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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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Short Report

Targeted next-generation sequencing in the diagnosis of neurodevelopmental disorders

Okamoto N., Miya F., Tsunoda T., Kato M., Saitoh S., Yamasaki M., Shimizu A., Torii C., Kanemura Y., Kosaki K.. Targeted next-generation sequencing in the diagnosis of neurodevelopmental disorders. Clin Genet 2014. © John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, 2014

We developed a next-generation sequencing (NGS) based mutation screening strategy for neurodevelopmental diseases. Using this system, we screened 284 genes in 40 patients. Several novel mutations were discovered. Patient 1 had a novel mutation in *ACTB*. Her dysmorphic feature was mild for Baraitser-Winter syndrome. Patient 2 had a truncating mutation of *DYRK1A*. She lacked microcephaly, which was previously assumed to be a constant feature of *DYRK1A* loss of function. Patient 3 had a novel mutation in *GABRD* gene. She showed Rett syndrome like features. Patient 4 was diagnosed with Noonan syndrome with *PTPN11* mutation. He showed complete agenesis of corpus callosum. We have discussed these novel findings.

Conflict of interest

The authors report no conflicts of interest.

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Key words: Baraitser-Winter syndrome – *DYRK1A* – *GABRD* – next-generation sequencing

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Despite many recent studies focusing on discovering the genetic basis of neurodevelopmental diseases, it is still largely unknown. We developed a next-generation sequencing (NGS) based mutation screening strategy. We screened 284 genes known or predicted to be associated with neurodevelopmental disorders with microcephaly/macrocephaly, central nervous system (CNS) anomalies and intellectual disability (ID).

Materials and methods

We studied 40 patients with neurodevelopmental disorders. They were negative for conventional cytogenetic studies and microarray analysis. With the approval of our institutional ethics committee, the patients were analyzed using this targeted sequencing. The genomic DNA of each patient was extracted from peripheral blood using extraction kit. Detail of the cell sample preparation was described in Supporting information.

Target gene sequencing

Three microgram of each sample DNA was sheared to 150–200 bp using the Covaris DNA Shearing System (Wobum, MA, USA). To capture the target exonic DNA, we used the SureSelectXT Custom capture library (Agilent, Santa Clara, CA) for 1.6 Mb of exons of neuronal gene capture. The sequence library was constructed with the SureSelect XT Target Enrichment System for Illumina Paired-End Sequencing Library kit (Agilent) according to the manufacturer's instructions. We performed DNA sequencing of either 76- or 101-bp paired-end reads using the Illumina Genome Analyzer IIx (Illumina, San Diego, CA) and HiSeq 2000 sequencer (Illumina, San Diego, CA).

Single nucleotide variation (SNV) calling

NGS reads were aligned to the Human reference genome (GRCh37/hg19). We then excluded polymerase chain reaction (PCR) duplicates, and extracted reads uniquely mapped to the reference genome that were properly paired within the insert size within mean ± 2 standard deviation (SD) of the mean. Base calling was performed in on-target regions, those regions within 100 bp upstream and downstream of the exon capture probes. SNV and insertion and deletion (indel) calling were performed using SAM TOOLS and GATK software. We excluded known variants found in database. We then narrowed the candidates to only non-synonymous, nonsense and splice site SNVs and frame shift indels. More details of method for variant calling are described in Supporting information.

NGS base-call quality check

To analyze the quality of our base-calling algorithm, we used genotypes from HapMap database (release #28, obtained from ftp://ftp.ncbi.nlm.nih.gov/hapmap/genotypes/2010-08_phaseII+III/). Sanger sequence validation of SNVs was performed using Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA).

Results

To identify the causal mutation for neuronal diseases, we designed custom capture probes for the exons of 284 neuronal genes (Table S1, Supporting information). We performed targeted genes sequencing using these probes and generated 1.7 Gb of sequence on average. The average read depth of the on-target regions was 608. To check the quality of our NGS base calls, we sequenced HapMap-JPT NA18943 using the same method as the other samples, and compared our NGS calls with the released genotype of the HapMap consortium. The genotypes for 3129 locations were comparable between the two data sets. All but 16 of the 3129 genotypes were concordant between our NGS calls and the HapMap data. We validated these mismatched 16 positions using Sanger sequencing and all 16 were consistent with our NGS calls (Tables S2 and S3). On the basis of this, we estimate the false positive and false negative rate of our SNV calling to be $<0.032\%$ ($<1/3129$).

Clinical reports

In all patients, developmental quotient (DQ) was measured using the Kyoto Scale of Psychological Development test.

Patient 1 with *ACTB* mutation

The 3-year-old female was born at 37 weeks of gestation by normal delivery. Her developmental milestones were markedly delayed. She sat unsupported at 18 months of age. Recently, she walked with support. She spoke several meaningful words. Her DQ was 39 at 2 years of age. Physical examination identified dysmorphic features, including a flat face, arched eyebrows, narrow palpebral fissures, low-set posteriorly rotated ears and a thin upper lip. Ophthalmological investigation revealed no colobomata. Her height was 86.3 cm (−0.8 SD), and weight was 12.3 kg (mean). Her head circumference was 50 cm (+1.2 SD) at 2 years and 6 months of age. Neuroradiological investigations revealed enlarged lateral ventricles, decreased white matter volume and pachygyria dominant in the frontal lobe (Fig. 1a,b).

A novel missense was identified in *ACTB*, c.733G>A, p.G245S. She was therefore diagnosed with Baraitser-Winter syndrome (BRWS) (1).

Patient 2 with *DYRK1A* mutation

The 7-year-old female patient was born at 39 weeks of gestation by induced delivery. Her developmental milestones were severely retarded. She could not walk independently. She had no communicative language. In addition, her visual acuity was disturbed by severe amblyopia. She could see and reach objects within 30 cm. She exhibited self-injurious behavior, temper tantrums and vocal tics by vibrating her palate. She

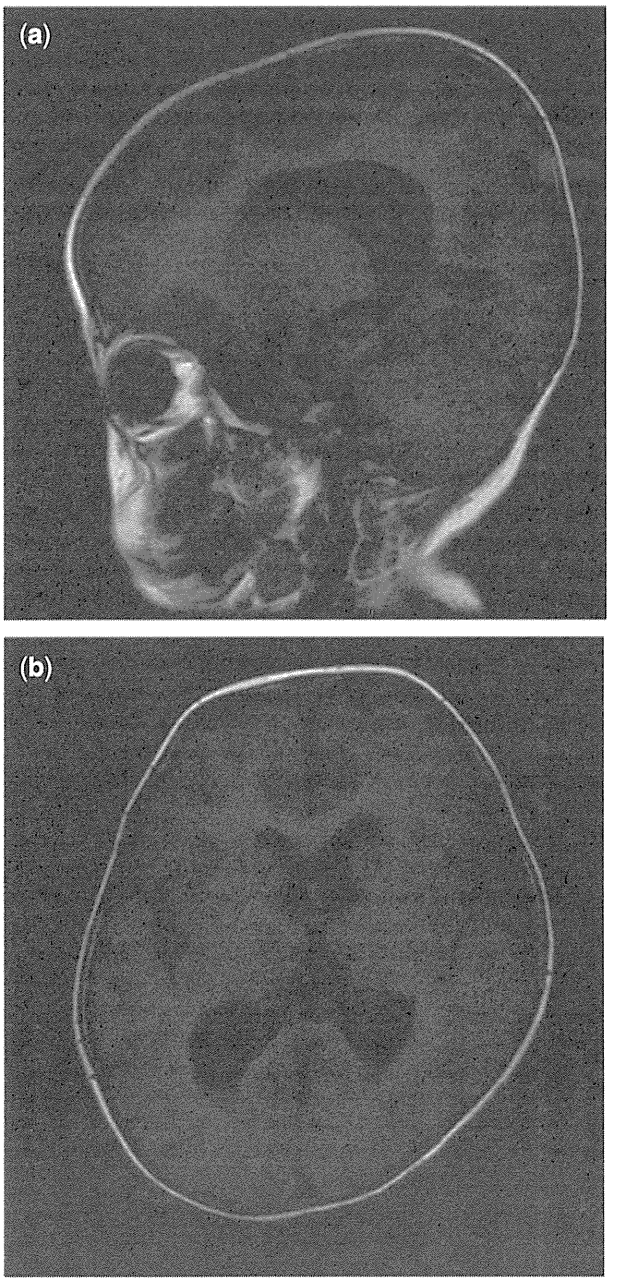

Fig. 1. (**a**, **b**) T1-weighted magnetic resonance image (MR) of patient 1 with *ACTB* mutation shows enlarged lateral ventricles, decreased white matter volume and pachygyria dominant in the frontal lobe.

was diagnosed with autism spectrum disorder (ASD) according to DSM-5. Her DQ was not properly assessed because of visual disturbance.

Physical examination identified dysmorphic features, including frontal bossing, hypertelorism, nystagmus, epicanthal folds, a flat nasal bridge, bilateral low-set ears, down-slanting palpebral fissures, a short philtrum, a high arched palate, downturned mouth and micrognathia. Her weight was 14.6 kg (−2.2 SD), height was 103.5 cm (−3.1 SD) and head circumference was 52 cm (+0.6 SD). She showed relative macrocephaly.

Brain computerized tomography (CT) and magnetic resonance imaging (MRI) were normal. Retinal abnormalities and optic nerve hypoplasia were not identified by fundoscopic investigations. Electroencephalography (EEG) showed no epileptic discharges. She had an early termination codon in exon 11 of the *DYRK1A* gene (c.C1699T: p.Q567*).

Patient 3 with *GABRD* mutation

The 12-year-old female was born at 41 weeks of gestation by induced delivery. Her development was severely retarded with generalized muscular hypotonia. She sat alone at 4 years of age. She cannot walk independently. She spoke no meaningful words. Her DQ was 12 at 9 years of age. She showed stereotyped behavior including hand gripping and bruxism. Purposeful hand skills were not obtained. She was diagnosed with Rett syndrome. EEG revealed bilateral occipital dominant high voltage slow spike and wave complex. Her height was 137 cm (−3.4 SD), weight was 35 kg (−2.1 SD) and head circumference was 51 cm (−1.8 SD). Brain CT and MRI were normal.

She had 2 bp insertion–deletion corresponding to two amino acids in *GABRD* gene (c.G498A:p.M166I and, c.G499A: p.D167N) (Fig. 2). This mutation was *de novo*.

Patient 4 with *PTPN11* mutation

The 4-year-old male was born at 40 weeks of gestation by normal delivery. Profound sensorineural hearing loss was confirmed. He was able to control his head at 4 months, roll over at 6 months of age. He could sit without support at 14 months of age. He started to walk without support at 3 years of age. His height was 90.7 cm (−1.8 SD), weight was 14.3 kg (−0.4 SD) and head circumference was 48.3 cm (−1.1 SD). Brain MRI at 4 years of age showed agenesis of corpus callosum (ACC) (Fig. 3). His DQ was 40. His dysmorphic features including hypertelorism, epicanthal folds, flat nasal bridge, low set ears, growth failure and ACC suggested the diagnosis of Mowat-Wilson syndrome. However, molecular analysis of *ZEB2* mutation was negative. Target gene sequencing revealed a heterozygous mutation in the *PTPN11* gene (c.A188G, p.Y63C). This mutation has been repeatedly reported in Noonan syndrome (NS) (2). We reevaluated his clinical features and concluded that the diagnosis of NS is appropriate. This is the first association of ACC and NS with *PTPN11* mutation.

Other patients

Three patients with cerebellar anomalies were diagnosed with mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH) due to *CASK* mutations. Another patient was homozygous for *AHI1* mutation. The diagnosis of Joubert syndrome was confirmed. They showed typical findings.

Discussion

ACTB mutation in patient 1 was predicted to be pathogenic in *in silico* analysis. BRWS is a rare

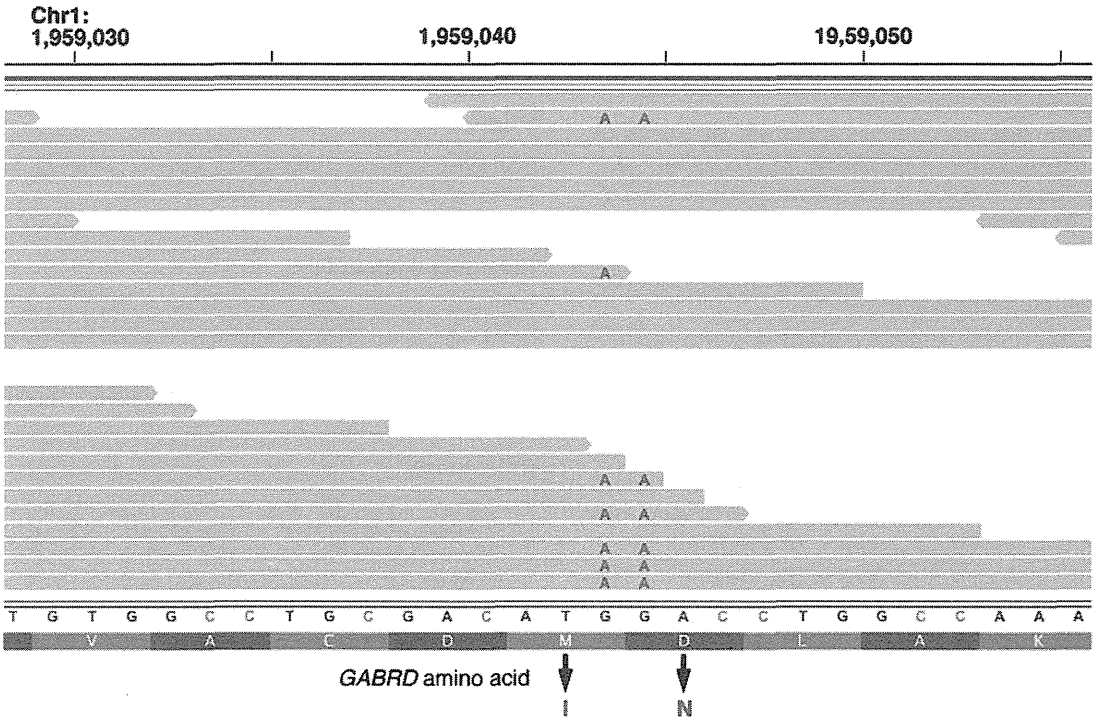

Fig. 2. Patient 3 had 2 bp insertion–deletion corresponding to two amino acids in *GABRD* gene (NM_000815: exon5: c.G498A: p.M166I and NM_000815: exon5: c.G499A: p.D167N).

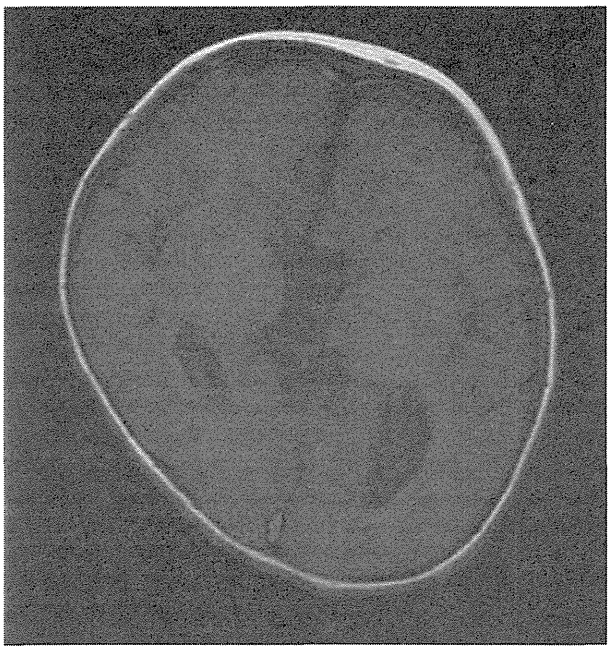

Fig. 3. Brain magnetic resonance imaging (MRI) of patient 4 with *PTPN11* mutation showed agenesis of corpus callosum

MCA/ID syndrome characterized by dysmorphic features, including ptosis, colobomata and neuronal migration anomalies (1). Rivière et al. (3) reported that mutations in *ACTB* and *ACTG1* cause BRWS. Clinical variability of BRWS is often discussed. Di Donato et al. (4) reported three patients with Fryns-Aftimos syndrome (FAS) who had a mutation in the *ACTB* gene. They suggested that mutations in *ACTB* cause a distinctly more severe phenotype than *ACTG1* mutations. They concluded that FAS is an early and severe manifestation of BRWS. Patient 1 did not show the typical features of BRWS. Her dysmorphic features were mild, and her head circumference was over average size. Recently, Verloes et al. (5) delineated the spectrum in 42 patients with BRWS. They reported that facial dysmorphism varies from mild to severe and evolves considerably over times. They suggested the designation of Baraitser-Winter cerebrofrontofacial syndrome.

Patient 2 had had severe ID, motor disturbance, autistic behavior and visual problems. She had truncating mutation of *DYRK1A*. She lacked microcephaly, which was previously assumed to be a constant feature of *DYRK1A* loss of function. *DYRK1A* is a protein kinase that belongs to the highly conserved dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family. *DYRK1A* is a highly conserved gene located in the Down syndrome critical region at 21q22. *DYRK1A* is involved in brain growth through neuronal proliferation and neurogenesis. *DYRK1A* overexpression has been implicated in ID and microcephaly in Down syndrome.

Haploinsufficiency of *DYRK1A* is associated with ID, epilepsy and microcephaly (6). So far, mutation analysis of *DYRK1A* has been carried out in patients with ID and microcephaly (7, 8). Courset et al. (9) studied the *DYRK1A* gene in a cohort of 105 patients with ID and Angelman syndrome-like symptoms, and they identified a *de novo* frameshift mutation in a patient with growth retardation, ID, and seizures. O'Roak et al. (10)

captured and sequenced 44 candidate genes in 2446 ASD probands. They discovered 27 *de novo* events in 16 genes including *DYRK1A*. The three patients with a *DYRK1A* mutation showed microcephaly.

We suppose that the clinical spectrum of *DYRK1A* mutations may have more variability. Microcephaly may not be a constant feature in the patients with *DYRK1A* mutations. Another novel finding in patient 2 was severe amblyopia. *Dyrk1A* (+/−) mice showed thin retina (11). We recommend ophthalmologic investigation for patients with *DYRK1A* mutations.

Patient 3 had a 2 bp insertion–deletion corresponding to two amino acids in *GABRD* gene. This is the first report of a *GABRD* mutation associated with Rett syndrome like features. *GABRD* encodes a subunit of the ligand-gated chloride channel for gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter (12). The majority of GABAA receptors contain two α-subunits, two β-subunits, and a γ- or δ-subunit. Mutations in inhibitory GABAA receptor subunit genes (*GABRA1*, *GABRB3*, *GABRG2* and *GABRD*) have been associated with genetic epilepsy syndromes including childhood absence epilepsy (CAE), juvenile myoclonic epilepsy (JME), pure febrile seizures (FS), generalized epilepsy with febrile seizures plus (GEFS+), and Dravet syndrome (or severe myoclonic epilepsy in infancy).

There have been some reports on the association of generalized epilepsies and *GABRD* mutations. *GABRD* gene is assigned to chromosome 1p36 (13). Patients with the 1p36 deletion syndrome often have epileptic seizures (14). Windpassinger et al. (12) found that *GABRD* is expressed most abundantly in the brain. They suggested that the *GABRD* is a good candidate for the neurodevelopmental and neuropsychiatric anomalies seen in the 1p36 deletion syndrome.

Patient 3 has been diagnosed with Rett syndrome. Heterozygous disruption of *GABRB3* produces increased epileptiform EEG activity and elevated seizure susceptibility in Angelman syndrome (15). We assume that mutant *GABRD* is likely to cause increased neuronal excitability in our patient. Further investigation is necessary to clarify mutations in Rett syndrome-like patients without known genetic causes.

Patient 4 was diagnosed with NS, the most common RASopathy characterized by short stature, distinct facial features, congenital heart defect, and ID of various degrees. Patient 4 showed ACC. So far association of NS and ACC is not known. Hypoplasia of corpus callosum is occasionally reported in cardio-facio-cutaneous syndrome, another RASopathy. We consider ACC to be an unusual manifestation of RASopathy.

Our NGS-based mutation screening strategy showed a certain success in the diagnosis of patients with neurodevelopmental disorders when conventional clinical genetic testing has proven negative. Presented patients showed unique or unexpected manifestations. We are planning whole-exome sequencing for the remaining unexplained patients.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Acknowledgements

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ORIGINAL ARTICLE

An emerging phenotype of Xq22 microdeletions in females with severe intellectual disability, hypotonia and behavioral abnormalities

Toshiyuki Yamamoto[1], Anna Wilsdon[2], Shelagh Joss[3], Bertrand Isidor[4,5], Anna Erlandsson[6], Mohnish Suri[2], Noriko Sangu[1], Shino Shimada[1], Keiko Shimojima[1], Cédric Le Caignec[4,5], Lena Samuelsson[6] and Margarita Stefanova[6,7,8]

The majority of Xq22 duplications seen in patients with Pelizaeus–Merzbacher disease (PMD) include *proteolipid protein 1* (*PLP1*), the gene responsible for PMD, and neighboring genes. Some cases result from larger duplications up to 7 Mb in size. In comparison, the deletions including *PLP1* seen in PMD patients are small. In this study, we present the genetic and clinical information for five female patients with deletions involving the Xq22 region, and review the correlation between the genotype and phenotype. Three of the five patients show similar large deletions (>3 Mb) ranging from Xq22.1 to Xq22.3 and all manifest severe intellectual disability, hypotonia and behavioral abnormalities. The most striking similarity among them are the behavioral problems, including poor eye contact and sleep disturbance. We propose that this represents an emerging distinctive microdeletion syndrome encompassing *PLP1* in female patients. The possible candidate region responsible for such distinctive features has been narrowed down to the neighboring region for *PLP1*, including the *interleukin 1 receptor accessory protein-like 2* (*IL1RAPL2*) gene and the clustered brain expressed X-linked (BEX) genes. The gene(s) responsible for severe neurological features in the patients in this study would be located in the regions proximate to *PLP1*; thus, males with the deletions involving the gene(s) would be lethal, and finally, the sizes of the deletions in PMD patients would be smaller than those of the duplications.
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Keywords: behavioral abnormality; brain expressed X-linked 3 (BEX3); intellectual disability; interleukin 1 receptor accessory protein-like 2 (IL1RAPL2); microdeletion Xq22; Pelizaeus–Merzbacher disease (PMD); *proteolipid protein 1* (*PLP1*)

INTRODUCTION

Generally, X-chromosome abnormalities result in a more severe phenotype in male patients, whereas females with the same abnormality are often asymptomatic because of heterozygosity of the X chromosome. Mutations and duplications of the *proteolipid protein 1* (*PLP1*) gene located at Xq22 are the major cause of Pelizaeus–Merzbacher disease (PMD; MIM #312080), an X-linked disease that causes congenital leukodystropy associated with spastic paraplegia.[1–3] The mutations and duplications of *PLP1* are often inherited from patients' healthy mothers. Comparatively, loss-of-function mutations, including nonsense mutations and deletions of *PLP1*, are rare and patients with these types of mutations demonstrate a milder phenotype.[4,5] Female carriers of such mutations can show some mild manifestations, such as mild spasticity,[6] without having skewed X-chromosome inactivation (XCI).[7] Although a majority of the duplications observed in PMD patients include genes neighboring *PLP1* and some of the patients have large duplications, up to 7 Mb in size,[8] there are no phenotypic differences between them. In comparison, deletions including *PLP1* observed in patients with PMD are smaller, indicating that larger deletions of this region are lethal in males.[4]

We studied five unrelated females with *de novo* deletions of Xq22 and three of them presented with severe intellectual disability and behavioral abnormalities, but without any features of PMD. The genotype–phenotype correlation in the five female patients with Xq22 deletions suggests a newly recognized microdeletion

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syndrome seen in females only, distinct from PMD. The severe presentation of the Xq22 deletions in these females could explain the lack of larger deletions of this region observed in male patients with PMD.

MATERIALS AND METHODS

Patients' blood samples were obtained with written informed consent based upon approval of each institution's ethical committee. DNA was extracted from the blood samples and used for subsequent testing. Metaphase spreads were also prepared from blood samples and used for fluorescence *in situ* hybridization analyses.

Copy number variants were identified using various microarray platforms from Agilent Technologies (Santa Clara, CA, USA), Affymetrix (Santa Clara, CA, USA), and BlueGnome (Cambridge, UK), according to the manufacturer's protocol (Table 1). Fluorescence *in situ* hybridization analysis was performed to confirm the results for patient 1, using the following human bacterial artificial chromosomes: RP11-832L2 (Xq22.2: 102 902 650–103 085 315) as a target and RP11-75D20 (Xp22: 13:18 314 474–18 506 931) as a marker; these were selected using the University of California Santa Cruz (UCSC) genome browser (http://www.genome.ucsc.edu). All of the genomic coordinates referred to in this paper are reported in build 19 nomenclature. The data obtained were uploaded onto the web-based database, DECIPHER (http://decipher.sanger.ac.uk/) in accordance with the policy to allow collaboration between centers and help establish new syndromes.

The XCI patterns were analyzed in the patients included in the present study by genotyping the CAG repeats in the androgen receptor gene, using the method described in a previous study.[9,10] Finally, the XCI patterns were classified as random (a ratio higher than 50:50 and lower than 75:25) or skewed (higher than 75:25). Linkage analysis was also performed using the ABI PRIMS Linkage Mapping Set Panel 28 (Life Technologies, Carlsbad, CA, USA) that includes the primer sets of 18 microsatellite markers, including DXS1106 (chrX: 102 731 932–102 732 317) that is located in the deletion region of patient 1; the analysis was performed as described previously.[11]

RESULTS

Genomic copy number aberrations

We identified copy number aberrations in the Xq22 region. The five patients with overlapping deletions were identified from five different institutions using the DECIPHER database. The genotype–phenotype correlation was then studied in detail. The details of the Xq22 deletions are summarized in Table 1 and depicted in the genome map (Figure 1). Although patient 5 had an additional duplication on chromosome 11 (chr11: 4 393 604–5 032 655), this was considered to be a possible benign copy number variant as it was inherited from a normal parent.

The absence of an Xq22 deletion was confirmed in the parents of all patients by use of either method (Table 1). Although the linkage study using DXS1106 was uninformative in patient 1, the deletions were confirmed as being *de novo* using fluorescence *in situ* hybridization (Supplementary Figure S1). The results of the linkage study showed no contradiction in biological relationship in the family of patient 1 (Supplementary Figure S2). From these results, all of the Xq22 deletions identified in this study were considered to be of *de novo* origin.

The XCI patterns

The XCI status was analyzed in patients 1, 2, 3 and 5 (Table 1). This analysis was uninformative in patient 1, who was homozygous for the CAG repeat length. Patients 2, 3 and 5 showed skewed XCI patterns.

Table 1 Summary of the genotypes related to the patients

No.	*DECIPHER ID*	*Karyotype*	*Platform*	*Region*[a]		*FISH*	*Inheritance*	*XCI*
Patient 1	257182	46,XX	Hmn CGH 60K (Agilent Technologies)	chrX: 101 365 862–105 847 036	Loss	Performed	*De novo*	Uninformative
Patient 2	264512	46,XX	GeneChip Human Mapping 250K Nsp I (Affimetrix)	chrX: 100 659 116–105 523 589	Loss	Not performed	*De novo*[b]	Skewed (79:21)
Patient 3	NA	46,XX	ISCA 60K (Agilent Technologies)	chrX: 100 907 884–103 982 269	Loss	Not performed	*De novo*[c]	Skewed[d]
Patient 4	265394	46,XX	ISCA 60K (Agilent Technologies)	chrX: 101 982 865–102 233 526	Loss	Not performed	*De novo*[b]	Not performed
Patient 5	265394	46,XX	Bluegnome Cytochip 8 × 60k ISCA v.2.0	chrX: 102 959 459–103 044 544	Loss	Performed (no further information)	*De novo*	Skewed (95:5)
Grillo *et al.*[14]				chrX: 100 935 125–102 041 438	Loss			
Inoue *et al.*[4] A (2002) = HOU542				chrX: 102 993 718–103 510 104	Loss			
Inoue *et al.*[4] B (2002) = HOU669				chrX: 102 957 289–103 314 254	Loss			
Torisu *et al.*[5]				chrX: 103 018 951–103 092 038	Loss			
Shimojima *et al.*[12]				chrX: 105 167 104–106 028 458	Loss			

Abbreviations: FISH, fluorescence *in situ* hybridization; NA, not applicable; XCI, X-chromosome inactivation.
[a]Genomic positions referred to build19.
[b]Inheritance was checked by microarray.
[c]Inheritance was checked by quantitative PCR.
[d]Raw data unavailable.

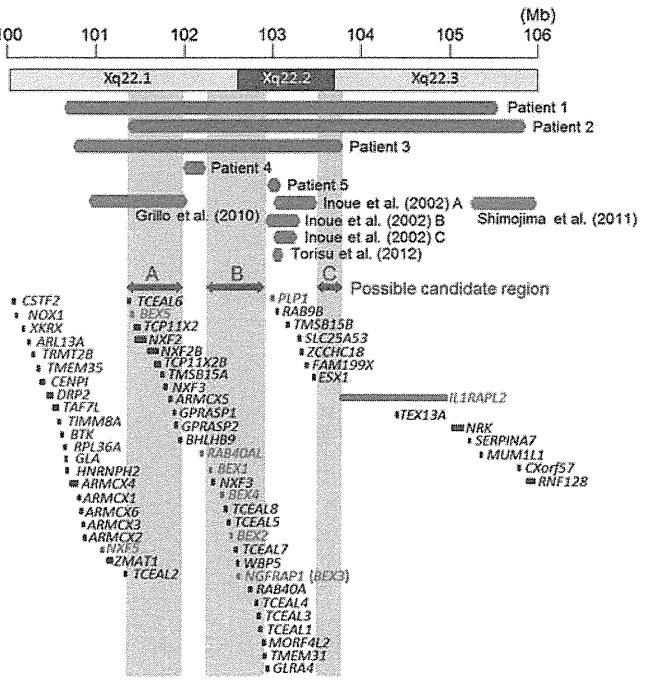

Figure 1 Results of microarray chromosomal testing and the genome map around Xq22. Red and gray hexagons indicate the deletion regions identified in the patients in this study as well as in previously reported patients. Rectangles indicate the locations of the genes. Genes mentioned in the text are highlighted by red. Possible candidate regions are shown by translucent blue bands.

CLINICAL REPORTS

The clinical features of the five patients are summarized in Table 2.

Patient 1

A 3-year-old girl (DECIPHER ID 264512) was born following a pregnancy complicated by polyhydramnios. Transient tachypnea was noted after birth, and the patient was described as being continually irritable when awake and vomiting frequently. Her parents noted poor eye contact and poor social smile at 2–3 months. At 9 months of age, she was diagnosed with developmental delay and underwent a laparoscopic Nissen fundoplication, having previously required nasogastric tube feeding. She had a single seizure in the neonatal period, and although an electroencephalogram demonstrated abnormalities, she had no further episodes. Brain magnetic resonance imaging (MRI) showed no definite abnormality. At her last review, she was underweight (11 kg; <3rd centile) and was still described as irritable and showed severe developmental delay and sleep disturbance. In addition, her eye contact was still poor and she demonstrated self-injury behaviors. There were no involuntary movements, pyramidal signs or stereotypical movements. She has a triangular face with wide nasal bridge, widely spaced eyes, strabismus, a long philtrum and a prominent jaw (Figure 2a).

Patient 2

A 6-year-old girl (DECIPHER ID 257182) was born at 42 weeks of gestation, after a pregnancy complicated by reduced fetal movements and polyhydramnios. Her birth weight was 4860 g (>97th centile), birth length was 54 cm (>97th centile) and occipitofrontal circumference was 38 cm (>97th centile). Her 37-year-old father is healthy, but her 36-year-old mother suffers from an undiagnosed neuromuscular disease with dominant inheritance and a clear anticipation pattern. The patient has severe developmental delay. She did not manage to support her head until she was over 1-year old. She has a distinct sleeping abnormality, exhibiting hypersomnia, and is described as sleeping 'most of the time'. There was a history of seizures. An electroencephalogram did not demonstrate any epileptiform activity, but did show increased slow wave activity. An MRI of her brain revealed generalized white matter hypoplasia, most pronounced in the occipital region, delayed myelination, hypoplasia of the corpus callosum and ventriculomegaly. A visual evoked potential indicated neurosensory visual impairment (severe attenuated N1-potential and normal P1-potential).

At present, she still has severe delay and no language skills, and cannot sit or walk. This patient's appearance is similar to that of patient 1, with a triangular face associated with strabismus and a prominent jaw (Figure 2b). Severe pes equinovarus required surgical correction. She has extremely short feet, corresponding to those of a 3-year-old child.

Patient 3

A 16-year-old girl was born at 43 weeks of gestation by cesarean section. The pregnancy had been complicated by polyhydramnios. Her birth weight was 4820 g (>97th centile). She is the only child of a non-consanguineous couple and there was no significant family history. She developed a social smile at 6 weeks, sat at 7 months and walked independently at 7 years. Her development is severely delayed; she reached the development level of a 7-month-old child when she was 16 months old, and reached a 10-month level when she was three and a half years old. Abnormal self-injury behaviors were noted later, including hitting herself and biting her fingers. She was hypotonic with a mild scoliosis, moderate bilateral hearing loss, constipation and an advanced bone age. Brain MRI examination at 1 year of age showed generalized cerebral atrophy that was slightly worse on the right side. A further MRI at the age of 6 years did not show any specific features, and there was no hypomyelination. Conventional G-banding showed a normal female karyotype.

Currently, she is aphasic and is incontinent. She has fine and slow-growing hair, bi-frontal narrowing, deep-set eyes, a prominent nasal bridge, full upper lip, a prominent jaw, deep palmer creases and prominent volar pads (Figure 2c). Her weight is 56.4 kg (between the 25th and 50th centiles), her height is 146.1 cm (<0.4th centile) and her occipitofrontal circumference is 56 cm (50th–75th centile), indicating short stature.

Patient 4

A 12-month-old girl (DECIPHER ID 265394) was born at term following a normal pregnancy. She is the third child of healthy unrelated parents. During the neonatal period, she had feeding difficulties and her early milestones were delayed. She showed mild motor developmental delay with sitting started from 12 months of age. Because of the bilateral moderate sensorineuronal deafness that was identified at 7 months of age, there was no meaningful word at that time. Social interaction was within normal limit. There was no facial dysmorphism. Behavioral abnormality was not noted.

Patient 5

A 7-year-old girl (DECIPHER ID 253614) weighed 3620 g at term (90–97th centile). From early infancy, she showed psychomotor developmental delay and was only able to sit unsupported at 18

Table 2 Summary of the clinical features of the patients presented in this study

	Patient 1	*Patient 2*	*Patient 3*	*Patient 4*	*Patient 5*
Demographics					
Current age	3 y	7 y	19 y	1 y	7 y
Gender	F	F	F	F	F
Ethnicity	Japanese	Swedish	White European	French/Caucasian	South Asian
Family history					
Consanguinity	–	–	–	–	–
Pregnancy					
Polyhydramnios	+	+	+	–	–
Others	Threatened premature delivery Premature ablation Fetal distress	Weak fetal movements	Episode of diarrhea and vomiting	–	–
Birth					
Gestation at birth	37 w	42 w	43 w	NA	40 w
Birth weight	2712 g	4860 g	4820 g	2200 g	3620 g
Complications	Transient tachypnea Hypoglycemia	Hypotonia	–	–	–
Growth					
Growth delay	–	–	–	–	–
Neurological features					
Development					
Milestone	Severe delay	Severe delay	Severe delay	Mild delay	Severe delay
Motor development	Immobile	Immobile	Sat 7 m and walked 7 y	NA	Sat 18 m and walked 6 y
Speech	Aphagic	Aphagic	Aphagic	Speech delay	Aphagic
Social	Poor eye contact	Poor eye contact	Initially delayed visual maturation	–	No react to pain
Sleep	Sleep disturbance	Hypersomnia	Poor sleep	–	Poor sleep
Behavioral problems	Ill humor Self-injury	–	Hitting self Biting fingers	–	Repetitive hands to mouth Hits face Air swallowing
Muscle tone	Severe hypotonia	Severe hypotonia	Hypotonia	–	–
Seizures	Only one episode	–	+	–	–
Visual function	Strabismus	Divergent strabismus	–	–	Strabismus
Excretion habit	Incontinence	NA	NA	–	Incontinence
Dysmorphic findings					
Facial findings					
Triangular face	+	+	+	–	–
Hypertelorism	+	+	–	–	–
Broad nasal bridge	+	+	–	–	–
Pointed chin	+	+	+	–	–
Others	Telecanthus Long philtrum Curly hair	Epicanthus inversus Irregular teeth Prominent medial incisors Posteriorly angulated ears	Mild facial asymmetry Frontal narrowing Prominent nasal bridge Deeply set eyes Thick upper lip Large ears	–	–
Extremities	–	Tapering fingers Proximal placement of thumb Hammer toes Pes equinovarus Extremely short feet (15 cm) Congenital dislocation of the hip	Deep palmar creases Prominent volar pads Overriding toes	–	–
Brain MRI					
Delayed myelination	–	+	–	NA	+
Abnormalities of the corpus callosum	–	Hypoplasia	Mild thinning of the posterior corpus callosum	NA	–
White matter hypoplasia	–	+	–	NA	–
Physical features					
Endocrinological	–	–	–	–	–
Cardiac	–	–	–	–	–
Respiratory	–	–	–	–	–
Gastrointestinal	Dysphagia Gastroesophageal reflux	–	Constipation	Gastroesophageal reflux	–

Abbreviations: F, female; M, male; m, months; MRI, magnetic resonance imaging; NA, not applicable; w, weeks; y, years.

months and walk independently at 6 years. An MRI of the brain showed delayed myelination. She is still aphasic and incontinent. She repeatedly brings her hands to her mouth, hits her own face and appears to have impaired pain perception. She also sleeps poorly. There is no definite facial dysmorphism, apart from strabismus (Figure 2d).