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研究課題名（和文）Investigation of novel genes responsible for intellectual disability and multiple congenital anomalies of unknown etiology

研究課題名（英文）Investigation of novel genes responsible for intellectual disability and multiple congenital anomalies of unknown etiology

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研究成果の概要（和文）：発達遅滞や多発奇形を合併する先天異常症は全人口の2-3%に存在する難治性疾患である。遺伝学的異質性によりその診断は容易でなく、診断未確定症例も数多く存在する。本研究は、診断未確定の発達遅滞・多発奇形に伴う新規遺伝子を同定することを目的とした。病因性copy number variationを陰性であった6症例を対象として全エクソーム解析を行い、3症例において原因遺伝子を検出した。その中から、2症例において新規候補遺伝子を同定した。

研究成果の学術的意義や社会的意義
This research was able to successfully identify the causative gene in 50% of the cases, and more importantly, two novel candidate genes. Besides providing a definitive molecular diagnosis, such results might help to guide treatment and better care of each patient.

研究成果の概要（英文）：Intellectual disability (ID) and multiple congenital anomalies (MCA) are complex neurodevelopmental disorders affecting 2-3% of the general population. Due to the high genetic heterogeneity, the diagnosis is challenging and remains largely unknown. This project aimed at detecting causative single nucleotide variants in undiagnosed ID/MCA patients previously negative for pathogenic copy number variants, with a focus on identifying novel candidate genes.
Whole-exome sequencing was performed in six parent-child trios, in which causative genes were detected in three cases. In two of the cases, rare mutations in genes that have never been associated to disorders were identified, making them potential novel candidate genes. We joined an international collaboration that had been investigating mutations in one of the novel genes, and this work was able to define a new ID/MCA disorder. Currently, functional assays are being carried out for the second novel candidate gene.

研究分野：Human Genetics

キーワード：Intellectual disability　Congenital anomalies　Sequencing　Candidate gene

様　式　C－１９、F－１９－１、Z－１９、CK－１９（共通）

１．研究開始当初の背景

Intellectual disability (ID) is a lifelong and complex neurodevelopmental disorder, clinically characterized by low intelligence and associated limitations in adaptive behavior (Salvador-Carulla et al., World Psychiatry 2011), and affecting approximately 2-3% of the population (de Vries et al., Am J Hum Genet 2005). ID can occur alone or be accompanied by other malformations collectively termed as multiple congenital anomalies (MCA). Due to the extensive genetic heterogeneity of ID, the diagnosis is challenging and remains unknown for a large subset of cases (~65%).

From 2005 to 2016, our group has carried out a project in collaboration with 23 medical institutes and hospitals in Japan (Japanese Array Consortium), with the aim of identifying pathogenic copy number variations (CNVs) responsible for ID/MCA in Japanese patients. A total of 645 subjects presenting with undiagnosed ID/MCA and a normal karyotype were recruited along 11 years of project. A three-stage microarray screening was performed, in which pathogenic CNVs were detected in 155 (24%) of the 645 cases (Hayashi et al., J Hum Genet 2011; Uehara et al., J Hum Genet 2016). Nevertheless, at least 60% of our cohort still remained undiagnosed.

Next, we performed a screening to detect disease-associated single nucleotide variants (SNVs) in 105 of the subjects previously negative for pathogenic CNVs, through targeted resequencing by a 75-gene custom panel. The panel was composed of the 61 most implicated genes in ID/MCA, and 14 candidate genes. In total, causative or likely pathogenic variants in known genes were identified in 20% of the cases (21/105).

２．研究の目的

This project aimed at detecting pathogenic SNVs, with the ultimate goal of identifying novel candidate genes responsible for ID/MCA of unknown etiology. The results may provide a definitive molecular diagnosis, eventually helping to guide treatment and better care of each patient towards a focus on precision medicine. Moreover, the identification of novel genes might provide insights into the underlying pathological mechanisms.

３．研究の方法

The research plan had the goal of identifying novel candidate genes responsible for ID/MCA of unknown etiology. This would be achieved as follows: (i) whole-exome sequencing (WES) of six selected parent-child trios, who have previously been negative for pathogenic CNVs or SNVs in known ID/MCA genes; (ii) data analysis with a focus on *de novo* variants, X-linked maternally inherited variants in male probands, and compound heterozygous variants; (iii) functional studies for the characterization of novel candidate genes by *in silico*, *in vitro* and *in vivo* analysis.

４．研究成果

Whole-exome sequencing was performed in six parent-child trios (Table 1). A causative or candidate gene could be detected in three cases (50%), in which one is a known ID/MCA gene (Patient 1) and two are novel candidate genes (Patients 2 and 3).

Table 1. Results of trio whole-exome sequencing in six undiagnosed ID/MCA cases.

Patient	Causative or candidate gene	Mutation	Classification
1	*GNB1* (1p36.33)	c.239T>C, p.Ile80Thr	Known
2	*OTUD5* (Xp11.23)	c.820C>T, p.Arg274Trp	Novel
3	*NUF2* (1q23.3)	c.371T>G, p.Ile124Ser	Novel
4	-	-	
5	-	-	
6	-	-	

1) Patient 1

Patient 1 is a 5-year-old male patient with severe ID, developmental delay, short stature

and brain ventriculomegaly. He was not able to speak meaningful words and had to walk with assistance. He also presented with hypotonia and a proximal muscular atrophy of lower limbs. WES detected a *de novo* missense variant (c.239T>C, p.Ile80Thr) in the *GNB1* gene.

GNB1 (G protein subunit beta 1) is located at 1p36.33 and encodes a beta subunit of the guanine nucleotide-binding proteins. *De novo* mutations in *GNB1* are the cause of a known ID/MCA syndrome, namely mental retardation autosomal dominant 42 (MRD42, OMIM# 616973). MRD42 is characterized by global developmental delay and intellectual disability. More variable features include hypotonia, seizures of various types, and poor overall growth. Table 2 shows a comparison between the features of Patient 1 and the main findings in other patients with *GNB1* mutations.

Table 2. Comparison between the clinical information of Patient 1 and the main findings in 46 other patients with *GNB1* mutations (adapted from Hemati et al., Am J Med Genet A. 2018).

Symptoms	Total literature	Patient 1
Global developmental delay	46/46 (100%)	+
Abnormal muscle tone	36/46 (78%)	+
Epilepsy	23/46 (50%)	-
Abnormal EEG	18/30 (60%)	N/A
Abnormal brain MRI	24/46 (52%)	+
Abnormal vision	27/46 (58%)	-
Growth delay (below -2SD)	10/46 (21%)	+
Microcephaly	1/33 (3%)	(+ when 11mo)
Macrocephaly	7/33 (21%)	N/A
Dysmorphic features	24/34 (70%)	N/A

EEG, electroencephalogram; MRI, magnetic resonance imaging; N/A, not available

To date, 46 cases with *GNB1* mutations have been reported, of which 11 cases (24%) have the same p.Ile80Thr mutation detected in Patient 1. Therefore, we conclude Patient 1 is a clear case of MRD42, being the first report of a *GNB1* mutation in a Japanese patient.

2) Patient 2

Patient 2 is a 15-year-old male patient presenting with a severe developmental delay, short stature, microcephaly, brain ventriculomegaly, hearing loss, micropenis, hypospadias and hypertrichosis of the back, buttocks and anterior tibia. He also presents with an autistic behavior, is not able to speak and eats with assistance. WES detected an extremely rare missense variant in the X-linked *OTUD5* gene (c.820C>T, p.Arg274Trp). This variant was inherited from the unaffected mother (Figure 1A). Androgen receptor methylation assay was performed in DNA from the mother to investigate the X inactivation pattern, which was shown to be completely skewed (Figure 1B), suggesting the mutation to be potentially pathogenic.

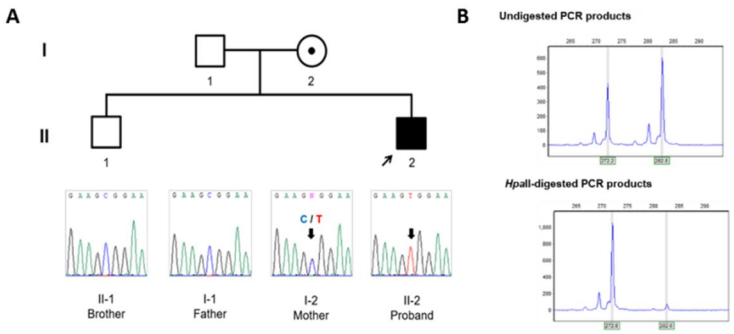

Figure 1. (A) Sanger sequencing validation of the c.820C>T *OTUD5* variant in the Patient 2, inherited from the unaffected mother. (B) Androgen receptor methylation assay in mother's DNA showed a completely skewed X inactivation pattern.

OTUD5 (OTU deubiquitinase 5), located at Xp11.23, encodes a member of the OTU (ovarian tumor) domain-containing cysteine protease superfamily, in which the OTU

domain confers deubiquitinase activity. The p.Arg274Trp mutation detected in Patient 2 is located at the OUT domain, specifically in the variable loop region. To date, there are no disease-associated reports of *OTUD5* in the literature, suggesting that *OTUD5* would be a candidate gene to a novel ID/MCA syndrome.

Through GeneMatcher (https://www.genematcher.org/), we established contact with a group from the National Human Genome Research Institute of the U.S. National Institutes of Health that had been studying *OTUD5* mutations detected in six other male cases, and decided to join the ongoing investigation. So far, *in vitro* and *in vivo* analyses using mouse and human pluripotent stem cell models have uncovered a novel regulatory circuit that coordinates chromatin remodeling pathways during early differentiation. Moreover, our collaborators were able to observe that OTUD5's Lys48-linkage specific deubiquitylation activity is essential for murine and human development and, if reduced, leads to aberrant differentiation. In conclusion, hypomorphic *OTUD5* mutations are responsible for a novel multiple congenital anomaly disorder. There results will be soon submitted for publication.

3) Patient 3

Patient 3 is a 12-year-old male patient born with intra-uterine growth retardation (IUGR) and microcephaly, with additional features including low-set ears, micrognathia, atrial septal defect and a wide foot. WES detected a rare *de novo NUF2* missense variant (c.371T>G, p.Ile124Ser) in Patient 3 (Figure 2A).

NUF2 (NUF2 component of NDC80 kinetochore complex), located at 1q23.3, encodes a component of the outer kinetochore-associated NDC80 complex, a highly conserved complex crucial for proper microtubule binding and spindle assemble checkpoint. Mutations in proteins involved with cell division and chromosome segregation, such as centrosome-associated and kinetochore proteins, have been reported to be associated with microcephaly and/or IUGR. Therefore, we speculated that the *NUF2* mutation might be related to the IUGR/microcephaly of Patient 3.

The Ile124 residue is located at the N-terminal domain superfamily of NUF2, which is known to interact with the N-terminus portion of the NDC80 protein. Interestingly, analysis with a patient-derived lymphoblastoid cell line showed that NUF2 is decreased at both mRNA and protein levels (Figure 2B-C). It is possible that the mutation might be lying in an exonic splicing enhancer site, hence the possibility of an aberrant splicing leading to a frameshift. Currently, a minigene assay is being carried out to investigate the effect of the c.371T>G mutation in splicing. Moreover, we observed that NDC80 protein levels are also downregulated in lysates from patient-derived cells (Figure 2C). This might be due to the fact that the p.Ile124Ser variant is predicted *in silico* to cause loss of hydrophobic interactions in the core of the protein, thereby the stability of the N-terminal interaction of NUF2-NDC80 might be impaired.

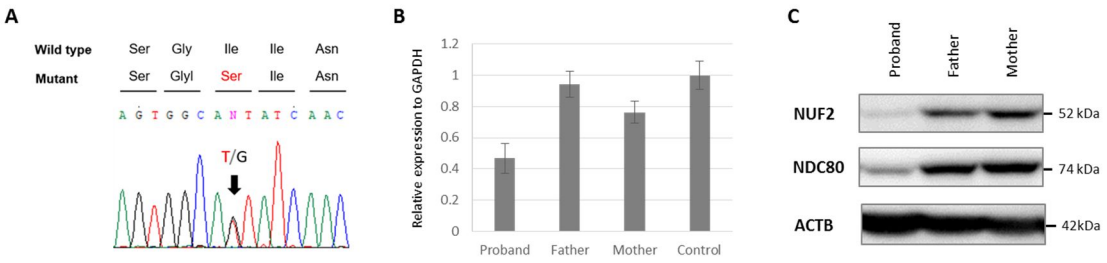

Figure 2. (A) Sanger sequencing validation of the c.371T>G *NUF2* variant in Patient 3. (B) qRT-PCR of NUF2 showing decreased mRNA levels in the proband. (C) Western blot of lysates from lymphoblastoid cell lines showed decreased levels for NUF2 and NDC80 in the Patient 3.

Currently, several functional *in vitro* assays are being carried out in order to establish a causal role of the p.Ile124Ser mutation in the phenotype of Patient 3. *NUF2* might be a candidate for IUGR/microcephaly, and if so, it would be the first component of the NDC80 complex to be implicated with such features.

In conclusion, this research was able to successfully identify the causative gene in 50% of the cases (3/6), and more importantly, two novel candidate genes.

５．主な発表論文等

〔雑誌論文〕（計　0　件）

〔学会発表〕（計　4　件）

1. Uehara DT, Tanimoto K, Inazawa J. Targeted next-generation resequencing analysis in 105 subjects with undiagnosed intellectual disability and multiple congenital anomalies. The 68th Annual Meeting of The American Society of Human Genetics, October 17, 2018, San Diego, USA.

2. Uehara DT, Tanimoto K, Inazawa J. Targeted resequencing in 105 subjects with undiagnosed intellectual disability and multiple congenital anomalies. The 63rd Annual Meeting of The Japanese Society of Human Genetics, October 12, 2018, Yokohama, Japan.

3. Uehara DT, Tanimoto K, Inazawa J. Targeted resequencing of 75 genes in subjects with undiagnosed intellectual disability and multiple congenital anomalies. The 62nd Annual Meeting of The Japanese Society of Human Genetics, November 17, 2017, Kobe, Japan.

4. Uehara DT, Tanimoto K, Inazawa J. Targeted next-generation sequencing of 75 genes in Japanese patients with intellectual disability and multiple congenital anomalies of unknown etiology. The 67th Annual Meeting of The American Society of Human Genetics, October 20, 2017, Orlando, USA.

〔図書〕（計　0　件）

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○出願状況（計　0　件）

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ホームページ等

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