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Article

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

Abstract (<250)

Corpus callosum anomalies (CCA) is a common congenital brain anomaly with various etiologies. Although one of the most important etiologies is genetic factors, the genetic background of CCA is heterogenous and diverse types of variants are likely to be causative. In this study, we analyzed 16 Japanese patients with corpus callosum anomalies to delineate clinical features and the genetic background of CCAs. We observed the common phenotypes accompanied by CCAs: intellectual disability (100%), motor developmental delay (93.8%), seizures (60%), and facial dysmorphisms (50%). Brain magnetic resonance imaging showed colpocephaly (enlarged posterior horn of the lateral ventricles, 84.6%) and enlarged supracerebellar cistern (41.7%). Whole exome sequencing revealed genetic alterations in 9 of the 16 patients (56.3%), including 8 *de novo* alterations (2 copy number variants and variants in *ARID1B*, *CDK8*, *HIVEP2*, and *TCF4*) and a recessive variant of *TBCK*. *De novo ARID1B* variants were identified in three unrelated individuals, suggesting that *ARID1B* variants are major genetic causes of CCAs. A *de novo TCF4* variant and somatic mosaic deletion at 18q21.31-qter encompassing *TCF4* suggest an association of *TCF4* abnormalities with CCAs. This study to analyze CCA patients using whole exome sequencing, demonstrating that comprehensive genetic analysis would be useful for investigating various causal variants of CCAs.

Keywords

Corpus callosum anomaly, colpocephaly, copy number variants, somatic mosaicism, whole exome sequencing

Introduction

The corpus callosum is the largest white matter tract in the forebrain, connecting the left and right cerebral hemispheres[1]. It spans most of the frontal and parietal lobes, from the anterior commissure to the end of the ventral hippocampal commissure, and comprises 200 to 300 million fibers[1]. The corpus callosum is an important mediator of the lateralization of brain functions. In the absence of cerebral hemispheric communication, complex sensory-motor control errors occur[2]. The function of the corpus callosum, in humans, was first investigated in patients with epilepsy, who had undergone callosotomy, in 1940[3]. Many studies have found that the corpus callosum is specialized to aid the transfer of visual, somatosensory, and motor information[4].

In humans, the genesis of corpus callosum begins at eight weeks of fetal life[5] and involves complex steps, including cell proliferation and migration, axon growth, midline glial pattern formation, telencephalic hemisphere formation, and commissural neuron birth[1]. The corpus callosum can be divided into four segments: genu, rostrum, body, and splenium. Corpus callosum anomalies (CCAs), such as agenesis of the corpus callosum (ACC) and dysplastic corpus callosum, are conditions in which all or part of these structures are congenitally lost or malformed due to disruption of any of the aforementioned processes during the developmental stage. Defects of the corpus callosum are rare, and most of them are associated with various underlying CCA syndromes[6]. ACC was first described in an autopsy in 1812, and its incidence rate is one per 4000–5000 live births[7]. Due to advances in magnetic resonance imaging (MRI) images and genetic analysis techniques, causal roles of 30%–40% of CCA syndromes have been established, of which about 10% have been attributed to chromosomal abnormalities, and 20%–35% have recognizable genetic factors. Most patients with identified pathogenic variants had

recognizable syndromes[5], however, the etiology of 55%–70% of patients with CCA syndrome had not been identified[5, 6, 8, 9]. Moreover, it was more difficult to find responsible genes in patients with isolated CCA[8].

In this report, we describe the genetic investigation of 16 patients with CCAs, using whole exome sequencing. We discussed the association between genetic alterations and a spectrum of brain malformations and clinical phenotypes accompanied with CCA syndrome.

Materials and Methods

Patients

A total of 16 individuals with CCAs and their parents were investigated in this study. These individuals were recruited from 10 medical institution in Japan within the period from Jan 2017 to Apr 2019 excluding one individual collected in Oct 2009. Written informed consent was obtained from the parents. Experimental protocols were approved by the Institutional Review Board of Showa University Faculty of Medicine and Hamamatsu University School of Medicine. Patients received an assessment protocol, which required information on family history and medical history and information from neurologic and physical examinations and general biochemical testing performed as part of the study. Upon participants selection, brain image analysis using MRI was performed on all the subjects and these images were evaluated by a doctor in charge (pediatric neurologists in most) and another independent pediatric neurologist. Electroencephalography was performed during seizures and evaluated by specialists of neurology.

Whole Exome Sequencing (WES)

Genomic DNA of the proband was extracted from peripheral blood leukocytes and processed using a SureSelect Human All ExonV6 kit (Agilent Technologies, Santa Clara, CA). Sequencing was performed on NextSeq500 sequencing system (Illumina, San Diego, CA), with 150 bp paired-end reads. Exome data processing, variant calling, and variant annotation were performed, as previously described[10] (Figure S1). Additionally, we examined copy number variants (CNVs) with the eXome-Hidden Markov Model and Nord method for 63 CCA associated genes registered in the Human Gene Mutation Database (Table S1), as previously described[11]. Variant detection for somatic single-nucleotide variant and small indels was performed by the MuTect2 algorithm in the tumor-only mode.

According to standards and guidelines of the American College of Medical Genetics (ACMG), candidate variants were classified into the recommended terminology as "pathogenic", "likely pathogenic", "uncertain significance", "likely benign", and "benign"[12]. Candidate nucleotide variants and CNVs detected via WES were confirmed with Sanger sequencing or quantitative polymerase chain reaction (PCR), respectively. Possible somatic mosaic variants were confirmed by high resolution melt PCR. Biological parentage was confirmed by analyzing 10 microsatellite markers.

Results

Clinical features of patients

The clinical details and summary of the 16 patients are presented in Table 1 and Table S2, respectively. In total, 3 males and 13 females, with a median age of 48 months (age range: 2 years 10 months to 21 years 6 months), were recruited in this study (Table 1).

We classified their CCA status into three categories based on midline sagittal MRI images: “total” ACC or the complete absence of the corpus callosum, “partial” or the absence of only the splenium and/or rostrum and “dysplasia” or condition of the corpus callosum is fully formed but morphologically abnormal. Fourteen patients showed total ACC, and two patients showed dysplasia of the corpus callosum. In this cohort no patients with partial ACC. Participants had neurological disorders, including intellectual disability (100%, 16/16), motor developmental delay (93.8%, 15/16), speech delay (93.8%, 15/16), seizures (60%, 9/15), hypotonia (11/16, 68.8%), spasticity (11%, 1/9), and hypotonic or spastic quadriplegia (43.8%, 7/16, Hypotonic:Spastic=5:2). None of patients showed ataxia. Other phenotypes including short stature (43.8%, 7/16), microcephaly (≤-2SD, 23.1%, 3/13), macrocephaly (≥2SD, 15.4%, 2/13), hypotonia (31.3%, 5/16), facial dysmorphism (50%, 8/16), and other dysmorphisms (50%, 8/16) were relatively common (Table1, TableS2). Brain MRI studies showed that colpocephaly (enlarged posterior horn of the lateral ventricles) was observed in almost all patients (84.6%, 11/13), and enlarged supracerebellar cistern was relatively common (41.7%, 5/12) (Figure 1 and Table 1). In the majority of the patients, seizures started within the first year of life (77.8%, 7/9) and was also found to disappear without treatment in two patients. Epileptic discharges were seen in seven of ten cases, three (42.9%) of whom had refractory seizures. Seizure occurrence was not different between types of CCA (9/14 in total ACC and 1/2 in dysplasia).

Identification of nine genetic alterations associated with CCAs

WES was used to identify the genetic causes of CCAs in the 16 patients. Due to the sporadic nature of CCAs, we focused on extremely rare *de novo* variants (not registered

in a public or an in-house database) and rare recessive variants (allele frequency below 0.01) and validated them via Sanger sequencing using trio DNA samples. We identified 10 possible pathogenic variants in 9 patients, comprising 6 *de novo* variants in *ARID1B*, *HIVEP2*, *TCF4*, and *CDK8*; 2 *de novo* CNVs; and 2 compound heterozygous variants in *TBCK* (Table 2). Four out of the six *de novo* variants were novel, and three unrelated individuals had the *de novo ARID1B* variants. The six *de novo* variants were absent from the gnomAD database and were predicted to be deleterious by *in silico* prediction tools, and therefore, these variants were classified as pathogenic or likely pathogenic based on ACMG guidelines. Two compound heterozygous variants in *TBCK* were predicted to be deleterious, and one of them was located in the TBC domain (Figure S2)[13, 14]. Allele frequency of the c.1588C>T variant is 0.0001475, and the variant is absent from the gnomAD. Although, both variants were classified as variants of uncertain significance, phenotypic overlap with *TBCK*-related disorders, including CCAs and pathogenicity predicted by *in silico* analysis, suggested that these *TBCK* variants are strongly associated with a patient's CCA phenotype. CNV analysis using WES data revealed *de novo* microdeletions in two individuals (12.5%, 2/16). Patient 5803 had a mosaic *de novo* microdeletion on the chromosome 18q21.32-qter encompassing *TCF4*, which has been previously reported[11]. Patient 5884 had a *de novo* 2.6 Mb microdeletion on the chromosome 1q44 (Figure S3). Among the nine patients with genetic associations with CCAs, seven patients showed total ACC (Figure 1A–D, I–T), and the other two patients showed dysplasia of the corpus callosum (Figure 1E–H).

Discussion

CCAs has been associated with several neurodevelopmental disorders and congenital

syndromes[6, 8], and various responsible genes have been identified. Our study revealed the genetic alterations in 9 of 16 patients with CCAs (56.3%), including 2 CNVs and 5 genes with nucleotide variants, further demonstrating the importance of genetic factors and their heterogeneity. Given the genetic heterogeneity of CCAs, making it difficult to perform a targeted genetic analysis. In this cohort, 10 patients showed syndromic CCA with multiple dysmorphic findings and 6 showed non-syndromic CCA, the diagnostic rate in each group was 60 % (6/10) and 50 % (3/6), respectively. This result indicated that genetic alterations might have important contribution to the etiology not only in syndromic CCA but in non-syndromic CCA.

We found three *de novo ARID1B* variants in three unrelated patients (18.8%, 3/16, two ACC patients and one dysplasia patient). The prevalence of *ARID1B* variants was consistent with a previous study, which reported a prevalence of 10%[15], supporting the fact that *ARID1B* variants are a major genetic cause of CCAs. Additionally, we identified a *CDK8* variant (c.185C>T, p.Ser62Leu) in a patient with ACC. Previous study described five patients with the same c.185C>T variant and 3 of five showed ACC[16], suggesting that ACC may be a major consequence of the c.185C>T variant. The spectrum of clinical presentations caused by variants in *TCF4*, *HIVEP2*, and *TBCK* include CCAs, but with variable expressivity[13, 14, 17, 18].

Here we identified two CNVs, 1q44 deletion and somatic mosaic 18q21.31-qter deletion. Previous studies have suggested that 10%–15% of ACC is caused by CNV[19, 20], and our results are consistent with their results (12.5%). These CNVs have been recurrently observed in individuals with ACC[8, 19], indicating that these regions may regulate key genes involved in corpus callosum development. In addition, a significantly higher rate of pathogenic CNVs were observed in patients with severe intellectual

disability or developmental delay than those with mild or moderate[21] and the larger size distribution of CNVs had a high risk of severe phenotypes[22]. Our patients with CNVs also showed profound developmental delay (DQ<10), supporting the association with CNVs and severity of the developmental delay. CNV analysis, using WES data, is of great value for elucidating the genes that play a causal role in CCAs and thus would contribute to the elucidation of molecular mechanisms of corpus callosum development in humans.

The 1q44 deletion spans candidate genes for autosomal-dominant neuropsychiatry diseases such as megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2 (MIM#615973) caused by variants in *AKT3*, mental retardation, autosomal dominant 22 (MIM#612337) caused by variants in *ZBTB18*, and epileptic encephalopathy, early infantile, 54 (MIM#617391) caused by variants in *HNRNPU*. Of note, *ZBTB18* and *AKT3* had been suggested to be strongly associated with a spectrum of structural CCAs[23, 24].

In this cohort, two patients had the missense *TCF4* variant (#6322) or the 18q21.31-qter deletion, encompassing *TCF4* gene (#5803), respectively. Patients with deletions containing *TCF4* were characterized by CCAs, small penis and accessory nipples and *TCF4* has been shown to play an important role in patients with 18q segmental deletions[25]. TCF4 mutant mice have been shown to have a reduced proportion of myelinated axons in the corpus callosum[26]. Interestingly, the 18q21.31-qter deletion was a low-prevalence mosaic deletion in our case[11], raising the possibility that even a small loss of *TCF4* function might result in ACC, in combination with dosage decreases of other genes. Thus, it is suggested that *TCF4* plays an important role not only in the cause of autism spectrum disorder but also in the corpus callosum.

Genetic defects in the mTOR pathway, which regulates both cell cycle and proliferation, is associated with dysregulation of autophagy, leading to several neurodevelopment

disorders with brain malformations, including ACC[27, 28]. Among the five genes identified in this study, biallelic *TBCK* variants have been reported to cause intellectual disability syndrome with variable severity[13, 14, 29]. The inhibition of mTORC1 activity cause induction of autophagy activity[30] and a recent study demonstrated that depletion of TBCK induced downregulation of mTOR signaling, leading to upregulation of autophagic activity and autophagosome-lysosomal dysfunction[29]. Interestingly, CCAs were frequently observed in two reports about *TBCK*: 100% (5/5) of patients in one report[13] and 75% (6/8) of patients in another[29]. Identification of a patient with ACC and biallelic *TBCK* variants in this study further support the idea that activation of mTOR-related autophagy may be one of the causes of CCAs.

The most common brain anomaly in this study is colpocephaly (84.6%, 11/13) [31, 32, 33]. Even more interesting from the brain MRIs of the nine cases, in whom causative variants were identified, we found an enlarged supracerebellar cistern in the three patients with *ARID1B* variants. ARID1B is a component of the BAF complex [31], which plays important roles in transcription, DNA repair, DNA replication, and chromosome division[34]. Heterozygous variants in genes encoding components of the BAF complexes causes Coffin-Siris syndrome[35] and lead to various brain defects[36]. It has been reported that patients with *ARID1B* variants showed CCAs (28.7%) and enlarged cisterna magna (14.9%)[37]. Interestingly, BCL11A is another member of the BAF complex, and haploinsufficiency of *BCL11A* is closely related to CCAs and to cerebellar anomalies including enlarged cisterna magna[38]. Some BAF genes including *ARID1B* and *BCL11A* may significantly contribute to genomic stability and DNA repair[39], suggesting a close association between impairment of BAF chromatin complex and corpus callosum and cerebellar anomalies.

Although our stepwise variant search strategy brings the higher identification rate than those of previously researches for CAAs [5, 6, 8, 9], we could not find any causative variants in the remaining 7 patients. For these undiagnostic cases, whole genome sequencing or RNA sequencing may be help to elucidate the responsible gene.

In conclusion, this study presented 15 new patients and 1 previously reported patient with ACC. We found 10 possible pathogenic variants, including 6 *de novo* nucleotide variants, 2 *de novo* CNVs, and 2 compound heterozygous variants. MRI of the brains of our patients supported the close relationship between colpocephaly and ACC. The genetic etiology of CCA is heterogeneous and various types of variants concern to its pathogeneses, therefore, a comprehensive genetic examination is beneficial to provide a high clinical diagnostic rate.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Reference

1. Raybaud C. Corpus Callosum: Molecular Pathways in Mice and Human Dysgeneses. Neuroimaging Clin N Am. 2019 Aug;29(3):445-59.
2. van der Knaap LJ, van der Ham IJ. How does the corpus callosum mediate interhemispheric transfer? A review. Behav Brain Res. 2011 Sep 30;223(1):211-21.
3. van Wagenen WP, Herren RY. Surgical division of commissural pathways in the corpus callosum. Arch Neurol Psychiatry. 1940;44(4):740-759.
4. Gazzaniga MS. Forty-five years of split-brain research and still going strong. Nat Rev Neurosci. 2005 Aug;6(8):653-9.
5. Al-Hashim AH, Blaser S, Raybaud C, MacGregor D. Corpus callosum abnormalities: neuroradiological and clinical correlations. Dev Med Child Neurol. 2016 05;58(5):475-84.
6. Edwards TJ, Sherr EH, Barkovich AJ, Richards LJ. Clinical, genetic and imaging findings identify new causes for corpus callosum development syndromes. Brain. 2014 Jun;137(Pt 6):1579-613.
7. Sotiriadis A, Makrydimas G. Neurodevelopment after prenatal diagnosis of isolated agenesis of the corpus callosum: an integrative review. Am J Obstet Gynecol. 2012 Apr;206(4):337.e1-5.
8. Schell-Apacik CC, Wagner K, Bihler M, Ertl-Wagner B, Heinrich U, Klopocki E, et al. Agenesis and dysgenesis of the corpus callosum: clinical, genetic and neuroimaging findings in a series of 41 patients. Am J Med Genet A. 2008 Oct 1;146A(19):2501-11.
9. Bedeschi MF, Bonaglia MC, Grasso R, Pellegri A, Garghentino RR, Battaglia MA, et al. Agenesis of the corpus callosum: clinical and genetic study in 63 young patients.

Pediatr Neurol. 2006 Mar;34(3):186-93.

10. Hiraide T, Nakashima M, Ikeda T, Tanaka D, Osaka H, Saitsu H. Identification of a deep intronic POLR3A variant causing inclusion of a pseudoexon derived from an Alu element in Pol III-related leukodystrophy. J Hum Genet. 2020 Oct;65(10):921-5.
11. Shiohama T, Nakashima M, Ikehara H, Kato M, Saitsu H. Low-prevalence mosaicism of chromosome 18q distal deletion identified by exome-based copy number profiling in a child with cerebral hypomyelination. Congenit Anom (Kyoto). 2020 May;60(3):94-6.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24.
13. Chong JX, Caputo V, Phelps IG, Stella L, Worgan L, Dempsey JC, et al. Recessive Inactivating Mutations in TBCK, Encoding a Rab GTPase-Activating Protein, Cause Severe Infantile Syndromic Encephalopathy. Am J Hum Genet. 2016 Apr 7;98(4):772-81.
14. Bhoj EJ, Li D, Harr M, Edvardson S, Elpeleg O, Chisholm E, et al. Mutations in TBCK, Encoding TBC1-Domain-Containing Kinase, Lead to a Recognizable Syndrome of Intellectual Disability and Hypotonia. Am J Hum Genet. 2016 Apr 7;98(4):782-8.
15. Mignot C, Moutard ML, Rastetter A, Boutaud L, Heide S, Billette T, et al. ARID1B mutations are the major genetic cause of corpus callosum anomalies in patients with intellectual disability. Brain. 2016 11 1;139(11):e64.

16. Calpena E, Hervieu A, Kaserer T, Swagemakers SMA, Goos JAC, Popoola O, et al. De Novo Missense Substitutions in the Gene Encoding CDK8, a Regulator of the Mediator Complex, Cause a Syndromic Developmental Disorder. Am J Hum Genet. 2019 04 4;104(4):709-20.
17. Goodspeed K, Newsom C, Morris MA, Powell C, Evans P, Golla S. Pitt-Hopkins Syndrome: A Review of Current Literature, Clinical Approach, and 23-Patient Case Series. J Child Neurol. 2018 03;33(3):233-44.
18. Srivastava S, Engels H, Schanze I, Cremer K, Wieland T, Menzel M, et al. Loss-of-function variants in HIVEP2 are a cause of intellectual disability. Eur J Hum Genet. 2016 Apr;24(4):556-61.
19. Sajan SA, Fernandez L, Nieh SE, Rider E, Bukshpun P, Wakahiro M, et al. Both rare and de novo copy number variants are prevalent in agenesis of the corpus callosum but not in cerebellar hypoplasia or polymicrogyria. PLoS Genet. 2013;9(10):e1003823
20. Heide S, Keren B, Billette de Villemeur T, Chantot-Bastaraud S, Depienne C, Nava C, et al. Copy Number Variations Found in Patients with a Corpus Callosum Abnormality and Intellectual Disability. J Pediatr. 2017 06;185:160-166.e1
21. Liao LH, Chen C, Peng J, Wu LW, He F, Yang LF, et al. Diagnosis of intellectual disability/global developmental delay via genetic analysis in a central region of China. Chin Med J (Engl). 2019 Jul 5;132(13):1533-40
22. Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, et al. A copy number variation morbidity map of developmental delay. Nat Genet. 2011 Aug 14;43(9):838-46.
23. van der Schoot V, de Munnik S, Venselaar H, Elting M, Mancini GMS, Ravenswaaij-

Arts CMA, et al. Toward clinical and molecular understanding of pathogenic variants in the ZBTB18 gene. Mol Genet Genomic Med. 2018 05;6(3):393-400.

24. Shetty M, Srikanth A, Kadandale J, Hegde S. Pre- and Postnatal Analysis of Chromosome 1q44 Deletion in Agenesis of Corpus Callosum. Mol Syndromol. 2015 Oct;6(4):187-92.

25. Hasi M, Soileau B, Sebold C, Hill A, Hale DE, O'Donnell L, et al. The role of the TCF4 gene in the phenotype of individuals with 18q segmental deletions. Hum Genet. 2011 Dec;130(6):777-87.

26. Phan BN, Bohlen JF, Davis BA, Ye Z, Chen HY, Mayfield B, et al. A myelin-related transcriptomic profile is shared by Pitt-Hopkins syndrome models and human autism spectrum disorder. Nat Neurosci. 2020 03;23(3):375-85.

27. Ebrahimi-Fakhari D, Saffari A, Wahlster L, Lu J, Byrne S, Hoffmann GF, et al. Congenital disorders of autophagy: an emerging novel class of inborn errors of neuro-metabolism. Brain. 2016 Feb;139(Pt 2):317-37.

28. Crino PB. mTOR signaling in epilepsy: insights from malformations of cortical development. Cold Spring Harb Perspect Med. 2015 Apr 1;5(4):a022442.

29. Ortiz-González XR, Tintos-Hernández JA, Keller K, Li X, Foley AR, Bharucha-Goebel DX, et al. Homozygous boricua TBCK mutation causes neurodegeneration and aberrant autophagy. Ann Neurol. 2018 01;83(1):153-65.

30. Wong PM, Puente C, Ganley IG, Jiang X. The ULK1 complex: sensing nutrient signals for autophagy activation. Autophagy. 2013 Feb 1;9(2):124-37.

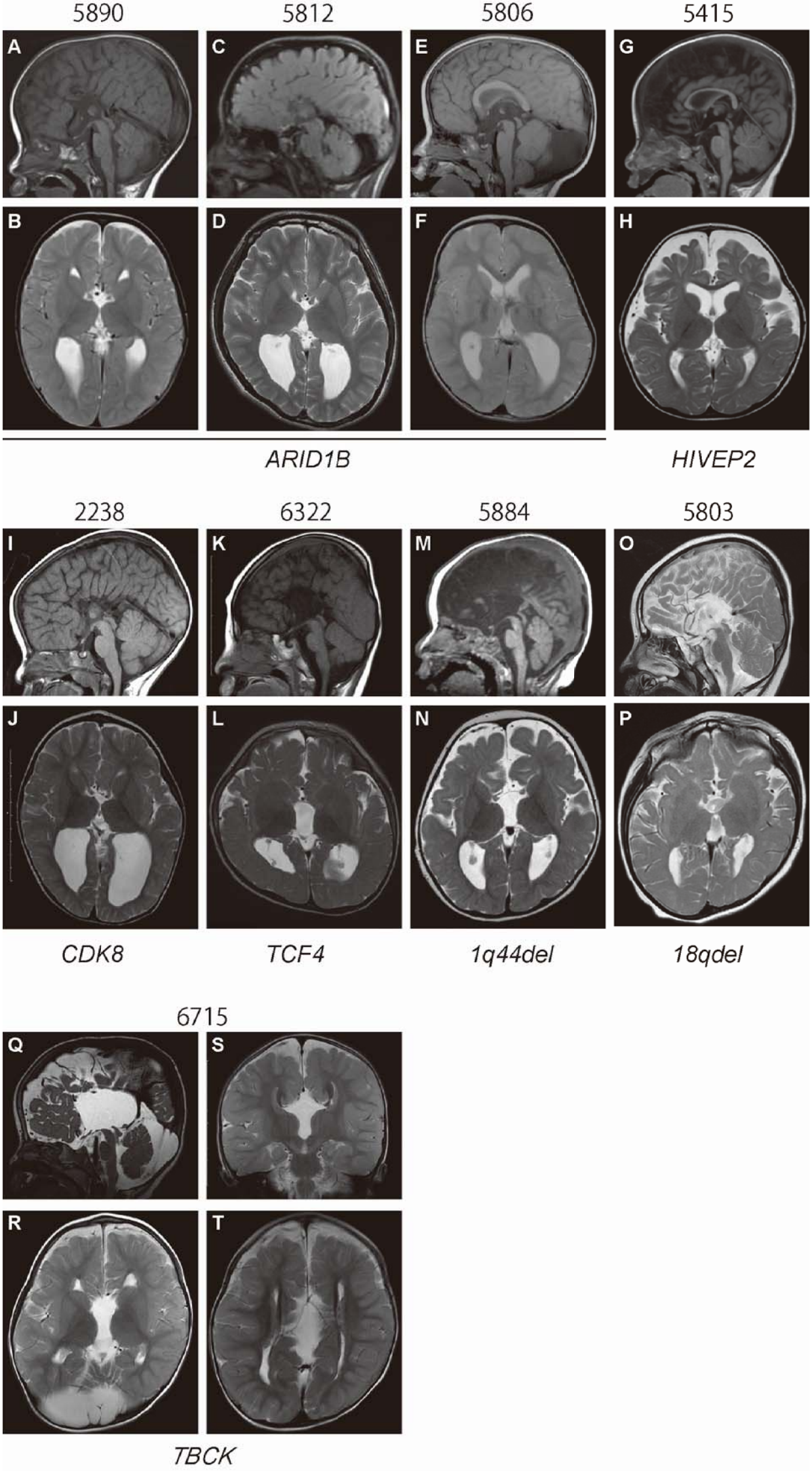
31. Moffat JJ, Jung EM, Ka M, Smith AL, Jeon BT, Santen GWE, et al. The role of ARID1B, a BAF chromatin remodeling complex subunit, in neural development and behavior. Prog Neuropsychopharmacol Biol Psychiatry. 2019 03 8;89:30-8.

32. Lieb JM, Ahlhelm FJ. [Agenesis of the corpus callosum]. Radiologe. 2018 Jul;58(7):636-45.
33. Dutta I, Sharma GN, Singh KP. Transsphenoidal encephalocele, colpocephaly and corpus callosum agenesis in a midline cleft lip and palate patient: A very rare case. Indian J Plast Surg. 2018 Sep-Dec;51(3):334-5.
34. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. Oncogene. 2009 Apr 9;28(14):1653-68.
35. Tsurusaki Y, Okamoto N, Ohashi H, Kosho T, Imai Y, Hibi-Ko Y, et al. Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. Nat Genet. 2012 Mar 18;44(4):376-8.
36. Filatova A, Rey LK, Lechler MB, Schaper J, Hempel M, Posmyk R, et al. Mutations in SMARCB1 and in other Coffin-Siris syndrome genes lead to various brain midline defects. Nat Commun. 2019 07 4;10(1):2966.
37. van der Sluijs PJ, Jansen S, Vergano SA, Adachi-Fukuda M, Alanay Y, AlKindy A, et al. The ARID1B spectrum in 143 patients: from nonsyndromic intellectual disability to Coffin-Siris syndrome. Genet Med. 2019 06;21(6):1295-307.
38. Shimbo H, Yokoi T, Aida N, Mizuno S, Suzumura H, Nagai J, et al. Haploinsufficiency of BCL11A associated with cerebellar abnormalities in 2p15p16.1 deletion syndrome. Mol Genet Genomic Med. 2017 Jul;5(4):429-37.
39. Huang HT, Chen SM, Pan LB, Yao J, Ma HT. Loss of function of SWI/SNF chromatin remodeling genes leads to genome instability of human lung cancer. Oncol Rep. 2015 Jan;33(1):283-91.

Figure legend

Figure 1. Brain magnetic resonance imaging (MRI) of patients with a variety of callosal dysgenesis

Midline sagittal (the top, third, and fifth rows) and axial (the second, fourth, and bottom rows) images using T1-weighted (A, E, G, I, K, M), T2-weighted (B, D, F, H, J, L, N, O-T), or localizer (TR 6ms, TE 3ms) (C) sequences. Patients #5890 at 14 months (A, B) and #5812 at 21 years of age (C, D) with the *ARID1B* variant show total agenesis of the corpus callosum (ACC) and Patient #5806 at 2 years of age (E, F) with the *ARID1B* variant shows a round-shaped corpus callosum (E) and an asymmetric low-intensity signal on the basal ganglia and thalamus in T2-weighted image (F). All three patients with the *ARID1B* variant show a mildly enlarged supracerebellar cistern and predominantly dilated posterior horn lateral ventricles or colpocephaly. Patient #5415 at 8 months of age (G, H) shows a mildly thin body of the corpus callosum, a hypoplastic frontal lobe, and a dilatation of the lateral ventricles. Patients #2238 at 18 months (I, J), #6322 at 23 months (K, L), #5884 at 9 months (M, N), and #5803 at 5 years of age (O, P) show total ACC and colpocephaly with no cortical malformation. Patient #5803 shows a drooped pons, cerebral hypomyelination, and an invisible anterior limb of internal capsule, similar to findings of tubulinopathies. Patient #6715 at 21 months of age shows total ACC, a dilated supracerebellar cistern, a malrotation of bilateral hippocampus, and periventricular nodular heterotopia (Q–T).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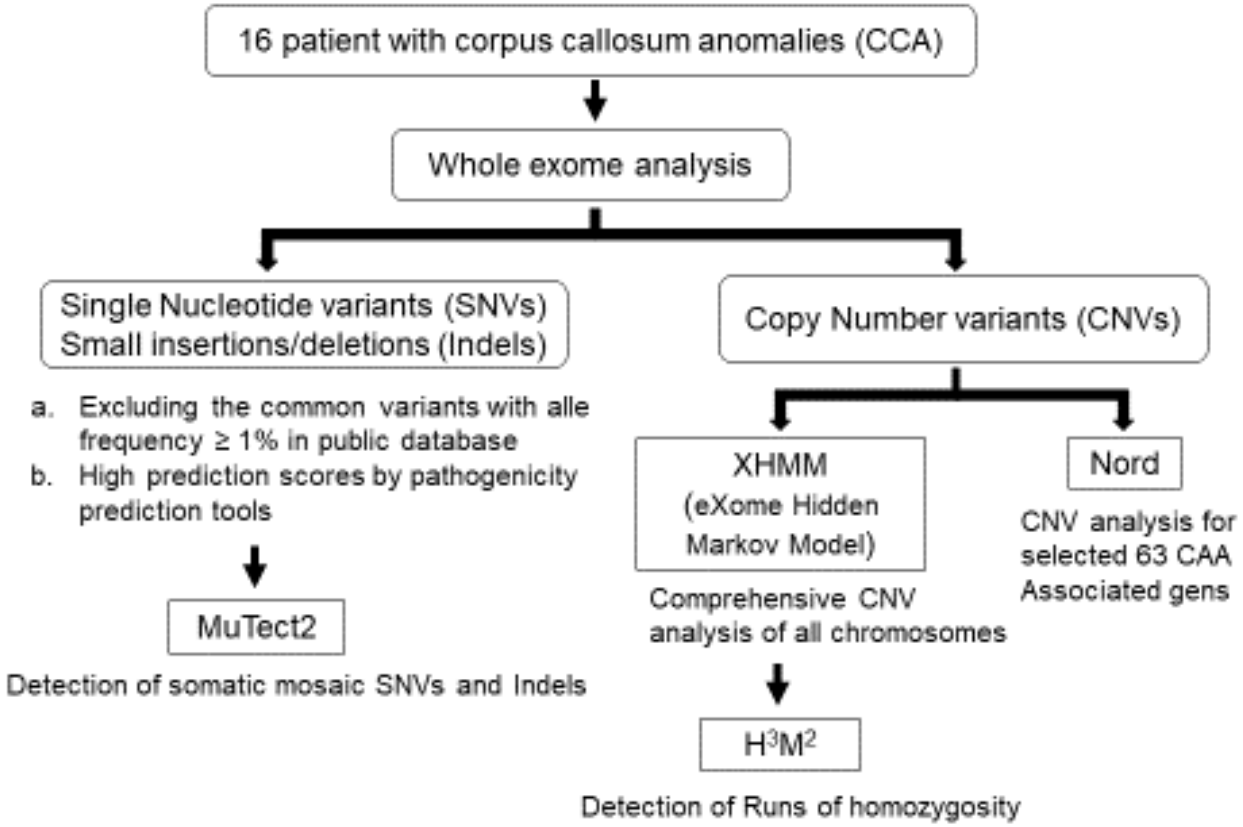
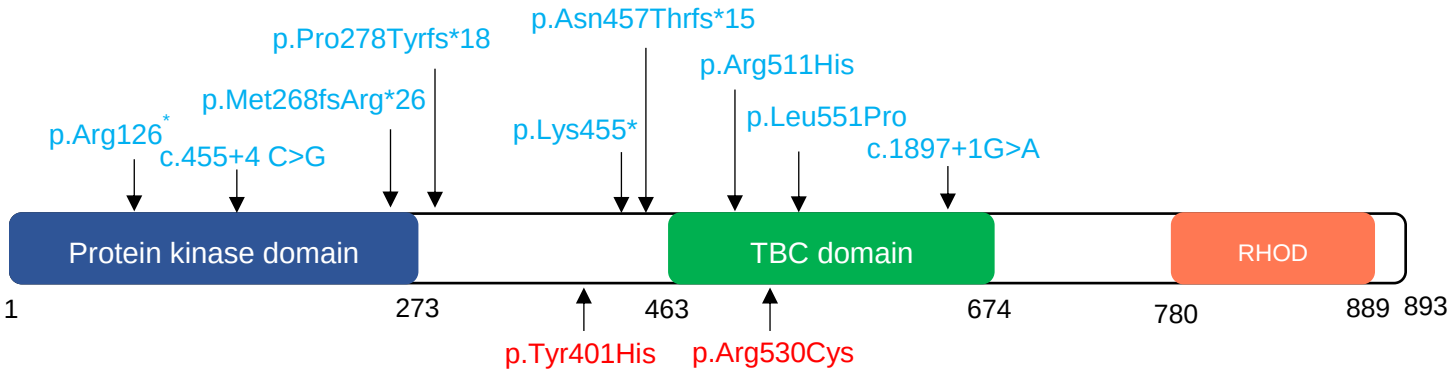
TBCK

Supplementary Figure S1



The strategy of this genetic analysis.

Supplementary Figure S2



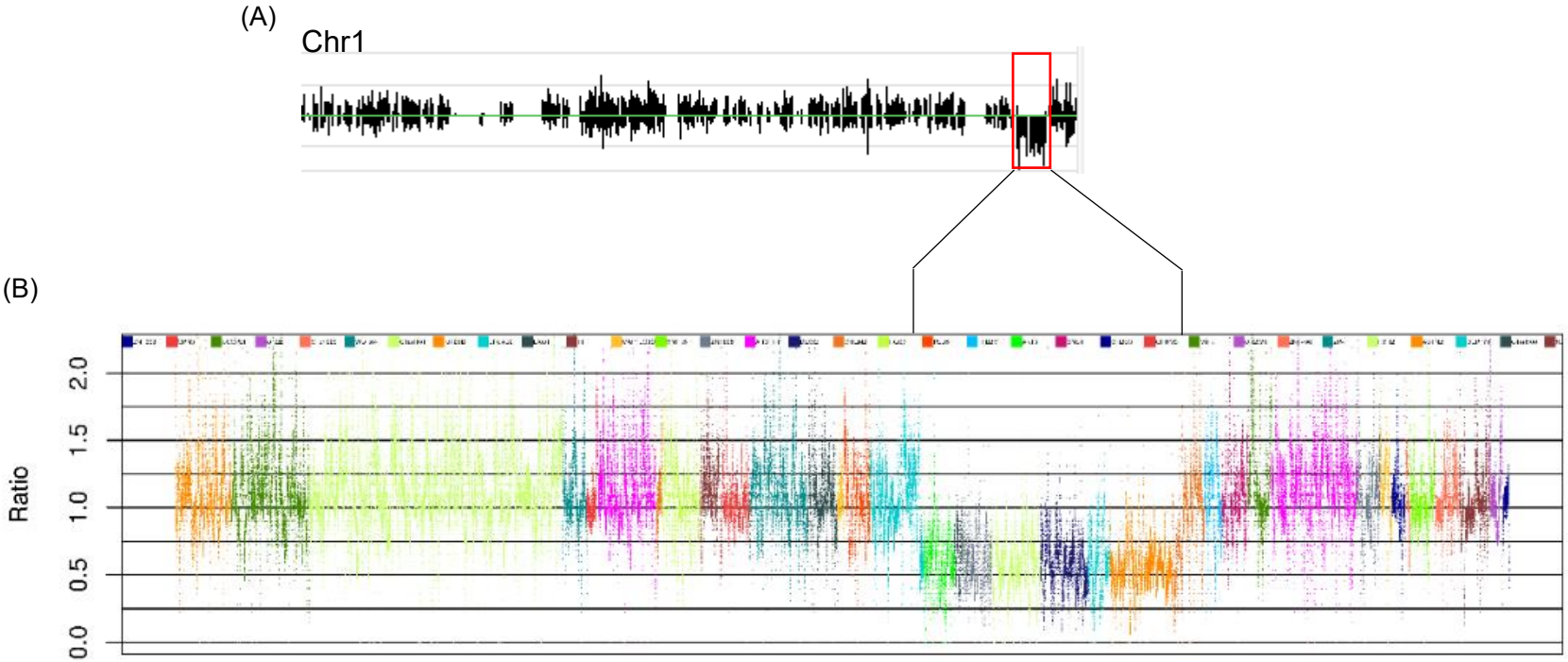
Previous reported variants: light blue

This case: red

Schematic protein structure of TBCK with reported and this case variants

Topological prediction of TBCK domains (InterPro). The blue, green and orange boxes indicate protein kinase domain, TBC domain and RHOD domain, repectively. The protein kinase domain harbors the ATP/GTP binding loop (45–53aa, pink), basic cluster (68-80aa, red), active site (156aa, black) and activation segment (175–201aa, orange). Arrows depicted above are previously reported variants. Two variants found in our case are shown below. The variant inherited from mother is localized in the outside of domain and the variant from father is localized in the TBC domain. Many of variants are predicted to be localized in protein kinase domain and TBC domain.

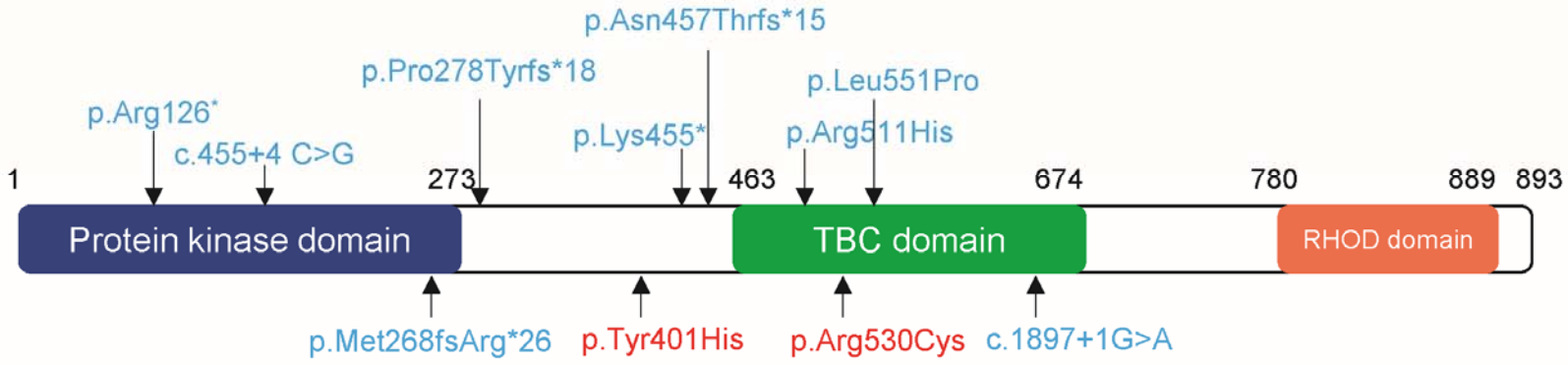
Supplementary Figure S3



The partial 1q43-q44 deletion detected by the XHMM and the Nord's method.

(A) A sample with a 2.6MB deletion at 1q43-q44 (Chr1:243384974-246027351) identified by XHMM analysis.

(B) Relative depth of coverage ratio for each target gene are indicated by different colors. A deletion including *SDCCAG8*, *ZBTB18*, *C1orf101*, *MIR4677*, *KIF26B*, *EFCAB2*, *CEP170*, *HNRNPU-AS1*, *DESI2*, *C1orf100*, *COX20*, *ADSS*, *AKT3*, *LOC339529*, *HNRNPU*, *SMYD3* are clearly observed.



Previous reported variant: light blue
This case: red

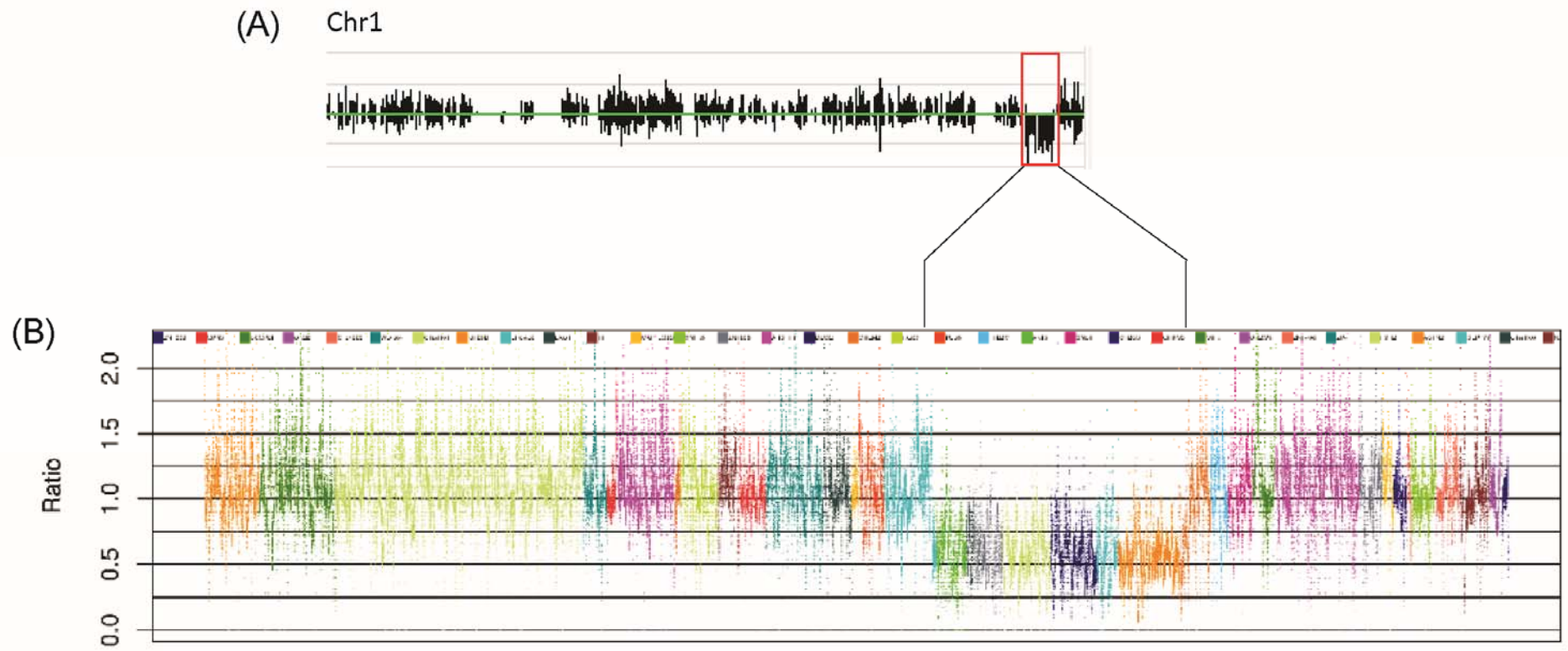

(A)
Chr1
(B)
Ratio
2.0
1.5
1.0
0.5
0.0

Table 1. Clinical characteristics of 16 individuals with CCAs

Individuals	2238	5254	5415	5658	5733	5803[I]	5806	5812	5839	5872	5884	5890	6046	6322	6393P	6715P
Age at final examination	10y10mo	3y6mo	4y	3y3mo	3y3mo	10y6mo	14y11mo	21y 6mo	5y0mo	3y	4y1mo	4y2mo	4y0mo	5y	2y11mo	3y3mo
Gender	F	F	F	F	F	F	F	F	M	M	F	F	F	M	F	F
Gene	*CDK8*		*HIVEP2*			18q21.31 -qter del	*ARID1B*	*ARID 1B*			1q44 del	*ARID1B*		*TCF4*		*TBCK*
Brain MRI																
CCAs	total	total	dysplasia	total	total	total	dysplasia	total	total	total	total	total	total	total	total	total
SCeC	Normal	N/A	Normal	Normal	N/A	Normal	Enlarged	Enlarged	N/A	Enlarged	Normal	Enlarged	N/A	Normal	Normal	Enlarged
CC	+	N/A	+	+	N/A	+	+	+	+	-	+	+	N/A	+	+	-
Other				PMG, heterotopia		Drooped pons, hypomyelination,	T2 hypointense thalamus			DWM						hippocampal malrotation , PVNH
Growth (At birth)																
Height (SD)	-1.76	0.13	0.58	-0.8	0.76	N/A	-1.62	N/A	-0.8	0.97	-0.27	-0.43	-1.92	0.69	0.05	-0.72
Weight (SD)	-0.6	1.44	1.49	-0.03	-0.67	-1.54	-1.13	0.07	-1.17	0.72	-0.99	-1.12	-1.46	0.27	-0.88	-0.57
HC (SD)	0.07	2.9	1.63	-0.43	1.86	N/A	0.42	0.26	-1.13	2.57	1.23	-2.35	-1.18	1.12	-1	-0.13
Growth (At most recent)																
Height (SD)	-1.18	-2.6	0.2	-1.74	-1.17	-4.17	-3.87	-0.4	-0.1	-1.4	-3.6	-2.36	-3.1	-1.5	-0.09	-1.57
Weight (SD)	-0.53	-2.3	-0.25	-2.28	-0.36	-6.35	-1.76	-2.3	0.8	-2.9	-0.4	-1.32	-1.9	-1.35	2.29	-0.73
HC (SD)	2.7	-1.8	NA	-1.71	1.4	-4.87	N/A	N/A	-1.9	2.9	-2.3	-1.67	-2.2	-4.5	-0.2	0.8
Epilepsy																
Seizure	+	+	-	+	+	+	+	N/A	+	-	+	-	-	+	-	-
Epileptic discharge	+	+	-	+	-	-	+	N/A	+	+ → -	-	-	-	+	-	-
Onset age	5d	7mo		2mo	3mo	1y8mo	2y11mo		2mo	-	5mo			1d		
Drug efficiency	effective	effective		-	N/A	-	N/A		Not so effective		effective			effective		

Intellectual disability[II]	IQ36	DQ<10	DQ 33	DQ 13	DQ 45	DQ＜10	IQ22	Mild ID	DQ<10	DQ 22	DQ<10	<DQ 50	DQ 57	DQ<22	DQ 66	DQ 69
Development																
Head control	5mo	-	4mo	-	3mo	11mo	12mo	3mo	-	-	-	6mo	3~4mo	+	3mo	6m
Sitting	12mo	-	9mo	-	16mo	-	18mo	10mo	-	-	-	12mo	12mo	-	7mo	13m
Walking	30mo	-	2y6mo	-	2y4mo	-	36mo	22mo	-	-	-	2y4mo	1y8mo	-	14mo	2y
Significant word	17mo	-	-	-	-	-	23mo	60mo	-	-	-	-	2y2mo	-	<1y6m	< 2y
Neurological findings																
Hypotonia	+→ -	+	-	-	+ (Truncal)	+	+	-	+	+	+	+	+	+	-	-
Spasticity	-	-	-	N/A	-	+	N/A	N/A	-	-	-	-	N/A	N/A	N/A	N/A
Quadriplegia	-	Hypotonic	-	Spastic	-	Spastic	-	-	Hypotonic	Hypotonic	Hypotonic	-	-	Hypotonic	-	-
Ataxia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Autism	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-
Dysmorphisms																
Facial	-	+	-	-	-	+	+	+	-	+	-	+	-	+	-	+
Digits	-	-	-	-	-	+	+	-	-	+	-	+	-	-	-	-
Genitalia	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-
Other	-	Chylothorax, marbled skin	-	-	Right esotropia	Curly hair	Cleft palate	-	Hypopigmented hair	Cleft lip, cleft palate	-	-	-	Pale skin	-	-
Other phenotypes	GHD	Hydrops fetalis	-	-	VSD, clumsiness	-	Sensorineural hearing loss	-	-	-	-	Dysphagia, laryngomalacia	-	-	-	-

y, year; mo, months; d. days; F, Female, M, Male; N/A, not assessed or not available; HC, Head circumference; SD, Standard deviation; CCAs, Corpus callosum anomalies; SCeC, Supracerebellar cistern; CC, Colpocehaly; PMG, Polymicrogyria; DWM, Dandy-Walker malformation; PVNH, Periventricular nodular heterotopia; Mild ID, mild intellectual disability; GHD, Growth hormone deficiency; VSD, Ventricular septal defect; IQ, Intelligence Quotient; DQ, Developmental Quotient; [I], Previously published (Ref. Dr.Shiohama)[11], [II], The attending physicians or clinical psychologists in each hospital administered developmental or intellectual assessment tests, mainly Japanese Enjoji Developmental scale or the

Kyoto Scale of Psychological Development for DQ and the Wechsler Intelligence Scale for Children-Fourth edition (WISC-IV) or Tanaka-Binet test for IQ, according to the age and developmental level of the patient.

Table2. Summary of the genetic variants discovered in our cohort

Individuals	Gender	Gene/locus	Variant		Origin	gnomAD	ACMG	
			DNA	Amino acid			Category	Classified
5415	F	*HIVEP2*	c.2827C>T	p.(Arg943*)	*de novo*	Absent	PS1, PS2, PM2	Pathogenic
5806	F	*ARID1B*	c.1832del	p.(Pro611Hisfs*44)	*de novo*	Absent	PVS1, PS2, PM2	Pathogenic
5803¶	F	18q21.32-qter	chr18:56,585,841-77,513,763 deletion		*de novo* (somatic mosaic)		Not applicable	
5812	F	*ARID1B*	c.6122A>C	p.(His2041Pro)	*de novo*	Absent	PS2, PM2	Likely Pathogenic
5884	F	1q44	chr1:243,384,987-246,027,303 deletion		*de novo*		Not applicable	
5890	F	*ARID1B*	c.5418_5434del	p.(Trp1806*)	*de novo*	Absent	PVS1, PS2, PM2	Pathogenic
6322	M	*TCF4*	c.1730A>G	p.(Glu577Gly)	*de novo*	Absent	PS2, PM2	Likely Pathogenic
2238	F	*CDK8*	c.185C>T	p.(Ser62Leu)	*de novo*	Absent	PS1, PS2, PM2	Pathogenic
6715P	F	*TBCK*	c.1201T>C	p.(Tyr401His)	maternal	0.0001475	PM3, PP3	Uncertain Significance
			c.1588C>T	p.(Arg530Cys)	paternal	Absent	PM2, PM3, PP3	Uncertain Significance

Transcripts: HIVEP2:NM_006734.3; ARID1B:NM_017519.2; TCF4:NM_001083962.1; CDK8:NM_001260.1; TBCK:NM_001163435.2

¶ Previously published patient (Ref. Dr.Shiohama)[11]

TableS1. The list of CCA associated genes

gene	Refseq Accession	Phenotype
AMPD2	NM_004037	Agenesis of corpus callosum, hypoplasia of bulbus and pons, blindness and protruding tongue
ASTN1	NM_004319	Agenesis of corpus callosum/developmental delay/microcephaly/seizures
DEGS1	NM_003676	Agenesis of corpus callosum, hydrocephalus, IUGR, short lower extremities
DISC1	NM_001164537	Agenesis of corpus callosum
KIF21B	NM_001252100	Agenesis of corpus callosum
DPYD	NM_002860	Microcephaly, developmental delay, agenesis of corpus callosum, neonatal seizures
MAGI3	NM_138425	Microcephaly, agenesis of corpus callosum, cerebellar hypoplasia, seizures and asymetric extremities
LRP2	NM_004525	Agenesis of corpus callosum and cardiac defects
TRIP12	NM_001284214	Agenesis of corpus callosum
DIS3L2	NM_004801	Microphthalmia and agenesis of corpus callosum
GLI2	NM_014191	Partial corpus callosum agenesis & severe intrauterine growth retardation
NRXN1	NM_000193	FG syndrome and ADHD
ZEB2	NM_133647	Intellectual disability, facial dysmorphia, speech delay, hydronephrosis, bicuspid aortic valve & absence of corpus callosum
ZIC1	NM_005631	Microcephaly, cortical malformation, callosal agenesis, cerebellar dysplasia, tethered cord & scoliosis
DCLK2	NM_001040260	Agenesis of corpus callosum, hypotonia, epilepsy, developmental delay, microcephaly, hypoplasia & autistic behaviour
CDH9	NM_001130438	Motor and speech delay, agenesis of corpus callosum, white matter abnormal signal, spasticity, muscle weakness
CHD1	NM_020759	Microcephaly, vision impairment, absent corpus callosum and epilepsy
TUBB2B	NM_015631	Microcephaly, corpus callosum agenesis, schizencephaly, polymicrogyria, and vermis & right third nerve hypoplasia
VIPR2	NM_003382	Agenesis of corpus callosum
GLI3	NM_000168	Acrocallosal syndrome
VIPR2	NM_003382	Agenesis of corpus callosum
COG5	NM_017775	Global developmental delay, microcephaly, cleft palate & agenesis of corpus callosum
SHH	NM_178012	Bilateral closed-lip schizencephaly, corpus callosum absence
SMO	NM_014795	Curry-Jones syndrome
CDK5RAP2	NM_018249	Agenesis of corpus callosum
NFIB	NM_001190737	Agenesis of corpus callosum
SPTAN1	NM_003412	Microcephaly, intellectual disability, seizures, hearing and vision loss, corpus callosum agenesis and cerebellar hypoplasia
ALDH18A1	NM_016279	Cutis laxa, autosomal recessive with corpus callosum agenesis
TCTN3	NM_001364113	Joubert syndrome with agenesis of corpus callosum
TEAD1	NM_021961	Aicardi syndrome
GRIP1	NM_021150	Agenesis of corpus callosum, subependymal heterotopia and lacking cryptophthalmos

C12orf57	NM_006348	Failure to thrive, agenesis of corpus callosum & intellectual disability; Temtamy syndrome
SCN8A	NM_152383	Intellectual disability, facial dysmorphia, speech delay, hydronephrosis,bicuspid aortic valve & absence of corpus callosum
DICER1	NM_177438	Agenesis of corpus callosum
FOXG1	NM_005249	Agenesis of corpus callosum
TMEM260	NM_017799	Agenesis of corpus callosum, systolic murmur and edema
BLM	NM_000057	Ventriculomegaly, agenesis of corpus callosum
KIF7	NM_198525	Acrocallosal syndrome; Acrocallosal syndrome with olfactory system abnormalities
SLC12A6	NM_000110	Agenesis of corpus callosum; Andermann syndrome; Andermann syndrome with motor neuronopathy; Motor & sensory neuropathy and corpus callosum agenesis; Peripheral neuropathy and corpus callosum agenesis
STARD9	NM_001083614	Intellectual disability, spasticity, agenesis of corpus callosum
CDK10	NM_052988	Agenesis of corpus callosum, retinopathy & deafness
EARS2	NM_001371271	Fatal neonatal lactic acidosis, recurrent hypoglycemia and agenesis of corpus callosum
ACTG1	NM_001199954	Agenesis of corpus callosum & neuronal heterotopia
HID1	NM_030630	Failure to thrive, growth retardation, intellectual disability, agenesis of corpus callosum
MAPT	NM_001142782	Developmental delay, hypotonia, agenesis of corpus callosum, dysmorphic facial features
MKS1	NM_016835	Joubert syndrome with agenesis of corpus callosum
TTC19	NM_017777	Macrocephaly, Gross motor delay, Agenesis of corpus callosum, Brain atrophy, Hypotonia
DCC	NM_005215	Agenesis of corpus callosum; Agenesis of corpus callosum and interhemispheric cyst; Agenesis of corpus callosum with mirror movements
EPG5	NM_020964	Vici syndrome; Vici syndrome, with severe central sleep apnea
OCEL1	NM_024578	Aicardi syndrome
ATRN	NM_139321	Agenesis of corpus callosum
SLC25A1	NM_005984	Agenesis of corpus callosum & optic nerve hypoplasia
FLNA	NM_001456	FG syndrome
L1CAM	NM_000425	Agenesis of corpus callosum
PAK3	NM_001128166	Intellectual disability and corpus callosum agenesis
MED12	NM_005120	Agenesis of corpus callosum; FG syndrome 1; Opitz-Kaveggia syndrome
EFNB1	NM_004429	Agenesis of corpus callosum
HUWE1	NM_031407	Ventricular septal defect, failure to thrive, speech delay, agenesis of corpus callosum, hypospadias, hydronephrosis, facial
CASK	NM_003688	FG syndrome; Opitz-Kaveggia syndrome
USP9X	NM_001039590	Agenesis of corpus callosum
ARX	NM_139058	ACC, epilepsy, abnormal genitalia; ACC, infantile spasms & abnormal genitalia; ACC, lissencephaly, seizures & genital hypoplasia; ACC, mental retardation, epilepsy & dyskinetic quadriparesis; Agenesis of corpus callosum and interhemispheric cyst; Epileptic encephalopathy, early infantile 1/Lissencephaly, X-linked 2/Partington syndrome/Proud syndrome;Severe intellectual disability, hypotonia, seizures infantile spasms and absence of corpus callosum
OFD1	NM_003611	Ventriculomegaly and agenesis of corpus callosum

TableS2. Clinical summary of 16 individuals in our cohort

General information	
Gender	Female:13, Male:3
Age	2years 10months~21years 6months (median; 48±62months)
<At birth>	
Height (SD)	-1.92 ~ 0.97 (median; -0.43±0.95)
Body Weight (SD)	-1.54~ 1.49 (median; -0.6±0.95)
Head circumference (SD)	-2.35 ~ 2.9 (median; 0.34±1.4)
Neurological phenotype	
Seizures	60% (9/15)
Seizure onset (age)	1day ~ 2year11months
Drug efficiency	57.1% (4/7)
Intellectual disability	100 % (16/16)
Motor developmental delay	93.8% (15/16)
Speech delay	93.8% (15/16)
Hypotonia	68.8% (11/16)
Spasticity	11.1% (1/9)
Quadriplegia	43.8% (7/16, Hypotonic:Spastic = 5:2)
Autism	18.8% (3/16)
Brain MRI findings	
corpus callosum anomalies	
total ACC	14
corpus callosum dysplasia	2
Enlarged supracerebellar cistern	41.7% (5/12)
Colpocpehaly	84.6% (11/13)
Other	31.3% (5/16)
Extra phenotype	
Short stature	43.8% (7/16)
Microcephaly (≤-2 SD)	23.1% (3/13)
Macrocephaly (≥2 SD)	15.4% (2/13)
Facial dysmorphism	50% (8/16)
Other dysmorphism	50% (8/16)

SD, standard deviation; ACC, agenesis of the corpus callosum