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Journal
American Journal of Medical Genetics Part A, 140A(17)

ISSN
1552-4825

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Publication Date
2006-09-01

DOI
10.1002/ajmg.a.31390

American Journal of Medical Genetics Part A 140A:1876–1879 (2006)

Research Letter

Identification of a Novel Polymorphism—The Duplication of the *NPHP1* (Nephronophthisis 1) Gene

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Received 21 November 2005; Accepted 9 June 2006

How to cite this article: Baris H, Bejjani BA, Tan W-H, Coulter DL, Martin JA, Storm AL, Burton BK, Saitta SC, Gajecka M, Ballif BC, Irons MB, Shaffer LG, Kimonis VE. 2006. Identification of a novel polymorphism—The duplication of the *NPHP1* (nephronophthisis 1) gene. Am J Med Genet Part A 140A:1876–1879.

To the Editor:

We have identified seven cases (Table I) of a microduplication of the nephronophthisis 1 (*NPHP1*) gene on chromosome 2q13 using targeted microarray comparative genomic hybridization (aCGH). Homozygous deletions of the *NPHP1* gene have been associated with familial juvenile nephronophthisis [Antignac et al., 1993; Konrad et al., 1995; Hildebrandt et al., 1997], Senior-Løken syndrome [Caridi et al., 1998], Joubert syndrome [Parisi et al., 2004], and ocular motor apraxia [Betz et al., 2000]. However, duplications of the *NPHP1* gene have not been previously reported. The SignatureChip™ aCGH (Signature Genomic Laboratories, LLC, Spokane, WA) was used for the identification of submicroscopic cytogenetic abnormalities. Version 2 of this targeted aCGH contains 831 clones placed around 63 loci of known microdeletion and microduplication syndromes, as well as all subtelomeric and pericentromeric regions; each locus is covered by at least three partially overlapping BAC clones, flanked by control contigs placed about 1 Mb on either side of the locus [Bejjani et al., 2005]. In 1,500 samples analyzed using this aCGH, 7 duplications, and 8 deletions of this region were identified (Shaffer et al., submitted). In addition, one case of *NPHP1* duplication was identified among 50 normal control samples during the validation of this aCGH. In this paper we provide evidence that this is a novel copy number variation.

Patient 1 was a 12-year-old adopted Japanese boy with pervasive developmental disorder, attention deficit-hyperactivity disorder (ADHD), obsessive compulsive disorder, language-based learning disability, speech delay, and dysmorphic features including mid-face hypoplasia, bilateral epicanthal folds, apparently long palpebral fissures, synophrys, upturned nose with a depressed nasal root and retrognathia. His height and weight were between the 3rd and 10th centiles, but his occipito-frontal circumference (OFC) was on the 50th centile. He had two 1-cm café-au-lait macules on his upper back and a 1-cm haemangioma on his nose. Both thumbs were adducted. Neurological examination was normal. Brain MRI and renal ultrasound scan were normal.

Patient 2 was a 26-month-old, former 33½-week-gestation South Indian boy with global developmental delay. His length was between the 25th and

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DOI 10.1002/ajmg.a.31390

TABLE I. Summary of Patients with Duplication of the *NPHP1* Gene

	Patients							
Features	1	2	3	4	5	6	7	Overall
Age/gender	12yo M	26mo M	7yo F	5yo F	10yo F	25mo M	18mo M	
Speech delay	+	+	−	+	+	+	+	6/7
Global developmental delay	−	+	−	+	+	+	+	5/7
>1 Dysmorphic feature	+	+	−	−	+	+	+	5/7
Relative macrocephaly	+	+	+	−	−	+	−	4/7
Microcephaly	−	−	−	+	+	−	+	3/7
Failure to thrive (post-natal)	−	−	+	−	−	+	+	3/7
Intrauterine growth retardation	−	−	−	OFC only[a]	+	+	−	2/7
Skin lesions	+	+	−	−	−	−	−	2/7
ADHD	+	−	−	−	+	−	−	2/7
Mother's genotype	N/A	Neg	Pos	N/A	N/A	N/A	N/A	
Father's genotype	N/A	Pos	Neg	N/A	N/A	N/A	N/A	

The table lists features, by frequency, that are seen in at least two patients.
[a]Occipitofrontal circumference <3rd centile; length and weight between 3rd and 10th centiles.
mo, months old; yo, years old; N/A, not available for testing; Neg, no duplication in *NPHP1* identified; Pos, same duplication in *NPHP1* gene; ADHD, attention-deficit hyperactivity disorder; PDD, pervasive developmental delay.

50th centiles, weight was on the 50th centile, and OFC was between the 75th and 98th centiles. He had a frontal upsweep to his hair, hypoplastic mid-face, bilateral epicanthal folds, apparently short palpebral fissures, apparent telecanthus, apparent hypertelorism, and mild synophrys consistent with increased forehead hair. He had a haemangioma on his anterior neck. He also had mild pectus excavatum and inverted nipples bilaterally. Renal function tests, renal ultrasound, and ophthalmological evaluation were normal. Head CT at 6 weeks of age was reportedly normal. His father had normal development except for "slurred speech" in early childhood that resolved spontaneously. He was not dysmorphic, but was macrocephalic (just above 98th centile), with height on the 50th centile. His mother was phenotypically normal.

Patient 3 was a 7½-year-old girl who was neither dysmorphic nor developmentally delayed. She had normal birth weight and length, but developed failure to thrive in infancy. Her height and weight were below the 3rd centile, while her OFC was on the 50th centile. Renal function tests and renal ultrasound were normal. Her parents were phenotypically normal with normal height.

Patient 4 was an adopted 5½-year-old girl who was born at term with birth weight and length between the 3rd and 10th centiles and OFC below the 3rd centile (31.5 cm). She had a positive meconium screen for alcohol. At 5½ years of age, she was functioning at a 3-year-old level. Her height and weight were on the 3rd centile and head circumference was on the 2nd centile. She had bushy, dark eyebrows with synophrys. There were no limb abnormalities. Her biological mother was short in stature, completed the 6th grade and was a "slow-learner", while her biological father completed the 11th grade.

Patient 5 was a 10½-year-old girl with developmental, particularly speech delay, behavioral problems, ADHD, and dysmorphic features. She was born at term with a birth weight on the 3rd centile (2.44 kg) and a birth length below the 3rd centile (43.2 cm) to a mother with a history of multi-substance abuse, as well as maternal stroke, diabetes mellitus and glycogen storage disease type III, and a father with a personality disorder. Her height was on the 10th centile, weight was between the 5th and 10th centiles, and OFC was below the 3rd centile (49 cm). Dysmorphic features included the presence of a frontal upsweep to the hair, downslanting palpebral fissures, apparently small posteriorly rotated ears, a bulbous nose with an apparently broad base, a minimal unilateral cleft lip, malar hypoplasia, and partial cutaneous syndactyly of the second and third toes. She had delayed tooth eruption and mild hyperextensibility of the elbows and knees. Neurological examination was normal. Brain MRI revealed punctate hyperintense foci in the left periventricular deep white matter.

Patient 6 was a 25-month-old Polish boy with global developmental delay, relative macrocephaly, mild dysmorphic features, and bilateral cataracts noted at 2 months of age. He was born at term with a birth weight below the 3rd centile. His height and weight were below the 3rd centile but OFC was on the 75th centile. He had simple pinnae, apparently small teeth, hypospadias, and generalized hypotonia. Brain MRI showed mild ventriculomegaly. Renal ultrasound was normal. The parents were phenotypically normal.

Patient 7 was an 18-month-old boy with gross motor delay, failure to thrive, microcephaly, and a family history of mental retardation. He was born at term with a birth weight on the 40th centile, length on the 50th centile, and OFC on the 5th centile. On

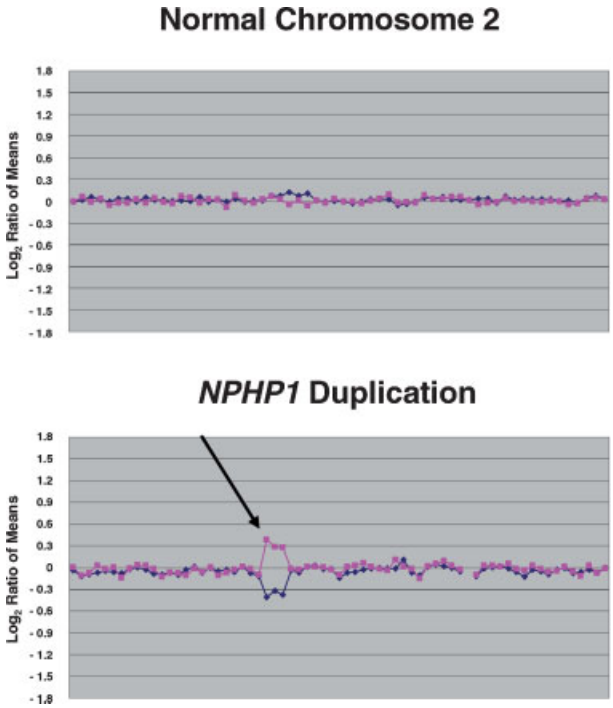

FIG. 1. Duplication of the *NPHP1* region of 2q13 detected using the aCGH. Each clone on the microarray is plotted along the x-axis according to its location on the chromosome with the most distal/telomeric p-arm clones on the left and the most distal/telomeric q-arm clones on the right. The blue line plot represents the $\log_2$ ratios from the first experiment (control Cy5/subject Cy3) and the pink line plot represents the $\log_2$ ratios obtained from the second experiment in which the dyes have been reversed (subject Cy5/control Cy3). The left panel is an aCGH plot for a normal chromosome 2. The right panel is a chromosome 2 plot from aCGH analysis on a subject showing duplication of the three clone contig (RP11-335A19, RP11-528G9, RP11-264O8) which spans the *NPHP1* gene (arrow).

exam, his height was on the 10th centile but his weight and OFC were below the 3rd centile. He was dolichocephalic, with downslanting palpebral fissures, protuberant ears with prominent cruri, a bulbous nose and high-arched palate. He had mild syndactyly of his second and third toes, bilaterally. He was mildly hypertonic in his lower extremities. Renal ultrasound and serum electrolyte levels were normal. Both his parents were mentally retarded, but further testing was not available.

All seven patients had normal chromosomal analysis. A single copy gain of the *NPHP1* gene region at chromosome locus 2q13 was detected in all patients (Fig. 1). The duplication was of the same size and involved three BACs (RP11-335A19, RP11-528G9, and RP11-264O8) spanning about 378 kb in all patients. The duplication was confirmed by fluorescent in situ hybridization (FISH) using standard methods in all patients except for patient 3 in whom the duplication was unclear by FISH [Shaffer et al., 1994]. The BAC clone used for the FISH confirmation was RP11-528G9, which covers *NPHP1* and partially covers MALL and a putative gene (Fig. 2a). We found that the father of patient 2 and the mother of patient 3 carried the same duplication (Table I). The pericentromeric (proximal) flanking clones were RP11-20G1, RP11-471N11, and RP11-265B3, covering about 402 kb; the telomeric (distal) flanking clones were RP11-259D8, RP11-768F19, and RP11-1144O16, covering about 375 kb. None of these pericentromeric and telomeric flanking clones demonstrated any copy number changes. Fiber FISH analysis using standard methods [Rautenstrauss et al., 1997] on patient 5 with two overlapping probes (RP11-528G9 and RP11-264O8) corresponding to the *NPHP1* gene demonstrated a tandem duplication (Fig. 2b), further confirming the aCGH finding of a duplication of this region.

The *NPHP1* gene is flanked by two 300–330 kb inverted repeats, as well as two 45 kb direct repeats, one of which lies 20 kb upstream of *NPHP1*, distal to the centromeric copy of the 330 kb repeat, the other copy lies downstream of *NPHP1* within the telomeric copy of the 330 kb repeat [Saunier et al., 2000]. This genomic architecture predicts that the reciprocal meiotic product of the deletion (i.e., duplication of the *NPHP1* gene) should be equally frequent, since large regions of genomic duplication are

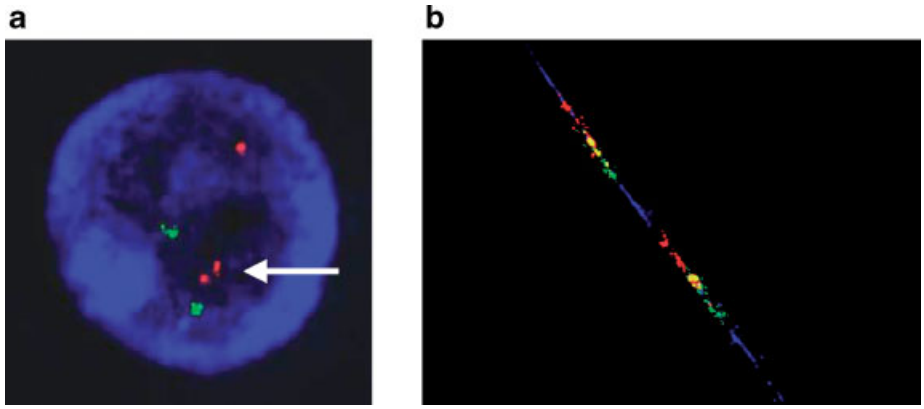

FIG. 2. **a**: Interphase FISH analysis showing duplication of the *NPHP1* locus. The central clone in the contig which contains the *NPHP1* gene (RP11-528G9) is labelled in red and shows duplication (arrow). The chromosome 2q telomere clone (RP11-367H11) is labeled in green as a control. **b**: Fiber FISH analysis. Probes corresponding to the *NPHP1* gene (RP11-528G9 labeled in red and RP11-264O8 labeled in green) demonstrate a tandem duplication (red-green-red-green pattern).

usually compatible with survival [Brewer et al., 1999]. However, to the best of our knowledge, this duplication has not been previously reported as a normal variant [Iafrate et al., 2004; Sebat et al., 2004]. The under-ascertainment of duplications may reflect the limitations of the technology used to visualize the genome [Shaffer and Lupski, 2000]. Newer cytogenetic techniques that allow one to survey the genome at higher resolutions such as aCGH are therefore likely to uncover more duplications, some of which may be of clinical significance [Shaffer and Bejjani, 2004].

In view of the lack of a consistent phenotype and the finding of the duplication in phenotypically normal parents and in 1 out of 50 control individuals, the *NPHP1* duplication is consistent with a normal population variant. However, the abnormal phenotypic features exhibited by the carriers of this duplication in our study, may be dependent on the genetic background of the individuals, or are manifested only when these carriers harbor a mutation in another, as yet unknown, locus. Identification of more individuals who carry this duplication will help to elucidate the significance of this finding.

COMPETING INTERESTS

The microarray CGH was performed using the SignatureChip™ developed by Signature Genomic Laboratories, LLC, Spokane, WA. Bassem Bejjani and Lisa Shaffer have ownership, receive consulting fees, and sit on the Board of Signature Genomic Laboratories, LLC. Blake Ballif is an employee of Signature Genomic Laboratories and has nothing to disclose.

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