Biallelic loss of human *APC2*, encoding adenomatous polyposis coli protein 2, leads to lissencephaly, subcortical heterotopia, and global developmental delay

Sangmoon Lee1,2, Dillon Y Chen1,2, Maha S. Zaki3, Reza Maroofian4, 5, Henry Houlden5, Nataliya Di Donato6, Dalia Abdin6, Heba Morsy7, Ghayda M. Mirzaa8,9,10, William B. Dobyns8,9, Jennifer McEvoy-Venneri1, Valentina Stanley1, Kiely N. James1, Grazia M.S. Mancini11, Rachel Schot11, Tugba Kalayci11,12, Umut Altunoglu12, Ehsan Ghayoor Karimiani4, Lauren Brick13, Mariya Kozenko13, Yalda Jamshidi4, M. Chiara Manzini14, Mehran Beiraghi Toosi15, Joseph G. Gleeson1,2,\*

1. Department of Neuroscience, Howard Hughes Medical Institute, University of

California, San Diego, CA, 92093, USA

2. Rady Children’s Institute for Genomic Medicine, Rady Children’s Hospital, San

Diego, CA 92123, USA

3. Clinical Genetics Department, Human Genetics and Genome Research Division,

National Research Centre, Cairo 12311, Egypt

4. Genetics Research Centre, Molecular and Clinical Sciences Institute, St. George’s, University, London, SW17 ORE, UK

5. Department of Neuromuscular Disorders, UCL Institute of Neurology, Queen Square, London, WC1N 3BG, UK

6. Institute for Clinical Genetics, TU Dresden, Fetscherstrasse 74, 01307 Dresden, Germany

7. Medical Research Institute, Alexandria University, Alexandria 21561, Egypt

8. Center for Integrative Brain Research, Seattle Children’s Research Institute, Seattle, WA, 98101, USA

9. Departments of Pediatrics and Neurology, University of Washington, Seattle Children’s Research Institute, Seattle, WA, 98101, USA

10. School of Medicine, University of Washington, Seattle, WA, 98101, USA

11. Department of Clinical Genetics, Erasmus University Medical Center, 3015 CN, Rotterdam, The Netherlands

12. Department of Medical Genetics, Istanbul University, Istanbul Faculty of Medicine, Istanbul, 34093, Turkey

13. Department of Genetics, McMaster Children’s Hospital, Hamilton, Ontario, L8S 4L8, Canada

14. Institute for Neuroscience, Dept. of Pharmacology and Physiology, The George

Washington University, DC 20037

15. Department of Pediatric Neurology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, 7HRJ+HQ, Iran

\*To whom correspondence should be addressed. E-mail: jogleeson@ucsd.edu  
Word Count: 2,361, Number of figures 3, Number of tables 1, Supplement 1 table and 1 figure  
Keywords: APC2, lissencephaly, agyria, pachygyria, band heterotopia, epilepsy, intellectual disability, epilepsy, neuronal migration

Email addresses:

sal004@ucsd.edu, rmaroofian@gmail.com, kozenko@mcmaster.ca, lauren.brick@gmail.com, g.mancini@erasmusmc.nl, chiara.manzini@gmail.com, tugba.kalayci@istanbul.edu.tr, Ghayda.Mirzaa@seattlechildrens.org, wbd@uw.edu, ualtunoglu@gmail.com, dr\_mahazaki@yahoo.com, dyc017@ucsd.edu, jmcevoyvenneri@ucsd.edu, kiely.n.james@gmail.com, vstanley@ucsd.edu, jogleeson@ucsd.edu, beiraghitm1@gmail.com, yjamshid@sgul.ac.uk, eghayoor@gmail.com, Nataliya.DiDonato@uniklinikum-dresden.de, h.houlden@ucl.ac.uk, Dalia.Abdin@uniklinikum-dresden.de, hebamorsi@alexu.edu.eg

**Abstract**

Lissencephaly is a severe brain malformation, where failure of neuronal migration results in agyria or pachygyria, in which the brain surface appears unusually smooth. Lissencephaly is often associated with microcephaly, profound intellectual disability, epilepsy and impaired motor abilities. Twenty-two genes are associated with lissencephaly, accounting for approximately 80% of disease. Here we report 12 individuals with a unique form of lissencephaly, from 8 unrelated families, with biallelic mutations in *APC2*, encoding Adenomatous Polyposis Coli 2 protein. Brain imaging studies demonstrate extensive posterior predominant lissencephaly, similar to LIS1-associated lissencephaly, as well as co-occurrence of subcortical heterotopia posterior to the caudate nuclei, ‘ribbon-like’ heterotopia in the posterior frontal region, and dysplastic in-folding of the mesial occipital cortex. The established role of APC2 in integrating the actin and microtubule cytoskeletons to mediate cellular morphological changes suggests shared function with other lissencephaly-encoded cytoskeletal proteins such as α-N-catenin (*CTNNA2*) and Lissencephaly-1 (*LIS1*). Our findings identify *APC2* as a radiographically distinguishable recessive form of lissencephaly.

**Text**

The development of the cerebral cortex is a complex dynamic process that occurs primarily between gestational weeks 6-20. The predominant steps include neural stem cell proliferation and differentiation, migration from the ventricular site of origin outward to the developing cortical plate, and cortical organization associated with synaptogenesis and neural network formation. Disruption of this process can lead to many different malformations of cortical development (MCD), with increasing diversity recognized from advances in both brain imaging and molecular genetics.1 MCDs collectively represent a major cause of neurodevelopmental disorders often associated with severe epilepsy, and contribute to morbidity and mortality in the first decade of life.2 The paradigm for MCD associated with defects in neuronal migration comprises the lissencephaly spectrum (LIS, agyria-pachygyria) and less severe MCDs including subcortical band heterotopia and more tubulinopathy-associated dysgyrias.

While some types of MCDs can result from environmental factors, LIS spectrum are almost always due to recessive, dominant or X-linked mutations, encoding proteins that regulate the neuronal cytoskeleton (both actin and microtubules) – a critical function as neurons migrate. To date genes associated with these disorders include: *ACTB* (MIM 102630), *ACTG1* (MIM 102560), *ARX* (MIM 300382), *CDK5* (MIM 123831), *CRADD* (MIM 603454), *CTNNA2* (MIM 114025), *DCX* (MIM 300121), *DYNC1H1* (MIM 600112), *KIF2A* (MIM 602591), *KIF5C* (MIM 604593), *LIS1* (MIM 601545), *MACF1* (MIM 608271), *MAST1* (MIM 612256), *NDE1* (MIM 609449), *RELN* (MIM 600514), *TUBA1A* (MIM 602529), *TUBB* (MIM 191130), *TUBB2A* (MIM 615101), *TUBB2B* (MIM 612850), *TUBB3* (MIM 602661), *TUBG1* (MIM 191135) and *VLDLR* (MIM 192977), and account for more than 80% of individuals with LIS and LIS variants.3-5 Differences in the gyral pattern and associated brain malformations, or other non-brain dysmorphisms, can distinguish some genetic forms of LIS. Some genes associate with a posterior-predominant (i.e. P>A) LIS (*LIS1*, *TUBA1A* and others) and others with anterior-predominant (A>P) LIS (*DCX*, *ACTB*, *RELN* and others). The LIS gradient and associated brain and other malformations allow for distinction of at least 21 different subtypes of LIS, and allow for prediction of likely mutant genes for newly identified individuals.5

In a collaborative effort to identify additional mechanisms underlying LIS, we recruited 8 families where previous phenotypic and molecular analysis suggested a novel cause. This cohort included families that could not be accurately classified into existing phenotypic categories, or where testing for mutations in existing genes was negative. This study was performed within an ethical framework set by the University of California San Diego IRB, and informed consent was obtained on each individual involved in this study. We recruited Family 1 from Egypt with documented parental 1st degree consanguinity with two affected male siblings showing a nearly identical clinical pattern of severe developmental delay and myoclonic seizures starting at 5 months of age, along with a radiographic pattern of P>A LIS (Figures 1A-1B). Genomic DNA from both affected individuals underwent whole exome sequencing with the SureSelect Human All Exome 50 Mb kit (Agilent Technologies, USA) with 125-bp paired-end read sequences generated on a HiSeq2500 (Illumina, Inc. USA), then analyzed with GATK best practices (see supplemental methods). We identified a homozygous truncating p.Gln361\* mutation in *APC2* (MIM 612034)encoding Adenomatous Polyposis Coli 2 protein, which is expressed throughout the central nervous system.6 Other homozygous variants in this family were either relatively common, were less likely to damage protein function or were already linked to other diseases among which *KCNMA1* (MIM 600150) is causal for autosomal dominant paroxysmal nonkinesigenic dyskinesia (MIM 609446) and *FBN1* (MIM 134797) causes autosomal dominant Marfan syndrome (MIM 154700) which affected individuals do not have (Table S1).

Two other families from our lissencephaly cohort of 75 families with predominantly recessive MCDs also showed homozygous truncating mutations in *APC2.* Family 2 and 3 both had documented parental 1st degree consanguinity. Brain MRIs in both families showed P>A LIS pattern that closely matched images in Family 1. Family 2 had a single affected child and demonstrated a homozygous p.Gln628\* mutation, whereas Family 3 had two affected and one healthy child and demonstrated a homozygous p.Ala2217Profs\*118 mutation in the affecteds. These data suggest that homozygous loss of function (LoF) mutations in *APC2* lead to fully penetrant P>A LIS. Through Matchmaker Exchange and correspondence with colleagues, we identified five additional families with truncating *APC2* mutations, in which the *APC2* variants were independently identified as likely most relevant to clinical presentation (Table S1), and where brain MRI showed P>A LIS. This included homozygous p.Thr565Argfs\*50, p.Ser246\*, p.Leu947Hisfs\*88 and c.1853+1G>C (splice donor), and a compound heterozygous p.Asp392Glyfs\*28;p.Pro1419Argfs\*157. None of the 8 families had damaging mutations in any known *LIS* genes. Thus, we identified a total of 8 families comprising 12 individuals with biallelic *APC2* LoF mutations and P>A LIS, suggesting biallelic loss of *APC2* function as a rare cause of P>A LIS.

All affected children were born full term without complications during pregnancy and delivery (Table 1). While length and weight at birth were normal, as a group, most affected individuals showed a trend toward smaller head circumference and only one individual met criteria for microcephaly, defined as a head size < 3 standard deviation (SD) below the mean, at the most recent measurement (Table 1). Most individuals presented at 3 months to 3 years of age with severe developmental delay, absent or delayed milestones, and had seizures starting at 3 months to 5 years of age. Seizures were typically myoclonic or generalized tonic clonic and occurred daily to monthly. Electroencephalogram for most individuals showed generalized epileptiform activity. Neurological findings included hypotonia of the trunk and hypertonia of the extremities, with alterations in deep tendon reflexes, features that are typical in severe LIS. None of the individuals were able to walk or had any language skills. Standard metabolic testing, visual evoked potentials, evaluation for dysmorphology and review of organ systems were unremarkable in all tested individuals. Thus, clinical features do not distinguish *APC2-*LIS from the reported spectrum of typical severe LIS.

In 5 of the individuals, high-resolution brain MRIs were available for review (Figure 2), which demonstrated features not common in other causes of LIS. A P>A gradient was evident in all, which has been reported with *LIS1, TUBG1, ARX, DYNC1H1*, and can also be seen in association with *TUBA1A, TUBB2B, KIF5C and KIF2A*.5 In addition, ventriculomegaly with stretched and thinned corpus callosum and an unusual posterior subcortical heterotopia just posterior to the caudate nuclei were noted in several individuals. In some individuals the subcortical heterotopia appeared to merge with the deep cellular layer of the posterior agyria, with almost no white matter visible. One child (6-III-2) had an undulating, ribbon-like deep cellular layer (Figure 2L) that began in the mid-frontal lobe and continued posteriorly to the parietal lobe, very different from the subcortical band heterotopia (MIM 300067) seen with *DCX* mutations. The ribbon shaped subcortical heterotopia of subject 6-III-2 was reminiscent of the ribbon-like heterotopia observed with *EML1* mutations (MIM 600348).7 However, *EML1* mutations are also associated with hydrocephalus, agenesis of the corpus callosum and diffuse polymicrogyria. Additionally, in *CENPJ* mutation (MIM 608393), a more discrete thin festooned heterotopia in the areas lateral and adjacent to the striatum has been seen (W.B.D. personal observation), and thin subcortical heterotopia band in the upper frontal area and parallel to the lateral ventricles is reported in *GPSM2* mutations causing Chudley-McCullough syndrome (MIM 604213).8 However, the pattern of subcortical heterotopia seen in these conditions is different from that of *APC2.* In addition, all subjects showed hippocampal defects (Figure S1) and striking dysplastic in-folding of the mesial occipital cortex, not seen in other forms of LIS (Figure 2; examples can be seen in Figures 2B, 2F, 2J, 2N, and 2R). In fact, the affected individual from family 8 was diagnosed clinically first through recognizable brain MRI findings (W.B.D.). Thus, we believe that the images can distinguish *APC2*-LIS from other forms of disease.

The Adenomatous Polyposis Coli gene family (not to be confused with the multisubunit Anaphase Promoting Complex) consists of two paralogues conserved to *Drosophila*, *APC* (MIM 611730) and *APC2*. *APC* was first identified as a human colon cancer tumor suppressor, associated with both sporadic and inherited forms of the disease,9 and APC functions as a negative regulator of Wnt signaling and in the organization and regulation of the actin and microtubule cytoskeletons.10 APC2 (also called APCL) is highly similar to APC in its N-terminal Armadillo-repeat containing half, but shares little sequence similarity to its C-terminal half. APC2 is not mutated in colon cancer, binds less efficiently to β-catenin than APC, and has not been implicated in Wnt signaling.11,12 APC2 localizes to actin and microtubule fibers, and *Apc2-/-* mice show disrupted neuronal migration leading to defects in lamination of the cerebral cortex and cerebellum,13 supporting *APC2* as a LIS candidate gene.

*APC2* is encoded on human chromosome 19, consisting of 15 coding exons and a 10.1 kb coding mRNA. Three alternative splice isoforms are described but the major isoform encodes a 2,303 amino acid protein. We identified truncating mutations in 4 of these exons, with most occurring in the largest and last exon 15 (Figure 1C). The four truncating mutations identified in the last exon were predicted to lead to a stable mRNA and potentially a C-terminally truncated protein, whereas mutations in earlier exons were predicted to lead to nonsense mediated decay and LoF. We considered the possibility that late truncating mutations might have a milder phenotype than early truncating mutations, but found no evidence of milder clinical or radiographic phenotypes. The locations of the truncating mutations occurred throughout the open reading frame (Figure 1D), and the lack of correlation of the location with severity of imaging phenotype suggested that most or all of these mutations are LoF. The variants were unique in our dataset of >5,000 exomes from individuals with neurodevelopmental phenotypes, were not represented in the Greater Middle Eastern Variome, 1000 Genomes, or gnomAD databases (Table S1). All variants were confirmed by Sanger sequencing and segregated according to a recessive mode of inheritance.

*APC2* has pLI value of 1.0 in gnomAD suggesting haploinsufficiency intolerance. However, heterozygous carriers in this study did not have any noticeable phenotype. Constraint metrics such as pLI, or the more recently introduced o/e ratio, represent a spectrum of tolerance to inactivation.14 Although pLI is generally accepted as an indicator of LoF intolerance, not all genes with high pLI score cause disease even if they have heterozygous LoF variants. Thus, pLI of 1.0 of *APC2* does not necessarily mean that heterozygous LoF variants of *APC2* cause haploinsufficiency or disease. In fact, 27 heterozygous high confident LoF variants of *APC2* were found in gnomAD in apparently healthy individuals. This means that heterozygous LoF variants of *APC2* are probably not sufficient to produce disease, may produce disease in specific genetic backgrounds, or may be subject to purifying selection on the population rather than the individual level.15 Therefore, heterozygous carriers in our study may have an unnoticeable phenotype, although they were not examined by brain MRI.

A recent publication identified a late truncating homozygous single base duplication (p.Lys1734Glnfs\*419; Figures 1C-1D) in exon 15 of *APC2,* in two affected children from a consanguineous marriage, displaying Sotos-like features but without noted brain malformation (MIM 617169).16 Sotos syndrome is a form of cerebral gigantism associated with intellectual disability and macrocephaly (MIM 117550). These children showed developmental delay and macrocephaly and brain MRIs showed only dilated brain ventricles. We reviewed the brain MRIs in the published paper and found no evidence of LIS. The reported variant was predicted to lead to replacement of the C-terminal 570 amino acid with 418 aberrant residues. Despite the fact that all of our affected individuals had biallelic truncating mutations throughout the protein, none of our subjects showed macrocephaly or Sotos-like features. This leaves the open question as to why this reported homozygous frameshift variant did not produce LIS. Possibilities include: 1] that the variant did not fully inactivate the protein, 2] that it produced a novel function, or 3] that it is an allele-specific association.17 Future work is needed to determine the full phenotypic spectrum associated with *APC2* mutations, as additional individuals and alleles are identified.

The role of APC2 in LIS remains to be established, but the phenotypes we report together with APC2’s published localization and binding partners support functional interaction with other LIS-related proteins. In migratory neurons, APC2 partially co-localizes with microtubules and F-actin at the leading edge of the growth cone.13 In *Apc2-/-* neurons, BDNF stimulation fails to increase the amount of F-actin at the leading edge, or effectively stabilize microtubules.

We recently reported homozygous *CTNNA2* mutations in LIS, and like APC2, the *CTNNA2* encoded protein (α-N-catenin), can interact with both β-catenin and actin. In *CTNNA2*-related LIS, defects in Wnt signaling were excluded; instead α-N-catenin competed with the Arp2/3 complex to suppress actin branching,18 leading to more stable leading neurites. Another recent report linked APC to cytoplasmic dynein through the cofactor AMER (APC-membrane recruitment) family of membrane-bound proteins.19 Furthermore, APC has been reported to have an important role in regulation of radial glial polarity and interneuron migration by modulating microtubule severing and thus to be essential for cortex development.20,21 APC2 may similarly serve as a microtubule regulator or form a complex with α-N-catenin or dynein, to mediate neurite stability or ‘minus-end’ directed dynein forces required during migration, although further experimental studies should follow to support these speculations, which are based upon APC. Interestingly, a genetic interaction between *Lis1* and *Apc* in murine neuronal migration was reported, but investigations of *Apc2* were not performed.22 Finally, in postmitotic neurons, APC2 controls dendritic development by promoting microtubule dynamics through two separate microtubule binding domains.23 It is possible that these domains function during neuronal migration to mediate leading-process organization.   
 In summary, we implicate *APC2* in a recessive form of P>A LIS, clinically characterized by severe intellectual disability, epilepsy and neuromotor involvement, and radiographically by stretched and thinned corpus callosum, subcortical thin and sometimes ribbon-shaped heterotopia in posterior perisylvian areas and dysplastic in-folding of gyri in the mesial occipital cortex. There are likely a range of developmental brain phenotypes resulting from loss of *APC2*, although our subjects are likely to be at the most severe end of the spectrum given the nature of the alleles. Future studies will be required to elucidate the full range of phenotypes, genotype-phenotype correlations, and mechanisms of pathogenicity.

**Description of Supplemental Data.** Supplemental Data include materials and methods, one figure and one table.

**Declaration of Interests.** The authors declare no competing interests.

**Acknowledgements.**  We thank the children and their families for their contributions to this study. This work was supported by NIH U01 MH108898, R01NS048453, R01 NS098004, the Simons Foundation Autism Research Initiative (SFARI), the Howard Hughes Medical Institute (J.G.G.), QNRF 6-1463 (J.G.G.) and the March of Dimes (M.C.M.). G.M.S.M. is supported by the ZonMW Top grant # 91217045. N.D.D., U.A., W.B.D., G.M.M., G.M.S.M. and M.S.Z. are members of the European Network on Brain Malformations (Neuro-MIG, COST Action CA16118). T.K. was supported by COST Action CA16118 (STSM grant # 41344). G.M. is supported by NIH K08NS092898 and Jordan’s Guardian Angels. One family was collected as part of the SYNaPS Study Group collaboration funded by The Wellcome Trust and strategic award (Synaptopathies) funding (WT093205 MA and WT104033AIA). This research was conducted as part of the Queen Square Genomics group at University College London, supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. We thank the Rady Children’s Institute for Genomic Medicine, Broad Institute (U54HG003067 to E. Lander and UM1HG008900 to D. MacArthur), the Yale Center for Mendelian Disorders (U54HG006504 to R. Lifton and Murat Gunel) for sequencing support, and the Matchmaker Exchange. We acknowledge M. Gerstein, S. Mane, A. B. Ekici, S. Uebe, E. S. Cauley, and UCSD IGM Genetics Center for sequencing support and analysis, the Yale Biomedical High-Performance Computing Center for data analysis and storage, the Yale Program on Neurogenetics, and the Yale Center for Human Genetics.

**Web resources**

OMIM <http://www.omim.org/>

1000 Genomes http://www.1000genomes.org/

GME http://igm.ucsd.edu/gme/

dbSNP http://www.ncbi.nlm.nih.gov/SNP/

GenBank <http://www.ncbi.nlm.nih.gov/genbank/>

gnomAD https://gnomad.broadinstitute.org/

UniProt <http://www.uniprot.org/uniprot/>

Matchmaker Exchange https://www.matchmakerexchange.org/

VEP

[https://www.ensembl.org/vep](https://www.ensembl.org/vep )

*APC2* GenBank ID: uc002lsr.1 NM\_005883.2

**References**

1. Guerrini, R., Dobyns, W.B., and Barkovich, A.J. (2008). Abnormal development of the human cerebral cortex: genetics, functional consequences and treatment options. Trends Neurosci 31, 154-162.

2. Parrini, E., Conti, V., Dobyns, W.B., and Guerrini, R. (2016). Genetic Basis of Brain Malformations. Mol Syndromol 7, 220-233.

3. Bahi-Buisson, N., and Cavallin, M. (1993). Tubulinopathies Overview. In GeneReviews((R)), M.P. Adam, H.H. Ardinger, R.A. Pagon, S.E. Wallace, L.J.H. Bean, K. Stephens, andA. Amemiya, eds. (Seattle (WA).

4. Di Donato, N., Kuechler, A., Vergano, S., Heinritz, W., Bodurtha, J., Merchant, S.R., Breningstall, G., Ladda, R., Sell, S., Altmuller, J., et al. (2016). Update on the ACTG1-associated Baraitser-Winter cerebrofrontofacial syndrome. Am J Med Genet A 170, 2644-2651.

5. Di Donato, N., Timms, A.E., Aldinger, K.A., Mirzaa, G.M., Bennett, J.T., Collins, S., Olds, C., Mei, D., Chiari, S., Carvill, G., et al. (2018). Analysis of 17 genes detects mutations in 81% of 811 patients with lissencephaly. Genet Med 20, 1354-1364.

6. van Es, J.H., Kirkpatrick, C., van de Wetering, M., Molenaar, M., Miles, A., Kuipers, J., Destree, O., Peifer, M., and Clevers, H. (1999). Identification of APC2, a homologue of the adenomatous polyposis coli tumour suppressor. Curr Biol 9, 105-108.

7. Kielar, M., Tuy, F.P., Bizzotto, S., Lebrand, C., de Juan Romero, C., Poirier, K., Oegema, R., Mancini, G.M., Bahi-Buisson, N., Olaso, R., et al. (2014). Mutations in Eml1 lead to ectopic progenitors and neuronal heterotopia in mouse and human. Nat Neurosci 17, 923-933.

8. Kau, T., Veraguth, D., Schiegl, H., Scheer, I., and Boltshauser, E. (2012). Chudley-McCullough syndrome: case report and review of the neuroimaging spectrum. Neuropediatrics 43, 44-47.

9. Groden, J., Thliveris, A., Samowitz, W., Carlson, M., Gelbert, L., Albertsen, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., et al. (1991). Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66, 589-600.

10. Aoki, K., and Taketo, M.M. (2007). Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. J Cell Sci 120, 3327-3335.

11. Schneikert, J., Vijaya Chandra, S.H., Ruppert, J.G., Ray, S., Wenzel, E.M., and Behrens, J. (2013). Functional comparison of human adenomatous polyposis coli (APC) and APC-like in targeting beta-catenin for degradation. PLoS One 8, e68072.

12. Zhou, M.N., Kunttas-Tatli, E., Zimmerman, S., Zhouzheng, F., and McCartney, B.M. (2011). Cortical localization of APC2 plays a role in actin organization but not in Wnt signaling in Drosophila. J Cell Sci 124, 1589-1600.

13. Shintani, T., Takeuchi, Y., Fujikawa, A., and Noda, M. (2012). Directional neuronal migration is impaired in mice lacking adenomatous polyposis coli 2. J Neurosci 32, 6468-6484.

14. Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alföldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., and Birnbaum, D.P. (2019). Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. BioRxiv, 531210.

15. Cassa, C.A., Weghorn, D., Balick, D.J., Jordan, D.M., Nusinow, D., Samocha, K.E., O'Donnell-Luria, A., MacArthur, D.G., Daly, M.J., Beier, D.R., et al. (2017). Estimating the selective effects of heterozygous protein-truncating variants from human exome data. Nat Genet 49, 806-810.

16. Almuriekhi, M., Shintani, T., Fahiminiya, S., Fujikawa, A., Kuboyama, K., Takeuchi, Y., Nawaz, Z., Nadaf, J., Kamel, H., Kitam, A.K., et al. (2015). Loss-of-Function Mutation in APC2 Causes Sotos Syndrome Features. Cell Rep 10, 1585-1598.

17. De Franco, E., Watson, R.A., Weninger, W.J., Wong, C.C., Flanagan, S.E., Caswell, R., Green, A., Tudor, C., Lelliott, C.J., Geyer, S.H., et al. (2019). A Specific CNOT1 Mutation Results in a Novel Syndrome of Pancreatic Agenesis and Holoprosencephaly through Impaired Pancreatic and Neurological Development. Am J Hum Genet 104, 985-989.

18. Schaffer, A.E., Breuss, M.W., Caglayan, A.O., Al-Sanaa, N., Al-Abdulwahed, H.Y., Kaymakcalan, H., Yilmaz, C., Zaki, M.S., Rosti, R.O., Copeland, B., et al. (2018). Biallelic loss of human CTNNA2, encoding alphaN-catenin, leads to ARP2/3 complex overactivity and disordered cortical neuronal migration. Nat Genet 50, 1093-1101.

19. Gao, F.J., Shi, L., Hines, T., Hebbar, S., Neufeld, K.L., and Smith, D.S. (2017). Insulin signaling regulates a functional interaction between adenomatous polyposis coli and cytoplasmic dynein. Mol Biol Cell 28, 587-599.

20. Yokota, Y., Kim, W.Y., Chen, Y., Wang, X., Stanco, A., Komuro, Y., Snider, W., and Anton, E.S. (2009). The adenomatous polyposis coli protein is an essential regulator of radial glial polarity and construction of the cerebral cortex. Neuron 61, 42-56.

21. Eom, T.Y., Stanco, A., Guo, J., Wilkins, G., Deslauriers, D., Yan, J., Monckton, C., Blair, J., Oon, E., Perez, A., et al. (2014). Differential regulation of microtubule severing by APC underlies distinct patterns of projection neuron and interneuron migration. Dev Cell 31, 677-689.

22. Hebbar, S., Guillotte, A.M., Mesngon, M.T., Zhou, Q., Wynshaw-Boris, A., and Smith, D.S. (2008). Genetic enhancement of the Lis1+/- phenotype by a heterozygous mutation in the adenomatous polyposis coli gene. Dev Neurosci 30, 157-170.

23. Kahn, O.I., Schatzle, P., van de Willige, D., Tas, R.P., Lindhout, F.W., Portegies, S., Kapitein, L.C., and Hoogenraad, C.C. (2018). APC2 controls dendrite development by promoting microtubule dynamics. Nat Commun 9, 2773.

**Figure Legends**

**Figure 1. *APC2* biallelicloss of function mutations in posterior-predominant (P>A) lissencephaly. (A)** Twelve affected individuals from eight families showed unique biallelic mutations in *APC2*. All families except Family 5 had documented parental consanguinity (double bars). Allele is listed below family number. Dot and question mark indicate heterozygous carriers and samples not tested, respectively. **(B)** Axial brain imaging in each family showed evidence of posterior-predominant lissencephaly. In Families 1-2 only brain CT was available, but for other families, brain MRI is shown. Scans showed more severe agyria in the posterior compared with the anterior cortex (arrows in first scan highlights posterior agyria). Scan from unaffected shown for comparison. T1-weighted image for 6-III-1 and 8-IV-1, T2-weighted images for families 4 and 5, and FLAIR images for families 3 and 7 and 6-III-2 are shown. **(C)** Gene organization of *APC2* with size bar 10 kb. *APC2* contains 15 exons, the first of which is non-coding. Mutations were scattered throughout the coding region of the protein, with 5 mutations present in large exon 15. Exon 15 also contains the homozygous c.5199dup Almuriekhi *et al.* mutation.16 **(D)** APC2 is a 2303aa multidomain scaffolding protein, containing an N-terminal coiled coil, Armadillo repeats, 20aa (FXVEXTPXCFSRXSSLSSLS) repeats and SAMP motifs. Affected individuals’ mutations were located throughout the open reading frame. The region of the protein encoded by the last exon is highlighted.

**Figure 2.** **Posterior-predominant (P>A) LIS with subcortical ribbon heterotopia associated with biallelic *APC2* mutations.** Individual identifier along left.Midline sagittal MRIs showed P>A LIS with stretched and thinned corpus callosum and relatively well-preserved anterior folding, brainstem and cerebellar architecture. Axial images all showed P>A LIS with mild (B-D, N-P, R-T) or moderate (F-H, J-L) frontal pachygyria, and posterior agyria (asterisks shown in 4th column only). 5-II-1: short comma-shaped subcortical heterotopias began just posterior to and at the same level as the tail of the caudate nuclei (between the red arrows in B-C). 7-III-3 and 7-III-4: same subcortical heterotopia began in the same place, but then merged with the deep cellular layer of the posterior agyria (thin red arrows in O, R-S). 6-III-1 and 6-III-2: (and in R) this same region appeared to have dysplastic cortex extending from the pial surface to the ventricular surface, with no white matter apparent (two-headed arrows in F-G, J-K and N). The ribbon heterotopia began at this level (two-headed arrow in K). All subjects showed striking dysplastic in-folding of one or several gyri in the mesial occipital region (thick blue or red arrows in all 5 images in 2nd column). The selected images include T1-weighted (A-D and M-T) and T2-weighted (E-L) images in midline sagittal (1st column) and multiple axial planes progressed from low to high slices (2nd to 4th columns).

**Figure 3. Schematic of major migrational defects in lissencephaly subtypes.** (A) Normal shows evenly spaced cortical gyri and sulci, with a thin mantle of grey matter. (B) P>A lissencephaly shows thickened cortical gray matter mantle in the neocortex (left) that is more severe in the posterior cortex (right). Ventriculomegaly also depicted (\*). (C) Double cortex shows a normally gyrated outer cortex with thin mantle, but additionally shows a band heterotopia in the subcortical white matter, evenly distributed anteriorly and posteriorly. (D) *APC2* lissencephaly shows P>A gradient with thickened cortical gray matter mantle posteriorly, and relatively preserved gyration anteriorly, with an abrupt transition (arrow). In the temporal region, the dysplastic cortex extends from the pia to the ventricle, resulting in reduce white matter (double arrow). Ribbon-like heterotopia is most noticeable in the perisylvian region and appears to connect with the tail of the caudate nuclei (red). In-folding of cortex in the mesial occipital region is often apparent (gray arrow).

**Table 1. Clinical features of individuals with *APC2* mutations.** Genomic position of allele is presented in hg19 reference. Abbreviations: cm: centimeter; F: female; M: male; HC: Head circumference; Kg: Kilograms; L: Left; MRI: Magnetic resonance imaging, NA: Not Available, R: Right; SD: Standard deviation; VEP/ERG: Visual evoked potential/electroretinogram.

**Table 1**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Family 1** | | **Family 2** | **Family 3** | | **Family 4** | **Family 5** | **Family 6** | | **Family 7** | | **Family 8** |
| Origin | Egypt | | Egypt | Egypt | | Iran | USA | Turkey | | Syria | | Egypt |
| **Variant** | | | | | | | | | | | | |
| Zygosity | Homozygous | | Homozygous | Homozygous | | Homozygous | Compound Heterozygous | Homozygous | | Homozygous | | Homozygous |
| Genomic (hg19) | chr19:g.1457116C>T | | chr19: 1465182C>T | chr19:g.1469945delC | | chr19:1462017\_1462018delCA | chr19:g.1457202del; g.1467547del | chr19:g.1456324C>A | | chr19:g.1466140\_1466146del | | chr19:g.1462177G>C |
| cDNA | c.1081C>T | | c.1882C>T | c.6645delC | | c.1694\_1695delCA | c.1167\_1180del; c.4247\_4259del | c.737C>A | | c.2840\_2846del | | c.1853+1G>C |
| Protein | p.Gln361\* | | p.Gln628\* | p.A2217fs\*118 | | p.T565Rfs | p.Q389fs; p.R1416fs | p.Ser246\* | | p.L947Hfs\*88 | | Splice donor |
| **Proband** | **1-III-1** | **1-III-2** | **2-III-1** | **3-III-1** | **3-III-3** | **4-III-1** | **5-II-1** | **6-III-1** | **6-III-2** | **7-III-3** | **7-III-4** | **8-IV-1** |
| Gender | M | M | M | M | F | F | M | F | M | M | F | F |
| Weight at birth (kg) | 3.2 (-0.5SD) | 3 (-1SD) | 3.5 (mean) | 3 (-1SD) | 3.2 (-0.3SD) | 3.9 (mean) | 10-25 centiles | 2.9 (-1.0SD) | 3 (-0.9SD) | ~ 2 | ~ 1 | NA (normal) |
| Length at birth (cm) | 50 (mean) | 49 (-0.2SD) | 49 (-0.2SD) | 50 mean | 48 (-0.2SD) | 50 (mean) | 10-25 centiles | 48 (-0.7SD) | 49 (-0.5SD) | NA | NA | NA (normal) |
| HC at birth (cm) | 35 (-0.5SD) | 34.5 (-0.8SD) | 34.5 (-0.8SD) | 35 (-0.5SD) | 34 (-0.8SD) | 37 (mean) | 90-95 centiles | 34 (-0.4SD) | 34 (-0.6SD) | NA | NA | NA (normal) |
| Age at last examination | 2y | 9m | 2y | 15y | 5y | 3y | 7.5y | 4y | 2y | 4y 7m | 6y | 7m |
| HC at last examination (cm) | 48.5 (-0.1SD) | 43.5 (-1.4SD) | 49 (mean) | 51 (-2.6SD) | 48.5 (-1.3SD) | 45 | NA | 47 (-2.1SD) | 44.5 (-3.1SD) | 47 | 47 | 90th centile |
| Diagnosis age | 3y | 3m | 2y | 2y | 6m | 3m | 19m | 18m | 8m | 6m | 4m | 7m |
| Intellectual Disability | Severe | Severe | Severe | Severe | Severe | Severe | Severe | Severe | Severe | Severe | Severe | Severe |
| **Psychomotor Development** | | | | | | | | | | | | |
| Gross motor | Delayed | Delayed | Delayed | Delayed | Delayed | Delayed | Delayed | Dealyed | Delayed | Delayed | Delayed | Delayed, no head control |
| Fine motor | Absent | Absent | Absent | Absent | Absent | Absent | Delayed | Delayed | Delayed | Delayed | Delayed | Delayed |
| Language | Delayed | Delayed | Delayed | Absent | Absent | Delayed | Delayed | Delayed | Delayed | Absent | Absent | NA |
| Social | Delayed | Delayed | Delayed | Delayed | Delayed | Delayed | Unknown | Delayed | Dealyed | Delayed | Delayed | Delayed |
| **Seizures** | **Y** | **Y** | **Y** | **Y** | **Y** | **N** | **N** | **Y** | **Y** | **Y** | **N** | **N** |
| Age of Onset | 5m | 3m | 4m | 6y | 4.5y | - | NA | 12m | 18m | 2y | - | - |
| Type | Generalized and myoclonic | Generalized and myoclonic | Myoclonic/infantile spasm | Generalized and myoclonic | Generalized and myoclonic | - | NA | Generalized and myoclonic | Generalized and myoclonic | Generalized and myoclonic | - | - |
| Frequency | Montly | Monthly | Daily | With fever | Daily | - | NA | Daily | Daily | Weekly | - | - |
| Controlled/Refractory | Fairly controlled | Fairly controlled | Refractory | Controlled | Refractory | - | NA | Refractory | Refractory | Controlled | - | - |
| EEG | Generalized epileptogenic activity involving midline structure | Generalized epileptogenic activity | Hypsarrhythmia | Bilateral tempro-pariental epileptogenic activity | Generalized epileptogenic activity | Normal | Normal | Generalized epileptogenic activity | Generalized epileptogenic activity | NA | NA | NA |
| **Neurological Findings** | | | | | | | | | | | | |
| Hypertonia | Y | Y | N | N | N | N | N | N | N | Y, peripheral | Y, peripheral | N |
| Hypotonia | N | N | Y | Y | Y | Y, truncal | Y | Y | Y | Y, central | Y, central | Y |
| Spastic tetraplegia | Y | Y | Tetraplegia but not spastic | N | N | Spastic dystonia | NA | N | N | Y | N | N |
| **Investigations** | | | | | | | | | | | | |
| Metabolic | Normal | Normal | Normal | Normal | Normal | Normal | NA | Normal | Normal | Normal | Normal | NA |
| VEP/ERG | Normal | Normal | Normal | Normal | Normal | Normal | NA | NA | NA | NA | NA | NA |
| **Neuroimaging** | CT | CT | CT | MRI | MRI | MRI | MRI | MRI | MRI | MRI | MRI | MRI |
| P>A Lissencephaly | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |
| Cerebral mantle thickening | >1cm | >1cm | Unknown | >1cm | >1cm | 8-10mm | NA | Y | Y | Y | Y | >1cm |
| Ribbon heterotopia | NA | NA | NA | N | N | N | Y | Y | Y | Y | Y | N |
| Corpus callosum hypogenesis | Y | Y | Y | Y | N | Y | Y | Y | Y | Y | Y | Y |
| Cerebellar hypoplasia | N | N | N | N | N | Very mild | N | Very mild | N | N | N | N |
| Brainstem hypoplasia | N | N | N | N | N | N | N | Very mild | Y | Small pons | Small pons | N |
| Ventriculomegly | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y mild |
| White matter paucity | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y | Y |