array-comparative genomic hybridization (CGH).<sup>6,7</sup> Among the screening, here we described two siblings showing resembling phenotypes. They had an identical microdeletion at 14q13.3 resulting from their paternal gonosomal mosaicism, and the deletion included *PAX9* and *NKX2-1*, which could adequately explain their phenotypes.

## MATERIALS AND METHODS

### Patients

Although the proband (II-2 in Figure 1a) was born at 40 weeks by normal delivery with Apgar score 9 at 1 min, she showed persistent pulmonary hypertension of the newborn (PPHN) and was treated with a ventilator for 23 days (Table 1). During the neonatal period, she was diagnosed with hypothyroidism and was treated with thyroid hormone. Her development was mildly retarded. She showed an apparent oligodontia and choreoathetosis (Supplementary Figure 1). Interestingly, her elder sister showed resembling phenotypes (II-1 in Figure 1a, Table 1). She was also treated with a ventilator for 9 days because of PPHN and presented hypothyroidism, oligodontia and choreoathetosis (Figure 1b, Supplementary Figure 1). Their parents were healthy including normal dental findings.

### Molecular genetic analysis

Regarding microarray analysis, first we analyzed the proband (II-2) using an in-house bacterial artificial chromosome (BAC) array,<sup>7</sup> and subsequently we

applied an oligonucleotide array (Roche-Nimblegen, Madison, WI, USA, Human CGH Array 2.1 Mb) to evaluate the precise size of the heterozygous deletion. We also performed SNP array (Illumina, San Diego, CA, USA, HumanOmniExpress) to estimate the mosaic states for the proband and her father (I-2). Each DNA was extracted from peripheral blood by the standard method. Fluorescence *in situ* hybridization (FISH) was performed as described elsewhere for chromosomes extracted from the peripheral blood.<sup>8</sup> Genomic PCR was performed using a primer combination distal and proximal to the deletion (5'-GTGACATTGGGCAAGACAGC-3' and 5'-TGTAGTTGGAGTC CCTTTTGAG-3', respectively).

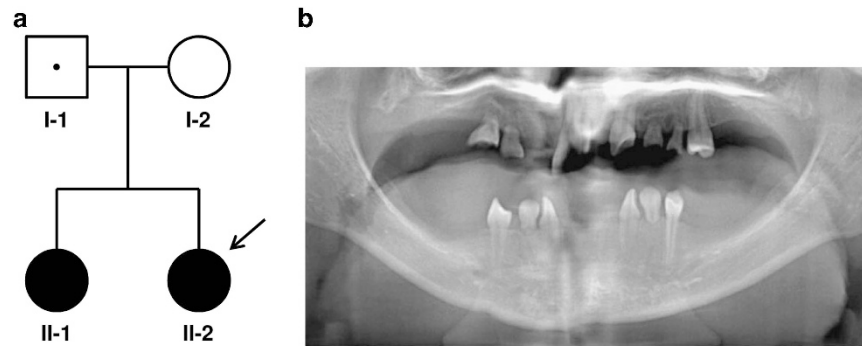
## RESULTS

First the BAC array analysis identified a heterozygous deletion at 14q13.3 by decreasing the ratio of two BAC clones, RP11-458A21 and RP11-81F13, in the proband (data not shown), and subsequently the oligonucleotide array determined the precise size of the deletion to be 884 kb. This result was described as follows: arr[hg18] 14q13.3 (35,922,011-36,805,702) × 1 (Figure 2a). The deletion was confirmed by FISH as follows: ish del(14)(q13.3q13.3)(RP11-56J17-) (Figure 2b). FISH also demonstrated that the sister shared the same deletion, while their father had the deletion in mosaic state. The ratio of the intact to the deleted chromosome was 12:8, that is, the percentage of the aberrant chromosome was 40%. This result is described as follows: mos ish del(14)(q13.3q13.3)(RP11-56J17-)[8]/ish 14q13.3(RP11-56J17x2)[12] (Figure 2c). This mosaicism was also confirmed by the SNP array (Figure 2d). These results suggested that the deletion in the siblings is derived from the father. Genomic PCR using the specific primer set encompassing the deletion showed amplified products only

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**Figure 1** (a) Pedigree of the present case. Filled circles denote the proband (II-2) and her affected sister (II-1), and the open square with a dot indicates the healthy father with mosaicism (I-1). The open circle indicates the healthy mother (I-2). (b) Panoramic radiograph of the dentition of the sister (II-1) at 14 years old.

**Table 1** Summary of clinical and molecular findings in the current cases and two literature cases

	Our cases		Patient 2 of Santen <i>et al.</i>	Family IT1 of Breedveld <i>et al.</i>
	II-1	II-2		
Gender	F	F	M	F
Age at last examination	14 years 11 months	7 years 4 months	4 years	9 years
Developmental delay	+	+	-	-
Speech delay	+	+	-	-
Motor delay	+	+	+	-
Holding one's head	6 months	12 months		
Walk alone	36 months	not yet		
Choreoathetosis	+	+	+	+
Hypothyroidism	+	+	+	+
Pulmonary problems	PPHN	PPHN	Pulmonary insufficiency	NA
Oligo/hypodontia	+	+	+	+
Congenital heart defect	-	-	NA	NA
Brain anomalies	-	-	NA	NA
Dysmorphisms	-	-	-	-
Other manifestations			Ataxia	-
Karyotyping	46,XX	46,XX		
Range of deletion at 14q13.3 <sup>a</sup>	35,922,011-36,805,702		36,010,235-36,727,597	35,532,864-36,708,716
Size of deletion (Mb)	0.884	0.884	0.717	1.176

Abbreviations: NA, not available; PPHN, persistent pulmonary hypertension of the newborn; +, present; -, absent.

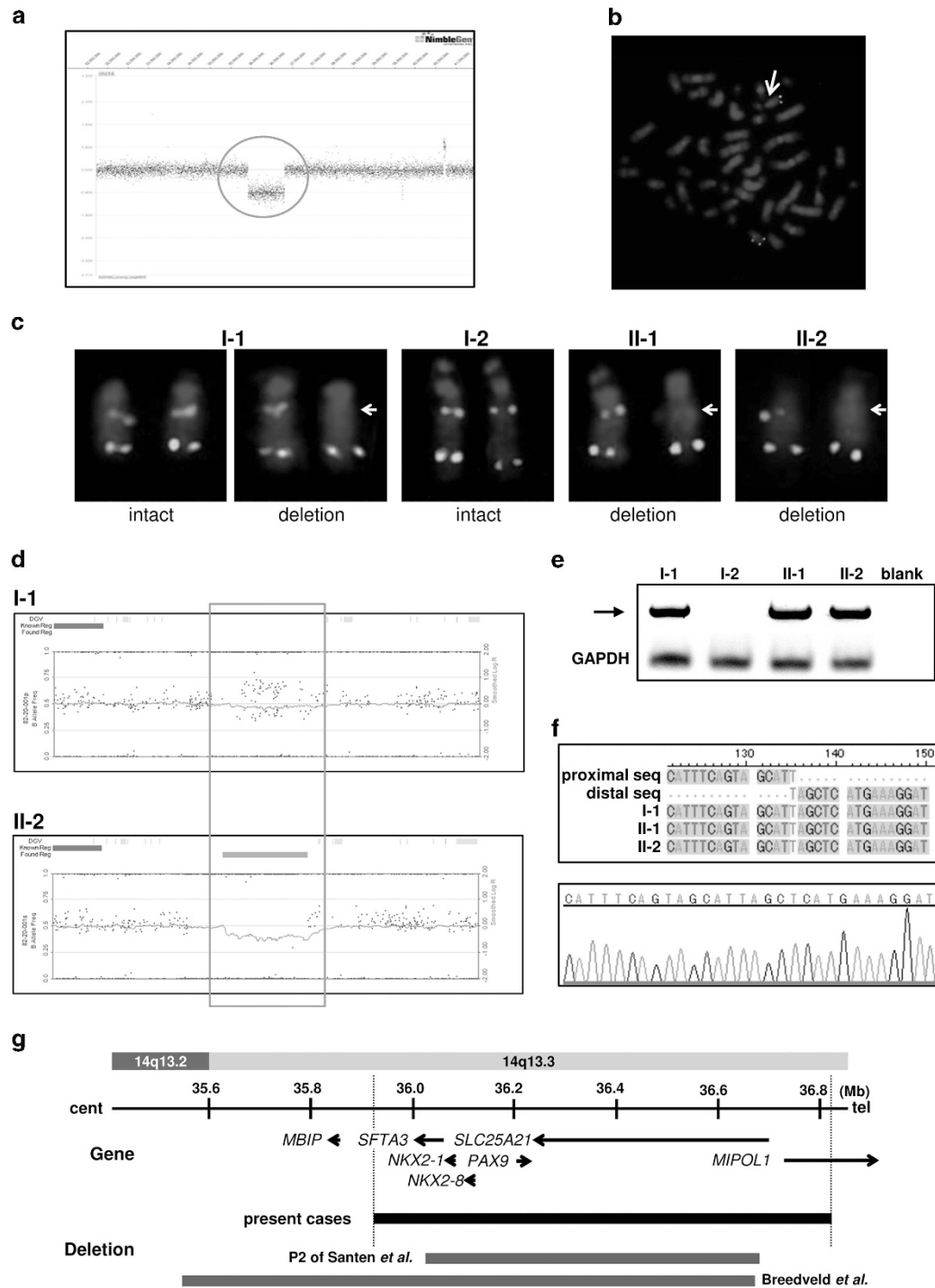
<sup>a</sup>The genomic coordinates are corresponding to the NCBI36/hg18 build of the human genome.

from the father, sister and the proband (Figure 2e). Sequencing around the breakpoint confirmed that the deletions were identical, and the breakpoint had only 1 bp homology between proximal and distal sequences (Figure 2f), suggesting that it resulted from microhomology-mediated break-induced replication.<sup>9</sup> It also suggested that the deletion occurred incidentally and did not result from a specificity of sequences, for example, low-copy repeats. A scheme of the deletions is shown in Figure 2g.

## DISCUSSION

In this study, we described an identical heterozygous deletion at 14q13.3 in two siblings with resembling phenotypes, which are oligodontia, hypothyroidism and PPHN. Among the six protein-coding

genes encompassed in the deletion, *PAX9* and *NKX2-1* appear to be good candidates to explain their phenotypes (Figure 2g). *PAX9* [OMIM: \*167416] is a member of the paired box (PAX) family of transcription factors, and it has been already reported that heterozygous mutations in *PAX9* cause selective tooth agenesis-3 (STHAG3) [#604625].<sup>10,11</sup> *NKX2-1* [\*600635] encodes a thyroid-specific transcription factor and heterozygous mutations in this gene cause choreoathetosis, hypothyroidism and neonatal respiratory distress [#610978].<sup>12-14</sup> Previous reports have described two patients with heterozygous deletions partially overlapping with our cases, and they also presented resembling phenotypes: choreoathetosis, hypothyroidism, pulmonary problems and oligo/hypodontia (Figure 2g, Table 1).<sup>15,16</sup> Although the previously reported cases



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**Figure 2** (a) Result of the oligonucleotide array. An 884 kb heterozygous deletion at 14q13.3 was detected (red circle). The genomic coordinates corresponds to the NCBI36/hg18 build of the human genome. (b) FISH using a probe at 14q13.3 (RP11-56J17, red) and a reference probe at 14q32.11 (RP11-79J20, green) delineated the heterozygous deletion in the proband (yellow arrow). (c) Enlarged chromosomes 14 of the FISH analyses in each member of the family. Both the siblings revealed a complete heterozygous deletion (yellow arrows), whereas the father had the deletion in mosaic state. The percentage of metaphase showing the deletion pattern was 40% (8/20). The mother had intact chromosomes 14 (not shown). (d) Results of the SNP array. The proband (II-2) showed the heterozygous deletion (red box) with a decreasing logR ratio (red line) and loss of heterozygosity (blue dot), whereas the father (I-1) showed slight decreasing of logR ratio and triplicate-like B allele frequency, confirming that he had the mosaicism. (e) Result of the genomic PCR. An amplified product was obtained only from the father, sister and proband. The arrow denotes deletion-specific products. (f) Chromatograms for the PCR products showed that the sequences around the breakpoint of the deletion were completely consistent. (g) A scheme of the region spanned by the deletion of the proband. Horizontal arrows indicate genes and their directions. Horizontal bars indicate the deletions of the proband (black) and the two cases in the literature (gray). A full color version of this figure is available at the *Journal of Human Genetics* journal online.

showed normal development unlike our cases despite their resembling deletion size, difference in penetrance may explain the discordant since patients with deletion or mutation of NKX2-1 frequently showed developmental delay.<sup>14</sup>

This study also demonstrated the possibility of recurrent CNV resulting from parental mosaicism. It was very interesting to observe that the siblings shared the identical deletion (Figures 2c, e and f). Although we made several hypotheses to explain the recurrence, their normal karyotyping denied a parental balanced chromosomal translocation, and an incidental recurrence is unlikely because the two deletions were completely consistent. We confirmed that the father has a somatic mosaicism of intact cells and cells with the deletion at 14q13.3 by FISH on chromosomes from his lymphocytes (Figures 2c and d). Although we did not estimate other tissues including sperm, the mosaicism was most likely to cause a mutation in his spermatozoa transmitted to his daughters, suggesting that the father probably had a gonosomal mosaicism.<sup>17</sup> The reason why the father is absolutely healthy may be explained by the possibility that the threshold for phenotypic expression is more than 40%. This possibility can be exemplified by the case of a patient with mosaicism at 64% of chromosomal deletion showing phenotypes,<sup>18</sup> although proportion of the mosaicism may differ depending on tissue or age.

The parental inheritance provides important information to assess the clinical relevance of a CNV, as CNVs inherited from healthy parents are likely to be benign.<sup>1,2</sup> Although the pathogenicity of the deletion that we identified was somewhat clear as it included a few morbid genes, generally the significance of CNVs inherited from healthy parents may be obscure because mosaicism generally shows an inheritance pattern contradicting Mendelian disorders. Actually, among the reported cases of siblings with identical genomic aberrations resulting from unaffected parental mosaicism, most of them are about well-known disorders; for example, *CREBBP* mutation for Rubinstein-Taybi syndrome,<sup>19</sup> maternal mosaicism for Down syndrome<sup>20</sup> and a microdeletion for 17q21.31 microdeletion syndrome.<sup>21</sup> Although CNV derived from a mosaicism may be overlooked when its significance is uncertain or the extent of mosaic population of cells is low, recent high-throughput techniques of genomic analysis may assist to identify cryptic mosaicism.<sup>17</sup> It is noteworthy that our cases reminded the importance of genetic counseling regarding the recurrence risk of pathogenic CNVs associated with parental mosaicism, even though most pathogenic CNVs are *de novo*.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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- Lee, C., Iafraite, A. J. & Brothman, A. R. Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. *Nat. Genet.* **39**, S48–S54 (2007).
- Miller, D. T., Adam, M. P., Aradhya, S., Biasecker, L. G., Brothman, A. R., Carter, N. P. *et al.* Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. *Am. J. Hum. Genet.* **86**, 749–764 (2010).
- D'Amours, G., Kibar, Z., Mathonnet, G., Fetni, R., Tihy, F., Désilets, V. *et al.* Whole-genome array CGH identifies pathogenic copy number variations in fetuses with major malformations and a normal karyotype. *Clin. Genet.* **81**, 128–141 (2012).
- Vissers, L. E., de Vries, B. B. & Veltman, J. A. Genomic microarrays in mental retardation: from copy number variation to gene, from research to diagnosis. *J. Med. Genet.* **47**, 289–297 (2010).
- Cooper, G. M., Coe, B. P., Girirajan, S., Rosenfeld, J. A., Vu, T. H., Baker, C. *et al.* A copy number variation morbidity map of developmental delay. *Nat. Genet.* **43**, 838–846 (2011).
- Inazawa, J., Inoue, J. & Imoto, I. Comparative genomic hybridization (CGH)-arrays pave the way for identification of novel cancer-related genes. *Cancer Sci.* **95**, 559–563 (2004).
- Hayashi, S., Imoto, I., Aizu, Y., Okamoto, N., Mizuno, S., Kurosawa, K. *et al.* Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies. *J. Hum. Genet.* **56**, 110–124 (2011).
- Hayashi, S., Kurosawa, K., Imoto, I., Mizutani, S. & Inazawa, J. Detection of cryptic chromosome aberrations in a patient with a balanced t(1;9)(p34.2;p24) by array-based comparative genomic hybridization. *Am. J. Med. Genet. A* **139**, 32–36 (2005).
- Hastings, P. J., Ira, G. & Lupski, J. R. A microhomology-mediated break-induced replication model for the origin of human copy number variation. *PLoS Genet.* **5**, e1000327 (2009).
- Stockton, D. W., Das, P., Goldenberg, M., D'Souza, R. N. & Patel, I. P. Mutation of PAX9 is associated with oligodontia. *Nat. Genet.* **24**, 18–19 (2000).
- Das, P., Stockton, D. W., Bauer, C., Shaffer, L. G., D'Souza, R. N., Wright, T. *et al.* Haploinsufficiency of PAX9 is associated with autosomal dominant hypodontia. *Hum. Genet.* **110**, 371–376 (2002).
- Devriendt, K., Vanhole, C., Matthijs, G. & de Zegher, F. Deletion of thyroid transcription factor-1 gene in an infant with neonatal thyroid dysfunction and respiratory failure. *New Eng. J. Med.* **338**, 1317–1318 (1998).
- Krude, H., Schütz, B., Biebertmann, H., von Moers, A., Schnabel, D., Neitzel, H. *et al.* Choreaethetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. *J. Clin. Invest.* **109**, 475–480 (2002).
- Carré, A., Szinnai, G., Castanet, M., Sura-Trueba, S., Tron, E., Broutin-L'Hermite, I. *et al.* Five new TTF1/NKX2.1 mutations in brain-lung-thyroid syndrome: rescue by PAX8 synergism in one case. *Hum. Mol. Genet.* **18**, 2266–2276 (2009).
- Breedveld, G. J., van Dongen, J. W., Danesino, C., Guala, A., Percy, A. K., Dure, L. S. *et al.* Mutations in TITF-1 are associated with benign hereditary chorea. *Hum. Mol. Genet.* **11**, 971–979 (2002).
- Santen, G. W., Sun, Y., Gijsbers, A. C., Carré, A., Holvoet, M., van Haeringen, A. *et al.* Further delineation of the phenotype of chromosome 14q13 deletions: (positional) involvement of FOXP1 appears the main determinant of phenotype severity, with no evidence for a holoprosencephaly locus. *J. Med. Genet.* **49**, 366–372 (2012).
- Biasecker, L. G. & Spinner, N. B. A genomic view of mosaicism and human disease. *Nat. Rev. Genet.* **14**, 307–320 (2013).
- Gamage, T. H., Godapitiya, I. U., Nanayakkara, S., Jayasekara, R. W. & Dissanayake, V. H. A child with mosaicism for deletion 14(q11.2q13). *Indian J. Hum. Genet.* **18**, 130–133 (2012).
- Chiang, P. W., Lee, N. C., Chien, N., Hwu, W. L., Spector, E. & Tsai, A. C. Somatic and germ-line mosaicism in Rubinstein-Taybi syndrome. *Am. J. Med. Genet. A* **149A**, 1463–1467 (2009).
- Gross, S. J., Tharapel, A. T., Phillips, O. P., Shulman, L. P., Pivnick, E. K. & Park, V. M. A jumping Robertsonian translocation: a molecular and cytogenetic study. *Hum. Genet.* **98**, 291–296 (1996).
- Koolen, D. A., Dupont, J., de Leeuw, N., Vissers, L. E., van den Heuvel, S. P., Bradbury, A. *et al.* Two families with sibling recurrence of the 17q21.31 microdeletion syndrome due to low-grade mosaicism. *Eur. J. Hum. Genet.* **20**, 729–733 (2012).

Supplementary Information accompanies the paper on Journal of Human Genetics website (<http://www.nature.com/jhg>)