of autosomal recessive diseases that manifest in the offspring of consanguineous relationships.

In conclusion, we rapidly identified a nonsense mutation in *MCT8* in a family with X-linked leucoencephalopathy using only a single lane of exome sequencing. This method is powerful for unbiased screening of disease-related mutations in X-linked or recessive conditions.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the institutional review board of Kanagawa Children's Medical Center and Yokohama City University School of Medicine.

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REFERENCES

1. **Shendure J,** Ji H. Next-generation DNA sequencing. *Nat Biotechnol* 2008;**26**:1135–45.
2. **Wheeler DA,** Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X, Tartaro K, Niazi F, Turcotte CL, Irzyk GP, Lupski JR, Chinault C, Song XZ, Liu Y, Yuan Y, Nazareth L, Qin X, Muzny DM, Margulies M, Weinstock GM, Gibbs RA, Rothberg JM. The complete genome of an individual by massively parallel DNA sequencing. *Nature* 2008;**452**:872–6.
3. **Ng SB,** Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, Shendure J. Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* 2009;**461**:272–6.
4. **Choi M,** Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloglu A, Ozen S, Sanjad S, Nelson-Williams C, Farhi A, Mane S, Lifton RP. Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proc Natl Acad Sci U S A* 2009;**106**:19096–101.
5. **Hodges E,** Xuan Z, Balija V, Kramer M, Molla MN, Smith SW, Middle CM, Rodesch MJ, Albert TJ, Hannon GJ, McCombie WR. Genome-wide in situ exon capture for selective resequencing. *Nat Genet* 2007;**39**:1522–7.
6. **Ng SB,** Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ. Exome sequencing identifies the cause of a mendelian disorder. *Nat Genet* 2010;**42**:30–5.
7. **Nannya Y,** Sanada M, Nakazaki K, Hosoya N, Wang L, Hangaishi A, Kurokawa M, Chiba S, Bailey DK, Kennedy GC, Ogawa S. A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res* 2005;**65**:6071–9.
8. **Li H,** Ruan J, Durbin R. Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res* 2008;**18**:1851–8.
9. **Dumitrescu AM,** Liao XH, Best TB, Brockmann K, Refetoff S. A novel syndrome combining thyroid and neurological abnormalities is associated with mutations in a monocarboxylate transporter gene. *Am J Hum Genet* 2004;**74**:168–75.
10. **Friesema EC,** Grueters A, Biebermann H, Krude H, von Moers A, Reeser M, Barrett TG, Mancilla EE, Svensson J, Kester MH, Kuiper GG, Balkassmi S, Uitterlinden AG, Koehrle J, Rodien P, Halestrap AP, Visser TJ. Association between mutations in a thyroid hormone transporter and severe X-linked psychomotor retardation. *Lancet* 2004;**364**:1435–7.
11. **Frints SG,** Lenzner S, Bauters M, Jensen LR, Van Esch H, des Portes V, Moog U, Macville MV, van Roozendaal K, Schrander-Stumpel CT, Tzschach A, Marynen P, Fryns JP, Hamel B, van Bokhoven H, Chelly J, Beldjord C, Turner G, Gecz J, Moraine C, Raynaud M, Ropers HH, Froyen G, Kuss AW. *MCT8* mutation analysis and identification of the first female with Allan-Herndon-Dudley syndrome due to loss of *MCT8* expression. *Eur J Hum Genet* 2008;**16**:1029–37.
12. **Vaurs-Barriere C,** Deville M, Sarret C, Giraud G, Des Portes V, Prats-Vinas JM, De Michele G, Dan B, Brady AF, Boespflug-Tanguy O, Touraine R. Pelizaeus-Merzbacher-Like disease presentation of *MCT8* mutated male subjects. *Ann Neurol* 2009;**65**:114–18.
13. **Bianco AC,** Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocr Rev* 2002;**23**:38–89.
14. **Friesema EC,** Ganguly S, Abdalla A, Manning Fox J, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. *J Biol Chem* 2003;**41**:40128–35.
15. **Lafreniere RG,** Carrel L, Willard HF. A novel transmembrane transporter encoded by the XPCT gene in Xq13.2. *Hum Mol Genet* 1994;**3**:1133–9.
16. **Dumitrescu AM,** Liao XH, Weiss RE, Millen K, Refetoff S. Tissue-specific thyroid hormone deprivation and excess in monocarboxylate transporter (mct) 8-deficient mice. *Endocrinology* 2006;**147**:4036–43.
17. **Maranduba CM,** Friesema EC, Kok F, Kester MH, Jansen J, Sertie AL, Passos-Bueno MR, Visser TJ. Decreased cellular uptake and metabolism in Allan-Herndon-Dudley syndrome (AHDS) due to a novel mutation in the MCT8 thyroid hormone transporter. *J Med Genet* 2006;**43**:457–60.
18. **Namba N,** Etani Y, Kitaoka T, Nakamoto Y, Nakacho M, Bessho K, Miyoshi Y, Mushiake S, Mohri I, Arai H, Taniike M, Ozono K. Clinical phenotype and endocrinological investigations in a patient with a mutation in the MCT8 thyroid hormone transporter. *Eur J Pediatr* 2008;**167**:785–91.
19. **Herzovich V,** Vaiani E, Marino R, Dratler G, Lazzati JM, Tilitzky S, Ramirez P, Iorcansky S, Rivarola MA, Belgorosky A. Unexpected peripheral markers of thyroid function in a patient with a novel mutation of the MCT8 thyroid hormone transporter gene. *Horm Res* 2007;**67**:1–6.

Hypomyelination with atrophy of the basal ganglia and cerebellum in an infant with Down syndrome

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List of key features

Hypomyelination
Atrophy of the basal ganglia
Atrophy of the cerebellum

Introduction

Hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) is a rare childhood-onset leukoencephalopathy and is diagnosed on the basis of MRI findings including hypomyelination of the cerebral white matter, atrophy of the basal ganglia, and cerebellum, accompanied by progressive pyramidal/extrapyramidal movement disorder and cognitive deficiency (Van der Knaap *et al.*, 2002). So far, 19 H-ABC patients have been reported in the literature (Matta and Ribas, 2007; Van der Knapp *et al.*, 2007). However, the genetic background of H-ABC and its diagnostic index marker and treatment regimen are not defined. We report the clinical, neurological, and genetic findings in the first case of Down syndrome with H-ABC.

Clinical summary

The patient was a 21-month-old Japanese boy born at 40 weeks of gestation as the first child to healthy nonconsanguineous parents. At 22 weeks of gestation, the mother was administered valacyclovir hydrochloride therapy for chicken pox. His birth weight, height, and head circumference were 3534 g (90th percentile), 51 cm (90th percentile), and 32.5 cm (25th percentile), respectively. He presented with hyperbilirubinemia, ventricular septal defect, and muscular hypotonia. Cytogenetic analysis showed a 47, XY, + 21 karyotype.

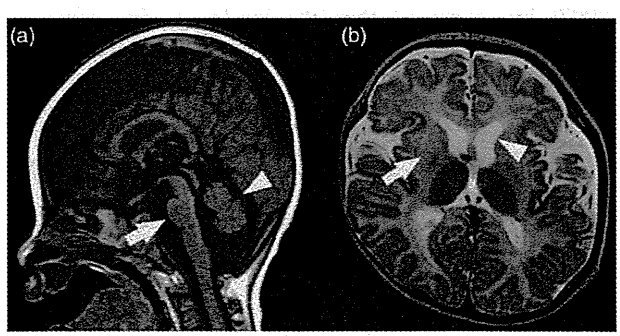
At the age of 5 months, he developed nystagmus and his eyes were unable to follow a moving object. At the age of 8 months, he presented with truncal hypotonia accompanied by bouts of opisthotonus, and limb hypertonia.

A brain MRI carried out at the age of 8 months showed hypoplasia of the cerebellar vermis and pons (Fig. 1a), diffuse hypomyelination of the cerebral white matter, a small caudate nucleus, and a barely visible putamen (Fig. 1b). A second brain MRI at the age of 14 months showed slightly advanced atrophy of the basal ganglia but no progression of myelination. These findings were consistent with the diagnosis of H-ABC.

The results of the routine hematological investigations and cerebrospinal fluid analysis were normal. Serum levels of neopterin and biopterin, amino acid analysis, and the cerebrospinal fluid levels of neopterin and biopterin were unremarkable.

Genetic analysis using a 385 k oligonucleotide microarray (Roche NimbleGen, Inc., Madison, Wisconsin, USA) was carried out with DNA extracted from leukocytes. Apart

Fig. 1



(a) Mid-sagittal T1-weighted image at the age of 8 months shows that the pons (arrow) and cerebellar vermis (arrow head) are somewhat hypoplastic. (b) Axial T2-weighted image at the age of 8 months shows that a mild dilatation of the lateral ventricles, the diffuse cerebral white matter hypomyelination, no visible putamen (arrow), and small caudate nucleus, almost lacking the normal low T2 signal intensity (arrow head).

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from the duplication of chromosome 21, the array comparative genomic hybridization (CGH) showed no copy number abnormalities.

Administration of diazepam and levodopa/carbidopa hydrate at the age of 8 and 10 months, respectively, had no favorable effect. After administration of dantrolene (40 mg/day) at the age of 16 months, opisthotonus and limb hypertonia improved gradually, however, the patient had attained no head control even at the age of 21 months.

Discussion

We report a 21-month-old boy with Down syndrome and H-ABC. MRI findings showed hypomyelination and atrophy of the basal ganglia and cerebellum; truncal hypotonia with bouts of opisthotonus and limb hypertonia was also observed.

Down syndrome is characterized by various clinical features, including hypotonia, cognitive deficiency, and visceral malformations. However, neither leukoencephalopathy nor atrophy of the basal ganglia and cerebellum has been reported earlier in Down syndrome. Compared with healthy individuals, patients with Down syndrome have a reduced cerebellar volume, whereas their basal ganglia are relatively normal (Lott and Dierssen, 2010).

The previously reported 19 patients with H-ABC showed initial clinical manifestations between the ages of 2 months and 3 years. Of these 19 patients, seven could not walk with or without support and one did not achieve head control (Matta and Ribas, 2007). Our patient showed initial clinical manifestations in middle infancy and did not achieve head control at the age of 21 months. He seemed to show characteristics of severe H-ABC.

The genetic background of H-ABC has not been determined. All the previously reported patients were sporadic cases and chromosome analyses and gene tests in several disorders associated with diffuse hypomyelination did not show genetic abnormalities. Van der Knapp *et al.* (2007) hypothesized that the mode of inheritance in H-ABC may be autosomal recessive or dominant manner with de novo mutations.

Owing to its relatively high prevalence, Down syndrome occasionally occurs in association with other syndromes (Vis *et al.*, 2009). Down syndrome and H-ABC might have occurred by coincidence in our patient. The alternative hypothesis is that submicroscopic copy number change, especially in chromosome 21, might be associated with H-ABC. Thus, we carried out array CGH analysis; however, except for trisomy 21, no aberration was found. Nevertheless, there is no denying that other genetic factors that are undetectable in array CGH may be associated with H-ABC. Further investigations involving genetic analysis of disorders associated with diffuse hypomyelination should be conducted. The observed association between Down syndrome and H-ABC may be a coincidence, which might be established with further studies.

We report the first case of a patient with Down syndrome and H-ABC. Down syndrome patients occasionally develop brain abnormalities such as Alzheimer's disease (Lott and Dierssen, 2010). Although Down syndrome patients usually show some degree of developmental delay, atypical features such as opisthotonus or limb hypertonia seen in our patient suggest that the further investigations, such as brain MRI, are warranted.

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References

Lott IT, Dierssen M (2010). Cognitive deficits and associated neurological complications in individuals with Down's syndrome. *Lancet Neurol* **9**:623–633.

Matta AP, Ribas MC (2007). Hypomyelination with atrophy of the basal ganglia and cerebellum: case report. *Arq Neurosiquiatr* **65**:161–163.

Van der Knaap MS, Naidu S, Pouwels PJ, Bonavita S, van Coster R, Lagae L, *et al.* (2002). New syndrome characterized by hypomyelination with atrophy of the basal ganglia and cerebellum. *AJNR Am J Neuroradiol* **23**:1466–1474.

Van der Knapp MS, Linnankivi T, Paetau A, Feigenbaum A, Wakusawa K, Haginoya K, *et al.* (2007). Hypomyelination with atrophy of the basal ganglia and cerebellum. *Neurology* **69**:166–171.

Vis JC, van Engelen K, Timmermans J, Hamel BC, Mulder BJM (2009). Marfan syndrome masked by Down syndrome? *Neth Heart J* **17**:345–348.

De novo mutations in epilepsy

Ohtahara syndrome and West syndrome are considered to be a continuum of early-onset epileptic encephalopathies, because the majority (75%) of Ohtahara syndrome cases evolve into West syndrome. Brain malformations are frequently associated with Ohtahara syndrome and West syndrome. The presence of cryptogenic cases suggests genetic factors may also be involved. However, most cases of these syndromes are sporadic, probably because of their poor clinical outcomes with severe psychomotor impairment.

De novo copy number variations (CNVs) and mutations are major causes of sporadic traits.[1] Their occurrence has been estimated to be 1.7×10^{-6} per locus and $2.2–4.0 \times 10^{-8}$ per nucleotide in a diploid embryo respectively, suggesting that an average newborn is expected to acquire approximately 0.86 amino acid altering mutations, and that de novo CNVs are more frequent than de novo mutations.[1–3] Increased availability of genomic microarrays, such as array comparative genomic hybridization, has facilitated the detection of de novo CNVs in which disease-causative genes may reside.

We have recently identified two disease-causative genes (*STXBP1* for Ohtahara syndrome and *SPTAN1* for a type of West syndrome) through identification of a de novo microdeletion at 9q33.3-q34.11 in one of four individuals (participant 1) with early-onset West syndrome with severe cerebral hypomyelination.[4–6] Among more than 40 genes within the deletion, we focused firstly on *STXBP1*, because it is involved in synaptic vesicle release and has brain-specific expression in both rodents and humans. Participant 1 was initially diagnosed with Ohtahara syndrome, which progressed to West syndrome at 3 months of age. *STXBP1* screening in other patients with Ohtahara syndrome with no brain anomalies led to the identification of four missense mutations, indicating that mutations in *STXBP1* cause cryptogenic Ohtahara syndrome.[4] To date, *STXBP1* abnormalities have been found in 19 out of 55 individuals (34.5%) with cryptogenic Ohtahara syndrome (including unpublished data).[7] Seventeen out of 19 deletions or mutations were confirmed as de novo events. The remaining two included an inherited mutation from the somatic-mosaic father, and an unconfirmed mutation due to unavailability of paternal DNA. Moreover, the clinical spectrum of *STXBP1* mutations has been shown to be broader. Aberrations of *STXBP1* were found in six out of 106 patients with early-onset epileptic encephalopathies (five mutations/deletions occured de novo).[8] Of note, the initial phenotype of five patients with *STXBP1* aberrations did not fit into either Ohtahara syndrome or West syndrome.[8] Furthermore, two de novo *STXBP1* mutations were also found in two out of 95 individuals with learning disability* and non-syndromic epilepsy.[9] These findings indicated that CNV and mutation screening in *STXBP1* should be considered in children with early-onset seizures.

Were there *STXBP1* mutations in the remaining three participants with early-onset West syndrome with severe cerebral hypomyelination? We could not find any *STXBP1* abnormalities in two of the remaining three participants (one was unavailable). While most patients with *STXBP1* mutations showed normal myelination, participant 1 additionally showed severe hypomyelination of the cerebral cortex and a thin corpus callosum at 12 months of age.[5] Therefore we hypothesized that another gene within the deletion may contribute to severe hypomyelination in participant 1. The *SPTAN1* gene encoding α-II spectrin appeared to be a primary candidate because zebrafish α-II spectrin mutants showed impaired myelination, and de novo in-frame mutations in *SPTAN1* were identified in the remaining two participants, suggesting that mutations in *SPTAN1* cause early-onset West syndrome with severe hypomyelination. Our identification of two disease genes from one de novo deletion in a single case may be a rare event; however, it should be emphasized that careful evaluation of clinical and molecular data in detail may reveal occult, yet important, findings.

In addition to *STXBP1* and *SPTAN1*, de novo mutations in *SCN1A*, *CDKL5*, and *ARX* genes have been reported in early-onset epileptic encephalopathies. Thus, it is likely that de novo mutations of known and unknown causative genes are a common cause of early-onset epileptic encephalopathies, which mostly occur sporadically. Recent progress in massively parallel DNA sequencing enables us to rapidly detect point mutations, and de novo mutations could be systemically identified by family-based exome sequencing (using trios: one patient and parents).[10] Together with genomic microarray, exome sequencing is likely to be provided as a clinical service in the near future, and will undoubtedly demonstrate important roles of de novo CNVs and mutations in early-onset epileptic encephalopathies.

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*North American usage: mental retardation.

REFERENCES

1. Lupski JR. Genomic rearrangements and sporadic disease. *Nat Genet* 2007; **39:** S43–7.
2. Lynch M. Rate, molecular spectrum, and consequences of human mutation. *Proc Natl Acad Sci USA* 2010; **107:** 961–8.
3. Roach JC, Glusman G, Smit AF, et al. Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science* 2010; **328:** 636–9.
4. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet* 2008; **40:** 782–8.
5. Tohyama J, Akasaka N, Osaka H, et al. Early onset West syndrome with cerebral hypomyelination and reduced cerebral white matter. *Brain Dev* 2008; **30:** 349–55.
6. Saitsu H, Tohyama J, Kumada T, et al. Dominant-negative mutations in alpha-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. *Am J Hum Genet* 2010; **86:** 881–91.
7. Saitsu H, Kato M, Okada I, et al. *STXBP1* mutations in early infantile epileptic encephalopathy with suppression-burst pattern. *Epilepsia* 2010; **51:** 2397–405.
8. Deprez L, Weckhuysen S, Holmgren P, et al. Clinical spectrum of early-onset epileptic encephalopathies associated with STXBP1 mutations. *Neurology* 2010; **75:** 1159–65.
9. Hamdan FF, Piton A, Gauthier J, et al. De novo STXBP1 mutations in mental retardation and nonsyndromic epilepsy. *Ann Neurol* 2009; **65:** 748–53.
10. Vissers LE, de Ligt J, Gilissen C, et al. A de novo paradigm for mental retardation. *Nat Genet* 2010; **42:** 1109–12.

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Short Report

Exome sequencing of two patients in a family with atypical X-linked leukodystrophy

Tsurusaki Y, Okamoto N, Suzuki Y, Doi H, Saitsu H, Miyake N, Matsumoto N. Exome sequencing of two patients in a family with atypical X-linked leukodystrophy.
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We encountered a family with two boys similarly showing brain atrophy with reduced white matter, hypoplasia of the brain stem and corpus callosum, spastic paralysis, and severe growth and mental retardation without speaking a word. The phenotype of these patients was not compatible with any known type of syndromic leukodystrophy. Presuming an X-linked disorder, we performed next-generation sequencing (NGS) of the transcripts of the entire X chromosome. A single lane of exome NGS in each patient was sufficient. Six potential mutations were found in both affected boys. Two missense mutations, including c.92T>C (p.V31A) in *L1CAM*, were potentially pathogenic, but this remained inconclusive. The other four could be excluded. Because the patients did not show adducted thumbs or hydrocephalus, the *L1CAM* change in this family can be interpreted as different scenarios. Personal genome analysis using NGS is certainly powerful, but interpretation of the data can be a substantial challenge requiring a lot of tasks.

Conflict of interest

None of the authors have any conflicts of interest to disclose.

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Key words: atypical phenotype – exome sequencing – *L1CAM* – X-linked leukodystrophy

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Focused/selected gene and genomic characterization has usually been carried out in clinically homogeneous groups of multiple affected samples to make identification of genetic abnormalities more efficient. Microarrays and next-generation sequencing (NGS) have provided new avenues for human genetic research (1–6). Using such new technologies, researchers are able to analyze small numbers of patients on a genome-wide scale. Even very rare cases (such as when only a few compatible patients are available or atypical patients showing no similar phenotypes) can be realistic targets of genetic research, as the new technologies can identify aberrations in a single gene from within virtually the whole genome; this could not be achieved with conventional techniques.

We encountered a family with two affected males showing atypical leukodystrophy. The phenotype of these patients did not match any known type of syndromic leukodystrophy. Because we presumed that abnormality of an X-linked gene caused the atypical leukodystrophy in this family, we performed exome sequencing of most of the X-chromosome transcripts and identified an unexpected gene mutation in these patients.

Materials and methods

A family with atypical X-linked leukodystrophy

Two brothers, II-1 currently aged 19 years and II-2 currently aged 17 years, who have unrelated healthy parents, presented with similar clinical

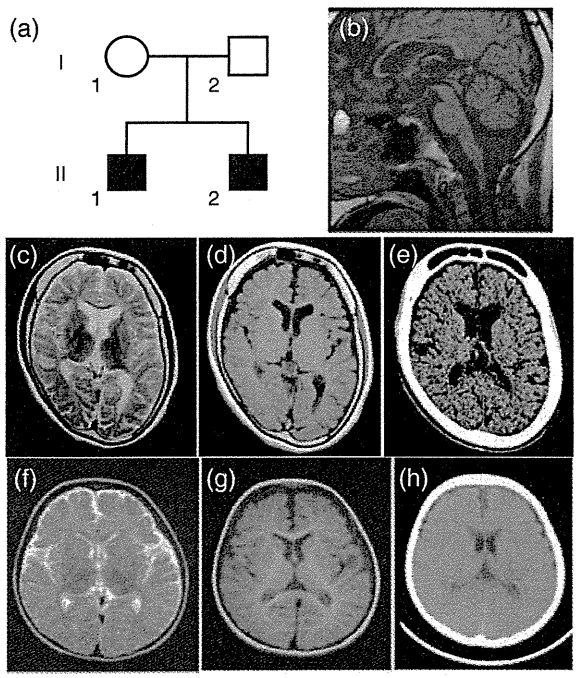

Fig. 1. Clinical features of the family. Familial pedigree (**a**). Brain magnetic resonance imaging (MRI) (**b**: T1-weighted image, **c**: T2-weighted image, **d**: T1-weighted image) of individual II-1 at 16 years old showing hypoplasia of the white matter, the brain stem and the corpus callosum. Brain computed tomographic (CT) images of individual II-1 at 19 years old (**e**) indicating a thick calvarium with enlarged frontal sinus as well as calcification of the choroid plexus in the atrophic brain. Brain MRI (**f**: T2-weighted image, **g**: T1-weighted image) of individual II-2 at 2 years old, also displaying hypoplasia of the white matter. Brain CT image of individual II-2 at 5 years old (**h**), also showing a thick calvarium.

features. Their mother did not show any neurological abnormalities (Fig. 1a).

Patient II-1

Patient II-1's birth weight was 2840 g at 40 weeks of gestational age. He had congenital nystagmus. He sat unsupported at 7 months old but after this his developmental milestones were delayed. He could creep at 18 months old. Spastic paralysis, especially in the lower extremities, became apparent. He was unable to stand unsupported. His mental development was severely delayed, and he needed special education from elementary school. He had suffered generalized epileptic seizures since he was 10 years old. He was confined to a wheelchair. He had severe mental retardation without speaking a word. His developmental quotient (DQ) at 9 years old was 19 by the Japanese standard method. Severe growth retardation [143 cm (<3%), 24 kg (<3%), occipitofrontal head circumference 49 cm (<3%) at 19 years] was also noted. He did not have dysmorphic features. Blood analysis revealed microcytic anemia [hemoglobin (Hb) 13.4 g/dl, mean corpuscular volume (MCV) (of red blood cell) 70.4 fl (normal: 89–99 fl), mean corpuscular hemoglobin (MCH) (of red blood cell) 23.1 pg (normal: 29–35 pg)] without any evidence of hemolysis or iron deficiency. Hormonal examination indicated that the levels of luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone were all low [0.9 mIU/ml (normal: 1.2–8.0 mIU/ml), 2.5 mIU/ml (normal: 2.3–15.1 mIU/ml), <0.01 μIU/ml (normal: 0.5–5.0 μIU/ml), respectively]. He showed delayed puberty with small testes. Pubic hair only appeared at 17 years old. His bone age at 18 years old was 12.6 years (67%). Brain magnetic resonance imaging (MRI) at 16 years old revealed brain atrophy associated with reduced white matter and hypoplasia of the brain stem and the corpus callosum (Fig. 1b–d). No hydrocephalus or adducted thumb was observed. Brain computed tomography (CT) at 19 years old showed a thick calvarium with enlarged frontal sinus as well as calcification of the cerebellar tentorium and the choroid plexus (Fig. 1e).

Patient II-2

Patient II-2's birth weight was 2910 g at 37 weeks of gestational age. Developmental delay was apparent since he was 10 months old. Spastic paralysis (especially in the lower extremities), confinement to a wheelchair, severe mental retardation without speaking a word (DQ = 5 at 17 years old), and severe growth retardation [130 cm (<3%) and 27 kg (<3%) at 17 years] were phenotypes shared with his brother (II-1). Blood analysis revealed microcytic anemia (Hb 12.0 g/dl, MCV 61.1 fl, MCH 19.0 pg) without any evidence of hemolysis or iron deficiency. Hormonal examination indicated that the levels of luteinizing hormone, follicle-stimulating hormone, and thyroid-stimulating hormone were relatively low (1.9 mIU/ml, 4.2 mIU/ml, <0.23 μIU/ml, respectively). He also showed delayed puberty with small testes. Pubic hair appeared only at 17 years old. His bone age at 17 years old was 11 years (65%). Brain MRI at 2 years old revealed brain atrophy associated with reduced white matter and hypoplasia of the brain stem and corpus callosum (Fig. 1f,g). Brain CT at 5 years old showed a thick calvarium (Fig. 1h). No hydrocephalus or adducted thumb was observed. Most of the clinical features were similar to those of his brother except for the absence of nystagmus in patient II-2.

Genome-wide SNP genotyping

Genome-wide single-nucleotide polymorphism (SNP) genotyping was performed on individuals I-2, II-1, and II-2 using a GeneChip™ Human Mapping 10K Array Xba 142 2.0 (Affymetrix, Inc., Santa Clara, CA), according to the manufacturer's protocols. Mendelian error in the pedigree to exclude conflicted SNPs was checked using GCOS 1.2 (GeneChip Operating Software; Affymetrix) and batch analysis in GTYPE 4.0 (GeneChip Genotyping Analysis Software; Affymetrix), with the default setting for the mapping algorithm. The linked region, with SNP genotypes shared between individuals II-1 and II-2, was checked manually.

Genomic partitioning, short-read sequencing, and sequence alignment

Three micrograms of genomic DNA from the affected brothers (II-1 and II-2) was processed using a SureSelect X Chromosome test kit (1582 transcripts covering 3053 kb) (Agilent Technologies, Santa Clara, CA), according to the manufacturer's instructions. Captured DNAs were analyzed using an Illumina GAIIx (Illumina, Inc., San Diego, CA). We used only one of the eight lanes in the flow cell (Illumina) for paired-end, 76-bp reads per sample. Image analysis and base-calling were performed using sequence control software (SCS) real-time analysis and off-line BASECALLER software v1.8.0 (Illumina). Reads were aligned to the human reference genome (UCSC hg19, NCBI build 37.1) using the ELANDv2 algorithm in CASAVA_v1.7.0 (Illumina). The ELANDv2 algorithm can align 100-bp reads to a reference sequence and split the reads into multiple seeds.

Mapping strategy and variant annotation

Approximately 57.5 million reads from individual II-1 and 71.1 million reads from individual II-2 that passed the quality control (Path Filter) were mapped to the human reference genome using mapping and assembly with quality (MAQ) (7) (Fig. 2). MAQ was able to align 51 720 952 and 65 990 660 reads to the whole genome for individuals II-1 and II-2, respectively; these were then statistically analyzed for coverage using a script created by BITS Co., Ltd. (Tokyo, Japan). SNPs and insertions/deletions were extracted from the alignment data using an original script created by BITS Co., Ltd., along with information on the registered SNPs (dbSNP 131). A consensus quality score of 40 or more was used for the SNP analysis in MAQ. SNPs in MAQ-passed reads were annotated using the SeattleSeq website (http://gvs.gs.washington.edu/SeattleSeqAnnotation/). Variants found by each informatics method were selected in terms of location on chromosome X, unregistered variants (excluding registered SNPs), variants in known genes, variants in coding regions, variants excluding synonymous changes, and variants with an allele frequency of at least 90% (assuming a homozygous mutation). NEXTGENE software v2.0 (SoftGenetics, State College, PA) was also used to analyze the reads, with a default setting. Variants found by both of the informatics methods were selected. The variants found in common between individuals II-1 and II-2 were focused on, and confirmed as true positives by Sanger sequencing of polymerase chain reaction (PCR) products amplified from patient genomic DNA, except for variants within genes at segmental duplications. The pathological significance of the variants was evaluated using four different websites: POLYPHEN (Polymorphism Phenotyping; http://genetics.bwh.harvard.edu/pph/index.html), POLYPHEN-2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), SIFT (http://sift.jcvi.org/) (output values less than 0.05 are deleterious), and MUTATIONTASTER (http://neurocore.charite.de/MutationTaster/).

Capillary sequencing

Possible pathological variants were confirmed by Sanger sequencing using an ABI 3500xl or ABI3100 autosequencer (Life Technologies, Carlsbad, CA), following the manufacturer's protocol. Sequencing data were analyzed using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI).

Expression studies

The relative mRNA levels of *TMEM187* in cDNA of various fetal and adult human tissues (Human MTC™ Panel I and Human Fetal MTC™ Panel; Clontech, Mountain View, CA) were determined by quantitative real-time reverse transcription–polymerase chain reaction (RT-PCR) using TaqMan gene expression assays (Hs01920894_s1 for *TMEM187* and Hs00357333_g1 for β-actin as a control) (Life Technologies).

Results and discussion

Our coverage analysis indicated that for individuals II-1 and II-2, 79.2% and 78.8%, respectively, of the entire X-chromosome coding sequence (CDS) were completely covered, and 88.5% and 88.5%,

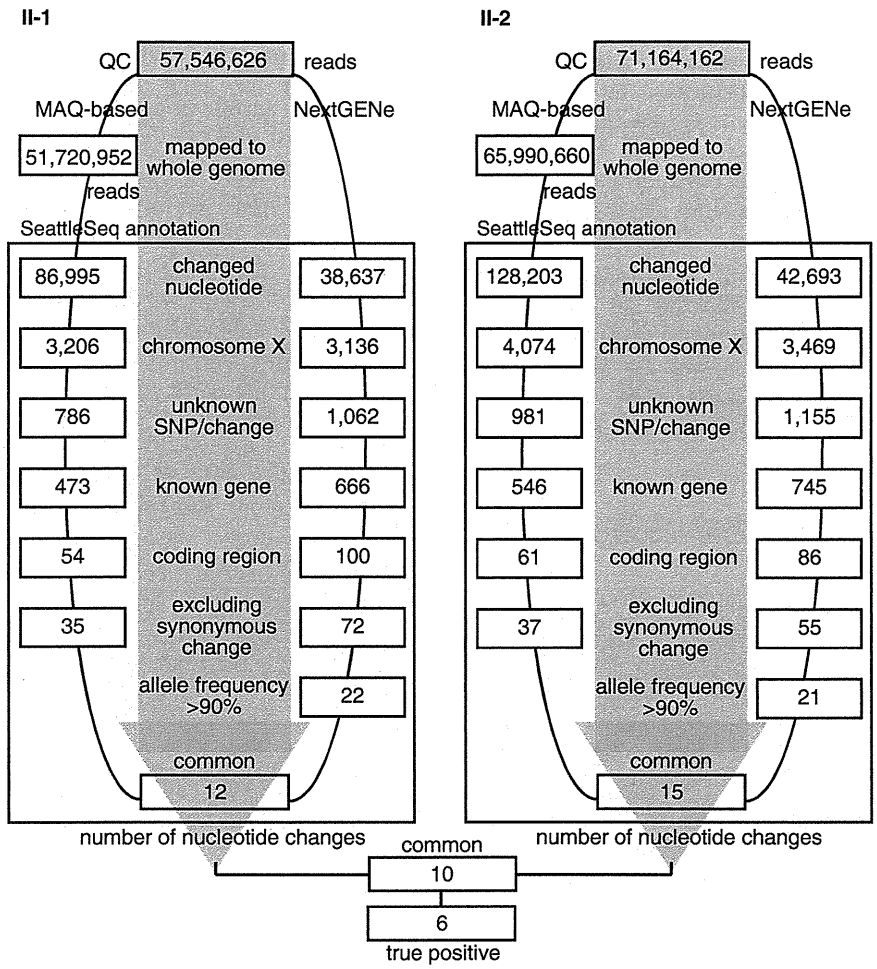

Fig. 2. Flow of informatics analysis. A MAQ-based method and NextGENe analysis were performed in individuals II-1 and II-2. The selection methods employed included variants compared with the human genome reference sequence, variants mapped to chromosome X, unknown variants [excluding registered single-nucleotide polymorphisms (SNPs)], variants in known genes, variants in coding regions, variants excluding synonymous changes, and variants common to the two informatics methods. Finally, the nucleotide changes in common between individuals II-1 and II-2 were focused on as potentially pathogenic mutations. True positive changes were confirmed by capillary sequencing of polymerase chain reaction (PCR) products amplified from genomic DNA.

respectively, of the CDS were at least 90% covered by reads. Using a single lane of sequencing per sample, the coverage with 20 reads or more comprised 89.6% and 89.7% of the CDS, and that with 100 reads or more comprised 87.6% and 89.7% of the CDS in individuals II-1 and II-2, respectively. SNP genotyping indicated that the region from rs727240 to rs721003 (UCSC genome browser hg19 assembly, chromosome X coordinates: 22131639–54454152; 32.2 Mb) was unlinked to the phenotype. Exome sequencing using two informatics methods successfully identified six potentially interesting changes as true positives in the linked region: *FAM123B* (RefSeq Gene ID NM_152424): c.85G>A (p.A29T), *FRMD7* (NM_194277): c.875T>C (p.L292P), *L1CAM* (NM_000425): c.92T>C (p.V31A), *TMEM187* (NM_003492): c.334G>A (p.A112T), *FLNA* (NM_001110556): c.1582G>A (p.V528M), and *LAGE3* (NM_006014): c.395G>A (p.R132Q).

The c.92T>C (p.V31A) variant in *L1CAM* was previously found in a patient with Hirschsprung disease, acrocallosal syndrome, and congenital hydrocephalus (8). *L1CAM* mutations cause a wide variety of clinical phenotypes: hydrocephalus due to stenosis of the aqueduct of Sylvius (MIM #307000), MASA syndrome (mental retardation, aphasia, shuffling gait, adducted thumb; MIM #303350), and X-linked agenesis of the corpus callosum (MIM #217990). Phenotypic variability, even within a family, has been noted, raising the caution that definite clinical diagnosis in single

Table 1. Characterization of nucleotide changes found by exome sequencing

	FAM123B	*FRMD7*	*L1CAM*	*TMEM187*	*FLNA*	*LAGE3*
Change	c.85G>A (p.A29T)	c.875T>C (p.L292P)	c.92T>C (p.V31A)	c.334G>A (p.A112T)	c.1582G>A (p.V528M)	c.395G>A (p.R132Q)
PolyPhen	Benign	Probably damaging	Benign	Benign	Possibly damaging	Benign
PolyPhen-2	Probably damaging	Probably damaging	Benign	Possibly damaging	Possibly damaging	Possibly damaging
SIFT	0.04	0.02	0.22	0.02	0.04	0.46
MutationTaster	Polymorphism	Disease causing	Disease causing	Polymorphism	Polymorphism	Polymorphism
Normal female	8/502[a]	2/502	2/502	1/502	15/502[a]	4/502
Normal male	1/118	0/117	0/118	0/118		1/86
Note		No nystagmus in II-2				

[a]Including one homozygous female. Underlining means that this result excludes the variant as potentially causative. Grayed shading indicates the variants that could not be excluded; between these two, the *L1CAM* variant is more likely to be causative.

cases is often impossible (9). Phenotypic features compatible with the *L1CAM* mutation in our patients include spastic paralysis, aphasia, severe mental and growth retardation, but atypical leukodystrophy and the absence of adducted thumbs were very rare or exceptional (9). A normal control study found that 2 of 251 normal females were heterozygous for this SNP, but none of 117 normal males carried the variant allele. One of the four web-based analyses of pathological significance (MutationTaster) indicated that this variant would be disease causing, while the others indicated that it would be benign (Table 1). X-linked hydrocephalus due to *L1CAM* mutations occurs in approximately 1/30 000 male births (10). Considering that the *L1CAM* mutation was found in 2/618 control alleles (0.32%), the change may be a rare polymorphism, a mutation causing lethality in the majority of affected males, or a mutation with low penetrance. Because we were unable to exclude this *L1CAM* change, its pathogenic status remains inconclusive.

We next examined c.85G>A in *FAM123B*, c.875T>C in *FRMD7*, c.1582G>A in *FLNA*, and c.395G>A in *LAGE3* in normal controls. The *FAM123B*, *FLNA*, and *LAGE3* variants were excluded as causative because a homozygous change was found in 1 of 251 female controls (*FAM123B* and *FLNA*) or a hemizygous change was found in 1 of 86 normal males (*LAGE3*). However, the thick calvarium in individuals II-1 and II-2 may be influenced by the *FAM123B* change, because it is causative for osteopathia striata with cranial sclerosis, an X-linked dominant disorder (MIM #300373) (11, 12). As the calvarium of the patients' mother having the heterozygous *FAM123B* change was not evaluated by CT, we could not confirm this possibility.

Only 2 of 251 control females carried the c.875T>C variant in *FRMD7* heterozygously, and none of 117 male controls carried this variant; thus, the pathogenicity of the *FMRD7* variant was inconclusive. Other *FRMD7* mutations cause X-linked congenital nystagmus 1 (MIM #310700) (13). However, the nystagmus found in individual II-1 was not observed in individual II-2, indicating that the variant in common between two brothers did not consistently cause nystagmus. Thus, it may not contribute to the phenotype in this family (Table 1).

We also evaluated the c.334G>A variant in *TMEM187*. Only 2 of 251 female controls carried this heterozygous change, and it was not found among 118 male controls. Two of the four programs (PolyPhen-2 and SHIFT) indicated that it would be pathogenic. By Taqman assay, *TMEM187* was ubiquitously expressed in various fetal and adult tissues, including the brain (data not shown), leaving the effect of this mutation on the phenotype in these patients inconclusive (Table 1).

In conclusion, we found two possible but inconclusive variants in this family with two boys affected by atypical leukodystrophy. High-throughput technologies are clearly powerful to detect genomic changes, but evaluation of the data can be very difficult and should be performed cautiously. More knowledge of rare SNPs and mutations is absolutely necessary before any conclusions can be drawn.

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References

1. Saitsu H, Kato M, Mizuguchi T et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat Genet 2008: 40: 782–788.
2. Check Hayden E. Genomics shifts focus to rare diseases. Nature 2009: 461: 458.
3. Biesecker LG. Exome sequencing makes medical genomics a reality. Nat Genet 2010: 42: 13–14.
4. Kuhlenbaumer G, Hullmann J, Appenzeller S. Novel genomic techniques open new avenues in the analysis of monogenic disorders. Hum Mutat 2011: 32: 144–151.
5. Miyake N, Kosho T, Mizumoto S et al. Loss-of-function mutations of CHST14 in a new type of Ehlers-Danlos syndrome. Hum Mutat 2010: 31: 966–974.
6. Ng SB, Bigham AW, Buckingham KJ et al. Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. Nat Genet 2010: 42: 790–793.
7. Li H, Ruan J, Durbin R. Mapping short DNA sequencing reads and calling variants using mapping quality scores. Genome Res 2008: 18: 1851–1858.
8. Nakakimura S, Sasaki F, Okada T et al. Hirschsprung's disease, acrocallosal syndrome, and congenital hydrocephalus: report of 2 patients and literature review. J Pediatr Surg 2008: 43: E13–E17.
9. Rietschel M, Friedl W, Uhlhaas S, Neugebauer M, Heimann D, Zerres K. MASA syndrome: clinical variability and linkage analysis. Am J Med Genet 1991: 41: 10–14.
10. Rosenthal A, Jouet M, Kenwrick S. Aberrant splicing of neural cell adhesion molecule L1 mRNA in a family with X-linked hydrocephalus. Nat Genet 1992: 2: 107–112.
11. Viot G, Lacombe D, David A et al. Osteopathia striata cranial sclerosis: non-random X-inactivation suggestive of X-linked dominant inheritance. Am J Med Genet 2002: 107: 1–4.
12. Jenkins ZA, van Kogelenberg M, Morgan T et al. Germline mutations in WTX cause a sclerosing skeletal dysplasia but do not predispose to tumorigenesis. Nat Genet 2009: 41: 95–100.
13. Tarpey P, Thomas S, Sarvananthan N et al. Mutations in FRMD7, a newly identified member of the FERM family, cause X-linked idiopathic congenital nystagmus. Nat Genet 2006: 38: 1242–1244.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Submicroscopic Deletion in 7q31 Encompassing *CADPS2* and *TSPAN12* in a Child With Autism Spectrum Disorder and PHPV

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We performed array comparative genomic hybridization utilizing a whole genome oligonucleotide microarray in a patient with the autism spectrum disorders (ASDs) and persistent hyperplastic primary vitreous (PHPV). Submicroscopic deletions in 7q31 encompassing *CADPS2* (Ca^{2+}-dependent activator protein for secretion 2) and *TSPAN12* (one of the members of the tetraspanin superfamily) were confirmed. The *CADPS2* plays important roles in the release of neurotrophin-3 and brain-derived neurotrophic factor. Mutations in *TSPAN12* are a relatively frequent cause of familial exudative vitreoretinopathy. We speculate that haploinsufficiency of *CADPS2* and *TSPAN12* contributes to ASDs and PHPV, respectively.

Key words: *CADPS2*; *TSPAN12*; autism; PHPV; CGH

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INTRODUCTION

Autism spectrum disorders (ASDs OMIM %209850) are complex neurodevelopmental conditions characterized by social communication disabilities, no or delayed language development, and stereotyped and repetitive behaviors. A number of studies have confirmed that genetic factors play an important role in ASDs.

About 10% of ASDs are associated with a Mendelian syndrome (e.g., fragile X syndrome, tuberous sclerosis and Timothy syndrome). Cytogenetic approaches revealed a high frequency of large chromosomal abnormalities (3–7% of patients), including the most frequently observed maternal 15q11-13 duplication (1–3% of patients). Association studies and mutation analysis of candidate genes have implicated the synaptic genes *NLGN3*(Neuroligin3 OMIM*300336), *NLGN4* (OMIM*300427) [Jamain et al., 2003], *SHANK3* (OMIM*606230)[Durand et al., 2007; Moessner et al., 2007], *NRXN1*(Neurexin1 MIM+600565) [Kim et al., 2008], *SHANK2* (OMIM*603290) [Berkel et al., 2010], and *CNTNAP2* (MIM*604569) [Alarcón et al., 2008; Arking et al., 2008] in ASDs. There is increasing evidence that the *SHANK3-NLGN4-NRNX1* postsynaptic density genes play important roles in the pathogenesis of ASDs.

Recently, on association between de novo copy number variation (CNV) and ASDs was revealed. Sebat et al. [2007] performed comparative genomic hybridization (CGH) on the genomic DNA from ASD patients and unaffected subjects to detect de novo CNV. As a result, they identified CNV in 12 out of 118 (10%) patients with sporadic ASD and confirmed de novo CNV were significantly associated with ASDs. Marshall et al. [2008] performed a genome-wide search for structural abnormalities in 427 unrelated ASD patients using SNP microarray analysis and karyotyping. De novo CNV were found in approximately 7% and approximately 2% of idiopathic families with one ASD child, or two or more ASD siblings, respectively. These authors discovered a CNV at 16p11.2 with an approximate frequency of 1%. Glessner et al. [2009] reported the results from a whole-genome CNV study of many European ASD patients and controls and found several new susceptibility genes encoding neuronal cell-adhesion molecules, including *NLGN1* and *ASTN2*, and genes involved in the ubiquitin pathways, including *UBE3A*, *PARK2*, *RFWD2*, and *FBXO40*. The investigators suggested that two gene networks, neuronal cell-

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adhesion and ubiquitin degradation, that are expressed within the central nervous system contribute to the genetic susceptibility of ASDs.

The International Molecular Genetic Study of Autism Consortium [1998] previously identified linkage loci on chromosomes 7 and 2, which were termed AUTS1 and AUTS5, respectively. Further genetic studies have provided evidence for AUTS1 being located on chromosome 7q [The International Molecular Genetic Study of Autism Consortium 2001]. Screening for mutations in six genes mapping to 7q, *CUTL1*, *SRPK2*, *SYPL*, *LAMB1*, *NRCAM*, and *PTPRZ1* in 48 unrelated individuals with autism led to the identification of several new coding variants in the *CUTL1*, *LAMB1*, and *PTPRZ1*genes [Bonora et al., 2005].

The human Ca^{2+}-dependent activator protein for secretion 2 (*CADPS2*: OMIM*609978) is also located on chromosome 7q31, which is within the AUTS1 locus [Cisternas et al., 2003]. It is a member of the CAPS/CADPS protein family that regulates the secretion of dense-core vesicles, which are abundant in the parallel fiber terminals of granule cells in the cerebellum and play important roles in the release of neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) [Sadakata et al., 2007a,b,c]. BDNF is indispensable for brain development and function, including the formation of synapses. Cisternas et al. [2003] studied *CADPS2* mutations in 90 unrelated autistic individuals, but identified no disease-specific variants. However, Sadakata et al. [2007a] reported that an aberrant, alternatively spliced *CADPS2* mRNA that lacks exon 3 (*CADPS2* Delta exon3) is detected in some patients with ASD.

Persistent hyperplastic primary vitreous (PHPV) is an ocular malformation caused by the presence of a retrolental fibrovascular membrane and the persistence of the posterior portion of the tunica vasculosa lentis and the hyaloid artery. It is often accompanied by microphthalmos, cataracts, and glaucoma. *NDP* (OMIM *300658, X-linked) and *FZD4* (OMIM *604579, dominant) were found to be mutated in unilateral and bilateral PHPV [Shastry, 2009]. These genes also cause Norrie disease and familial exudative vitreoretinopathy (FEVR), which share some clinical features with PHPV. FEVR is a genetically heterogeneous retinal disorder characterized by abnormal vascularization of the peripheral retina, which is often accompanied by retinal detachment. Mutations in the genes encoding *LRP5* (OMIM *603506, dominant and recessive) also cause FEVR. Junge et al. [2009] showed that *Tetraspanin12* (*Tspan12*) is expressed in the retinal vasculature, and loss of *Tspan12* phenocopies defects are seen in Fzd4, *Lrp5*, and *Norrin* mutant mice. *TSPAN12* is one of the members of the tetraspanin superfamily, characterized by the presence of four transmembrane domains. It constitutes large membrane complexes with other molecules. Nikopoulos et al. [2010] applied next-generation sequencing and found a mutation in *TSPAN12* (MIM*613168). Poulter et al. [2010] described seven mutations that were identified in a cohort of 70 FEVR patients without mutations in three known genes. Mutations in *TSPAN12*, which is at 7q31, are a relatively frequent cause of FEVR.

We performed array comparative genomic hybridization (array-CGH) utilizing a 44K whole genome oligonucleotide microarray in a patient with the ASDs and PHPV. Submicroscopic deletions in 7q31 encompassing *CADPS2* and *TSPAN12* were confirmed. We speculate that haploinsufficiency of *CADPS2* and *TSPAN12* contributes to ASD and PHPV, respectively.

CLINICAL REPORT

The patient, a 3-year-old boy, was born to nonconsanguineous healthy Japanese parents. His family history was unremarkable. He was born at 40 weeks' of gestation, his birth weight was 3,100 g, and his birth length was 50.0 cm. After birth, congenital nystagmus was noted, and he did not pursuit objects. An ophthalmological examination revealed bilateral PHPV. Cataract, glaucoma, and FEVR were not present. His gross motor development was normal, and his verbal development was delayed.

At 3 years of age, he came to our hospital for evaluation because of developmental delay. On examination dysmorphic features included a round face, low-set ears, broad eyebrows, apparent hypertelorism, blepharophimosis, hypoplastic alae nasi, a long philtrum, and a small mouth. His visual acuity was low, but he could perform daily activities with some support. In addition, impairment of social interaction, poor social skills, and strict adherence to routine behaviors were noted. He showed stereotypic movements and hyperactivity in his day care room. He was diagnosed as having an ASD according to the DSM-VI criteria. His DQ was 76 according to standard Japanese method. At 3 years and 8 months of age, his height, weight, and head circumference were 88.6 cm (−2.4 SD), 11.7 kg (−1.8 SD), and 46.8 cm (−2.4 S.D), respectively.

The results of routine laboratory tests were unremarkable. G-banded karyotype analysis revealed the following karyotype: 46,XY,inv(4)(p14;q21). Electroencephalography (EEG) showed occipital epileptic discharges. He was free from epileptic seizures.

Ultrasound evaluation revealed echogenic bands in the posterior segments of both globes. Magnetic resonance brain imaging also showed bilateral fibrous intraocular tissue (Fig. 1). However, no specific findings were found in the CNS including the cerebellum.

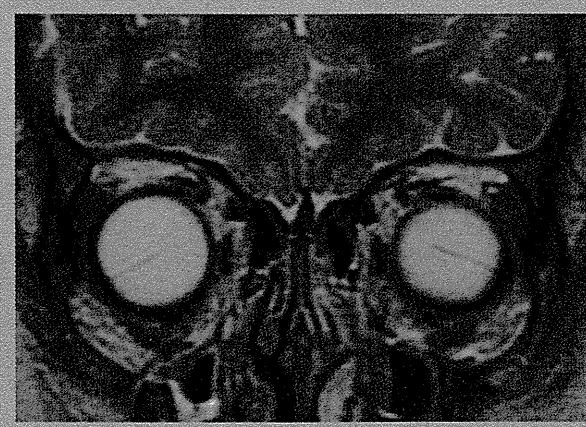

FIG. 1. MR coronal image, T2-weighted. Magnetic resonance imaging also showed fibrous intraocular tissue in the eye. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833]

MATERIALS AND METHODS

After obtaining informed consent based on a permission approved by the institution's ethical committee, peripheral blood samples were obtained from the patient and his parents. Genomic DNA was extracted using the QIAquick DNA extraction kit (QIAgen, Valencia, CA).

Array-CGH analysis was performed using the Human Genome CGH Microarray 44K (Agilent Technologies, Santa Clara, CA), as described previously [Shimojima et al., 2009].

Metaphase nuclei were prepared from peripheral blood lymphocytes using standard methods and were used for FISH analysis with human BAC clones selected from the UCSC genome browser (http://www.genome.ucsc.edu), as described elsewhere [Shimojima et al., 2009]. Physical positions refer to the March 2006 human reference sequence (NCBI Build 36.1).

RESULTS

Using array-CGH analysis, genomic copy number loss was identified in the 7q31.31 region (Fig. 2). The deletion was 5.4 Mb in size and included *CADPS2* and *TSPAN12*, but not *FOXP2*. There were no copy number changes in chromosome 4. FISH analyses confirmed the above deletion (Fig. 3). There were no deletions in either parent indicating de novo occurrence.

DISCUSSION

We described a patient with an ASD and PHPV who demonstrated submicroscopic deletion in chromosome 7q31.31. The deletion resides in the AUTS1 locus on chromosome 7q. The deleted region contained about 20 genes including *CADPS2* and *TSPAN12*. Little data are available about the association of other genes with developmental and ophthalmological disorders. We posit that haploinsufficiency of *CADPS2* and *TSPAN12* contributes to ASDs and PHPV, respectively.

Our patient fulfilled the DSM-VI criteria for an ASD. Poor eye contact, impairment of social interaction, poor social skills with strict adherence to routine, stereotypic movements, and hyperactivity were noted. However, his intellectual disability was mild. Ataxic movement was not observed.

There have been several reports of small deletions on chromosome 7q. Lennon et al. [2007] reported a young male with moderate intellectual disability, dysmorphic features, and language delay who had a deletion in the 7q31.1-7q31.31 region, which included the *FOXP2* gene. The patient demonstrated language impairment, including developmental verbal dyspraxia, but did not meet the criteria for autism. Cukier et al. [2009] reported a chromosomal inversion spanning the region from approximately 7q22.1 to 7q31 in autistic siblings. They suggested that an autism susceptibility gene is located in the chromosome 7q22–31 region. Dauwerse et al.

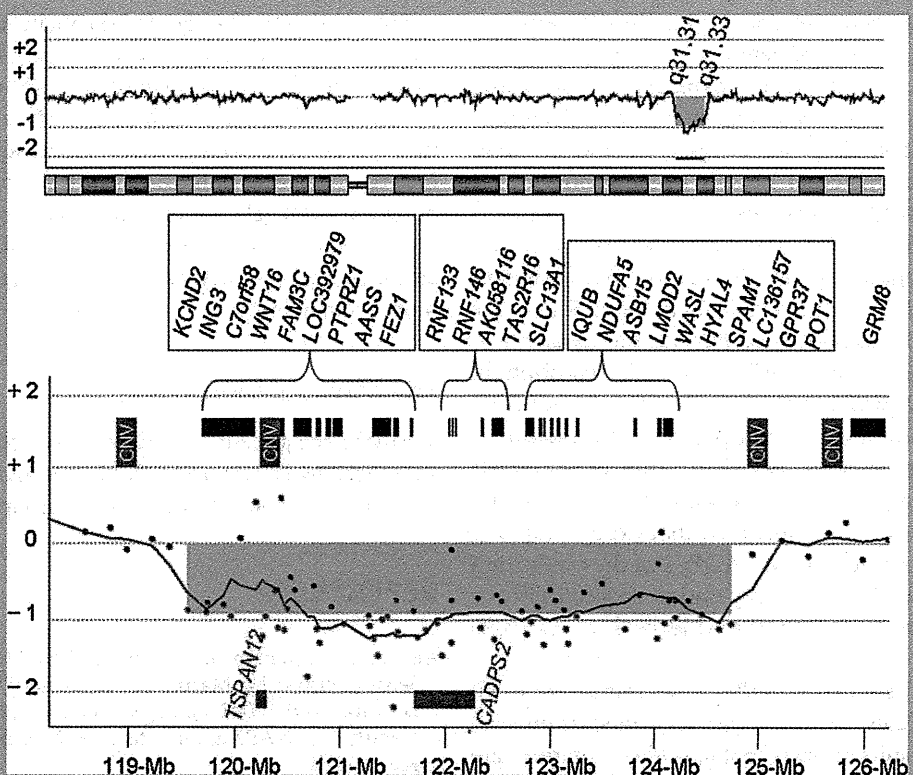

FIG. 2. Array-CGH of the patient. Loss of the genomic copy numbers was identified in the region of 7q31.31. The deletion size was 5.4 Mb and included *CADPS2* and *TSPAN12*.

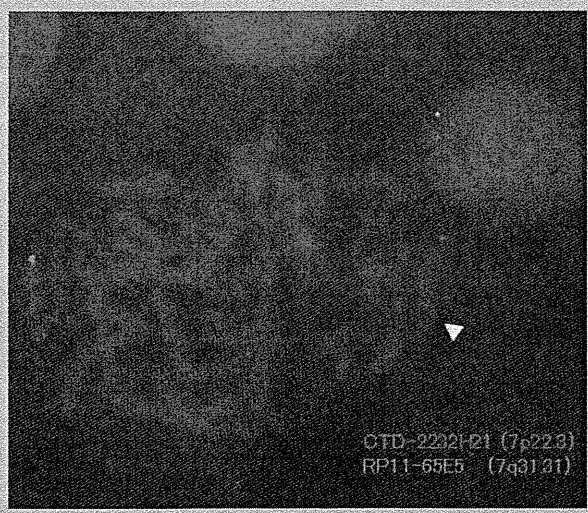

FIG. 3. FISH analyses of the patient. Arrow head indicates the deletion at 7q31.31.

[2010] characterized a de novo complex rearrangement of the long arm of chromosome 7 in a female patient with moderate mental retardation, anxiety disorder, and autistic features and suggested that disruption of the *C7orf58* gene contributed to the anxiety disorder, and autistic features of their patient. The *C7orf58* gene was also deleted in our patient. However, there have been no basic studies on the association of the *C7orf58* gene and brain function. Further studies are necessary on the role of the *C7orf58* gene.

Sadakata et al. [2007b] studied the behavior of *Cadps2−/−* mice. They showed impaired social interaction, hyperactivity, decreased exploratory behavior, and/or increased anxiety in a novel environment and deficits in intrinsic sleep–wake regulation and circadian rhythmicity. In addition, maternal neglect of newborns was a striking feature. They identified that *Cadps2−/−* mice show deficient release of NT-3 and BDNF. Cerebellar development was impaired in the mice. Sadakata et al. [2007a] found an aberrant alternatively spliced *CADPS2* mRNA that lacks exon 3 in some autistic patients. Exon 3 was shown to encode the dynactin 1-binding domain and affect axonal CADPS2 protein distribution. Exon 3-skipped CADPS2 protein possesses almost normal BDNF releasing activity but is not properly transported into the axons of neocortical or cerebellar neurons. However, Eran et al. [2009] observed no difference in prevalence of exon 3 skipping between ASDs and control samples. They concluded that exon 3 skipping represents a normal, minor isoform of CADPS2 in the cerebellum and is likely not a mechanism underlying autism susceptibility or pathogenesis. Our result may reinforce the evidence that *CADPS2* is associated with ASDs.

Cisternas et al. [2003] studied *CADPS2* gene mutations in 90 unrelated autistic individuals. However, they identified no disease-specific variants. Their results indicate that *CADPS2* mutations are not a major cause of ASDs. However, although small deletions of *CADPS2* as found in the present patient, might be rare, they support the idea that *CADPS2* abnormalities are associated with autism susceptibility.

Nikopoulos et al. [2010] reported two missense mutations in five of 11 FEVR families, indicating that mutations in *TSPAN12* are a relatively frequent cause of FEVR. Both residues are completely conserved throughout vertebrate evolution. These authors suggested that both haploinsufficiency and a dominant-negative effect of the mutant TSPAN12 on the wild-type protein should be considered as underlying disease mechanisms. Poulter et al. [2010] described mutations in the *TSPAN12* gene in FEVR patients and suggested that haploinsufficiency of *TSPAN12* causes FEVR because at least four of the seven mutations are predicted to lead to transcripts with premature-termination codons that are likely to be targeted by nonsense-mediated decay.

Recently, the Norrin/Frizzled4 signaling pathway that acts on the on the surface of developing endothelial cells and controls retinal vascular development is highlighted [Ye et al., 2010]. This pathway is composed of Norrin, its transmembrane receptor, Frizzled4, coreceptor, Lrp5, and an auxiliary membrane protein, Tspan12. The resulting signal controls a transcriptional program that regulates endothelial growth and maturation. PHPV and FEVR are associated with their pathogenesis. Our findings indicate that haploinsufficiency of *TSPAN12* is a plausible causative mechanism for PHPV. It will be interesting to study *TSPAN12* abnormalities in PHPV without *NDP* and *FZD4* mutations.

Singh et al. [2006] reported a voltage-gated potassium channel gene mutation in a temporal lobe epilepsy patient, namely a Kv4.2 truncation mutation lacking the last 44 amino acids in the carboxyl terminal. Kv4.2 channel is encoded by the *KCND2* gene. We suggest that the epileptic discharges on EEG reflect neuronal excitability caused by haploinsufficiency of *KCND2*.

Shen et al. [2010] suggested that using chromosomal microarray analysis to test for submicroscopic genomic deletions and duplications should be considered as part of the initial diagnostic evaluation of patients with ASDs. Miller et al. [2010] suggested that the use of chromosomal microarray is recommended as the first-tier cytogenetic diagnostic test for patients with unexplained developmental delay/intellectual disability, ASDs, or multiple congenital anomalies. In patients with ASDs and other anomalies, chromosomal microarray may be the useful method to clarify the underlying defect.

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REFERENCES

Alarcón M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, Sebat J, Wigler M, Martin CL, Ledbetter DH, Nelson SF, Cantor RM, Geschwind DH. 2008. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. Am J Hum Genet 82:150–159.

Arking DE, Cutler DJ, Brune CW, Teslovich TM, West K, Ikeda M, Rea A, Guy M, Lin S, Cook EH, Chakravarti A. 2008. A common genetic variant in the neurexin superfamily member CNTNAP2 increases familial risk of autism. Am J Hum Genet 82:160–164.

Berkel S, Marshall CR, Weiss B, Howe J, Roeth R, Moog U, Endris V, Roberts W, Szatmari P, Pinto D, Bonin M, Riess A, Engels H, Sprengel R, Scherer SW, Rappold GA. 2010. Mutations in the SHANK2 synaptic scaffolding gene in autism spectrum disorder and mental retardation. Nat Genet 42:489–491.

Bonora E, Lamb JA, Barnby G, Sykes N, Moberly T, Beyer KS, Klauck SM, Poustka F, Bacchelli E, Blasi F, Maestrini E, Battaglia A, Haracopos D, Pedersen L, Isager T, Eriksen G, Viskum B, Sorensen EU, Brondum-Nielsen K, Cotterill R, Engeland H, Jonge M, Kemner C, Steggehuis K, Scherpenisse M, Rutter M, Bolton PF, Parr JR, Poustka A, Bailey AJ, Monaco AP, International Molecular Genetic Study of Austism Consortium. 2005. Mutation screening and association analysis of six candidate genes for autism on chromosome 7q. Eur J Hum Genet 13:198–207.

Cisternas FA, Vincent JB, Scherer SW, Ray PN. 2003. Cloning and characterization of human CADPS and CADPS2, new members of the Ca^{2+}-dependent activator for secretion protein family. Genomics 81: 279–291.

Cukier HN, Skaar DA, Rayner-Evans MY, Konidari I, Whitehead PL, Jaworski JM, Cuccaro ML, Pericak-Vance MA, Gilbert JR. 2009. Identification of chromosome 7 inversion breakpoints in an autistic family narrows candidate region for autism susceptibility. Autism Res 2: 258–266.

Dauwerse JG, Ruivenkamp CA, Hansson K, Marijnissen GM, Peters DJ, Breuning MH, Hilhorst-Hofstee Y. 2010. A complex chromosome 7q rearrangement identified in a patient with mental retardation, anxiety disorder, and autistic features. Am J Med Genet Part A 152A:427–433.

Durand CM, Betancur C, Boeckers TM, Bockmann J, Chaste P, Fauchereau F, Nygren G, Rastam M, Gillberg IC, Anckarsäter H, Sponheim E, Goubran-Botros H, Delorme R, Chabane N, Mouren-Simeoni MC, de Mas P, Bieth E, Rogé B, Héron D, Burglen L, Gillberg C, Leboyer M, Bourgeron T. 2007. Mutations in the gene encoding the synaptic scaffolding protein SHANK3 are associated with autism spectrum disorders. Nat Genet 39:25–27.

Eran A, Graham KR, Vatalaro K, McCarthy J, Collins C, Peters H, Brewster SJ, Hanson E, Hundley R, Rappaport L, Holm IA, Kohane IS, Kunkel LM. 2009. Comment on "Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients". J Clin Invest 119: 679–680.

Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, Zhang H, Estes A, Brune CW, Bradfield JP, Imielinski M, Frackelton EC, Reichert J, Crawford EL, Munson J, Sleiman PM, Chiavacci R, Annaiah K, Thomas K, Hou C, Glaberson W, Flory J, Otieno F, Garris M, Soorya L, Klei L, Piven J, Meyer KJ, Anagnostou E, Sakurai T, Game RM, Rudd DS, Zurawiecki D, McDougle CJ, Davis LK, Miller J, Posey DJ, Michaels S, Kolevzon A, Silverman JM, Bernier R, Levy SE, Schultz RT, Dawson G, Owley T, McMahon WM, Wassink TH, Sweeney JA, Nurnberger JI, Coon H, Sutcliffe JS, Minshew NJ, Grant SF, Bucan M, Cook EH, Buxbaum JD, Devlin B, Schellenberg GD, Hakonarson H. 2009. Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature 459:569–573.

International Molecular Genetic Study of Autism Consortium. 1998. A full genome screen for autism with evidence for linkage to a region on chromosome 7q. Hum Mol Genet 7:571–578.

International Molecular Genetic Study of Autism Consortium (IMGSAC). 2001. Further characterization of the autism susceptibility locus AUTS1 on chromosome 7q. Hum Mol Genet 10:973–982.

Jamain S, Quach H, Betancur C, Råstam M, Colineaux C, Gillberg IC, Soderstrom H, Giros B, Leboyer M, Gillberg C, Bourgeron T. Paris Autism Research International Sibpair Study. 2003. Mutations of the X-linked genes encoding neuroligins NLGN3 and NLGN4 are associated with autism. Nat Genet 34:27–29.

Junge HJ, Yang S, Burton JB, Paes K, Shu X, French DM, Costa M, Rice DS, Ye W. 2009. TSPAN12 regulates retinal vascular development by promoting Norrin- but not Wnt-induced FZD4/beta-catenin signaling. Cell 139:299–311.

Kim HG, Kishikawa S, Higgins AW, Seong IS, Donovan DJ, Shen Y, Lally E, Weiss LA, Najm J, Kutsche K, Descartes M, Holt L, Braddock S, Troxell R, Kaplan L, Volkmar F, Klin A, Tsatsanis K, Harris DJ, Noens I, Pauls DL, Daly MJ, MacDonald ME, Morton CC, Quade BJ, Gusella JF. 2008. Disruption of neurexin 1 associated with autism spectrum disorder. Am J Hum Genet 82:199–207.

Lennon PA, Cooper ML, Peiffer DA, Gunderson KL, Patel A, Peters S, Cheung SW, Bacino CA. 2007. Deletion of 7q31.1 supports involvement of FOXP2 in language impairment: Clinical report and review. Am J Med Genet A 143A:791–798.

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW. 2008. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488.

Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. 2010. 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.

Moessner R, Marshall CR, Sutcliffe JS, Skaug J, Pinto D, Vincent J, Zwaigenbaum L, Fernandez B, Roberts W, Szatmari P, Scherer SW. 2007. Contribution of SHANK3 mutations to autism spectrum disorder. Am J Hum Genet 81:1289–1297.

Nikopoulos K, Gilissen C, Hoischen A, van Nouhuys CE, Boonstra FN, Blokland EA, Arts P, Wieskamp N, Strom TM, Ayuso C, Tilanus MA, Bouwhuis S, Mukhopadhyay A, Scheffer H, Hoefsloot LH, Veltman JA, Cremers FP, Collin RW. 2010. Next-generation sequencing of a 40 Mb linkage interval reveals TSPAN12 mutations in patients with familial exudative vitreoretinopathy. Am J Hum Genet 86:240–247.

Poulter JA, Ali M, Gilmour DF, Rice A, Kondo H, Hayashi K, Mackey DA, Kearns LS, Ruddle JB, Craig JE, Pierce EA, Downey LM, Mohamed MD, Markham AF, Inglehearn CF, Toomes C. 2010. Mutations in TSPAN12 cause autosomal-dominant familial exudative vitreoretinopathy. Am J Hum Genet 86:248–253.

Sadakata T, Washida M, Iwayama Y, Shoji S, Sato Y, Ohkura T, Katoh-Semba R, Nakajima M, Sekine Y, Tanaka M, Nakamura K, Iwata Y, Tsuchiya KJ, Mori N, Detera-Wadleigh SD, Ichikawa H, Itohara S, Yoshikawa T, Furuichi T. 2007a. Autistic-like phenotypes in Cadps2-knockout mice and aberrant CADPS2 splicing in autistic patients. J Clin Invest 117:931–943.

Sadakata T, Kakegawa W, Mizoguchi A, Washida M, Katoh-Semba R, Shutoh F, Okamoto T, Nakashima H, Kimura K, Tanaka M, Sekine Y, Itohara S, Yuzaki M, Nagao S, Furuichi T. 2007b. Impaired cerebellar development and function in mice lacking CAPS2, a protein involved in neurotrophin release. J Neurosci 27:2472–2482.

Sadakata T, Washida M, Furuichi T. 2007c. Alternative splicing variations in mouse CAP S2: Differential expression and functional properties of splicing variants. BMC Neurosci 8:25.

Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J, Leotta A, Pai D, Zhang R, Lee YH, Hicks

J, Spence SJ, Lee AT, Puura K, Lehtimäki T, Ledbetter D, Gregersen PK, Bregman J, Sutcliffe JS, Jobanputra V, Chung W, Warburton D, King MC, Skuse D, Geschwind DH, Gilliam TC, Ye K, Wigler M. 2007. Strong association of de novo copy number mutations with autism. Science 316:445–449.

Shastry BS. 2009. Persistent hyperplastic primary vitreous: Congenital malformation of the eye. Clin Experiment Ophthalmol 37:884–890.

Shen Y, Dies KA, Holm IA, Bridgemohan C, Sobeih MM, Caronna EB, Miller KJ, Frazier JA, Silverstein I, Picker J, Weissman L, Raffalli P, Jeste S, Demmer LA, Peters HK, Brewster SJ, Kowalczyk SJ, Rosen-Sheidley B, McGowan C, Duda AW III, Lincoln SA, Lowe KR, Schonwald A, Robbins M, Hisama F, Wolff R, Becker R, Nasir R, Urion DK, Milunsky JM, Rappaport L, Gusella JF, Walsh CA, Wu BL, Miller DT. Autism Consortium Clinical Genetics/DNA Diagnostics Collaboration. 2010. Clinical genetic testing for patients with autism spectrum disorders. Pediatrics 125:e727–e735.

Shimojima K, Páez MT, Kurosawa K, Yamamoto T. 2009. Proximal interstitial 1p36 deletion syndrome: The most proximal 3.5-Mb microdeletion identified on a dysmorphic and mentally retarded patient with inv(3)(p14.1q26.2). Brain Development 31:629–633.

Singh B, Ogiwara I, Kaneda M, Tokonami N, Mazaki E, Baba K, Matsuda K, Inoue Y, Yamakawa K. 2006. A Kv4.2 truncation mutation in a patient with temporal lobe epilepsy. Neurobiol Dis 24:245–253.

Ye X, Wang Y, Nathans J. 2010. The Norrin/Frizzled4 signaling pathway in retinal vascular development and disease. Trends Mol Med 16:417–425.

Reduced expression by *SETBP1* haploinsufficiency causes developmental and expressive language delay indicating a phenotype distinct from Schinzel –Giedion syndrome

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Reduced expression by *SETBP1* haploinsufficiency causes developmental and expressive language delay indicating a phenotype distinct from Schinzel–Giedion syndrome

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ABSTRACT

Background Mutations of the SET binding protein 1 gene (*SETBP1*) on 18q12.3 have recently been reported to cause Schinzel–Giedion syndrome (SGS). As rare 18q interstitial deletions affecting multiple genes including *SETBP1* correlate with a milder phenotype, including minor physical anomalies and developmental and expressive speech delay, mutations in *SETBP1* are thought to result in a gain-of-function or a dominant-negative effect. However, the consequence of the *SETBP1* loss-of-function has not yet been well described.

Methods Microarray-based comparative genomic hybridisation (aCGH) analyses were performed to identify genetic causes for developmental and expressive speech delay in two patients. SETBP1 expression in fibroblasts obtained from one of the patients was analysed by real-time RT-PCR and western blotting. A cohort study to identify nucleotide changes in *SETBP1* was performed in 142 Japanese patients with developmental delay.

Results aCGH analyses identified submicroscopic deletions of less than 1 Mb exclusively containing *SETBP1*. Both patients show global developmental, expressive language delay and minor facial anomalies. Decreased expression of SETBP1 was identified in the patient's skin fibroblasts. No pathogenic mutation of *SETBP1* was identified in the cohort study.

Conclusion SETBP1 expression was reduced in a patient with *SETBP1* haploinsufficiency, indicating that the *SETBP1* deletion phenotype is allele dose sensitive. In correlation with the exclusive deletion of *SETBP1*, this study delimits a milder phenotype distinct from SGS overlapping with the previously described phenotype of del(18)(q12.2q21.1) syndrome including global developmental, expressive language delay and distinctive facial features. These findings support the hypothesis that mutations in *SETBP1* causing SGS may have a gain-of-function or a dominant-negative effect, whereas haploinsufficiency or loss-of-function mutations in *SETBP1* cause a milder phenotype.

Mutations in the SET binding protein 1 gene (*SETBP1*) have recently been shown to cause Schinzel–Giedion syndrome (SGS, MIM #269150).[1] Whole-exome sequencing for four patients with SGS identified nucleotide alterations in the conserved region of *SETBP1*. Further analyses by standard Sanger sequencing for nine patients with SGS were performed, and eight of the nine patients showed *SETBP1* mutations. All five identified mutations were missense mutations, rather than nonsense mutations or truncations. As previously reported, rare chromosomal deletions in 18q including *SETBP1* correlate with a milder phenotype, and the severe SGS phenotype was proposed to be the consequence of a gain-of-function or dominant-negative effect of the mutations. However, the exact function of the gene is not known, and the consequences of an exclusive *SETBP1* loss-of-function or haploinsufficiency are not well described.

We identified de novo heterozygous microdeletions containing exclusively *SETBP1* in two patients with developmental, expressive language delay and distinctive facial features. The phenotypes are milder and differ significantly from the severe clinical appearance of SGS. Genotype–phenotype correlations of *SETBP1* haploinsufficiency are demonstrated in this study and discussed.

PATIENTS AND METHODS

Patients

After informed consent based on permission from the ethics committee of the institutions or individual written consent had been obtained, peripheral blood samples were taken from patients with developmental delay of unidentified aetiology to investigate potential genomic copy number aberrations.

Patients' reports

Patient 1 (DECIPHER #TWM253969) is a 7-year old boy, the second child of non-consanguineous parents (https://decipher.sanger.ac.uk/). His 10-year-old sister is healthy and normally developed. He was born with a birth weight of 2504 g (3–10th centile), length of 47 cm (10–25th centile), and occipitofrontal circumference (OFC) of 33.5 cm (=50th centile). At the time of his birth, his father and mother were 34 and 40 years old, respectively. His development was moderately delayed with crawling at 1 year, free walking at 2 years, and the first word at 5 years. He suffered febrile seizures several times, but EEG and brain MRI showed no abnormal findings. At 7 years, his height was 115 cm (25–50th centile), weight was 15.0 kg (<3rd centile), and OFC was 49.3 cm (3–10th centile). He showed distinctive facial features with an inverted triangle face, prominent forehead, ptosis with periorbital fullness, epicanthus and

pointed chin (figure 1A). He can walk by himself and can speak only a few words. The Kyoto developmental scale measured his developmental quotient as 40, which indicated moderate developmental delay. Visual acuity examination showed a refractive error of +8D in both eyes, indicating hyperopia. Previously performed conventional chromosomal analysis showed a normal male karyotype of 46,XY.

Patient 2, the 3rd child of non-consanguineous healthy parents, was born at 38 weeks by caesarean section for breech presentation after an uneventful pregnancy. In the neonatal period, the boy was hypotonic, sleepy and passive and rarely cried. He showed significantly delayed motor development, with sitting at 14 months and walking at 2 years, as well as delayed pincer grip. Initially, a discrete hemiparesis of the left part of his body manifested only while running with a slight spastic posture of his left hand and gait asymmetry suggested a perinatal or prenatal stroke. Cerebral MRI at the age of 4 years was normal except an unspecific T2 hyperintense infratentorial lesion in the right cranial paramedian cerebellum. The patient still exhibits coordination deficits in fine motoricity. His growth parameters are in the normal range (75th–90th centile), and OFC is within the 10th–25th centile. Hearing was found to be normal. Interestingly, the boy has not developed any expressive speech at all to date, whereas receptive language abilities are intact. He actively communicates using gestures illustrating his demands and ideas, but well understands his interlocutor, permitting a bidirectional exchange. He exhibits kind and social behaviour but at the same time a restless search for interactive communication. He has difficulty concentrating and has no sense of danger or pain. Facial dysmorphisms include frontal upsweep, a lighter blond hair corona in the front, hypertelorism, ptosis of eyelids predominantly on the left, periorbital fullness, straight and sparse eyebrows, flat nasal bridge, short nose, thin upper lip, short fingers and broad distal phalanges (figure 1B–D). No major malformations have been found. Microcytic hypochrome anaemia remains unexplained; the search for HbH inclusion bodies which would indicate X-linked α-thalassaemia/mental retardation syndrome was negative.

Microarray-based comparative genomic hybridisation (aCGH)

aCGH analyses were performed using the Human Genome CGH Microarray 44K (Agilent Technologies, Santa Clara, California, USA) and the whole genome tiling NimbleGen CGH array (Human CGH 2.1M WG-T v2.0; NimbleGen; Roche NimbleGen Inc, Madison, Wisconsin, USA) for patient 1 and patient 2, respectively, according to the manufacturer's protocols.

Fluorescence in situ hybridisation

Identified aberrations were confirmed by fluorescence in situ hybridisation (FISH) using locus-specific BAC clones as probes. In patient 1, two clones, CTD-3236P11 on 18q12.3 (chr18:40 779 351–40 864 576) as a target and RP11-105C15 on 18p11.31 (chr18:5 910 725–60 63 460) as a marker, were selected from the UCSC genome browser (http://www.genome.ucsc.edu). In patient 2, the locus-specific probe RP11-24L5 (BlueGnome, Cambridge, UK) in the region 18q12.3 (chr18:40 588 784–40 776 858) was used on metaphase spreads. Physical positions refer to the March 2006 human reference sequence (NCBI Build 36.1).

Expression analysis of SETBP1

Total RNAs were extracted from cultured skin fibroblasts from patient 1 and the control individual using the ISOGEN RNA extraction kit (Wako, Osaka, Japan), reverse-transcribed to complementary DNA (cDNA) using the SuperScript VILO cDNA Synthesis Kit (Life Technologies, Carlsbad, California, USA) according to the manufacturer's instructions, then used as templates for real-time PCR using Power SYBR Green PCR master mix (Life Technologies). Primers for SETBP1 mRNA were designed in the coding region (SETBP1 nt374F; 5′-GTCCA CCTGAGATCAAGATC-3′ and SETBP1 nt663R; 5′-GTCCATGT GGTTCTGGCTGC-3′). Beta actin primers (5′-GGCACCCAGCA CAATGAAGATC-3′ and 5′-AAGTCATAGTCCGCCTAGAAGC-3′)

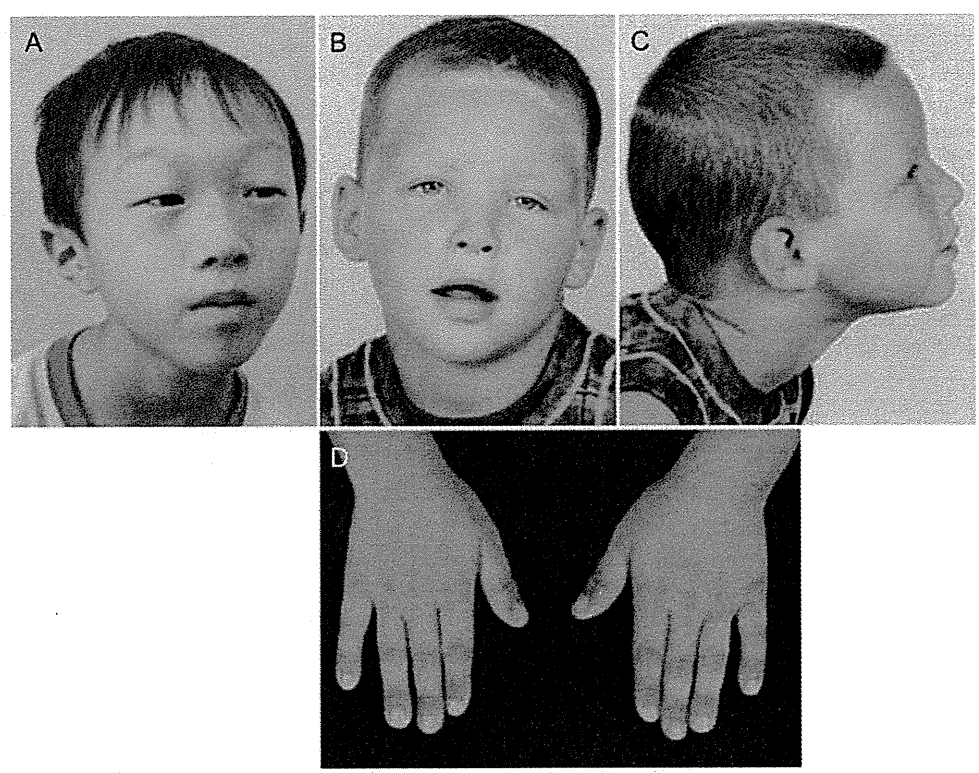

Figure 1 Phenotypes of the patients. (A) Patient 1; (B,C) frontal and lateral views of patient 2; (D) both hands of patient 2.

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