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Original article

Genetic analysis of undiagnosed ataxia-telangiectasia-like disorders

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Abstract

Objectives: Defects in DNA damage responses or repair mechanisms cause numerous rare inherited diseases, referred to as "DNA-repair defects" or "DNA damage deficiency", characterized by neurodegeneration, immunodeficiency, and/or cancer predisposition. Early accurate diagnosis is important for informing appropriate clinical management; however, diagnosis is frequently challenging and can be delayed, due to phenotypic heterogeneity. Comprehensive genomic analysis could overcome this disadvantage. The objectives of this study were to determine the prevalence of ataxia-telangiectasia (A-T) and A-T-like DNA-repair defects in Japan and to determine the utility of comprehensive genetic testing of presumptively diagnosed patients in facilitating early diagnosis.

Methods: A nationwide survey of diseases presumably caused by DNA-repair defects, including A-T, was performed. Additionally, comprehensive next-generation sequencing (NGS) analysis, targeting known disease-causing genes, was conducted.

Results: Sixty-three patients with A-T or other diseases with characteristics of DNA-repair defects were identified. Thirty-four patients were genetically or clinically definitively diagnosed with A-T (n = 22) or other DNA-repair defects (n = 12). Genetic analysis of 17 presumptively diagnosed patients revealed one case of ataxia with oculomotor apraxia type 1 (AOA1); one ataxia with oculomotor apraxia type 2 (AOA2); two types of autosomal dominant spinocerebellar ataxia (SCA5, SCA29); two *CACNA1A*-related ataxias; one microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR); and one autosomal dominant *KIF1A*-related disorder with intellectual deficit, cerebellar atrophy, spastic paraparesis, and optic nerve atrophy. The diagnostic yield was 58.8%.

Conclusion: Comprehensive genetic analysis of targeted known disease-causing genes by NGS is a powerful diagnostic tool for subjects with indistinguishable neurological phenotypes resembling DNA-repair defects.

Keywords: DNA-repair defects; Ataxia-telangiectasia; Spinocerebellar ataxia; Cerebellar ataxia; Microcephaly; Next-generation sequencing

Abbreviations: AFP, alpha-fetoprotein; A-T, ataxia-telangiectasia; AOA1, ataxia with oculomotor apraxia type 1; AOA2, ataxia with oculomotor apraxia type 2; ATLD1, ataxia-telangiectasia-like disorder 1; DDR, DNA damage response; ExAC, Exome Aggregation Consortium; MCLMR, microcephaly with or without chorioretinopathy, lymphedema, or mental retardation; NBS, Nijmegen breakage syndrome; NGS, next-generation sequencing; SCA, spinocerebellar ataxia; SCAR1, spinocerebellar ataxia, autosomal recessive 1; T2DM, type 2 diabetes mellitus; WES, whole-exome sequencing.

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1. Introduction

Defects in the DNA damage response (DDR) or DNA repair cause numerous rare inherited diseases, referred to as "DNA-repair defects" or "DNA-repair deficiency". DNA-repair defects are a heterogeneous group of diseases with diverse clinical phenotypes, including neurological degeneration, immunodeficiency, and/or cancer predisposition.

Ataxia-telangiectasia (A-T, Mendelian Inheritance in Man (MIM) #208900) is a representative DNA-repair defect that causes progressive cerebellar ataxia, telangiectasia, immunodeficiency, and cancer predisposition. Cells derived from patients with A-T exhibit radiosensitivity, and *ATM*, the gene mutated in A-T, is central to DDR signaling [1].

Numerous DDR- or DNA repair-associated genes have been identified as causative for monogenic diseases. Pathogenic variants of the *MRE11A* and *NBN* (*NBS1*) genes, which are involved in the DDR pathway, cause ataxia-telangiectasia-like disorder 1 (ATLD1, MIM #604391) and Nijmegen breakage syndrome (NBS, MIM #251260), respectively. Although the neurological symptoms of ATLD1 are similar to those of A-T, the associated immunodeficiency is less severe. NBS typically exhibits immunodeficiency, similar to A-T; however, there is no ataxia. Hence, these DNA-repair defects can have overlapping features, including neurological symptoms, immunodeficiency, and/or cancer predisposition [2]. However, given the rarity of these diseases, most physicians will almost never encounter them. Moreover, many pediatric neurological diseases manifest non-specific phenotypes in multiple organs. These factors make it difficult for physicians to distinguish DNA-repair defects from other neurological diseases. Patients with DNA-repair defects exhibit genomic instability; therefore, administration of X-rays or radiomimetic drugs should be avoided in these cases. Moreover, surveillance for cancer development should be properly scheduled not only for patients, but also for carriers in the case of A-T. Dysregulation of the immune system can lead to opportunistic, or recurrent severe, infections, such as aspiration pneumonia, while prophylaxis for opportunistic infections or management of recurrent infections improves survival [3]. Hence, early diagnosis and intervention are important for patients with DNA-repair defects.

Comprehensive genome analysis, such as whole-exome sequencing (WES) analysis via next-generation sequencing (NGS), is expected to be a technological breakthrough that helps to unravel undiagnosed diseases; however, the diagnostic yield of WES is 34.3% for trio studies and 26.5% for those using singleton samples [4]. By contrast, targeted exome sequencing of samples from patients with phenotypically homogeneous diagnoses generates a higher diagnostic yield, because it involves deep sequencing ($\geq$500–1000$\times$ coverage) of key genes or regions of interest.

With the aim of facilitating early diagnosis and intervention to improve clinical management, we conducted a nationwide survey of A-T and A-T-like DNA-repair defects in Japan and carried out comprehensive genetic testing for patients with clinically presumptive diagnoses.

2. Materials and methods

2.1. Participants

For this nationwide survey, a list of diseases involving DNA-repair defects, including A-T and A-T-like DNA-repair defects, was generated (Table 1). A-T was definitively diagnosed according to the criteria of the Project for Rare/Intractable Diseases "Establishment of diagnostic and therapeutic approach, elucidation of etiology for ataxia telangiectasia and DNA damage response associated diseases" (Supplementary Data 1) and/or molecular analysis. A-T-like DNA-repair defects were definitively diagnosed by genetic testing.

To identify patients with DNA-repair defects, including A-T and symptoms resembling A-T, patients with disorders characterized by more than two of the following symptoms were recruited: cerebellar ataxia, microcephaly, ocular abnormalities (e.g., ocular telangiectasia and/or abnormal eye movements such as oculomotor apraxia), immunodeficiency (defined in Supplementary Data 2), and/or medical history of malignant tumors. Cases who did not fulfil any diagnostic criteria or who were temporarily diagnosed without genetic testing were defined as having clinically presumptive diagnoses.

Primary questionnaires (n = 1250), soliciting information about patients with DNA-repair defects, including A-T and A-T-like diseases (Table 1), were sent to departments of neurology, pediatric neurology, and pediatrics in Japan. The response rate was 53% (n = 642). A secondary questionnaire (n = 54), requesting details of the number of patients, their clinical diagnoses, and intentions to perform genetic testing, was sent to attending physicians who managed the care of candidates for inclusion in the study. The response rate was 91% (n = 49). Seventeen patients with clinically presumptive diagnoses underwent genetic testing. Medical reports and blood samples were obtained with the written informed consent of the patients or their legal guardians. This research was approved by the ethical committee at Tokyo Medical and Dental University (Approval Number, 196).

Table 1
List of DNA-repair deficiency-associated genes and diseases included in the nationwide survey.

Disease	Gene	Phenotypes
Ataxia-telangiectasia (A-T)	*ATM*	Cerebellar ataxia, telangiectasia, immune defects, predisposition to malignancy, elevated α-fetoprotein
Seckel syndrome	*ATR*	Microcephaly, primordial dwarfism, dysmorphic facial features
Nijmegen breakage syndrome (NBS)	*NBS1*	Microcephaly, immunodeficiency, cancer predisposition
DNA ligase IV syndrome	*LIG4*	Microcephaly, immunodeficiency, developmental and growth delay
Ataxia-telangiectasia-like disorder 1 (ATLD1)	*MRE11*	Mild A-T-like features, possible cancer predisposition
Nijmegen breakage syndrome-like disorder (NBS-LD)	*RAD50*	NBS-like phenotype
Fanconi anemia, complementation group D1	*BRCA2*	Microcephaly, progressive bone marrow failure, developmental and growth delay
Ataxia with oculomotor apraxia type 1 (AOA1)	*APTX*	Ataxia, neurodegeneration, oculomotor apraxia, peripheral neuropathy, hypoalbuminemia
Ataxia with oculomotor apraxia type 2 (AOA2) and spinocerebellar ataxia, autosomal recessive 1 (SCAR1)	*SETX*	Ataxia, neurodegeneration, oculomotor apraxia, dystonia, peripheral neuropathy, elevated α-fetoprotein
Spinocerebellar ataxia, autosomal recessive with axonal neuropathy (SCAN1)	*TDP-1*	Ataxia, neurodegeneration, peripheral axonal motor and sensory neuropathy
Microcephaly 1, primary, autosomal recessive (MCPH1)	*BRIT1*	Microcephaly, mental retardation
RIDDLE syndrome	*RNF168*	Dysmorphic features, learning difficulties, immunodeficiency
Severe combined immunodeficiency with microcephaly, growth retardation, and sensitivity to ionizing radiation	*NHEJ1*	Microcephaly, immunodeficiency, growth retardation
Microcephaly, seizures, and developmental delay (MCSZ)	*PNKP*	Microcephaly, seizures, developmental delay

2.2. Targeted sequencing

Genomic DNA was extracted from peripheral blood mononuclear cells using SepaGene (Sekisui Medical, Co., Ltd, Tokyo, Japan). Comprehensive targeted sequencing was performed using TruSight One sequencing panels (Illumina, San Diego, CA), which included 4813 genes associated with known clinical phenotypes, on the Illumina MiSeq platform with paired-end reads. For bioinformatic analyses, data were processed using VariantStudio software (Illumina). Variants of unknown significance were filtered using information from the 1000 genomes, Exome Aggregation Consortium East Asian cohort (ExAC East Asian), Human Genetic Variation Database (HGVD), and Integrative Japanese Genome Variation Database (IJGVD). The pathogenicity of missense variants was predicted using the Polymorphism Phenotyping v2 database (Polyphen-2) and Protein Variation Effect Analyzer (PROVEAN). Identified variants were confirmed by Sanger sequencing.

2.3. Sanger sequencing

Exons of specific genes were amplified by PCR using Power SYBR® Green PCR Master Mix and a Veriti 96-Well Thermal Cycler (Applied Biosystems, Carlsbad, CA). Nucleotide sequencing was performed by cycle sequencing using the BigDye® Terminator v3.1 Reaction Kit (Applied Biosystems) followed by capillary electrophoresis on an Applied Biosystems 3130xl Genetic Analyzer.

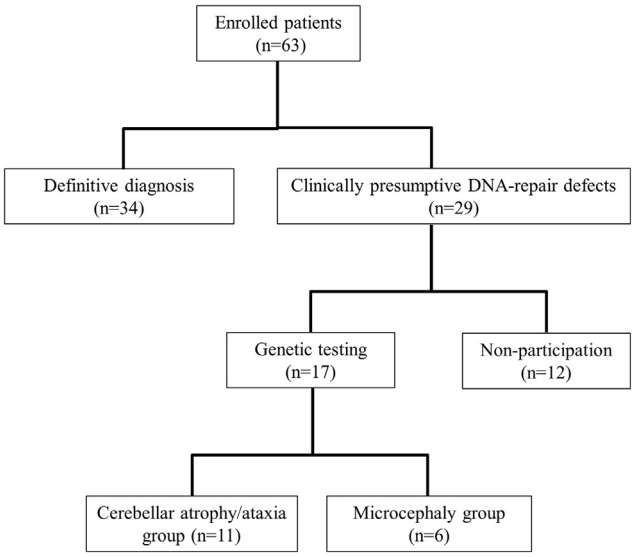

Fig. 1. Flow diagram illustrating patient selection.

3. Results

3.1. Nationwide survey of DNA-repair defects

Sixty-three patients were identified from the nationwide survey. A flow diagram illustrating patient selection is presented in Fig. 1. Thirty-four patients, including 22 with A-T, were definitively diagnosed (Table 2). Twenty-nine patients had clinically presumptive diagnoses of A-T or A-T-like DNA-repair defects, or a clinically uncertain consensus diagnosis. Among them, 17 patients agreed to provide samples for genetic

Table 2
Cases with definitive diagnoses.

Disease	Gene	Number of patients
Ataxia-telangiectasia (A-T)	*ATM*	22
Ataxia with oculomotor apraxia type 1 (AOA1)	*APTX*	5
Ataxia with oculomotor apraxia type 2 (AOA2)	*SETX*	3
Fanconi anemia, complementation group D1	*BRCA2*	2
Seckel syndrome	*ATR*	1
Ataxia-telangiectasia-like disorder 1 (ATLD1)	*MRE11A*	1

analysis, while 12 patients declined genetic testing. A summary of the clinical features and data from patients included in the genetic analysis is provided in Table 3. All patients (except for Patient 2) were born from non-consanguineous parents. There were 4 males and 13 females with an age range of 2–51 years and they had no family history of A-T or A-T-like diseases.

We divided patients into two groups: the cerebellar atrophy and ataxia (atrophy/ataxia) group (Patients 1–11) and the microcephaly group (Patients 12–17). In the cerebellar atrophy/ataxia group, developmental delay or intellectual disability was noted in six patients (54%). Two patients showed oculomotor apraxia and nystagmus, two had telangiectasia, and one had IgA deficiency. Elevated alpha-fetoprotein (AFP) was noted in one patient. In the microcephaly group, all patients had mild to severe microcephaly and intellectual disability, and one patient developed acute leukemia. None of the patients were immunodeficient or exhibited ocular abnormalities.

The presumptive clinical diagnoses of attending physicians are provided in Table 3. In the cerebellar atrophy/ataxia group, 5 of 11 patients were presumptively diagnosed with A-T, one patient was presumptively diagnosed with each of ataxia with oculomotor apraxia type 1 (AOA1) and type 2 (AOA2), and the remaining four patients were undiagnosed. In the microcephaly group, four of six patients were diagnosed with Seckel syndrome and two patients were not diagnosed with any specific disorder.

3.2. Gene variants

Overall, 10 presumptive disease-causing variants were identified in the 17 patients (Supplementary Fig. 1), representing a diagnostic yield of 58.8%. Final definitive diagnoses based on genetic testing are presented in Table 3. In the cerebellar atrophy/ataxia group, six gene variants (*APTX, SETX, SPTBN2, KIF1A, ITPR1,* and *CACNA1A*) associated with neurodegenerative conditions were identified in 11 patients (54.5%). Two other disease-associated gene variants (*PAX4* and *TNFRSF13B*) were also identified. By contrast, in the microcephaly group, only one variant (*KIF11*) was identified among the six patients (16.6%). The parents of seven cases refused to undergo genetic testing.

4. Discussion

Patient 1 was diagnosed with AOA1 in this study, based on targeted sequencing data. AOA1 is characterized by childhood-onset, slowly progressive cerebellar atrophy/ataxia, followed by oculomotor apraxia and severe primary peripheral motor axon neuropathy, and is caused by autosomal recessive pathogenic variants in *APTX*. AOA1 is the most frequent cause of autosomal recessive ataxia in Japan [5]. The mean age of ataxia onset is 4.3 years (range, 2–10 years). Other clinical features include nystagmus, dysarthria, and cognitive impairment. Oculomotor apraxia may be present at disease onset; however, hypoalbuminemia, hypercholesterolemia, and peripheral neuropathy become apparent during the later stages of the condition [6]. Homozygotes for the *APTX* p.Q246Gfs variant are relatively common in Japan [7]; this variant causes severe phenotypes, relative to other missense variants [8]. In our case, oculomotor apraxia was not prominent and the specific laboratory findings were not apparent at the age at which the patient was tested. Therefore, the attending physician could not nominate AOA1 as a differential diagnosis for this patient. Moreover, diagnosis based on symptoms such as hypoalbuminemia, hypercholesterolemia, and peripheral neuropathy is difficult during childhood.

We diagnosed Patient 2 with AOA2, based on homozygous mutation of *SETX*. Juvenile amyotrophic lateral sclerosis 4, (ALS4, MIM #602433) is caused by autosomal dominant inheritance of *SETX* variants, while autosomal recessive inheritance of mutations in this gene leads to spinocerebellar ataxia, autosomal recessive 1 (SCAR1, MIM #606002), also referred to as AOA2 [9]. AOA2 is characterized clinically by cerebellar atrophy, axonal sensorimotor neuropathy, oculomotor apraxia, and elevated AFP [9]. The onset of ataxia is between 10 and 22 years of age, and it progresses slowly, leading to severe disability [10]. The phenotypes caused by SETX pathogenic variants are highly variable, as mentioned above; therefore, it is difficult to ascertain the causative gene through phenotypic evaluation. Oculomotor apraxia is a distinctive feature of A-T, ATLD1, AOA1, and AOA2 that is present in almost 50% of patients; however, this condition is occasionally hard to diagnose during infancy and early childhood.

Table 3
Clinical summary and annotated gene variants of 17 patients with presumptive DNA-repair defects.

Group	No.	Presumptive diagnosis	Definitive diagnosis	Gene	Age (years)	Sex	Ocular involvement	ID	AFP	Otherclinical features	Other MRI findings	Variant	Zygosity	Varianttype	In silico predictionPolyphen-2PROVEAN	ExAC-VAFToMMo-VAFHGVD-VAF	Inheritance	Ref.
Cerebellar atrophy/ ataxia	1	Uncertain	AOA1	*APTX*	7	F	OMA Strabismus	+	Normal	–		NM 001195248.1: c.731dupT [p. E246Gfs*38]	Hom	Frameshift		0.0001156 – –	Inherited	[8]
	2	AOA2	AOA 2	*SETX*	51	F	–	+	Elevated	Peripheral neuropathy Epilepsy		NM_015046.5: c.7025T>G [p. I2342S]	Hom	Missense	Damaging (0.942) Deleterious (−4.94)	– – –	Unknown	–
	3	A-T	SCA 5	*SPTBN2*	6	F	–	+	Normal	Abnormal EEG		NM_006946.2: c.1309C>G [p. R437G]	Het	Missense	Damaging (0.926) Deleterious (−6.30)	– – –	De novo	–
	4	Uncertain	*KIF1A*-related disorder	*KIF1A*	12	F	Optic atrophy	+	Normal	Hypothyroidism Chilblains		NM_001244008.1: c.650C>T [p. S217F]	Het	Missense	Damaging (0.997) Deleterious (−5.41)	– – –	Unknown	–
	5	A-T	*CACNA1A*-relatedataxia	*CACNA1A*	39	F	Telangiectasia Nystagmus Exophthalmos	+	Normal	Adult-onset ataxia Papillary thyroid carcinoma		NM_023035.2: c.2044G>T [p. G682W]	Het	Missense	Damaging (0.998) Deleterious (−7.30)	– – –	Unknown	–
	6	AOA1	*CACNA1A*-related congenital ataxia	*CACNA1A*	2	F	OMA	–	Normal	Dysarthria		NM_023035.2: c.1954G>A [p. D652N]	Het	Missense	Damaging (0.998) Deleterious (−4.58)	– – –	Unknown	–
	7	Uncertain	SCA29	*ITPR1*	10	F	Nystagmus	+	Normal	Euryopia Prominent forehead Epicanthic fold	Pontine tegmental atrophy	NM_001168272.1: c.800C>T [p. T267M]	Het	Missense	Damaging (1) Deleterious (−5.47)	– – –	De novo	[28]
	8	A-T	DM, type 2, susceptibility	*PAX4*	15	F	Telangiectasia	N/A	Normal	Obesity, diabetes Hyperlipidemia Hypopituitarism		NM_006193.2: c.361C>T [p. R121W]	Het	Missense	Damaging (1) Deleterious (−5.90)	0.002771 0.0233 0.027	Unknown	[4]
	9	A-T	CVID	*TNFRSF13B*	4	M	–	+	Normal	Hypogammaglobulinemia (IgA)		NM_012452.2: c.214C>T [p. R72C]	Het	Missense	Damaging (0.928) Deleterious (−3.77)	0.0001157 – –	Unknown	–
	10	Uncertain	N/A	N/A	10	F		–	Normal									
	11	A-T	N/A	N/A	47	M	–	–	Normal		Middle cerebellarpeduncle atrophy							
Microcephaly	12	Seckel syndrome	MCLMR	*KIF11*	10	F	–	+	NE	Epilepsy		NM_004523.3: c.700C>T [p. R234C]	Het	Missense	Damaging (1) Deleterious (−7.98)	– – –	Unknown	[32]
	13	Seckel syndrome	N/A	N/A	9	M	–	+	NE	Short stature Cryptorchidism								
	14	Seckel syndrome	N/A	N/A	7	M	–	+	NE	Epilepsy	Ventriculomegaly Cortical dysplasia							
	15	Uncertain	N/A	N/A	N/A	F	–	+	NE									
	16	Seckel syndrome	N/A	N/A	3	F	–	+	NE	Short stature								
	17	Uncertain	N/A	N/A	8	F	–	+	NE	Short stature Leukemia								

Abbreviations: cerebellar atrophy/ataxia, cerebellar atrophy and ataxia; N/A, not applicable; M, male; F, female; ID, intellectual disability; OMA, oculomotor apraxia; NE, not examined; Uncertain, uncertain clinical consensus diagnosis; Hom, homozygote; Het, heterozygote; ExAC, Exome Aggregation Consortium (East Asian); ToMMo, Tohoku Medical Megabank Organization; HGVD, Human Genetic Variation Database; VAF, variant allele frequency; A-T, ataxia-telangiectasia; AOA1, ataxia with oculomotor apraxia type 1; AOA2, ataxia with oculomotor apraxia type 2; SCA, spinocerebellar ataxia; DM, diabetes mellitus; MCLMR, microcephaly with or without chorioretinopathy, lymphedema, or mental retardation; CVID, common variable immunodeficiency; Ref., reference.

Serum AFP levels are consistently elevated during the disease, and usually also before disease onset [10]. Therefore, increased AFP level is a feature that can assist diagnosis. Ataxia accompanies increased AFP in A-T, AOA2, and RNF168 deficiency.

Heterozygous pathogenic variants of *SPTBN2* cause autosomal dominant spinocerebellar ataxia 5 (SCA5, MIM #600224). *SPTBN2* encodes beta-III spectrin, and is highly expressed in Purkinje cells [11]. SPTBN2 stabilizes the glutamate transporter, EAAT4; therefore, defects in SPTBN2 function lead to disturbances in the localization and function of mGluR1α [12]. The p.R437G variant identified in Patient 3 was a de novo missense variant observed in neither parent and likely to be pathogenic. Clinically, SCA5 is a slowly progressive cerebellar ataxia with juvenile to adult onset, whereas our case had infantile onset, and exhibited non-progressive ataxia [13], and was therefore considered to have ataxic cerebral palsy, which is primarily caused by peri- to neo-natal asphyxia [14,15]. Genetic investigations are rarely performed in patients with cerebral palsy; therefore, once a patient is diagnosed with this condition, a genetic diagnosis will be delayed.

Heterozygosity for pathogenic variants in *KIF1A* causes autosomal dominant mental retardation (MRD9, MIM #614255), whereas homozygosity results in spastic paraplegia 30 (SPG30, MIM #610357) and hereditary sensory neuropathy, Type IIC (HSN2C, MIM #614213). De novo mutations in the motor domain of *KIF1A* cause cognitive impairment, spastic paraparesis, axonal neuropathy, and cerebellar atrophy [16]. *KIF1A*, a member of the kinesin gene family, is strongly expressed in neurons and encodes a motor protein involved in the anterograde transport of synaptic-vesicle precursors along axonal microtubules [17,18]. In the present study, we identified a novel heterozygous variant encoding p.S217F in *KIF1A*; in the absence of functional or familial analyses, it is not clear whether this variant is truly pathogenic. Nonetheless, the clinical features of Patient 4 closely resembled those of previously reported cases carrying neighboring pathogenic variants (p.S215R, p.R216P) [16]. As in the previously described cases, this case was clinically diagnosed with cerebral palsy, because they developed periventricular leukomalacia; therefore, genetic diagnosis was delayed.

The *CACNA1A* gene encodes the transmembrane pore-forming subunit of a CaV2.1P/Q-type voltage-gated calcium channel expressed in Purkinje cells [19]. Pathogenic variants of *CACNA1A* result in clinically variable neurological defects, including familial hemiplegic migraine (FHM, MIM #141500) and episodic or progressive adult-onset ataxia (EA2, MIM #108500; and SCA6, MIM #183086). The development of SCA6 is primarily caused by CAG trinucleotide repeat expansions within the *CACNA1A* gene [20]; however, missense pathogenic variants can also cause *CACNA1A*-related ataxia, which closely resembles SCA6 [21,22]. Two *CACNA1A*-associated variants, p.G682W and p.D652N, were identified in Patients 5 and 6. A previously described cerebellar ataxia case with a *CACNA1A* mutation (p.G686S) exhibited progressive adult onset [22], and the clinical phenotype was concordant with that of our case. The case carrying p.G682W was unique in presenting with telangiectasia and cancer development, mimicking the characteristic features of A-T, whereas Patient 6 with the p.D652N variant exhibited oculomotor apraxia from 3 months old, and an unsteady walk at 17 months old. Recently, *CACNA1A* variants have been identified in patients with non-progressive congenital ataxia with epileptic seizure or hemiplegic migraine attacks [23]. Patients 5 and 6 have no history of seizure or migraine attacks to date, and diagnosis is difficult during the early pediatric period. Again, these are novel variants; therefore, it is unclear whether they are truly pathogenic without functional or familial analyses.

Variants in the *ITPR1* gene cause spinocerebellar ataxia type 15 (SCA15, MIM #606658) and spinocerebellar ataxia type 29, congenital non-progressive (SCA29, MIM #117360). The *ITPR1* gene encodes inositol 1,4,5-triphosphate receptor type 1, an intracellular calcium channel, expressed at high levels in Purkinje cells [24]. The *ITPR1* p.T267M variant has been previously reported as a causative variant in SCA29 [25]. Associated symptoms include infantile-onset delayed motor development, mild cognitive delay, and non-progressive cerebellar ataxia with cerebellar atrophy. Patient 7 had distinctive dysmorphic facial features, including euryopia, prominent forehead, and epicanthic fold. These characteristic features have not been reported previously and likely obfuscated the diagnosis based on clinical features.

Human microcephaly comprises a heterogeneous group of conditions characterized by failure of normal brain growth. Variants in *KIF11*, a homotetramer kinesin motor protein of the kinesin-5 family [26], cause autosomal dominant microcephaly with or without chorioretinopathy, lymphedema, or mental retardation (MCLMR: MIM #152950). The *KIF11* variant, p.R234C, has been previously reported as causative [27]. Patient 12 had no sign of chorioretinopathy; therefore, it was difficult to identify the causative gene.

This study targeted cases with ataxia and some additional phenotypes. Through our analysis, two presumptive disease-causing variants, which are not directly associated with neurodegeneration, were identified. One, encoding *PAX4* p.R121W, which is associated with type 2 diabetes mellitus (T2DM) [28], was identified in Patient 8 who exhibited ataxia and T2DM, while the other (*TNFRSF13B* p.R72C) was in a gene associated

with common variable immunodeficiency and was identified in Patient 9 who exhibited ataxia and immunodeficiency. These observations suggest that ataxia with complex phenotypes may have an underlying bi-genic cause. We previously reported a case of ataxia with immunodeficiency and speculated that the etiology of the ataxia was chronic viral infection of the central nervous system caused by immunodeficiency [29]. As described in this case, environmental factors may also modify monogenic disorders.

A limitation of this study is the lack of functional analysis of newly identified, presumably pathogenic, variants. Further functional analysis is required to consolidate our findings; however, we believe that the variants identified in this study are likely to be pathogenic, as discussed above.

This nationwide survey identified 22 A-T patients with genetically or clinically definitive diagnoses. The overall prevalence of A-T patients is 1:100,000–150,000 live births. The percentage of *ATM* variant carriers in the population is estimated as 0.5–1% [30]. The number of patients identified by our survey was lower than expected, suggesting that many patients may remain undiagnosed for years, exposing them to the risk of long-term complications.

In this study, we performed genetic analysis to facilitate early diagnosis and improve clinical management for patients with A-T and A-T-related DNA-repair defects; however, childhood-onset cerebellar ataxia and congenital microcephaly are rare and have heterogeneous clinical phenotypes and etiologies [31,32], and hence definitive diagnoses based on clinical presentation remain challenging, despite improved diagnostic methods, such as neuroimaging and metabolic tests. Recent advances in genomic technologies allow the identification of pathogenic gene variants in patients with indistinguishable neurological phenotypes. A targeted sequencing approach, using the TruSight One sequencing panel, allowed us to investigate a selected set of disease-causing genes and variants within several days; however, causative variants were not detected in 9 of 17 patients (53%). Further genetic studies, such as array comparative genomic hybridization, investigation of expanded trinucleotide repeats, and WES, including analysis of family members, are required to identify primary disease-causing variants. Our results indicate that targeted sequencing is a useful approach to identify genetic variants in patients with rare DNA-repair defects. This approach will facilitate accurate diagnosis at an early stage of disease and improve clinical management of severe complications.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2018.09.007.

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