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Original article

Genomic backgrounds of Japanese patients with undiagnosed neurodevelopmental disorders

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Abstract

Background: Recently, many genes related to neurodevelopmental disorders have been identified by high-throughput genomic analysis; however, a comprehensive understanding of the mechanism underlying neurodevelopmental disorders remains to be established. To further understand these underlying mechanisms, we performed a comprehensive genomic analysis of patients with undiagnosed neurodevelopmental disorders.

Methods: Genomic analysis using next-generation sequencing with a targeted panel was performed for a total of 133 Japanese patients (male/female, 81/52) with previously undiagnosed neurodevelopmental disorders, including developmental delay (DD), intellectual disability (ID), autism spectrum disorder (ASD), and epilepsy. Genomic copy numbers were also analyzed using the eXome Hidden Markov Model (XHMM).

Results: Thirty-nine patients (29.3%) exhibited pathogenic or likely pathogenic findings with single-gene variants or chromosomal aberrations. Among them, 20 patients were presented here. Pathogenic or likely pathogenic variants were identified in 18 genes, including *ACTG1*, *CACNA1A*, *CHD2*, *CDKL5*, *DNMT3A*, *EHMT1*, *GABRB3*, *GABRG2*, *GRIN2B*, *KCNQ3*, *KDM5C*, *MED13L*, *SCN2A*, *SHANK3*, *SMARCA2*, *STXBP1*, *SYNGAP1*, and *TBL1XR1*.

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Conclusion: A diagnostic yield of 29.3% in this study was nearly the same as that previously reported from other countries. Thus, we suggest that there is no difference in genomic backgrounds in Japanese patients with undiagnosed neurodevelopmental disabilities. Although most of the patients possessed *de novo* variants, one of the patients showed an X-linked inheritance pattern. As X-linked recessive disorders exhibit the possibility of recurrent occurrence in the family, comprehensive molecular diagnosis is important for genetic counseling.

Keywords: X-linked recessive; Genomic copy number variations; single nucleotide variants; Dysmorphism

1. Introduction

Neurodevelopmental disorders are a consequence of inadequate brain development associated with a range of quad neurological features, including developmental delay (DD), intellectual disability (ID), autism spectrum disorder (ASD), and epilepsy. Given that structures and functions of the human brain are complex and precise, it is clear that a large number of biomolecules are involved in its development. Patients with chromosomal microdeletions often present with neurodevelopmental disorders except in rare cases, suggesting that many genes distributed throughout the chromosomes are related to brain development and/or neurological functions [1]. Recent innovations in analytical equipment have allowed for high-throughput genomic analysis [2]. As a result, many genes related to these conditions have been identified [3]. The predicted functions of such genes are varied, and the mechanisms responsible for neurodevelopmental disorders remains to be fully elucidated.

To further understand the underlying mechanisms of neurodevelopmental disorders, we performed a genomic analysis on 133 Japanese patients suffering from undiagnosed neurodevelopmental disorders.

2. Materials and methods

2.1. Patients

The study included Japanese patients with undiagnosed neurodevelopmental disorders who fulfilled one of the following conditions: 1) DD and/or ID with intellectual quotient (IQ) or developmental quotient (DQ) less than 70, 2) ASD diagnosed by Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5). Patients who were suspected to possess known congenital disorders were excluded. This study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the institution. After receiving written informed consent from patients' families, we obtained blood samples from the patients and their family members, and we then extracted genomic DNA. Detailed clinical information of the patients was also acquired.

2.2. Methods

Clinical exome sequencing was performed using the TruSight One v1.0 sequencing panel (Illumina, San Diego, CA) as previously described [4]. The extracted data was mapped to the GRCh37/hg19 reference genome, annotated, and filtered using the Variant Studio software (Illumina). Prediction scores were obtained through WANNOVAR (http://wannovar.wglab.org/). Obtained variants were filtered by the following strategies: 1) variants with synonymous changes, variants with more than 1% general population frequency, and variants registered in the Human Genetic Variation Database (HGVD), which includes genetic variations obtained from a cohort of Japanese individuals [5], were eliminated; 2) variants in genes which showed no functional relevance to patient phenotypes were eliminated; 3) variants with combined annotation-dependent depletion (CADD) scores under 20 were eliminated (http://cadd.gs.washington.edu/). Candidate variants were checked against the databases ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/). Possible pathogenic variants were curated manually by inspections of genomic information in comparison with clinical features. For evaluation of loss-of-function intolerant, pLI scores of genes were obtained through ExAC Browser Beta (http://exac.broadinstitute.org/).

For all samples, the existence of genomic copy number variations (CNVs) was firstly investigated by the eXome Hidden Markov Model (XHMM) using the obtained next-generation sequencing data [6]. Any identified possible pathogenic CNVs were confirmed by other methods, including chromosomal microarray testing and fluorescence in situ hybridization.

It is easy to draw final conclusions when only a small number of candidate variants exist, even if we analyze only patient sample. We defined this pattern as "Solo" sample usage. Conversely, we further analyzed samples from both parents when there were too many candidate variants after filtering of patient results. We defined this as "Trio" analysis. This diagnostic flowchart is demonstrated in Fig. 1. Subsequently, the results obtained from the parent samples were compared to determine

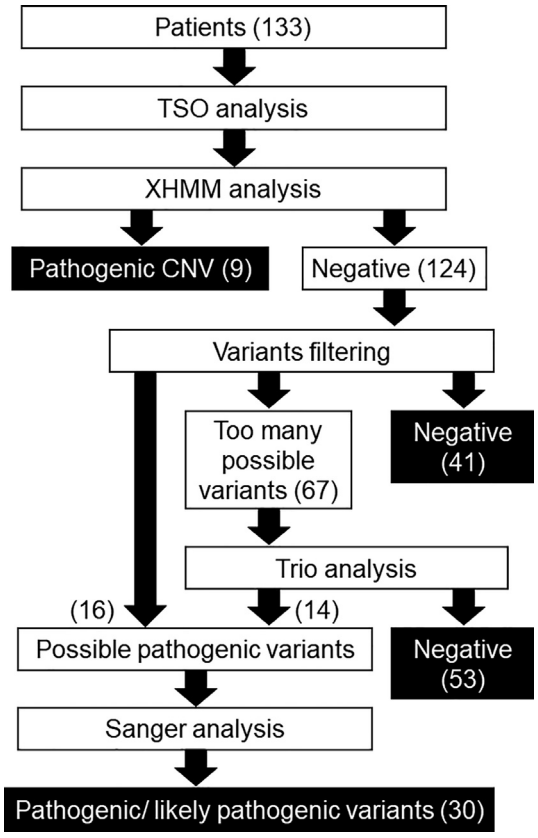

Fig. 1. Diagnostic flow chart in this study. Black squares show the final conclusion. Arabic numerals represent the number of adaptation samples. TSO, TruSight One panel; XHMM, eXome Hidden Markov Model; CNV, copy number variation.

if they matched any pattern of Mendelian inheritance, including *de novo* origins, autosomal recessive traits, and X-linked recessive traits. Finally, DNA samples obtained from all patients and their parents were re-analyzed by Sanger sequencing to provide confirmation (Fig. 2). Conclusive assessment for molecular diagnosis was evaluated according to the recommendations of the American College of Medical Genetics and Genomics [7]. Descriptions of the variants were checked using Mutalyzer web-based software (https://mutalyzer.nl/).

Biological relationships between patients and their parents were checked through "Trio" analysis. For families of the patients with "Solo" sample usage, biological relationships were checked using the ABI Prism Linkage Mapping Set Panel (Thermo Fisher Scientific, Waltham, MA) with at least 3 loci, as described previously [8].

Statistical analysis was performed by chi square test.

3. Results

3.1. Clinical features of patients in this study

A total of 133 patients (male/female, 81/52; median age, 4.0 years) were included in this study. All patients included in this study were new patients who had not been previously analyzed for genomic variants. Among the four findings of neurodevelopmental disorders (DD, ID, ASD, and epilepsy), DD and/or ID were observed in all patients (Table 1). Percentages to satisfy ASD, epilepsy, and other complications such as microcephaly and distinctive features are also shown in Table 1. Diagnostic yields when patients are associated with such findings in addition to DD/ID are listed in Table 1. As shown, there was no significant difference between them.

Among the 133 patients, 39 patients (29.3%) exhibited genomic findings related to molecular diagnosis (Tables 1 and 2). Nine patients showed no possible candidate variants; however, they did exhibit chromosomal aberrations determined by XHMM (Table 2). Two of them were typical Xq28 duplications [9]. The main clinical features of the present patients are summarized in Supplemental Table S1. More detailed clinical features for each patient are described in Supplemental information.

Of the 133 patients, 67 patients (50.4%) were analyzed through "Trio" analysis (Fig. 1). Accordingly, the other 66 patients (49.6%) were analyzed by "Solo" sample usage. Diagnostic yield for samples analyzed through "Trio" analysis was 14/67 (20.1%). Diagnostic yield of "Solo" sample usage approach was higher than that of "Trio" analysis (25/66 [37.9%]); however, all nine chromosomal aberrations were included in "Solo" sample usage approach. Thus, diagnostic yield of single gene variants (excluding chromosomal aberrations) through "Solo" sample usage was 16/57 (28.1%). Although it is higher than that through "Trio" analysis, there was no significant difference between them.

The biological relationships between patients and their parents were examined, and there were no contradictions in any of the families.

3.2. Identified variants

Thirty-two patients possessed single-gene variants. Twelve patients were reported previously [4,10], and

Table 1
Percentage to satisfy each condition and respective diagnostic yields.

Classification	Patients	Ratio[$]	Diagnostic yield[#]
DD/ID	133	100%	29.3%
Autism spectrum disorder	54	40.6%	31.5%
Epilepsy	45	33.8%	28.9%
Other complication(s)[*]	43	32.3%	37.2%
Total	133	100%	29.3%

DD/ID; developmental delay and/or intellectual disability.
[*] Complications include major/minor anomalies.
[$] Percentage of patients who showed other findings in addition to DD/ID.
[#] Diagnostic yields when patients are associated with other findings in addition to DD/ID.

Table 2
Number of identified variants in this study.

	Male	Female	Total
Patients	81	52	133
Chromosomal aberrations	5	4	9
Variants previously reported elsewhere	8	2	10
Pathogenic single nucleotide variants*	5	5	10
Likely pathogenic single nucleotide variants*	6	4	10
Overall detection	24	15	39
Overall detection ratio	29.6%	28.8%	29.3%

* Variants reported in this manuscript.

the remaining 20 patients are presented here. 20 variants in 18 genes (*ACTG1*, *CACNA1A*, *CHD2*, *CDKL5*, *DNMT3A*, *EHMT1*, *GABRB3*, *GABRG2*, *GRIN2B*, *KCNQ3*, *KDM5C*, *MED13L*, *SCN2A*, *SHANK3*, *SMARCA2*, *STXBP1*, *SYNGAP1*, and *TBL1XR1*) were assessed as "pathogenic" or "likely pathogenic" (Supplemental Table S1). Nineteen patients showed *de novo* variants, and one exhibited X-linked recessive variants (Fig. 2). There was no variant related to autosomal recessive traits.

In the case of the *SHANK3* variant, Mutalyzer did not show any predicted amino-acid change. This signifies a possible problem in the annotation of the reference sequence. The final description of the amino acid change derived from the identified variant was manually confirmed.

Variants in *CACNA1A*, *CHD2*, *CDKL5*, *GABRB3*, *GABRG2*, *GRIN2B*, *KCNQ3*, *MED13L*, *SCN2A*, *STXBP1*, and *SYNGAP1* are often observed in non-specific neurodevelopmental disorders or epileptic encephalopathy [11–14]. As the clinical features of the patients (patients 2, 3, 4, 5, 6, 9, 10, 11, 12, 14, 15, 18, and 19) harboring these gene variations were similar to those reported in previous studies, there is no contradiction in the molecular diagnosis for these patients.

ACTG1, *DNMT3A*, *EHMT1*, *KDM5C*, *SHANK3*, *SMARCA2*, and *TBL1XR1* are related to syndromic ID, specifically Baraitser-Winter cerebrofrontofacial syndrome (MIM #614583), Tatton-Brown-Rahman syndrome (MIM #615879), Kleefstra syndrome (MIM #610253), X-linked syndromic ID Claes-Jensen-type (MIM #300534), Phelan-McDermid syndrome (MIM #606232), Nicolaides-Baraitser syndrome (MIM #601358), and Pierpont syndrome (MIM #602342), respectively.

Two genes, *ACTG1* and *DNMT3A*, showed pLI scores less than 0.9, indicating that they are loss-of-function tolerant. An *ACTG1* variant identified in patient 1 was a missense variant, and thus it was expected to exert a gain-of-function effect. Indeed, patient 1 with the *ACTG1* missense variant showed characteristic gestalts. Therefore, this novel variant was considered responsible for Baraitser-Winter cerebrofrontofacial syndrome (MIM #614583) [15]. Conversely, patient 7 showed a loss-of-function variant in *DNMT3A*. Previously, many loss-of-function variants in *DNMT3A* have been reported as pathogenic. Chromosomal deletions, including *DNMT3A*, can cause Tatton-Brown-Rahman syndrome, as previously demonstrated by our research group [16]. Thus, haploinsufficiency of *DNMT3A* is the main cause of this syndrome [17]. Indeed, patient 7 showed ID associated with a distinctive facial appearance and overgrowth. Thus, the identified loss-of-function variant in *DNMT3A* was considered pathogenic.

An *EHMT1* variant identified in patient 8 is related to Kleefstra syndrome (MIM #610253) [18]. Patient 16 possessing a *de novo* variant of *SHANK3* showed characteristics of Phelan-McDermid syndrome [19,20]. Thus, clinical diagnosis of this syndrome was made only from clinical features in preference to molecular diagnosis. Clinical features of patient 17 possessing a *SMARCA2* variant were compatible with Nicolaides-Baraitser syndrome [21]; however, patient 20 with a *TBL1XR1* variant did not show characteristic bilateral congenital fat pads. Thus, patient 20 was diagnosed as "autosomal dominant mental retardation 41" (MIM #616944) [22] rather than Pierpont syndrome [23].

Among the variants identified in this study, only one variant in *KDM5C* was identified as X-linked recessive and not as *de novo*. *KDM5C*, previously known as *JARID1C*, is related to X-linked ID Claes-Jensen-type (MIM #300534), which is characterized by severe ID associated with abnormal behaviors. Patients with this syndrome often show other variable features, such as slowly progressive spastic paraplegia, seizures, microcephaly, facial dysmorphism, and hypothyroidism [24,25]. Patient 13 possessing the *KDM5C* variant also showed spasticity and hypothyroidism from early infancy, and given this observation, the molecular diagnosis is compatible with the clinical findings.

4. Discussion

Recently, a number of reports have presented evidence that most of the patients exhibiting neurodevelopmental disorders suffered *de novo* variants in related genes [3,26]. In this study, we obtained molecular

Table 3
Diagnostic yields of comprehensive genomic analyses in neurodevelopmental disorders.

Authors	Published year	Country	Method	Diagnostic yield	Subjects	References
Srivastava *et al.*	2014	USA	WES	41.0%	Neurodevelopmental disability	35
Yang *et al.*	2014	USA	WES	27.2%	Neurological phenotype	33
Wright *et al.*	2015	UK	Microarray + WES	27.0%	Developmental disorders	37
Thevenon *et al.*	2016	France	WES + XHMM	32.5%	Severe neurodevelopmental disorders	28
Lazaridis *et al.*	2016	USA	WES	29.0%	Diagnostic odyssey	30
Monroe *et al.*	2016	The Netherlands	Trio WES	29.4%	Intellectual disability	31
Cherot *et al.*	2017	France	Trio/duo/solo TSO	25.9%	Neurodevelopmental disorders	36
Snoeijen-Schouwenaars *et al.*	2018	The Netherlands	Trio/solo WES	25.0%	Epilepsy and intellectual disability	32
Cordoba *et al.*	2018	Argentina	WES	40.0%	Neurogenetic odysseys	34
Gieldon *et al.*	2018	Germany	Trio/quartet/solo TSO	34.0%	Intellectual disability	27
Nambot *et al.*	2018	France	Solo WES + CNV analysis	27.9%	Congenital anomalies and/or intellectual disability	29
Present study	NA	Japan	Trio/solo TSO + XHMM	29.3%	Neurodevelopmental disorders	NA

WES, whole exome sequencing; XHMM, eXome Hidden Markov Model; TSO, TruSight One; CNV, copy number variation; NA, not applicable.

diagnoses in 39/133 patients (29.3%). The diagnostic yields reported from other countries were 25–41% (Table 3), and our results were nearly identical [27–37]. Given this, we suggest that there is no difference in genomic backgrounds in Japanese patients with undiagnosed neurodevelopmental disabilities.

In this study, two genes, *CHD2* and *CDKL5*, showed recurrent involvements. Patients 3/4 and patients 5/6 showed different *CHD2* and *CDKL5* variants, respectively. These four patients exhibited epileptic encephalopathy in association with DD/ID. The *MED13L* variant identified in patient 14 was the third *MED13L* variant reported from our laboratory [4]. Thus, these three genes are considered as the major contributors to neurodevelopmental disorders, in addition to Xq28 duplications. The other variants were identified only in a single patient.

Finally, 29.3% of the patients with undiagnosed neurodevelopmental disorders were successfully diagnosed through comprehensive genomic analysis. Among the 30 patients who were diagnosed to have pathogenic or likely pathogenic variants in single genes, more than half of the patients (16/30) were diagnosed through a "Solo" sample usage. This may indicate that variants that were strongly suggested as "pathogenic" are easily identified by "Solo" sample usage. Although approximately half of the patients were diagnosed as having syndromic ID, these could not be diagnosed from clinical features, and were instead diagnosed through comprehensive genomic analysis. Therefore, comprehensive genomic analysis is necessary not only for molecular diagnosis of non-syndromic developmental disorders but also for syndromic ID.

Regarding the inheritance pattern, 19 patients showed *de novo* variants, and only one patient showed X-linked inheritance of a *KDM5C* variant. X-linked recessive disorders have possibility of recurrent occurrence in the family, and given this, molecular diagnosis is important for genetic counseling.

A weakness of this study is that genes were analyzed by targeted panel analysis and not by whole exome sequencing (WES). The TruSight One panel includes approximately 5000 genes. In comparison, WES would include about 20,000 genes. Thus, some pathogenic variants may be underdiagnosed. Diagnostic yield in this study was 29.3%; however, this is nearly the same as that observed in studies performed with WES [28–37] (Table 3). Therefore, we consider that the ratio of underdiagnosed variants may not be significantly high.

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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.2019.05.007.

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