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Bi-allelic mutations of *CCDC88C* are a rare cause of severe congenital hydrocephalus

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Abstract

Congenital or infantile hydrocephalus is caused by genetic and non-genetic factors and is highly heterogeneous in etiology. In recent studies, a limited number of genetic causes of hydrocephalus have been identified. To date, recessive mutations in the *CCDC88C* gene have been identified as a cause of non-syndromic congenital hydrocephalus in three reported families. Here, we report the fourth known family with two affected individuals with congenital hydrocephalus due to a homozygous mutation in the *CCDC88C* gene identified by whole exome sequencing. Our two newly described children, as well as the previously published ones, all shared several features including severe infantile-onset hydrocephalus, mild to severe intellectual delay, varying degrees of motor delay, and infantile onset seizures. All identified homozygous mutations in *CCDC88C* abolish the PDZ binding site necessary for proper *CCDC88C* protein function in the Wnt signaling pathway. Our report further establishes *CCDC88C* as one of the few known recessive causes of severe prenatal-onset hydrocephalus. Recognition of this syndrome has important diagnostic and genetic implications for families identified in the future.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Keywordsautosomal recessive; *CCDC88C*; DAPLE; hydrocephalus**1 | INTRODUCTION**

Hydrocephalus results from progressive cerebrospinal fluid accumulation in the ventricular system. Under normal conditions, cerebrospinal fluid is produced by the choroid plexus and absorbed into the vascular and lymphatic systems (Damkier, Brown, & Praetorius, 2013). In communicating (non-obstructive) hydrocephalus, the four interconnected cavities, or ventricles, expand with increasing fluid pressure, and secondary cerebral abnormalities ensue (McAllister, 2012). Congenital or infantile onset hydrocephalus is highly heterogeneous in etiology, with a prevalence of 1 in 1,000 births (Jeng, Gupta, Wrensch, Zhao, & Wu, 2011; Tully et al., 2016). Genetic etiologies of congenital hydrocephalus are rarely identified and include mutations in *L1CAM*, *APIS2*, and *POMT1* (Tully & Dobyns, 2014). However, there continue to be significant gaps in our knowledge of these genetic causes due to absence of systematic data, and the paucity of known genes associated with isolated hydrocephalus (i.e., excluding well-studied syndromes in which hydrocephalus is a feature, such as the dystroglycanopathies). In 2010, recessive mutations of the *CCDC88C* gene were first identified in a large consanguineous multiplex family, with only two additional families identified since (Drielsma et al., 2012; Ekici et al., 2010; Tsoi et al., 2014). Here, we report a family of two affected children with congenital hydrocephalus due to a novel homozygous mutation in the *CCDC88C* gene, and we further characterize the spectrum of *CCDC88C*-associated hydrocephalus.

2 | CASE REPORT

Our family consists of two affected children (LR12-112a1 and LR12-112a2) born to healthy non-consanguineous parents. The first affected girl (LR12-112a1) was the product of a full-term pregnancy complicated by prenatal diagnosis of massive hydrocephalus that necessitated delivery by cesarean section. Birth weight was approximately 4.1 kg (93rd percentile for age and gender), and birth occipitofrontal circumference (OFC) was +9.83 standard deviations (SD) above the mean for age and gender. A ventriculo-peritoneal (VP) shunt was placed at five days of age, with marked reduction in ventricular volume. Her OFC decreased to 40 cm (+2 SD) at age 2 months at that time. Post-operative imaging by CT scan showed that the brain parenchyma had expanded, with subdural fluid accumulations and residual ventriculomegaly. Shunting also led to collapse of the calvarial plates, resulting in retrusive frontal bones and acquired craniosynostosis. Subsequently, the patient underwent calvarial reconstruction at age 2 years. At age 3 years, her head shape became normal. No developmental delays were noted at that time.

The child had her first seizure at age 1.5 years. On examination at age 12 years, weight was 68.5 kg (+2 SD), height was 145.8 cm (−0.7 SD), and OFC was 52.9 cm (53rd percentile). She was able to write her first and last name, and perform basic arithmetic. She had trouble with simple tasks such as bathing and dressing. Her seizures were treated with topiramate,

with the last seizure observed at 9 years of age. She also had polyarticular juvenile arthritis which was treated with remicade infusions and methotrexate.

In the following years, she continued to have developmental delays. She was enrolled in small group special education classes. On last examination at age 14 years, she had been seizure-free for four years, and therefore the doses of her antiepileptic medications were reduced. During this time, she had a shunt obstruction resulting in rapid rise in intracranial pressure leading to cardiorespiratory arrest. Urgent shunt revision was performed; however, cerebellar herniation and severe, irreversible ischemic brain injury had already occurred, and the patient passed away 6 days later.

Ten years after the birth of LR12-112a1, parents give birth to a male child (LR12-112a2), with congenital hydrocephalus that had first been recognized at 5–6 months gestation. This boy was delivered by cesarean section at 38.5 weeks' gestation. Prenatal history was complicated by gestational diabetes. Birth weight was 4.35 kg (94th percentile), length 55 cm (97.2nd percentile), OFC was 46 cm (+8 SD). A VP shunt was placed on day of life 2, and two shunt revisions were performed at 1 month and 3 years of age.

Seizure onset was at one year of age. Seizures consisted of jerking movements, with several clusters since then. The child was placed on topiramate, initially with relatively good response, worsening seizures led to the addition of lacosamide, lamotrigine plus a rescue medicine. He has moderate global developmental delays. He sat at 6–7 months, and walked at 3 years of age. He began using his first two words, the Spanish equivalents of “mom” and “dad,” at age 1 year. At age 3 years, he could communicate approximately 20 single words, and a few phrases. On evaluation at age 7.5 years, the patient's OFC was at the 58th percentile. He is now in the 2nd grade and is enrolled in special education classes. He had secondary abnormalities of skull shape and cranial reconstruction surgery was recommended but not pursued.

Brain imaging of both siblings showed severe enlargement of the lateral ventricles with secondary compression of the supratentorial and infratentorial compartments causing deviation of brain tissue across the midline and cerebellar tonsillar ectopia. The corpus callosum was thin and stretched. The cerebral aqueduct was completely effaced in both patients on all studies, implying congenital aqueductal stenosis. LR12-112a2 also had a subdural hematoma along the midline and left side of the posterior falx with a porencephalic cyst, which were presumably consequences of rapid decompression of the lateral ventricles after shunting (Figure 1).

Besides these two affected children, the family consists of two other healthy brothers (aged 11 months and 22 years). The mother had one spontaneous miscarriage. Parents are of Hispanic ancestry. The mother and father are not known to be related to each other. However, all eight great grandparents were raised in the same area in Santiago Johannes, Mexico (Pedigree shown in Figure 2a).

Trio-based exome sequencing was performed on the first affected child (LR12-112a1) and parents, and a homozygous *CCDC88C* mutation was identified in the affected child (NM_001080414.3, c.5553dupC, p.Ser1852GlnfsX4). Targeted mutation analysis for this

mutation was performed on the second affected child (LR12-112a2) by Sanger sequencing and confirmed the presence of this homozygous mutation as well.

3 | METHODS

3.1 | Human subject ascertainment

Patients were enrolled in the Developmental Brain Disorders Research Program at the Seattle Children's Research Institute with IRB approval. The investigators reviewed all clinical and neuroimaging data, as well as analyzed the whole exome sequencing data.

3.2 | Whole exome sequencing (WES)

WES was performed on the first affected child (LR12-112a1) and parents using the Agilent SureSelect 50 Mb capture method. Paired end reads were mapped to the human genome hg19 using BWA-MEM with default parameters. The Genome Analysis Toolkit (GATK) was used to realign reads around known indels, and recalibrate quality scores to reduce artifacts caused by the sequencing chemistry, and Picard was used to mark duplicate reads. Variants were identified using haplotype caller within GATK and Freebayes. The intersection of the two variant callers were annotated with SnpEff and loaded into a database using the GEMINI framework. Annotations included predicted functional effect (e.g., splice-site, nonsense, missense), protein position, known clinical associations (OMIM, CLINVAR), mouse phenotypes (MGI), conservation score (PhastCons, GERP), and effects on protein function (PolyPhen, CADD scores), and population allele frequencies (Exome Variant Server and Exome Aggregation Consortium data). Tools within GEMINI were used to identify variants conforming to a number of disease models. We identified variants that are rare in the population (MAF <0.01 or <0.05), are predicted to have a high impact on protein function and are de novo or transmitted in an autosomal recessive, compound heterozygote, or x-linked manner. All exome variants that passed our filtering criteria are shown in Supplementary Table S1. The homozygous *CCDC8CC* variant in LR12-112a1 was within one of three regions of homozygosity identified by WES (Supplementary Table S2). Exome coverage of all other hydrocephalus-associated genes was checked (shown in Supplementary Table S3).

4 | DISCUSSION

Congenital hydrocephalus is an extremely heterogeneous disorder with genetic and non-genetic etiologies, with only a few large series systematically looking at single gene causes for congenital hydrocephalus (Tully & Dobyns, 2014) (Supplementary Table S4). Mutations in the X-linked gene, *LICAM*, have emerged from these studies as the most common cause of congenital hydrocephalus, typically associated with aqueductal stenosis (Schrandt-Stumpel, 2004). More recently, de novo/dominant mutations of several other genes have been identified in disorders of brain overgrowth (megalencephaly) and other multi-system features in which hydrocephalus may be a component, such as *AKT3*, *PIK3R2*, *PIK3CA*, and *CCND2* (Tully & Dobyns, 2014).

Recessive mutations of *CCDC88C* have been identified in only four multiplex families to date, three of whom were from in-bred populations (two consanguineous families of

Palestinian and Algerian origin) and one Ashkenazi Jewish family (Drielsma et al., 2012; Ekici et al., 2010) (Supplementary Table S5). Altogether, affected children had congenital hydrocephalus, seizures, and varying degrees of intellectual and/or motor delays, but no other major organ-system involvement. Hydrocephalus was diagnosed in utero or at birth in all mutation-positive individuals, including the siblings in our report. In two previously published families, a total of six pregnancies were terminated due to prenatal findings of severe ventriculomegaly (Drielsma et al., 2012; Ekici et al., 2010). All patients underwent VP shunting within the first few weeks of life (mean age at shunt placement 5.8 days, range 5–21 days of age). All patients had variable types of infantile onset epilepsy (mean age of onset 1.4 years, range of 7 weeks to 3 years at onset). Interestingly, one previously published patient with congenital hydrocephalus did not show signs of motor or developmental delay when last assessed at age 3 years (Ekici et al., 2010). Schizencephaly (Ekici et al., 2010) and biparietal polymicrogyria (Drielsma et al., 2012) have been reported in association with *CCDC88C* mutations. We feel it is difficult to diagnose neuronal migrational anomalies with confidence following decompression from such severe ventriculomegaly, as the gyral folding pattern of the cerebral hemispheres can be distorted substantially following shunting.

In summary, the key clinical features of *CCDC88C* related congenital hydrocephalus, including the family reported in this study, include the following: (i) severe prenatal-onset hydrocephalus (seen in seven out of seven living individuals on whom clinical data are available, to date); (ii) mild to severe intellectual delay, with a wide range of motor delays (6/7); (iii) infantile onset epilepsy (7/7); (iv) additional brain MRI abnormalities including stretching of the corpus callosum and distortion of the cerebral/cerebellar structures secondary to increased intra-cranial pressure (4/7). At least one individual had closed lip schizencephaly as well; diagnosis of schizencephaly in the two siblings reported here is limited by the distortion following decompression of the ventricles.

CCDC88C gene (Coiled coil domain containing 88C gene, also known as Dvl- associating protein with a high frequency of leucine residues, DAPLE) encodes DAPLE, a key regulator of the non-canonical Wnt signaling pathway. The transcript has several regulatory protein binding motifs near its C-terminus. At amino acid positions 2,026–2,028, the carboxy terminus contains a Gly-Cys-Val motif for Disheveled protein association (also known as the PDZ binding domain) (Oshita et al., 2003), as well as another motif for Gαi binding (GBA domain), for subsequent trimeric G protein activation (Aznar et al., 2015). DAPLE has been shown to function as an inhibitor of B-catenin and the canonical Wnt pathway through its interaction with Disheveled protein (Oshita et al., 2003). Simultaneously, the protein enhances the non-canonical pathway by facilitating trimeric G protein activation (Aznar et al., 2015). The non-canonical Wnt signaling pathway has a role in establishing planar cell polarity in ependymal cells that line the ventricular zone (Del Bigio, 2010; Ohata et al., 2014). Ependymal motile cilia are necessary for the proper flow of CSF (Tissir et al., 2010). Recent studies by Takagishi confirm that DAPLE protein is required for translational and rotational polarity of ependymal cilia. They found that *daple*^{-/-} mice experience a disruption in ependymal flow and exhibit communicating hydrocephalus (Takagishi et al., 2017). Loss of the PDZ and GBA binding motifs in DAPLE disrupts pathway signaling. The resulting disruption of CSF fluid flow could be a contributing mechanism to congenital hydrocephalus in patients with mutations in the *CCDC88C* gene. However, the pathophysiological

mechanisms of severe hydrocephalus remain poorly understood and require further investigation (Tully & Dobyns, 2014).

The *CCDC88C* mutations identified in the three previously published families were truncating. Here, we report the fourth, novel homozygous variant in the *CCDC88C* gene. Altogether, the four mutations are distributed throughout the transcript, affecting both PDZ and GBA domains which are crucial for proper Wnt signaling. The first variant (R312X) from family I (Drielsma et al., 2012), is a nonsense mutation that leads to the loss of the coiled-coil, PDZ, and GBA domains. The other recessive mutations also result in a truncated transcript further downstream. The E1949G mutation in family II published by Drielsma (Drielsma et al., 2012) caused a frameshift and inclusion of an early stop codon in exon 30, affecting the PDZ motif. The novel homozygous mutation in our family was also a frameshift in exon 30 resulting in truncation of the transcript and loss of PDZ binding site. Conversely, the mutation reported by Ekici et al. (2010) was a substitution in the donor splice site of intron 29, resulting in loss of exons 29 and 30. In summary, all hydrocephalus associated mutations in *CCDC88C* result in the destruction of the PDZ binding site necessary for DAPLE-DVL association.

Interestingly, a heterozygous mutation of *CCDC88C* has been reported in one multiplex family demonstrating autosomal dominant inheritance. All affected individuals in the family had adult-onset spinocerebellar ataxia (SCA) rather than congenital hydrocephalus (Tsoi et al., 2014). The SCA-associated *CCDC88C* mutation was a missense variant (p.R464H) in the coiled coil domain. Western blot analysis from patient fibroblasts suggest a gain of function effect of this variant on the JNK apoptosis pathway, in contrast to the loss of function effect of hydrocephalus associated *CCDC88C* mutations.

In conclusion, we report the fourth known family with a rare recessive form of congenital hydrocephalus caused by mutations of the *CCDC88C* gene. All *CCDC88C* mutations to date are recessive homozygous predicted to cause loss of protein function through truncation of binding motifs vital to the non-canonical Wnt pathway. Our data provide further evidence that mutations of this critical Wnt regulatory gene are associated with congenital hydrocephalus requiring early shunt placement, infantile onset epilepsy, and variable degrees of intellectual and motor delays. MRI findings, though striking, overlap with other forms of severe congenital hydrocephalus such as *L1CAM*-associated hydrocephalus. Our study helps characterize a rare recessive form of congenital hydrocephalus which has important implications for accurate diagnosis and genetic counseling for affected families. This study further highlights the contribution of genetic factors to congenital hydrocephalus overall, and emphasizes the importance of genomic studies, such as whole exome and whole genome sequencing, in children with hydrocephalus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Brain MR images of two siblings with congenital hydrocephalus. MRI of LR12-112a1 at age 4 years includes T1-weighted mid-sagittal (a), and T2-weighted axial (b) and coronal (c) images showing hydrocephalus status post shunting. Note thinning and distortion of the corpus callosum (white arrow in a), as well as the suggestion of closed-lip schizencephaly (white arrowhead in b) attributable to distortion of cerebral folding by shunting following severe hydrocephalus. No CSF signal is visible through the cerebral aqueduct (black arrow in a). Also note crowding of the cerebellum with mild cerebellar tonsil ectopia. (d) Axial head CT image of patient LR12-112a2 on day one of life shows severe enlargement of the lateral ventricles (*) and thinning of the cerebral parenchyma. (e) Calvarial three-dimensional reconstructions show marked diastasis of the sutures (double white arrows) due to hydrocephalus. (f) Coronal T2-weighted brain MR image of LR12-112a2 at age 33 months shows a cystic collection in the midline with a fluid-hematocrit level secondary to shunting complication. At age 5 years, this patient underwent another brain MRI, shown here with sagittal volumetric T2 (g), and axial (h) and coronal (i) T2-weighted images. Similar to the sibling, note the thinning and distortion of the corpus callosum (white arrow in g), absence of CSF in the cerebral aqueduct, ventriculomegaly with distortion following shunting (*), and resolution of the previously seen intraventricular hemorrhagic cyst. [Color figure can be viewed at wileyonlinelibrary.com]

FIGURE 2.

(a) Pedigree of family LR12-112 showing the two affected children (arrows), as well as unaffected members of the family. Label^{+/+} indicates the patient is homozygous for the frameshift variant p.S1852QfsX4, while label^{+/-} indicates heterozygosity. (b) Diagram of the CCDC88C protein showing the distribution of identified CCDC88C mutations. Our family's mutation is shown in red. All mutations are homozygous truncating mutations, except for R464H which was identified in heterozygous form in a family with SCA. [Color figure can be viewed at wileyonlinelibrary.com]