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Whole genome sequencing of 45 Japanese patients with intellectual disability

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Abstract

Intellectual disability (ID) is characterized by significant limitations in both intellectual functioning and adaptive behaviors, originating before the age of 18 years. However,

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the genetic etiologies of ID are still incompletely elucidated due to the wide range of clinical and genetic heterogeneity. Whole genome sequencing (WGS) has been applied as a single-step clinical diagnostic tool for ID because it detects genetic variations with a wide range of resolution from single nucleotide variants (SNVs) to structural variants (SVs). To explore the causative genes for ID, we employed WGS in 45 patients from 44 unrelated Japanese families and performed a stepwise screening approach focusing on the coding variants in the genes. Here, we report 12 pathogenic and likely pathogenic variants: seven heterozygous variants of *ADNP*, *SATB2*, *ANKRD11*, *PTEN*, *TCF4*, *SPAST*, and *KCNA2*, three hemizygous variants of *SMS*, *SLC6A8*, and *IQSEC2*, and one homozygous variant in *AGTPBP1*. Of these, four were considered novel. Furthermore, a novel 76 kb deletion containing exons 1 and 2 in *DYRK1A* was identified. We confirmed the clinical and genetic heterogeneity and high frequency of *de novo* causative variants (8/12, 66.7%). This is the first report of WGS analysis in Japanese patients with ID. Our results would provide insight into the correlation between novel variants and expanded phenotypes of the disease.

KEYWORDS
intellectual disability, intellectual disability-associated gene, likely pathogenic variant, pathogenic variant, whole genome sequencing

1 | INTRODUCTION

Intellectual disability (ID), defined as significant limitations in both intellectual functioning and adaptive behaviors originating before the age of 18 years, is present in 1–3% of the global population (Hu et al., 2019; Maulik, Mascarenhas, Mathers, Dua, & Saxena, 2011; Ropers, 2010). The development of a subset of IDs is caused by genetic defects. To date, 1535 ID-associated genes have been described using the Human Gene Mutation Database (HGMD), Professional 2019.2, of which inheritance modes include autosomal dominant, autosomal recessive, X-linked recessive, and *de novo*. Autosomal *de novo* variants have been observed in 60–70% IDs with a definite molecular diagnosis (Fitzgerald et al., 2015). In addition, variants causing monogenic forms of X-linked ID are also frequently responsible and explain up to 10–12% ID in males (Musante & Ropers, 2014; Vissers, Gilissen, & Veltman, 2016). In the Caucasian population, autosomal recessive ID (ARID) has been also estimated to account for 10–12% ID (Musante & Ropers, 2014), but the majority of causative ARID genes remain unidentified. Other genetic causes that cannot be identified by analysis of nuclear genes, such as imprinting abnormalities and mitochondrial abnormalities, also have been identified.

Despite the essential roles of genetic factors in ID etiology, delineation of the genetic causes in each patient with ID remains largely unsolved due to the requirement of extensive multi-step investigations, including chromosome analysis for numerical and large structural abnormalities and array comparative genomic hybridization (aCGH) analysis for submicroscopic structural abnormalities, followed by panel or exome sequencing for pathogenic variants and insertions/deletions (indels) in single genes. The potential of whole genome sequencing (WGS) as the first-line clinical diagnostic tool has been widely discussed (Bowling et al., 2017; Gilissen et al., 2014; Lindstrand et al., 2019; Ostrander et al., 2018; Zahir et al., 2017). WGS enables a comprehensive single-step genome evaluation and the cost per genome is continuously decreasing. The use of different algorithms on WGS data enables the detection of pathogenic variants, small indels, and structural variants from a single source; moreover, WGS detects alterations in coding and noncoding regions more precisely than exome sequencing (Bowling et al., 2017; Gilissen et al., 2014; Zahir et al., 2017). Recently, variants in noncoding regions have also been highlighted as ID causes (Devanna, van de Vorst, Pfundt, Gilissen, & Vernes, 2018; Niemi et al., 2018; Short et al., 2018; Smedley et al., 2016). Theoretically, WGS can replace all these procedures as a single-step clinical diagnostic tool for ID (Lindstrand et al., 2019).

In this pilot study, we explored the genetic causes of ID in Japanese individuals by WGS and identified 12 pathogenic and likely pathogenic variants of the ID-associated genes.

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

This study was approved by the ethical committee of the National Center of Neurology and Psychiatry (NCNP). Written informed consent was obtained from all participants and their parents.

2.2 | Patient cohort

Forty-five patients with ID from 44 families and their parents were enrolled from the NCNP Biobank (Tokyo, Japan) after obtaining

informed consent. We collected clinical information as well as extracted genomic DNA from the peripheral blood.

2.3 | Whole genome sequencing

We enrolled all patients without *MECP2* pathogenic variants. We performed WGS using HiSeq 2500 (Illumina; San Diego, CA, USA) on the DNA extracted from the patients and their parents, as described previously (Okada et al., 2018). The mean depth was 32×, with >99.4% bases covered at 5×. We additionally performed whole exome sequencing (WES) on the DNA extracted from the sibling of MR464 using DNBseq (Beijing Genomics Institute; Guangdong, Shenzhen, China). The mean depth was 96.5×, with >99.7% bases covered at 5×. Reads were aligned to the hs37d5 reference genome, which includes data from GRCh37, the rCRS mitochondrial sequence, human herpesvirus 4 type 1, and concatenated decoy sequences, using Burrows-Wheeler aligner (BWA) (Li & Durbin, 2009). The duplicate reads were removed using Picard (http://broadinstitute.github.io/picard/). Variants, including small insertions and deletions, were called according to the Genome Analysis Toolkit (GATK) (McKenna et al., 2010) version 3.2 in the individuals whose DNA was used to perform WGS and GATK version 3.5 in the individual whose DNA was used to perform WES. Annotation was performed by Annovar (K. Wang, Li, & Hakonarson, 2010).

2.4 | Verification of SNVs identified by WGS and WES

The variants were filtered according to the following strategies: (1) nonsense, missense, or frameshift variants located in the exonic or canonical splicing regions; (2) absent from common variants in Exome Sequencing Project 6500 (ESP6500, https://evs.gs.washington.edu/EVS/), the 1000 Genomes Project (1000genome, https://www.internationalgenome.org/), the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/), if autosomal *de novo* or X-linked recessive; (3) allele frequency < 0.001 and no homozygotes in these public databases if autosomal recessive; (4) predicted to be damaging by at least three of the following four algorithms: SIFT (Kumar, Henikoff, & Ng, 2009), PolyPhen2 (Adzhubei et al., 2010), MutationTaster (Schwarz, Rödelsperger, Schuelke, & Seelow, 2010), and CADD (Kircher et al., 2014) with a cutoff value of 20. The average number of qualified variants was 3.38 per individual, with a maximum of 10 and a minimum of 0. These variants were curated manually by using the following three conditions: (1) variants of a gene that has been reported to be associated with ID; (2) matching clinical phenotypes with previously reported cases; (3) not registered in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/) if autosomal *de novo* or X-linked recessive, or registered as variants with allele frequency of 0.001 or less if autosomal recessive. In parallel, variants in the list of ID-associated genes based on HGMD Professional 2015 were extracted regardless of the mode of inheritance (Supplementary Table 1). In addition, the Human Genetic Variation Database (HGVD, http://www.hgvd.genome.med.kyoto-u.ac.jp/) and Tohoku Medical Megabank Organization (ToMMo, https://jmorp.megabank.tohoku.ac.jp/202001/) were utilized to evaluate the specific variants in the Japanese population. The extracted variants were classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015). All variants were confirmed using Sanger sequencing. The verified variants were submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) and the accession numbers were registered as SCV001438314 to SCV001438325.

2.5 | Verification of SVs identified by WGS

We investigated the structural variants (SVs) larger than 50 bp using our original pipeline (manuscript in preparation) developed based on our previous study (Kosugi et al., 2019). Briefly, SVs were called with 14 existing SV detection tools, including BreakDancer (K. Chen et al., 2009), CNVnator (Abyzov, Urban, Snyder, & Gerstein, 2011), DELLY (Rausch et al., 2012), forestSV (Michaelson & Sebat, 2012), inGAP-sv (Qi & Zhao, 2011), Lumpy (Layer, Chiang, Quinlan, & Hall, 2014), Manta (X. Chen et al., 2016), MATCHCLIP (Wu, Tian, Pirastu, Stambolian, & Li, 2013), MELT (Gardner et al., 2017), MetaSV (Mohiyuddin et al., 2015), Mobster (Thung et al., 2014), Pindel (Ye, Schulz, Long, Apweiler, & Ning, 2009), SoftSV (Bartenhagen & Dugas, 2016), Wham (Kronenberg et al., 2015). For each individual, we obtained overlap calls that were shared between the selected pairs of tools for each SV type, as previously shown (Kosugi et al., 2019). The overlap call sets for each individual were merged to generate a single vcf file. Among them, we extracted the exon-overlapped *de novo* SVs and, in the case of males, the maternally inherited SVs on X chromosome. We confirmed all these SVs by Integrative Genomics Viewer (IGV, https://software.broadinstitute.org/software/igv/). Furthermore, we excluded 13 SVs registered in the Database of Genomic Variants (DGV, http://dgv.tcag.ca/dgv/app/home). Remaining SVs were manually curated by reference to clinical features associated with genes contained in each SV via PubMed (https://pubmed.ncbi.nlm.nih.gov/) and OMIM (https://www.omim.org/). A direct sequence was performed for the case classified as pathogenic or likely pathogenic according to the standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants (Kearney, Thorland, Brown, Quintero-Rivera, & South, 2011). In addition, to detect cases developed by loss of heterozygosity, we evaluated the variants on the counter allele when an SV involves the causative genes of ARID.

2.6 | Statistical analysis

Statistical differences were analyzed using the Chi-square test by GraphPad Prism5.

3 | RESULTS

3.1 | Clinical overview of the Japanese ID cohort

Forty-five patients (26 males and 19 females) with ID from 44 unrelated families were included. A summary of the clinical information of the patients is shown in Table 1. Speech delay, epilepsy, hypotonia, brain abnormalities, and growth retardation were observed at various frequencies. Imaging the brain of the patients showed abnormal findings including white matter atrophy, cerebellum hypoplasia, and corpus callosum thinning. There were no meaningful differences ($p > 0.05$) in the sex ratio (male versus female, $p = 0.52$), ID severity (borderline and mild versus moderate and severe disability, $p = 0.45$), and the incidence of hypotonia ($p = 1$) and abnormal findings in brain magnetic resonance imaging (MRI) ($p = 0.51$) between the patient group with pathogenic or likely pathogenic variants and the group without pathogenic or likely pathogenic variants.

3.2 | Overview of the variants identified in this study

We found nine coding variants in the nine known ID-associated genes, *SATB2* (NM_015265), *SMS* (NM_004595), *AGTPBP1* (NM_001330701), *PTEN* (NM_000314), *TCF4* (NM_001083962), *IQSEC2* (NM_001111125), *SLC6A8* (NM_005629), *SPAST* (NM_014946), and *KCNA2* (NM_004974), and two coding deletions, a 2 bp deletion in *ANKRD11* (NM_001256182), and a 10 bp deletion in *ADNP* (NM_001282531). The clinical information of each patient with the pathogenic or likely pathogenic variant is summarized in Table 2.

TABLE 1 Clinical characteristics of 45 ID individuals

Characteristic	Individuals		
	Total	Positive[a]	Negative[b]
Sex			
Male	26	8	18
Female	19	4	15
Age (years) at registration			
0–2	19	7	12
3–6	16	4	12
7–12	6	1	5
13–18	3	0	3
19+	1	0	1
Neurological abnormalities			
Borderline intellectual disability (79–70)	3	3	0
Intellectual disability, mild (DQ69–50)	8	1	7
Intellectual disability, moderate (DQ49–35)	7	4	3
Intellectual disability, severe (DQ35–20)	9	3	6
Intellectual disability, most severe (DQ20–)	17	1	16
Intellectual disability (not evaluated)	1	0	1
Speech delay	40	12	28
Epilepsy	17	1	16
Autism spectrum disorder	9	1	8
Sleep disturbance	7	2	5
Hypotonia	10	3	7
Abnormal findings in brain MRI[c]	22	7	15
Growth retardation	13	4	9
Congenital malformations			
Cardiac	4	2	2
Renal	2	0	2
Genital	4	2	2
Skeletal	6	1	5

[a]Individuals with pathogenic or likely pathogenic variants.
[b]Individuals without pathogenic or likely pathogenic variants.
[c]MRI findings include white matter atrophy, cerebellum hypoplasia, or corpus callosum thinning.

TABLE 2 Clinical phenotypes and genotypes of the patients with the identified variants

Individual	Sex	Age at registration	Gene	Variant	Mutation type	Predicted pathogenicity				Inheritance	Parental age at conceptionin sporadic cases		Clinical findings	DQ	Brain abnormalities	Reference
						SIFT (score)	Polyphen2 (HDIV score)	Mutation taster(score)	CADD Phred score		Father	Mother				
MR391	F	2y 10 m	*ADNP*	c.642_651del p.(N214Kfs*31)	Frameshift	-	-	-	-	Sporadic	38	40	Autism, hypotonia, ventricular septal defect (spontaneously closed), bilateral double ureters	DQ is unknown but is low	NA	This study
MR456	F	1y 6 m	*SATB2*	c.1174G > A p.(G392R)	Missense	Damaging (0.001)	Damaging (1)	Disease (1)	32	Sporadic	37	33	Strabismus, hypotonia	9	Mild volume loss of bilateral frontal lobe	This study
MR464	M	6y	*ANKRD11*	c.2615_2616del p.(S872Cfs*43)	Frameshift	-	-	-	-	Sporadic	31	35	Amblyopia	73	No abnormal findings	This study
MR483	M	3y 3 m	*SMS*	c.587 T > C p.(I196T)	Missense	Damaging (0.001)	Damaging (0.999)	Disease (1)	25.1	X-linked	-	-	Bilateral cryptorchidism, stenosis of peripheral pulmonary artery, osteoporosis	20	No abnormal findings	This study
MR398	M	2y 6 m	*AGTPBP1*	c.2552C > T p.(T851M)	Missense	Damaging (0)	Damaging (0.899)	Disease (0.81)	33	Autosomal Recessive	-	-	Scoliosis, high palate, micropenis	15	Hypoplasia of cerebellar vermis, atrophy of caudate putamen	Shashi et al., 2018
MR408	F	1y 5 m	*TCF4*	c.1738C > T p.(R580W)	Missense	Damaging (0)	Damaging (1)	Disease (1)	35	Sporadic	26	26	Hypotonia, apnea	41	Thinning of corpus callosum, volume loss of white matter, ventriculomegaly	Amiel et al., 2007
MR421	M	1y 7 m	*IQSEC2*	c.854delC p.(P285Lfs*21)	Frameshift	-	-	-	-	Sporadic	28	28	Epilepsy	less than 10	Mild volume loss of white matter	Mignot et al., 2019
MR425	F	12y	*KCNA2*	c.1120A > G p.(T374A)	Missense	Damaging (0)	Damaging (0.989)	Disease (1)	24.2	Sporadic	24	27	Scoliosis, hypertonia	DQ is unknown but is low	Atrophy of cerebellum, cerebrum, and brainstem	Hundallah et al., 2016, Masnada et al., 2017
MR447	M	1y 11 m	*SLC6A8*	c.1145C > T p.(P382L)	Missense	Damaging (0)	Damaging (1)	Disease (1)	31	X-linked	-	-	Sleep disturbance, short stature	45	Mild volume loss of white matter	Mercimek-Mahmutoglu et al., 2009
MR506	M	3y 11 m	*PTEN*	c.253dupG p.(V85Gfs*7)	Frameshift	-	-	-	-	Sporadic	37	36	Relative macrocephaly	20	White matter hyperintensities on T2 weighted image	rs876660082 (dbSNP150)
MR516	M	4y	*SPAST*	c.1496G > A p.(R499H)	Missense	Damaging (0)	Damaging (1)	Disease (1)	34	Autosomal dominant	-	-	Restricted motion of foot joints, abnormal gait	51 (verbal)	NA	Park et al., 2005, Polymeris et al., 2016
MR508	M	1y	*DYRK1A*	Partial deletion including exon 1 and 2	Deletion	-	-	-	-	Sporadic	34	33	Hypertonia, irritability, sudden death at the age of 1 year and 7 months old	DQ is unknown but is low	NA	This study

Of these 11 individuals, four were female and seven were male. The inheritance modes were autosomal dominant in one, *de novo* in six, X-linked recessive in two, X-linked *de novo* in one, and autosomal recessive in one individual. Three heterozygous variants in *ADNP*, *SATB2*, and *ANKRD11*, and one hemizygous variant in *SMS* were considered novel in comparison with those mentioned in public databases. Pedigrees and electropherograms of the families with the novel variants are shown in Figure 1. No other potentially pathogenic variants were identified in any of the 11 families. Finally, the evaluation of variations based on the guidelines for the classification of genetic variants from ACMG is shown in Supplementary Table 2.

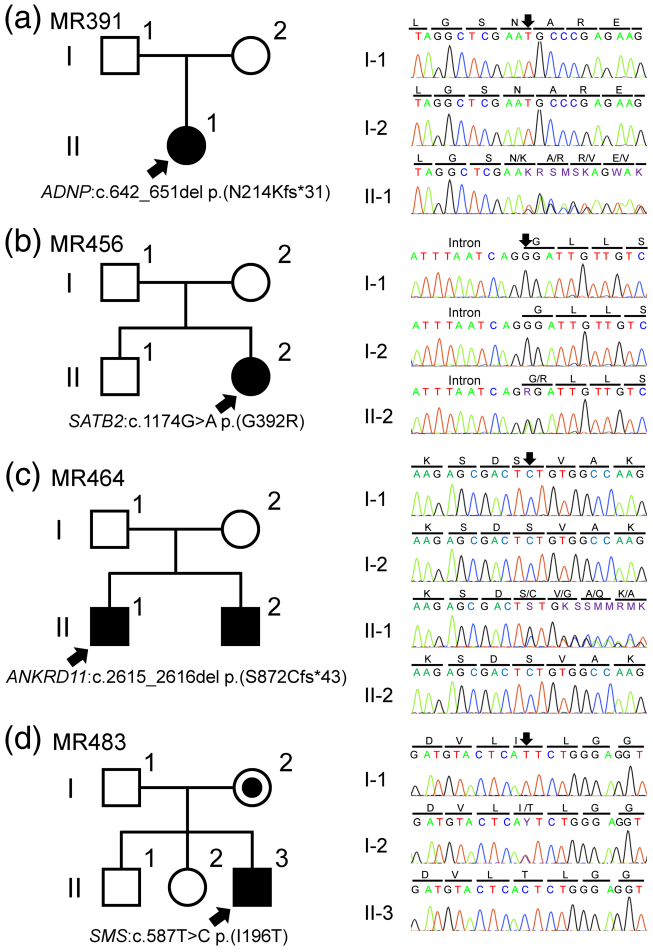

FIGURE 1 Pedigree charts and electropherograms of the four families with affected individuals carrying the novel variants identified by WGS. (a) Pedigree and electropherogram of family MR391. A heterozygous frameshift variant c.642_651del (p.N214Kfs*31) in *ADNP* was identified in the proband (II-1). (b) Pedigree and electropherogram of family MR456. A heterozygous missense variant c.1174G > A (p.G392R) in *SATB2* was identified in the proband (II-2). (c) Pedigree and electropherogram of family MR464. A heterozygous frameshift variant c.2615_2616del (p.S872Cfs*43) in *ANKRD11* was identified in the proband (II-1). His father (I-1), mother (I-2), and affected brother (II-2) did not carry the same variant. (d) Pedigree and electropherogram of family MR483. A hemizygous variant c.587 T > C (p.I196T) in *SMS* was identified in the proband (II-3). His mother (I-2) was heterozygous for the variant and his father (I-1) was wild type at the same site [Color figure can be viewed at wileyonlinelibrary.com]

3.3 | Case presentation with novel variants

3.3.1 | Case 1

MR391 with a novel *de novo* variant c.642_651del (p.N214Kfs*31) in *ADNP* was born to non-consanguineous parents as their first child after 41 weeks of gestation through an uneventful delivery when her father was 39 and her mother was 41 years old (Figure 1(a) and Table 2). Her birth weight was 2200 g (−3.0 SD). After birth, echocardiography showed a ventricular septal defect, which closed spontaneously. Bilateral double ureters were also identified. She showed depressed nasal bridge and tented upper lips. Although her motor development was initially within normal range, holding her head at the age of 3 months, sitting at the age of 6 months, and standing with support at the age of 10 months; thereafter, her psychomotor development remained unchanged. At the age of 12 years, her height was 132 cm (−3.4SD), weight 31 kg (−2.1SD), and head circumference 50 cm (−3.1SD) suggesting short stature and microcephaly. She walked with support and spoke no meaningful words. She was hypotonic and hyperactive. She also showed stereotypic behaviors and was diagnosed as autism spectrum disorder.

3.3.2 | Case 2

MR456 with c.1174G > A (p.G392R) in *SATB2* was born after 40 weeks of uneventful gestation to non-consanguineous patients as their second child when her father was 38 and her mother was 34 years old (Figure 1(b) and Table 2). She showed hypotonia and developmental delay since early infancy. At the age of 9 years, she could move only by rolling and was unable to sit without support. She sometimes spoke some words, "no", "good", and "hello", but it was rare. Her physical examination showed strabismus, but no craniofacial abnormalities, including palatal and dental anomalies. Her head circumference was 53 cm (0.6SD), not microcephaly or macrocephaly. Brain MRI revealed mild volume loss of the bilateral frontal lobes.

3.3.3 | Case 3

MR464 with c.2615_2616del (p.S872Cfs*43) in *ANKRD11* was born after 41 weeks of uncomplicated gestation to non-consanguineous patients as their first child when her father was 32 and her mother was 36 years old (Figure 1(c) and Table 2). He revealed amblyopia. Although his milestones were initially delayed, he gradually developed head control at the age of 6 months, sat without support at the age of 12 months, and walked at the age of 1 year and 6 months. He spoke meaningful words at the age of 2 years and 7 months, and two-word sentences at the age of 4 years. He suffered from complex febrile seizures at the age of 1 year, but both MRI and electroencephalogram revealed normal findings. His developmental quotient (DQ) was 73, within borderline intelligence, and his younger brother revealed borderline intelligence. However, his brother did not have the same variant.

3.3.4 | Case 4

MR483 with c.587 T > C (p.I196T) in *SMS*, which was inherited from his mother, was born after 39 weeks of gestation to non-consanguineous parents as their third child (Figure 1(d) and Table 2). He has a healthy brother and a sister with normal development. His birth weight was 2580 g (−1.9 SD). He showed bilateral cryptorchidism and peripheral pulmonary arterial stenosis. His development was initially normal: he smiled and followed objects with his eyes at the age of 3 months and held his head at the age of 4 months. However, a delay in his developmental milestones became apparent. He achieved sitting without support at the age of 2 years and walking with support at the age of 7 years. At the age of 9 years, he was unable to walk or speak meaningful words. His physical examination revealed growth delay with a body weight of 16.3 kg (−2.0 SD), and height of 116 cm (−2.5SD). He had no history of bone fractures, but his plain radiographs suggested diminished bone density. Dual-energy X-ray absorptiometry (DEXA) scan Z score was −7.1 in the lumber spine, and his bone density 0.249 g/cm^2. He was diagnosed as osteoporosis.

3.4 | Case presentation with a partial deletion in *DYRK1A*

We identified a 75,820 bp deletion (hg19:chr21:38,720,165–38,795,984) involving the exons 1 and 2 of *DYRK1A* in MR508 (Table 2). The breakpoint and flanking sequences are shown in Figure 2. The patient was born to non-consanguineous parents as their first child after *in vitro* fertilization accompanied with velamentous insertion when her father was 35 and her mother was 34 years old. His birth weight was 2374 g (−2.2 SD), and his head circumference was 29.5 cm (−2.9 SD). He had hypertonia and irritability from birth. He followed objects and was able to hold his head at the age of 2 months. He was able to sit at the age of 9 months, but thereafter his development stopped. At the age of 1 year and 1 month, he did not stand with support or walk. He also showed growth retardation: his height and weight were 66 cm (−3.8 SD) and 8.2 kg (−1.5 SD), respectively. He died of unknown cause at the age of 1 year and 7 months.

4 | DISCUSSION

We identified 12 pathogenic or likely pathogenic variants involving ID-associated genes in 12 out of 44 families (27%). Of these variants, eight occurred *de novo* (66.7%), two showed maternally inherited X-linked patterns (16.7%), one was autosomal dominant (8.3%), and one was transmitted in an autosomal recessive (AR) manner (8.3%). The proportion of inheritance patterns of pathogenic or likely pathogenic variants in previous studies using high-throughput DNA sequencing analysis differs in frequency study by study from other East Asian and Western European countries, but *de novo* dominant variants were the most common (55–90%), while maternally inherited X-linked variants (5–22%) and variants inherited in AR manner (0–22%) have variable frequencies, second only to *de novo* variants (Chérot et al., 2018; Fitzgerald et al., 2015; Gieldon et al., 2018; Han, Jang, Park, & Lee, 2018; Nambot et al., 2018; Wright et al., 2015; Yamamoto et al., 2019; Yan et al., 2019). Our study in a Japanese ID cohort also showed a high frequency of *de novo* variants and a similar frequency in X-linked and AR variants. Moreover, this high prevalence of *de novo*

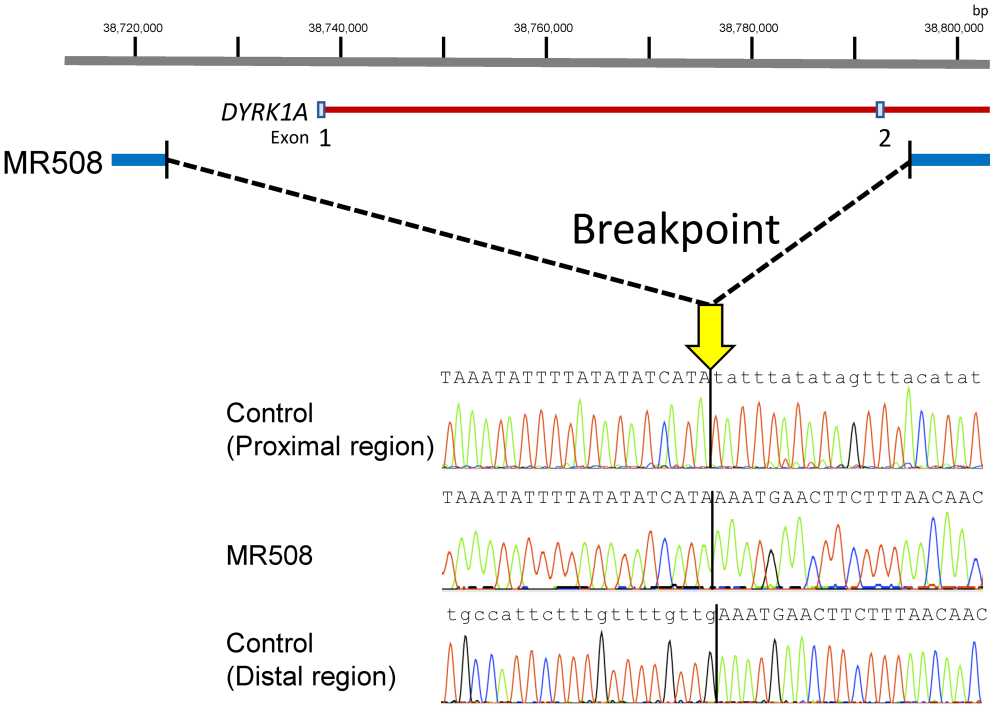

FIGURE 2 Breakpoint sequence of *DYRK1A* identified in MR508. Sequence analysis revealed a *de novo* 75,820 bp deletion and no apparent homology between the proximal and distal sequences of the junction fragment [Color figure can be viewed at wileyonlinelibrary.com]

pathogenic variants was similar to the result of the previous targeted gene analysis in the Japanese population (Yamamoto et al., 2019). It should be noted that the homozygous variant c.2552C > T (p.T851M) found in *AGTPBP1* was not registered in either HGVD or ToMMo, suggesting that this variant is likely a private mutation rather than a common founder mutation in the Japanese population. These findings suggest that the distribution of genetic causes for ID in the Japanese population is similar to that in other outbred population, and the analysis of *de novo* variants is particularly important in the Japanese population as well. Common founder mutations for ARID were not evident in our Japanese cohort.

Overall, our detection rate for the pathogenic and likely pathogenic variants were limited to 27% because we scanned for variants only in the coding regions. Previous studies on ID using high-throughput DNA sequencing revealed a conclusive diagnostic rate as 8.0–34.0% (Chérot et al., 2018; Fitzgerald et al., 2015; Gieldon et al., 2018; Han et al., 2018; Lindstrand et al., 2019; Nambot et al., 2018; Wright et al., 2015; Yamamoto et al., 2019; Yan et al., 2019). Of these, one study using WGS revealed a total diagnostic rate of 27%, which was the same as that reported in this study (Lindstrand et al., 2019). In the current *post hoc* pipeline of variant detection, WGS showed no higher diagnostic rate in ID than WES and panel analysis. WGS has some technical limitations. Evaluating the intergenic and deep intronic genomic regions is challenging due to the absence of algorithms to predict variant pathogenicity in such regions. Short-read WGS cannot detect triplet repeat expansions, which are involved in triplet repeat diseases that results in ID (Budworth & McMurray, 2013; Wright, FitzPatrick, & Firth, 2018). Although many algorithms for SV detection have been developed, it is difficult to evaluate the results correctly due to the differences in the SVs detected by each algorithm, and standardized WGS-based detection of chromosomal abnormalities is still challenging (Kosugi et al., 2019). In addition, mosaic variants can be missed due to the low coverage depth (Wright et al., 2018). However, some of these issues with WGS will be resolved in the near future by developed public databases of non-coding regions and SVs, empowerment of prediction tools, and effective combination of SV detection tools (Collins et al., 2020; Cummings et al., 2020; Karczewski et al., 2020). Accordingly, the diagnostic rate of ID using WGS will increase.

The interpretation of pathogenicity of the five novel variants is discussed below. We identified a heterozygous frameshift variant, c.642_651del (p.N214Kfs*31), in *ADNP* in MR391 (Table 2). *ADNP* is a homeodomain-containing zinc finger transcription factor important for brain formation, axonal transport, dendritic spine plasticity, and autophagy (Gozes, 2007; Gozes et al., 2017; Helsmoortel et al., 2014) and is associated with Helsmoortel-Van der Aa syndrome (HVDAS; OMIM#615873). A total of 96 pathogenic or likely pathogenic variants have been reported (HGMD Professional 2020.2), 30 of which are small deletions. HVDAS is characterized by ID, stereotypic behaviors, attention deficit/hyperactivity disorder (AD/HD), and hypotonia. The average age for achieving independent walking and speaking meaningful words in the patients with HVDAS is 2 years and 6 months (Van Dijck et al., 2019), while MR391 did not reach these developmental milestones at 12 years, suggesting that she had severe HVDAS. In MR391, the identified deletion in the fifth and last exon is predicted to escape nonsense-mediated decay. This deletion presumably results in a truncated protein lacking both the N-terminal NAP motif and multiple C-terminal domains, including the DNA-binding domain, which interacts with the chromatin remodeling complex. HVDAS onset is strongly related to the abnormalities in multiple C-terminal domains, but the genotype–phenotype correlation is still unclear (Gozes, Ivashko-Pachima, & Sayas, 2018; Helsmoortel et al., 2014; Van Dijck et al., 2019; Vandeweyer et al., 2014). In this study, pathogenic variants predicted to lack both the motif and the domains have been reported, but no difference in severity of clinical symptoms due to the range of lacking regions was apparent. Thus, the factors affecting HVDAS severity are still unclear, confirming a high degree of genetic heterogeneity. Cardiovascular problems are observed in approximately one-third of patients (Gozes et al., 2017; Helsmoortel et al., 2014; Van Dijck et al., 2019). These include atrial septal defect, patent ductus arteriosus, mitral valve prolapse, tetralogy of Fallot, and ventricular septal defect, as seen in this study. As these features require appropriate medical management, providing genetic diagnosis in the patients with HDVAS is critical for their medical care.

We identified a heterozygous missense variant, c.1174G > A (p.G392R), of *SATB2* in MR456. *SATB2* is associated with GLASS syndrome (OMIM#612313), which is characterized by developmental delay and craniofacial anomalies including dental and palatal anomalies, and strabismus (Zarate & Fish, 2017; Zarate et al., 2018). *SATB2* encodes a highly conserved transcription factor that organizes chromatin remodeling by binding to genomic nuclear matrix-attachment regions and regulates gene regulatory networks associated with brain and craniofacial patterning development (Dobreva et al., 2006; Gyorgy, Szemes, de Juan Romero, Tarabykin, & Agoston, 2008). The SATB2 protein has two DNA-binding domains: CUT1 and CUT2. Of the 89 pathogenic variants, missense variants are enriched in the CUT1 domain (17 out of 31 missense variants); such variants may affect its DNA binding activity by changing the protein structure (Bengani et al., 2017; Zarate et al., 2019). Interestingly, MR456 with c.1174G > A (p.G392R) did not show palatal or dental anomalies, which were the typical features of GLASS syndrome. Conversely, the previously reported patient with c.1174G > C (p.G392R), carrying a different nucleotide substitution but the same residual alteration, had shown all the typical features. This study further confirms the clinical heterogeneity caused by *SATB2* pathogenic variants.

The c.2615_2616del (p.S872Cfs*43) variant of *ANKRD11* was identified in MR464. *ANKRD11* encodes a protein interacting with nuclear receptor complexes and functions as both a transcription inhibitor by interacting with histone deacetylases and histone molecules, and a transcription activator by interacting with histone acetyltransferases in different cellular contexts (Gallagher et al., 2015; Ka & Kim, 2018; A. Zhang et al., 2004). It is generally recognized that *ANKRD11* haploinsufficiency seems to cause KBG syndrome (OMIM#148050) characterized by mild ID in most, macrodontia, and short stature (Gallagher et al., 2015; Isrie et al., 2012; Sirmaci et al., 2011; Walz et al., 2015) with 200 reported pathogenic or likely

pathogenic variants (HGMD Professional 2020.4). This variant results in frameshift and premature termination, and presumably causes haploinsufficiency. However, his younger brother with borderline ID did not have the same variant. One potential explanation for this genetic discordance is genetic heterogeneity, as reported in familial ASD with or without ID, due to which about 70% of the affected siblings had different genetic causes from those of probands (Yuen et al., 2015). The second explanation is the complex etiologies in mild IDs. Mild ID is more prevalent and may involve more complex or multiple genetic factors than severe IDs (Vissers et al., 2016). This family illustrated a genetic discordance between siblings with borderline ID, representing a challenge in delineating their genetic causes.

c.587 T > C (p.I196T), an *SMS* variant, was identified in MR483. Loss-of-function *SMS* variants cause Snyder-Robinson syndrome (SRS; OMIM#309583), which is characterized by mild to profound ID, osteoporosis, genital anomalies, hypotonia, and seizures (Z. Zhang et al., 2013). SMS is a polyamine biosynthesis enzyme that converts spermidine to spermine. Both spermine and spermidine play essential roles in protein and nucleic acid synthesis, and the correct spermine: spermidine ratio is important for normal cell growth and development (Pegg, 2016). In the central nervous system, spermine binds to the ion channels involved in synaptic transmission and plasticity, and regulates ionic flux (Pegg, 2016), probably underlying the ID phenotype. Along with the typical features of SRS, MR483 also revealed peripheral pulmonary artery stenosis, a cardiovascular problem, which has not been reported as a symptom associated with *SMS* variants. In adults, polyamines are also associated with cardiovascular disease. For example, excess spermine exacerbates abnormal vascular remodeling, which is involved in pulmonary arterial hypertension, and spermine regulation by SMS inhibition is a potential therapeutic method (He et al., 2020). In addition, spermidine, another polyamine, improves age-related vascular disorders such as arteriosclerosis (J. Wang et al., 2020). *SMS* is expressed in the cardiovascular system during both the perinatal and adult periods; thus, it is possible that his abnormality of the pulmonary artery is associated with the *SMS* variant.

DYRK1A haploinsufficiency causes mental retardation, autosomal dominant 7 (MRD7; OMIM #614104), which is characterized by ID, autism spectrum disorder, and microcephaly often at birth or early infancy (van Bon et al., 2011). Intrauterine growth retardation is also common. Microcephaly and hypertonia beginning at birth seen in our patient were concordant with the typical features of MRD7. *DYRK1A* encodes a member of the dual-specificity tyrosine phosphorylation-regulated kinase family, which has both serine/threonine and tyrosine kinase activities and functions in cellular processes including proliferation and development. *Dyrk1A*$^{+/-}$ mice show growth retardation, developmental delay, learning deficits, and impaired recognition ability (Arque et al., 2008; Fotaki et al., 2002). *DYRK1A* is located in the Down syndrome critical region of chromosome 21 and is regarded as a candidate gene for ID in Down syndrome, in which *DYRK1A* is overexpressed due to an extra copy of chromosome 21. A total of 96 variants, including whole or partial *DYRK1A* deletions, have been reported as pathogenic or likely pathogenic variants (HGMD Professional 2020.4). The 75.8 kb deletion removes the putative promoter region and the first two exons of *DYRK1A*, which are constitutive in most transcripts, thereby presumably causing a loss-of-function allele and resulting in haploinsufficiency. Analyses of genome sequences flanking the deletion breakpoints identified no stretch of homologous sequences or palindromic structures that potentially stimulate recurrent genomic rearrangement at this locus (Figure 2). Thus, non-homologous end joining is likely the recombination mechanism for the genomic rearrangement in this case.

In conclusion, the WGS analysis of 45 Japanese patients with ID identified 12 pathogenic and likely pathogenic variants in clinically undiagnosed patients. We confirmed the diverse genetic etiology with a high frequency of *de novo* causative variants.

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AUTHOR CONTRIBUTIONS

Chihiro Abe-Hatano, Aritoshi Iida, Ken Inoue, and Yu-Ichi Goto designed the study. Chihiro Abe-Hatano, Aritoshi Iida, Keiko Ishikawa, Yukihide Momozawa, Shunichi Kosugi, Chikashi Terao, Naomichi Matsumoto, Yoichiro Kamatani, and Michiaki Kubo acquired and interpreted the genome data. Mariko Okubo, Yasuo Hachiya, Hiroya Nishida, Kazuyuki Nakamura, Rie Miyata, Chie Murakami, Kan Takahashi, Kyoko Hoshino, Haruko Sakamoto, Sayaka Ohta, Masaya Kubota, Eri Takeshita, Akihiko Ishiyama, Eiji Nakagawa, Masayuki Sasaki, and Mitsuhiro Kato acquired and interpreted the clinical information from affected individuals. Yoshiyuki Takahashi and Jun Natsume revised the manuscript critically. Chihiro Abe-Hatano, Aritoshi Iida, Shunichi Kosugi, and Ken Inoue drafted the manuscript. Yu-Ichi Goto supervised the study. All authors have read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

Variant data was submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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