Regulation of human cerebral cortical development by EXOC7 and EXOC8, components of the exocyst complex, and roles in neural progenitor cell proliferation and survival

Michael E. Coulter, MD, PhD1,2, Damir Musaev, BSc3, Ellen M. DeGennaro, BA1,4, Xiaochang Zhang, PhD1,5, Katrin Henke, PhD6, Kiely N. James, PhD3, Richard S. Smith, PhD1, R. Sean Hill, PhD1, Jennifer N. Partlow, MS1, Muna Al-Saffar, MBChB, MSc1,7, A. Stacy Kamumbu, BA1, Nicole Hatem, BA1, A. James Barkovich, MD8, Jacqueline Aziza, MD3, Nicolas Chassaing, MD, PhD10,11, Maha S. Zaki, MD, PhD12, Tipu Sultan, MD13, Lydie Burglen, MD, PhD14,15, Anna Rajab, MD, PhD16, Lihadh Al-Gazali, MBChB, MSc2, Ganeshwaran H. Mochida, MD, MMSc1,17, Matthew P. Harris, PhD6, Joseph G. Gleeson, MD3 and Christopher A. Walsh, MD, PhD1

Purpose: The exocyst complex is a conserved protein complex that mediates fusion of intracellular vesicles to the plasma membrane and is implicated in processes including cell polarity, cell migration, ciliogenesis, cytokinesis, autophagy, and fusion of secretory vesicles. The essential role of these genes in human genetic disorders, however, is unknown.

Methods: We performed homozygosity mapping and exome sequencing of consanguineous families with recessively inherited brain development disorders. We modeled an EXOC7 splice variant in vitro and examined EXOC7 messenger RNA (mRNA) expression in developing mouse and human cortex. We modeled exoc7 loss-of-function in a zebrafish knockout.

Results: We report variants in exocyst complex members, EXOC7 and EXOC8, in a novel disorder of cerebral cortex development. In EXOC7, we identified four independent partial loss-of-function (LOF) variants in a recessively inherited disorder characterized by brain atrophy, seizures, and developmental delay, and in severe cases, microcephaly and infantile death. In EXOC8, we found a homozygous truncating variant in a family with a similar clinical disorder. We modeled exoc7 deficiency in zebrafish and found the absence of exoc7 causes microcephaly.

Conclusion: Our results highlight the essential role of the exocyst pathway in normal cortical development and how its perturbation causes complex brain disorders.

Keywords: exocyst; EXOC7; EXOC8; microcephaly; developmental delay

INTRODUCTION

Eight genes in the human genome, EXOC1–EXOC8, encode the exocyst complex, a multimeric, evolutionarily conserved complex that traffics vesicles within the cell to the plasma membrane for fusion. The exocyst complex has been shown to play a role in several cellular processes, including cell polarity, cell migration, ciliogenesis, cytokinesis, autophagy, and fusion of secretory vesicles,1 but human disorders associated with definitive loss-of-function variants in any of these components have not yet been reported. Although a missense variant in EXOC82 was reported in a single case of Joubert syndrome (MIM 213300), and a missense variant in EXOC43 was reported in a case of Meckel–Gruber syndrome (MIM 249000), the pathogenicity of these two variants has not yet been confirmed. As such, the essential role of individual proteins of the exocyst complex remains unclear.

1Division of Genetics and Genomics and Howard Hughes Medical Institute, Boston Children’s Hospital, Departments of Pediatrics and Neurology, Harvard Medical School, Boston, MA, USA; 2Program in Neuroscience and Harvard/MIT MD-PHD Program, Harvard Medical School, Boston, MA, USA; 3Department of Neurosciences and Howard Hughes Medical Institute, University of San Diego, La Jolla, CA, USA; 4Harvard–MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, USA; 5Department of Human Genetics, University of Chicago, Chicago, IL, USA; 6Division of Orthopedic Research, Boston Children’s Hospital, Department of Genetics, Harvard Medical School, Boston, MA, USA; 7Department of Paediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates; 8Benioff Children’s Hospital, Departments of Radiology, Pediatrics, Neurology, and Neurological Surgery, University of California San Francisco, San Francisco, CA, USA; 9Département de Pathologie, Institut Universitaire du Cancer de Toulouse–Oncopole–CHU Toulouse, Toulouse, France; 10Service de Géniétique Médicale, CHU Toulouse, Toulouse, France; 11UDEAR; UMR 1056 Inserm–Université de Toulouse, Toulouse, France; 12Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt; 13Department of Paediatrics, Institute of Child Health & The Children’s Hospital, Lahore, Pakistan; 14Centre de référence des malformations et maladies congénitales du cervelet, Département de génétique, AP–HP, Sorbonne Université, Paris, France; 15Hôpital Trousseau and Developmental Brain Disorders Laboratory, Imagine Institute, INSERM UMR 1163, Paris, France; 16National Genetics Center, Directorate General of Health Affairs, Ministry of Health, Muscat, Oman; 17Department of Neurology, Massachusetts General Hospital, Boston, MA, USA. Correspondence: Joseph G. Gleeson (jogleeson@ucsd.edu) or Christopher A. Walsh (christopher.walsh@childrens.harvard.edu)

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Several of the reported functions of the exocyst complex, cell polarity and migration, cytokinesis, and ciliogenesis, are integral processes during cerebral cortical development and so motivated us to test the hypothesis that variants in exocyst encoding genes cause brain development disorders. First, establishing cell polarity and cell migration are essential for cortical development. Variants in radial glial cell progenitors (RGC) polarity genes, such as Pal1 and Par3, disrupt cortical development through massive cell death or premature cell cycle exit, respectively. In addition, variants in genes required for neuronal migration can cause one of several cortical malformations, including lissencephaly (MIM 607432, PAFAH1B1/LIS1), double cortex syndrome (MIM 300067, DCX10,11), and cortical dysplasia (MIM 610031, TUBB3,12 TUBB5,13 KIF5C,14 KIF2A15). Second, robust and rapid cell division of cortical progenitors is essential for cortical development and several genetic causes of microcephaly (MIM 251200) exhibit disrupted cytokinesis as a result of supernumerary (CDK5RAP2,15,16 KATNB117,18), or missing spindle poles (ASP,19 WDR6220) stemming from dysfunctional centrosomes. Finally, defects in cilia formation lead to ciliopathy syndromes (MIM 209900), complex syndromes with disrupted brain development (INPP5E22 C2CD3,23 BBS124). Here we provide a systematic analysis of variants in two exocyst components, by defining several variants in EXOC7 and EXOC8. We identify four independent, partial loss-of-function variants in EXOC7, associated with developmental brain disorders of variable severity characterized by developmental delay, seizures, brain atrophy, microcephaly, and infantile death. We also describe one loss-of-function variant in EXOC8 similarly associated with severe developmental delay, seizures, brain atrophy, microcephaly, and premature death. We further provide a zebrafish genetic model of EXOC7 loss-of-function and offer genetic evidence that EXOC7 is required for neuron survival.

MATERIALS AND METHODS

Human subjects
This study was conducted with the approval of institutional review boards and according to the ethical standards of the participating institutions: Boston Children’s Hospital; University of California–San Diego; the Faculty of Medicine, United Arab Emirates University; and AP-HP Sorbonne Université. Informed consent was received from all participants. Permission was received to publish patient photographs.

IACUC approval of zebrafish housing and experiments
A complete description of the husbandry and environmental conditions in housing for the fish used in these experiments is available as a collection in protocols.io (https://doi.org/10.17504/protocols.io.mrjc54n). All animals were cared for humanely and all experiments were approved by Boston Children’s Hospital Institutional Animal Care and Use Committee (IACUC).

Exome sequencing
DNA was extracted from whole blood and exome sequencing was performed (See Supplement). We filtered out variants with allele frequency >10% in the Middle Eastern population.

Sanger sequencing
Primers surrounding the reported variant in each family were used for polymerase chain reaction (PCR) and subsequent Sanger sequencing to confirm genotype from exome sequencing and determine segregation within the family.

Minigene cloning and expression
A ~5-kb section of human EXOC7 locus was amplified with primers (F: AAGGACTGAAGGAGCATTTC, R: CAGGGA GTGGAAGGTCTTCT) from a BAC and cloned into pcAG expression vector. The splice acceptor variant from family I was introduced with site-directed mutagenesis. Wild-type (WT) or splice variant containing vector was transfected into mouse N2A cells and after 48 hours RNA was isolated and retrotranscribed into complementary DNA (cDNA). N2A cells were cultured at 37°C and 5% CO2 in high-glucose DMEM (GIBCO) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin.

HAP1 mutant cell line
HAP1 human cell line was cultured at 37°C and 5% CO2 in high-glucose DMEM (GIBCO) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. The splice acceptor variant from family I was introduced into a HAP1 cell line as a hemizygous variant using CRISPR/Cas9 mutagenesis (Supplemental Methods)26 (Horizon Discovery). Immunoblot was performed using human EXOC7 antibody (Abcam, ab118792).

Exoc7 alternative splicing in developing cortex
Alternative splicing analysis of EXOC7 in developing human (GW13–16) and mouse (E14.5) cortex was performed as described previously.27 Aligned BAM files from RNA sequencing data sets were analyzed with the MISO pipeline (version 0.4.6) to determine the inclusion frequency of alternatively spliced exons.

Generation of exoc7 mutant zebrafish lines
Exoc7 mutant zebrafish were generated by CRISPR/Cas9 mutagenesis. Cas9 messenger RNA (mRNA) (250 ng/μl) and exoc7 targeting guide RNA (target: CCGTCCTCA TCTGGAGAGCC, 80 ng/μl) were injected into 1-cell embryos. Embryos developed to adulthood and then Sanger sequencing was used to identify potential heterozygous exoc7 mutant carriers in F1 progeny. A 1-bp frameshift deletion in exon 5 was identified and this fish was backcrossed to WT to generate heterozygous carriers. This allele is mh111.
Toluidine blue staining of zebrafish
Five days postfertilization (dpf) embryos were fixed in 4% PFA overnight at 4 °C and then embedded in JB-4 resin according to manufacturer’s protocol (Polysciences Inc). Fish were sectioned at 2 μm, and then matching sections were stained with toluidine blue and imaged with a bright-field microscope.

Immunostaining of zebrafish progenitor cells
Five dpf embryos were fixed in 4% PFA overnight at 4 °C, embedded in OCT, and sectioned coronally at 20 μm on a cryostat. Matched sections were stained with a primary antibody against Sox2 (Abcam, ab97959). Tissue was permeabilized and blocked in 3% BSA, 0.3% Triton X-100, 0.3% sodium azide in PBS. Primary antibodies were incubated overnight at 4 °C. Sections were stained with Alexa secondary antibodies and Hoechst. Imaging was done on Zeiss 510 confocal microscope. Sox2-positive nuclei in telencephalon were counted.

TUNEL staining in developing zebrafish
Five dpf embryos were fixed in 4% PFA overnight at 4 °C, embedded in OCT, and cryosectioned coronally. Apoptotic cells in matched sections were labeled with TUNEL staining using the Apoptag kit (Millipore) according to the manufacturer’s instructions. Imaging was done on Zeiss 510 confocal microscope. TUNEL positive cells in telencephalon were counted.

RNAscope
RNAscope on human fetal brain tissue was performed according to manufacturer’s protocol (ACDBio). Tissue was fixed in 4% PFA, frozen, and sectioned at 20 μm on a cryostat.

Quantification and statistical analysis
In all analyses, mean values are presented for pooled data and errors bars are SEM. For all quantifications, statistical significance was determined using a two-tailed, unpaired t test (GraphPad Prism).

RESULTS

EXOC7 and EXOC8 variants in recessive developmental disorders
In mapping developmental disorders affecting the cerebral cortex, we identified variants in EXOC7 and EXOC8 associated with recessive brain development syndromes with a range of symptom severity including developmental delay, seizures, brain atrophy, microcephaly, and infantile death (Table 1).

Family I is a consanguineous family with the most severely affected children, who have infantile lethality with neonatal microencephaly, seizures, and arthrogryposis (Fig. 1a, S1A). The family had two daughters who were born with myoclonic seizures and arthrogryposis multiplex. One had documented microcephaly (−2.7 SD), and both died within the first months of life. Imaging of both siblings showed a cerebrum

Table 1  Variant summary for each family.

| Family | Maximum LOD score | Gene | Variant type | Variant | Segregates in family | gnomAD frequency |
|--------|------------------|------|--------------|---------|----------------------|-----------------|
| I      | 1.93             | EXOC7| In-frame deletion | Exon 7 splice acceptor (c.809-2A>G) & exon 10: c.1212_1226 delTGGGCTG ATGCTTGA | Yes | Absent |
| II     | 2.9              | EXOC7| Splice variant & in-frame deletion | Exon 7 splice acceptor (c.809-2A>G) | Yes | 2/277,066 alleles, heterozygous |
| III    | 2.5              | EXOC8| Missense | Ala523Thr (C>T) | Yes | 2/276,426 alleles, heterozygous |
| IV     | 2.5              | EXOC7| Frame shift deletion | Asp607Ter (C>T) | Yes | 2/251,414 alleles, heterozygous |
| V      | 2.5              | EXOC8| Splice variant | c.1534-1535delCCT | Yes | 2/251,414 alleles, heterozygous |

LOD logarithm of the odds.
EXOC7 variants cause a recessive brain development disorder. (a) Left, pedigree of family I showing consanguineous parents and recessive inheritance of lethal microcephaly in I-01 and I-05. Middle, coronal and axial brain magnetic resonance image (MRI) (I-01) or computed tomography (CT) (I-05) show extremely simplified gyral pattern, small cortex, and fluid accumulation with age-matched normal MRI for comparison. Right, Sanger sequencing of EXOC7 intron 6/exon 7 boundary shows intronic A>G variant that mutates the canonical splice acceptor (aglG to ggG). This variant is homozygous in affected individuals and segregates with disease. (b) Left, pedigree of family II showing consanguineous parents and recessive inheritance of brain atrophy, microcephaly, and seizures in II-04. Middle, Sanger sequencing of EXOC7 exon 3 reveals a homozygous 3-base-pair ATC deletion in the affected individual that segregates with disease. Right, this deletion removes amino acid Serine 48, which is located in the EXOC7 N-terminal region responsible for binding to EXOC8.30 (c) Left, pedigree of family III showing recessive inheritance of fetal microcephaly and cerebellar hypoplasia in III-03, III-04. Middle, Sanger sequencing showing EXOC7 exon 7 homozygous splice acceptor variant. Right, diagram of 15-bp heterozygous deletion in EXOC7 exon 10. (d) Left, pedigree of family IV showing consanguineous parents and recessive inheritance of brain atrophy and seizures in IV-05, IV-07, and IV-09. Middle, Sanger sequencing showing EXOC7 exon 15 reveals a homozygous G>A variant in affected individuals that segregates with disease. Right, this variant changes amino acid 523, a highly conserved amino acid from humans to zebrafish, from alanine to threonine. (e) Family V has recessive inheritance of a syndrome of developmental regression and delay, seizures, brain atrophy, and early death. Homozygosity mapping and exome sequencing reveals a homozygous 2-base-pair deletion in EXOC8 that causes early protein truncation. aa amino acid.
Asp607Ter, Fig. 1e), which is absent from normal controls (gnomAD). This frameshifting deletion creates a premature stop codon at amino acid 607, short of the full-length protein (725 aa), and segregates perfectly with disease in this family. One additional homozygous variant was detected in the family (stop-gain in RP1L1). In gnomAD, this variant is present as a homozygote in one individual and RP1L1 is associated with adult-onset retinitis pigmentosum (OMIM 613587), so this variant is unlikely to contribute to microcephaly in this family.

In total, we report four novel missense and splice site variants in EXOC7 and one novel truncating variant in EXOC8 in families with a recessive syndrome of brain atrophy, seizures, and developmental delay, and in more severe cases microcephaly and infantile death (Table 1). The presence of cerebral atrophy in all families indicates neurodegeneration and suggests EXOC7 and EXOC8 are required for neuronal survival. Loss-of-function variants in EXOC7 have not been previously linked to human disease, and we have recently reported one homozygous missense variant in EXOC8 in a single case of Joubert syndrome.2

**EXOC7 splice variant disrupts mRNA splicing patterns and reduces protein expression**

EXOC7 is an alternatively spliced gene with 5 verified transcripts;3 two of which include exon 7. We generated a minigene assay to model the exon 7 splice acceptor variant found in both families I and III and found this variant disrupts splicing and decreases EXOC7 protein level. The minigene was constructed using 5 kb of genomic DNA from the human EXOC7 locus spanning exon 6 to exon 9 (Fig. 2a).

Reverse transcription PCR (RT-PCR) of mRNA transcribed from the minigene plasmid expressed in mouse N2A cells revealed three splicing disruptions caused by the human variant (Fig. 2a). First, cDNA encoding a high-abundance transcript (including exons 6, 7, and 9) was isolated from wild-type minigene but completely absent from the variant (Fig. 2a). A low-abundance larger product that could not be subcloned for sequencing was found in wild-type and likely encodes a transcript including exons 6, 7, 8, and 9 (*, Fig. 2a).

Second, two novel out-of-frame splice forms that are predicted to encode early truncations were found exclusively in variant minigene cDNA (Fig. 2a). Form A splices in the last

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**Fig. 2** EXOC7 exon 7 splice acceptor variant disrupts splicing. (a) Top, diagram of EXOC7 human minigene construct with splice acceptor variant. Blue arrows mark reverse transcription polymerase chain reaction (RT-PCR) primers used to generate complementary DNA (cDNA) products shown in gel image. Arrows from each band in gel point to splicing diagram determined by Sanger sequencing of the cDNA. Wild-type (WT) minigene generated two in-frame isoforms (left), whereas variant minigene generated one in-frame isoform and two novel out-of-frame isoforms (right). Two variant isoforms encode novel stop codons that lead to premature protein truncation. Asterisk (*) indicates low-abundance product that could not be subcloned for sequencing. (b) Immunoblot of EXOC7 protein in WT and variant HAP1 cells showing reduction of two EXOC7 isoforms. GAPDH is a protein loading control. (c) Quantification of (b) showed significant 50% reduction of larger EXOC7 band (band 1, \( p = 0.045 \)), while the lower weight band (band 2) was not significantly reduced. \( P \) values calculated by two-tailed t test. Error bars represent SEM.
Similarly, Exoc7 (MGI). RNA sequencing analysis of human fetal cortex showed abundant hybridization using RNAscope in human fetal cortex zone (adjacent to the ventricle and coexpressing 3a, b cortical plate (adjacent to the pial surface) (Fig. TBR2 outer subventricular zone (coexpressing the location of postmitotic neurons. (d) differential inclusion of exon 7 in CP vs. VZ.

Immunoblot of EXOC7 protein showed reduced expression in HAP1 cell line to encode the patient variant (hemizygous). To assess the variant’s effect on EXOC7 protein we mutated a HAP1 cell line to encode the patient variant (hemizygous). Immunoblot of EXOC7 protein showed reduced expression in variant HAP1 cells (Fig. 2b, c). We detected two isoforms: (1) a larger isoform was significantly reduced by 50% (two-tailed t test, p = 0.045) and (2) a smaller isoform showed a trend towards reduction (two-tailed t test, p = 0.088). Together, these results support the pathogenicity of this variant.

**EXOC7 is highly expressed in developing cerebral cortex**

EXOC7 is highly expressed in the developing human cortex, consistent with the affected individuals’ phenotypes. In situ hybridization using RNAscope in human fetal cortex showed abundant EXOC7 expression in the ventricular zone (adjacent to the ventricle and coexpressing VIM), the outer subventricular zone (coexpressing TBR2) and in the cortical plate (adjacent to the pial surface) (Fig. 3a, b). Similarly, Exoc7 is expressed in developing mouse cortex (MGI). RNA sequencing analysis of human fetal cortex confirms this expression pattern with expression in the ventricular zone and the inner and outer subventricular zones at a similar level as ASPM, a canonical microcephaly gene, and in the cortical plate (Fig. 3c). EXOC7 expression in both progenitors and postmitotic neurons suggests it has important roles in both cell types during cortical development. Notably, exon 7 of EXOC7, which contains a splice acceptor variant in families I and III, is differentially spliced in cortical progenitors compared with postmitotic cortical neurons. Using a previously published method, we identified differentially spliced exons in the developing cortex with RNA sequencing of separated cortical progenitors and postmitotic neurons isolated from both developing mouse and human brain tissue. During fetal human cortical development (GW15), exon 7 is included in 93% of transcripts from the cortical plate and 47% of transcripts from ventricular zone (Fig. 3d). Similarly, during cortical development in mice (E14.5), exon 7 is included in 87% of transcripts isolated from the cortical plate and 35% of transcripts from the ventricular zone (Fig. 3d). The role of this differential splicing in the regulation of EXOC7 function in development is unknown, but our evidence that the splicing variant identified in families I and III alters this differential expression, by eliminating expression of the exon 7 included isoform (Fig. 2), suggests this variant is pathogenic by disrupting cortical development.

**Exoc7 is essential for vertebrate embryonic development**

EXOC7 protein is highly conserved among vertebrates (Fig. 4a). To further characterize the function of EXOC7 in

Fig. 3  **EXOC7 is highly expressed in developing cortex.** (a) Diagram of cortical section of human fetal cortex indicating locations of RNAscope imaging in (b) and RNA sequencing in (c). (b) RNAscope imaging of fetal human cortex shows EXOC7 expression in VZ and OSVZ, two progenitor zones, and in CP, the location of postmitotic neurons. (c) EXOC7 is highly expressed in developing human cortex and shown in comparison with ASPM. Expression levels measured based on RNA sequencing. (d) RNA sequencing data from developing human fetal cortex (GW15) and mouse cortex (E14.5) showing differential inclusion of exon 7 in CP vs. VZ. FPKM fragments per kilobase of transcript per million mapped reads.
brain development, we examined Exoc7 loss in both zebrafish and mouse. Mice deficient for Exoc7 have been created by Lexicon Pharmaceuticals and are reported to be homozygous lethal (personal communication). This is consistent with the phenotype for loss-of-function variants in other exocyst components also reported to be early embryonic lethal,\(^3\),\(^4\),\(^5\) and suggests that the three human alleles identified retain some function and are partial loss-of-function.

To facilitate analysis of Exoc7 function during development, we took advantage of the ease of genome editing in the zebrafish. We created an exoc7 mutant zebrafish with predicted loss of function of the encoded protein that

| Adj. head dia. (um) | Control | Null |
|---------------------|---------|------|
| 3.00                | 28      | 24   |
| 3.50                | 5       | 5    |
| 4.00                | 0       | 0    |

| Body length (mm) | Control | Null |
|------------------|---------|------|
| 2.00             | 5       | 5    |

| Apoptotic cells | Control | Null |
|-----------------|---------|------|
| 0.00            | 5       | 5    |

| Sox2+ cells     | Control | Null |
|-----------------|---------|------|
| 0.00            | 5       | 5    |

Fig. 4 Exoc7 is essential for zebrafish telencephalon development. (a) Exoc7 amino acid sequence is highly conserved between human, mice, and zebrafish. (b) exoc7 1-bp frameshift deletion variant in exon 5 is confirmed by DNA sequencing and predicted to cause a frameshift and subsequently a protein truncation through a premature stop codon at amino acid 186. gRNA guide RNA. (c) exoc7 homozygous mutant fish have gross developmental abnormalities by 5 dpf, notably small eyes and head edema. Green line shows measurement of adjusted head diameter calculated by subtracting edema (red lines). (d) Heterozygous exoc7 zebrafish crosses generated mutant fish (small eye/edema or dead) at expected Mendelian ratio. Genotyping confirmed that phenotypically mutant larvae were homozygous for the exoc7 variant. (e) Quantification of adjusted head diameter, which is significantly reduced in homozygous mutant fish. (f) Body length is not significantly changed in homozygous mutant fish. (g) Toluidine blue stain of 5-dpf wild-type and exoc7 mutant zebrafish. (h) Apoptag staining shows a significant increase of apoptotic cells in the exoc7 mutant telencephalon. (i) Immunohistochemical staining of neuronal progenitors using Sox2. The number of Sox2+ progenitors is significantly decreased in the exoc7 mutant telencephalon. P values calculated with two-tailed t test. Error bars represent SEM.
allowed us to examine the function of *exoc7* in development. We used CRISPR/Cas9 mutagenesis to create a 1-bp deletion in exon 5 of *exoc7* that generates a predicted frameshift and subsequent nonsense variant. This variant is predicted to lead to a prematurely truncated protein 185 amino acids long, well short of the full-length peptide at 735 (Fig. 4b).

We found that *exoc7* is essential for zebrafish development. At 5 dpf, approximately 25% of progeny from a heterozygous incross showed head edema and small eyes, consistent with Mendelian inheritance (Fig. 4c, d, g). Genotyping confirmed that these phenotypes were associated with loss of *exoc7* and demonstrated that the phenotype was highly penetrant (Fig. S5A). The mutant fish from the incross (edema and small eyes) die shortly after day 5, showing *exoc7* is essential for early zebrafish survival.

Quantification of the small eye phenotype revealed that the eye area was reduced by 26% in mutant fish (two-tailed *t* test, 3.6 × 10−24, Fig. SSB). Broader characterization of the phenotype in *exoc7* mutant fish revealed general defects in head size although this was partially masked with the present edema. To account for the changes caused by the edema, we measured the distance from the outside of one eye to the other and then subtracted the regions of edema (Fig. 4e). Even with this conservative measure, head diameter was 4% smaller in *exoc7* null fish compared with clutch controls (two-tailed *t* test, *p* = 6.8 × 10−5, Fig. 4e). We detected no difference in body length in mutant fish, suggesting that these defects are specifically caused by the loss of *exoc7* and not simply by developmental delay or allometric changes (two-tailed *t* test, *p* = 0.21, Fig. 4f).

**Cellular defects in *exoc7* null developing telencephalon**

To identify cellular mechanisms underlying microphthalmia and microcephaly in *exoc7* mutant zebrafish (Fig. 4g), we measured apoptosis and counted progenitor cells in the developing telencephalon. At 5 dpf, we found a threefold increase in the number of apoptotic cells (TUNEL stain) in the telencephalon of *exoc7* null zebrafish (control *n* = 5, *exoc7* knockout [KO] *n* = 5, two-tailed *t* test, *p* = 0.0023, Fig. 4h). At the same age, we also found a 53% decrease in the number of Sox2-positive telencephalon progenitor cells (control *n* = 4, *exoc7* KO *n* = 5, two-tailed *t* test, *p* = 0.0019, Fig. 4i). The number of Sox2-positive neuroprogenitors was also decreased in the retina of *exoc7* null fish, with a 76% decrease compared with controls (control *n* = 11, *exoc7* KO *n* = 10, two-tailed *t* test, *p* = 3.0 × 10−6, Fig. S5C).

We examined Hoechst-stained mitotic figures in developing telencephalon and did not find a detectable increase in abnormal mitoses in *exoc7* null fish, suggesting normal cytokinesis (Fig. S5E). Together, these results highlight specific cellular defects that drive microcephaly in zebrafish in the absence of *exoc7*, and further suggest that the atrophy and microcephaly observed in humans with *EXOC7* variants reflect loss of proliferating progenitor cells and postmitotic neurons.

**DISCUSSION**

We identified four independent presumably hypomorphic variants in *EXOC7* and one predicted loss-of-function variant in *EXOC8* causing a recessive human brain development disorder characterized by brain atrophy, seizures, and developmental delay and in more severe cases, microcephaly and infantile death. We show that *EXOC7*, a member of the mammalian exocyst complex, is highly expressed in developing human cortex. In addition, a zebrafish model of *Exoc7* deficiency recapitulates the human disorder with increased apoptosis and decreased progenitor cells during telencephalon development, suggesting that the brain atrophy in human cases reflects neuronal degeneration. These findings provide key inroads into understanding the role of the exocyst complex in normal cortical development and complex neurodegenerative disorders.

**Exocyst variants cause a range of brain development disorders**

Our work represents the first systematic genetic analysis of the role of exocyst components in human genetic disease. The four distinct alleles that we describe in *EXOC7* show a range of severity consistent with the degree to which they likely damage the protein, with the splicing variant being most severe, a 5-amino acid deletion having similar severity, a 1-amino acid deletion being less severe, and an amino acid substitution being the mildest. However, all families share central nervous system (CNS) disease and specifically cortical atrophy.

We also report a null variant in *EXOC8* associated with severe phenotypes within this spectrum. Interestingly, we previously reported that a single affected individual with Joubert syndrome had a homozygous missense variant (E265G) in *EXOC8*. This variant occurred at a highly conserved amino acid and was predicted to be damaging to protein function. Careful clinical review of the affected individuals here confirms that they do not have classic signs of Joubert syndrome. It is possible that these two alleles, E265G and Asp607Ter, lead to different clinical syndromes based on differing variant severity, where a hypomorphic missense variant causes Joubert syndrome and a null variant causes cortical atrophy and microcephaly. Consistent with this idea, homozygous null *Exoc8* mice are reported to have early embryonic lethality (MGI, Mouse Phenotyping Consortium). Here we report that loss-of-function variants in either *EXOC7* or *EXOC8* lead to highly overlapping clinical features, suggesting perhaps that disruption of the exocyst complex broadly impairs normal cortical development. This is supported by previous work reporting an individual with Meckel–Gruber syndrome and microcephaly had a homozygous missense variant (Gln578Arg) in another exocyst component, *EXOC4*. The exact mechanism for exocyst dysfunction causing microcephaly and cortical atrophy is unknown, but previous work and our current results suggest the exocyst may be essential for multiple molecular processes during cortical development. Joubert syndrome
and Meckel–Gruber syndrome both have features of ciliopathies, and the exocyst is reported to localize to the primary cilium where some members are required for normal ciliogenesis (EXOC3S). In developing zebrafish, a ciliopathy phenotype of abdominal or cardiac edema, upward tail curvature, and small eyes has been observed with loss of exoc5, and knockdown of Joubert syndrome gene arl13b (OMIM 612291). We find that exoc7 mutant zebrafish have a phenotype with some ciliopathy features including small eyes with edema but missing other features such as abdominal edema or upward tail curvature (Fig. S5D). Further investigation will be required to determine if loss of exoc7 causes mild cilia dysfunction. Interestingly, the patients we report with EXOC7 hypomorphic variants do not have classic ciliopathy features. We find that loss of EXOC7 leads to apoptosis, cell loss, and atrophy in the developing brain and further studies will determine which cellular processes (or combination thereof) are disrupted including RGC polarity and cilia function.

**Exocyst complex in cortical development**

Our results agree with previous studies of Exoc7 function in neurons and add new details for its role in brain development. Previous work reported that expression of dominant-negative Exoc7 in developing mouse cortex impaired neuron migration and in vitro Exoc7 knockdown in cultured neurons disrupted polarization and prevented process outgrowth. A conditional mouse knockout of Arp2/3, an actin binding protein that interacts with Exoc7, in neuroprogenitors showed cortical disorganization characterized by radial glia process truncation, impaired neuron migration, and abundant apoptosis. Here we find exoc7 deficiency in the zebrafish developing telencephalon is also associated with abundant apoptosis.

We report the first identification of human variants in an exocyst member, EXOC7, and show these variants and a null variant in another exocyst member, EXOC8, cause a neurodevelopmental syndrome of brain atrophy, seizures, and developmental delay with microcephaly and infantile death. This study exposes key, shared properties of two neurodevelopmental syndromes of brain atrophy, seizures, and death. This study exposes key, shared properties of two neurodevelopmental syndromes of brain atrophy, seizures, and death.

**SUPPLEMENTARY INFORMATION**

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**DISCLOSURE**

The authors declare no conflicts of interest.

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