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ARTICLE

Homozygous loss of *DIAPH1* is a novel cause of microcephaly in humans

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The combination of family-based linkage analysis with high-throughput sequencing is a powerful approach to identifying rare genetic variants that contribute to genetically heterogeneous syndromes. Using parametric multipoint linkage analysis and whole exome sequencing, we have identified a gene responsible for microcephaly (MCP), severe visual impairment, intellectual disability, and short stature through the mapping of a homozygous nonsense alteration in a multiply-affected consanguineous family. This gene, *DIAPH1*, encodes the mammalian Diaphanous-related formin (mDia1), a member of the diaphanous-related formin family of Rho effector proteins. Upon the activation of GTP-bound Rho, mDia1 generates linear actin filaments in the maintenance of polarity during adhesion, migration, and division in immune cells and neuroepithelial cells, and in driving tangential migration of cortical interneurons in the rodent. Here, we show that patients with a homozygous nonsense *DIAPH1* alteration (p.Gln778*) have MCP as well as reduced height and weight. *diap1* (mDia1 knockout (KO))-deficient mice have grossly normal body and brain size. However, our histological analysis of *diap1* KO mouse coronal brain sections at early and postnatal stages shows unilateral ventricular enlargement, indicating that this mutant mouse shows both important similarities as well as differences with human pathology. We also found that mDia1 protein is expressed in human neuronal precursor cells during mitotic cell division and has a major impact in the regulation of spindle formation and cell division.
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INTRODUCTION

Microcephaly (MCP) is a neurodevelopmental disorder characterized by a small cranium with a significantly reduced occipito-frontal head circumference. The reduced brain volume is particularly evident within the cerebral cortex and is often associated with some degree of intellectual disability (ID). MCP is categorized as either primary (MCP vera) or syndromic, indicating the presence of other physical or neurological deficits or gross structural brain abnormalities. Mendelian forms of MCP have been identified, representing both dominant and recessive forms of inheritance.[1–3] In the present study, we report a consanguineous Saudi Arabian pedigree with a novel syndromic form of MCP, involving small head size, ID, seizures, short stature, and blindness. Genome-wide linkage analysis identified a maximum LOD score of 3.7 under a recessive model of inheritance mapping to chromosome *5q31.3*. Whole-exome sequencing (WES) and targeted sequencing analyses identified a rare, nonsense, homozygous sequence variant c.2332C>T (p.Gln778*, RefSeq NM_005219.4) in the gene *DIAPH1* (mouse symbol is *Diap1*) (MIM 602121) encoding the mammalian diaphanous-related formin, mDia1. The encoded protein has previously been implicated in neuronal migration of cortical interneurons,[4] autosomal hearing loss,[5] and myelodysplasia.[6] Depending upon the cell type and position in the cell cycle, mDia1 has been shown to localize to the cell cortex, trafficking endosomes, cleavage furrow, mid-bodies, and centrosomes, the cytoplasmic microtubule-organizing center crucial for cell division.[7] These properties are shared with several other primary microcephaly genes, including *WDR62* (*Homo sapiens* WD repeat domain 62),[2,8,9] *CENPJ* (centromere protein J),[10] *CEP152* (centrosomal protein 152 kDa),[11] *ASPM* (abnormal spindle-like MCP-associated (*Drosophila*)),[12] and *CEP135* (centrosomal protein 135 kDa).[13] Interestingly, analysis of *Diap1*-deficient mice revealed cerebral ventricular enlargement but not the reduction in brain volume and blindness observed in the human phenotype. Thus, *DIAPH1* has a crucial role in brain development and exhibits species differences in its function.

MATERIALS AND METHODS

The study protocol was approved by the Yale Human Investigation Committee (HIC). Approval from the institutional review board for genetic studies and

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informed written consent from the family were obtained by the referring physician in the Alhada Military Hospital, Taif, Saudi Arabia. Nucleotide numbers are in reference to cDNA (NM_005219.4 where A of the ATG translational start site is designated as +1) and amino acid number is in reference to protein (O60610) coordinates.

Neuroimaging

Magnetic resonance images (MRIs) containing axial, sagittal, and coronal TI- and T2-weighted images were obtained from patient IV:7 in this pedigree. The corpus callosum, posterior fossa structures (the cerebellum, midbrain, pons, and medulla), deep gray matter, degree of myelination, and presence of any associated malformations were assessed on each MRI.

Genotyping, targeted sequencing, and WES

Genomic DNA derived from whole blood was genotyped using the 1Mv1 Duo Bead array chips (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. SNPs showing Mendelian inconsistency in transmission were removed[14] and data were pruned to 42 703 SNPs that are in minimal linkage equilibrium with each other using linkage disequilibrium (LD)-based SNP pruning with the following criteria: pairwise correlation coefficient $r^2 < 0.23$ within a 50-SNP window; proportion of missing genotypes <5%, relative minor allele frequency >5%, and Hardy–Weinberg equilibrium 0.0001 among the controls and cases. Family relatedness was ascertained through estimation of identity by descent. The LOD score was calculated with pruned SNPs using Allegro 1.2 (DeCode genetics Inc., Reykjavik, Iceland).

DNA from both patient IV:4 and the unaffected father were sequenced using a custom-designed NimbleGen Sequence Capture 385K array (>60 bp probes to sequence the target linkage region; Roche Nimblegen, Inc., Madison, WI, USA) and later data from patient IV:4 using WES according to the manufacturer's protocol. WES was conducted as previously described.[2] The sequence reads were mapped to the human genome (NCBI/hg19). Variants were confirmed by Sanger sequencing. *DIAPH1* sequence alteration and other variations (Supplementary Tables 4 and 6) found in patient IV:4 were submitted to the LOVD public database (http://databases.lovd.nl/shared/individuals/00004216).

DIAPH1 screening in independent cases and control groups

All 28 exons comprising *DIAPH1* were evaluated in the following individuals: (1) 62 Turkish and Middle Eastern subjects found to have regions of homozygosity corresponding to chromosome 5q31, presenting one or more of the following conditions: MCP, lissencephaly, polymicrogyria, cerebellar hypoplasia, schizencephaly, corpus callosum hypogenesis, demyelination, psychosomatic motor delay, ID, epilepsy, and autism; (2) 136 Turkish probands, including 120 from consanguineous and 16 from non-consanguineous families presenting with MCP; and (3) 100 neurologically normal control individuals from Saudi Arabia. PCR primer sequences and conditions are available upon request.

Quantitative PCR and western blot analysis

DIAPH1 mRNA expression in Epstein–Barr virus (EBV)-transformed lymphoblastoid cells (LCLs) available from four affected individuals and both parents was assessed by real-time-PCR (RT-PCR) using *DIAPH1* and the reference gene *TBP* (TATA box-binding protein) primers with the PCR efficiency of >90% (slope = −3.2 and −3.6) and R^2 99%. Relative changes in gene expression were analyzed with the ΔCT method.[15] Cell lysates from LCLs of four affected individuals, parents, and an independent control were used for western blot analysis. We used primary rabbit anti-DIAPH1 antibodies from N-terminus (1:10 000, Abcam-ab11172, Cambridge, MA, USA) and C-terminus (1:5000, Bethyl Labs-A300–078, Montgomery, TX, USA). Anti-GAPDH (1:200) was used as the loading control.

Cellular localization of mDia1

ReNcell CX cells from the immortalized fetal human cortical neural progenitor cell line (Millipore-SCC007, Billerica, MA, USA) were fixed in 4% PFA and blocked by blocking solution (5% normal donkey serum, 1% BSA, 0.1% glycine, 0.1% lysine, and 0.3% Triton X-100 in PBS). Fixed cells were incubated with mouse anti-mDia1 (1:50, Santa Cruz-sc-373807, Dallas, TX, USA) and rabbit anti-pericentrin (1:500, Covance-PRB-432C, Princeton, NJ, USA). Alexa Fluor 488 Donkey Anti-Mouse IgG and Alexa Fluor 594 Donkey Anti-Rabbit IgG were used as secondary antibodies (Invitrogen, Grand Island, NY, USA). DNA was visualized with DAPI. Cells were imaged with an Aperio microscope (Aperio Technologies, Vista, CA, USA).

Human and mouse brain specimens

The study protocol was approved by the HIC. All human tissues were collected under guidelines approved by the Yale HIC and were part of the tissue collection at the Department of Neurobiology. For each tissue donation, appropriate written, informed consent and approval were obtained. All experiments with mice were carried out in accordance with protocols approved by the Institutional Animal Care and Use Committee at Yale University, School of Medicine. *diap1*-knockout mice are a gift from A S Alberts (Van Andel Institute, MI, USA). The genotype of the *diap1*-deficient allele was analyzed by PCR using the following primers: Wt F-5′-TAGATAGGGATAAAGTTG GTGGGCTGTG-3′, Wt R-5′-GAGATGTCCTGCAATGACTGAGCAGTGG-3′, and Mut R-5′-GCATCACCTTCACCCTCTCCACTGACAG-3'. The morning of a detectable vaginal plug and the first neonatal day were considered to be embryonic day 0.5 (E0.5) and postnatal day 0 (P0), respectively.

In situ hybridization

For embryonic stages, pregnant females were anesthetized, and pups at E12.5, E14.5, and E17.5 stages were extracted from the uterus. Embryonic brains were dissected and fixed by immersion in 4% PFA. At all postnatal stages (P3, P7, P14), mice were anesthetized with injectable anesthetics and intracardially perfused with 4% PFA. Brains were postfixed overnight in 30% sucrose/4% PFA, and sectioned in the coronal plane on a Leica sledge cryomicrotome at 36 μm. Sections were processed for nonradioactive *in situ* hybridization. The RNA probes complementary to mouse *diap1* (bases 3062–3861 of the mouse *diap1* cDNA, NM_007858.2) and human *DIAPH1* (bases 2241–3960 of the human *DIAPH1* cDNA, NM_005219.4) were labeled with digoxigenin-11-UTP. Sections were analyzed with a Zeiss Stemi dissecting microscope or a Zeiss AxioImager with an AxioCam MRc5 digital camera (Zeiss, Oberkochen, Germany).

Immunostaining

For immunostaining, fixed E14.5 and P0 brain tissues were embedded in 4% agarose and sectioned at 50 μm with a Vibratome VT1000S (Leica, Wetzlar, Germany). Tissue sections were incubated in primary antibodies (Supplementary Table 5) and with appropriate donkey Alexa Fluor-conjugated secondary antibodies (1:1000, Invitrogen). Nissl staining was performed using the standard method.

Gene co-expression network

To identify any possible correlation of the *DIAPH1* gene with MCP genes (*MCPH1* (MIM 607117), *WDR62* (MIM 613583), *CDK5RAP2* (MIM 608201), *ASPM* (MIM 605481), *CENPJ* (MIM 609279), *STIL* (MIM 181590), and *CEP135* (MIM 611423)), we performed co-expression analyses using the previously generated human brain transcriptome data set.[16] For each MCP gene, we used Pearson's correlation analysis with other genes, and then chose its top 100 highly correlated genes. Cytoscape software[17] was used to visualize the co-expression network, in which genes are shown by circles and the correlated genes are connected by lines. The 'un-weighted force-directed layout' parameter was used to optimize the network visualization. The human brain data set is generated by exon arrays and is available from the Human Brain Transcriptome database and the NCBI Gene Expression Omnibus under accession number GSE25219. It covers 16 brain regions over 15 periods, ranging in stage from embryonic development to late adulthood.[16] To test the significance of overlapping eight genes between *DIAPH1* and *CDK5RAP2*, permutation analysis was performed. Briefly, we randomly picked two groups with 100 genes in the whole human gene list. We set up the threshold that the number of genes shared by these two groups should be larger than eight. We repeated this operation 10 000 000 times and

counted the number of operations that passed the preset threshold. The *P* value was estimated by the ratio of successful operations.

RESULTS

A new MCP syndrome with temporal pole atrophy, callossal hypotrophy, seizure, and blindness

We ascertained a consanguineous family from Saudi Arabia (Pedigree SAR1008) including five (three female and two male) children that came to clinical attention initially at ~3 months of age because of seizures and were found to have severe MCP (head circumferences more than 3 SD below the mean for age) (Figure 1a and Supplementary Data).

The mother had a history of a single second-trimester miscarriage (IV:1) prior to delivering her first-born child (IV:2). It is not known whether the fetus had MCP. The second offspring (IV:3) was full term, but died at 3 months of age, despite hospitalization in a NICU, because of congenital heart disease. In addition, patient IV:5 recently died at the age of 18 as a result of chest infection.

Clinical examinations of the four oldest affected individuals showed moderate to severe ID, delays in both motor and language development, and severe bilateral visual impairment. Their hearing was grossly normal bilaterally, as was audiological examination. The youngest affected individual, patient IV:8, was assessed at 3 months and 3 weeks of age. He was found to be microcephalic (head circumference less than 3rd percentile); his weight was 5 kg (3rd percentile), and he suffered from seizures and bilateral visual impairment. Additional details of the medical history are presented in the Supplementary data.

T1- and T2 weighted, non-contrast, 1.5T MR imaging revealed that the brain of affected individual IV-7 exhibited a substantial reduction in the size of the cerebral cortex without obvious evidence of abnormal neuronal migration or grossly abnormal architecture

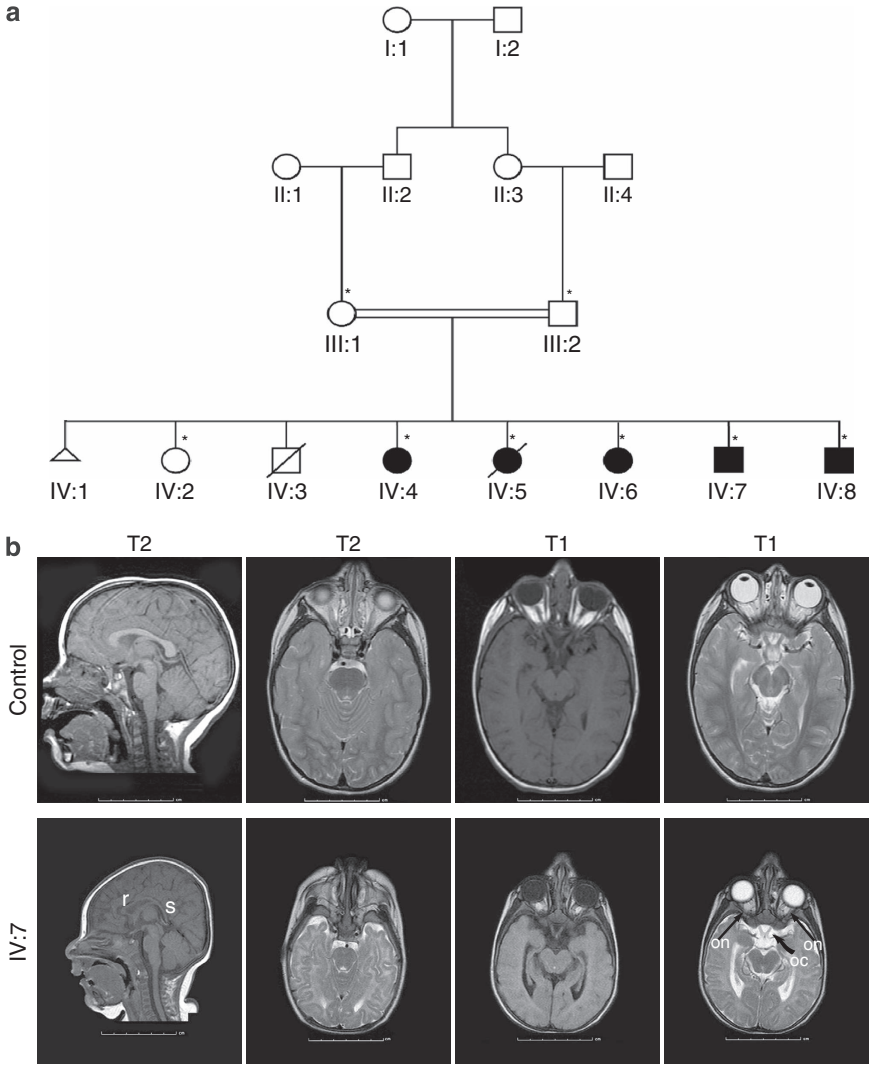

Figure 1 The pedigree structure and radiographic features of the SAR1008 family. (**a**) The patients described in this study are numbered. Parents are first cousins and have three affected female (IV:4, IV:5, IV:6), two affected male (IV:7, IV:8) and one unaffected female (IV:2) children. Squares and circles indicate males and females, respectively. Affected individuals are shown as filled symbols. One pregnancy that resulted in miscarriage is shown as a triangle. A diagonal line through a symbol denotes that the individual is deceased. The individuals from whom DNAs were obtained are indicated by *. (**b**) Representative MRIs of individual IV:7 at 4 months of age and control subject at 3 years of age. Axial T2 and T1 at the level of the temporal lobe, T2 and T1 at the level of the chiasm, and T1 sagittal images are shown (left to right). The MRI reveals temporal pole atrophy with normal cortical thickness. The optic nerves (on) and chiasm (oc) appear normal in size (indicated by arrows). Midline sagittal T1 shows hypoplasia of the rostrum (r) and splenium (s) of the corpus callosum. The scale bars are in cm.

(MCP vera). He also had volume loss of the anterior end of the temporal lobe (the temporal pole) with normal cortical thickness suggesting atrophy rather than hypoplasia. There was also loss of volume of the rostrum and splenium of the corpus callosum on midline sagittal T1-weighted images. The optic nerves and chiasm, cerebral aqueduct, and fourth ventricle were normal on midline sagittal T1 (Figure 1b). The subject's small weight and height indicated that body size was also affected.

A homozygous nonsense sequence variant in *DAPH1* causes MCP

We performed whole-genome genotyping of all members of the pedigree SAR1008 and confirmed the reported parental relationship of first cousin (found pi-hat = 0.15, consistent with a first-cousin union) (Supplementary Table 1). We then performed parametric linkage analysis using both affected and unaffected individuals and specifying a recessive mode of inheritance, penetrance of 99%, a phenocopy rate of 0.01, and a disease allele frequency of 0.0001 using a pruned set of 42 703 SNPs selected on the basis of LD information derived from the Saudi Arabian and Middle Eastern populations.[14] We identified a single linkage peak on chromosome 5q31.3 reaching the maximal theoretical LOD score of 3.7 under the specified parameters. This locus is flanked by SNPs *rs2907308* and *rs164078* and constitutes an approximately 724 kb region containing 38 annotated genes (Figure 2a and Supplementary Table 2). No other region of the genome was found to reach genome-wide significance (Supplementary Figure 1).

Next, in order to identify the disease-causing sequence variant, using the gDNA from proband IV:4, we performed WES as well as targeted sequencing of the LOD-2 confidence interval (138.8–160 cM), achieving a very high level of coverage (Supplementary Table 3). For targeted sequencing, we also used the gDNA from the father, focusing on the LOD-2 confidence interval (138.8–160 cM), using a custom-designed NimbleGen Sequence Capture 385K array based on the Illumina Genome Analyzer IIx platform.

Within the targeted linkage interval, we identified only a single homozygous, nonsense sequence variant affecting the *DIAPH1* gene. No other novel homozygous coding insertion or deletion sequence variants were observed within this interval. We identified a total of 15 homozygous missense variants that were reported previously at dbSNP with their minor allele frequencies between 0.086 and 47.2% (Supplementary Table 4). The nonsense sequence variant in *DIAPH1*, c.2332C>T (r.2473c>u), was predicted to terminate translation at amino acid 778 of 1272 (p.Gln778*, Q778*), leading to a truncated 86 kDa protein lacking the carboxyl-terminus bearing

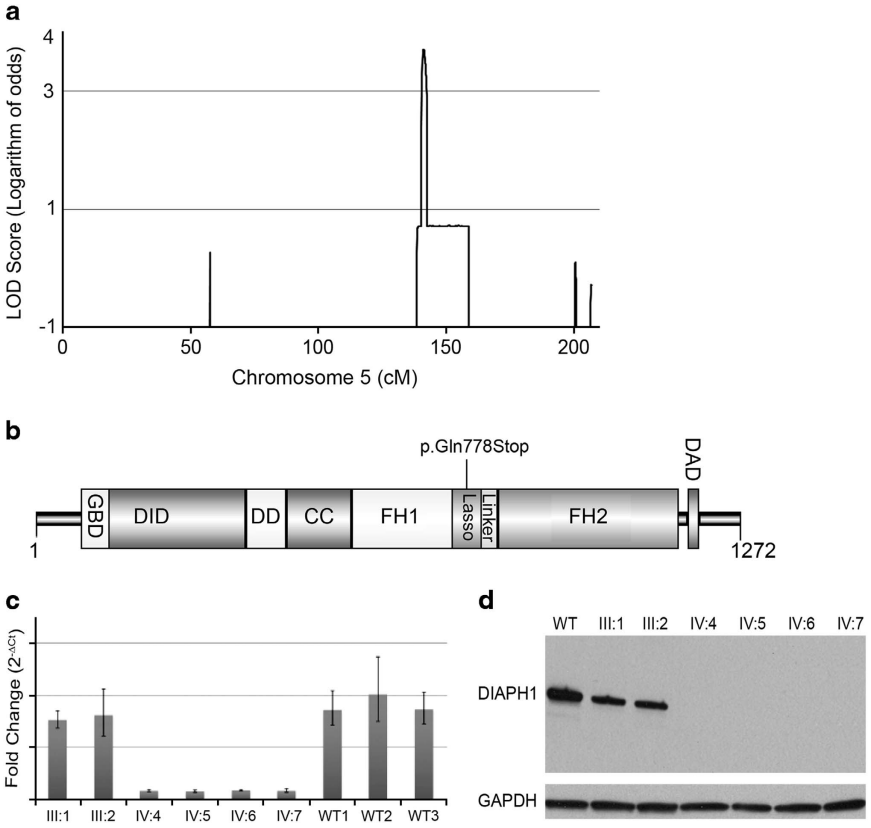

Figure 2 Identification of a nonsense homozygous sequence variant in the *DIAPH1* gene in a family with MCP. (**a**) Panel shows the results of the parametric linkage analysis for chromosome 5q, expressed as LOD scores. The maximum theoretical LOD score for this family is 3.7 using the genotyping data from IMv1 Duo Bead array chips. The analysis is modeled under the recessive mode of inheritance, penetrance of 99%, a phenocopy rate of 0.01, and a disease allele frequency of 0.0001. (**b**) Domain organization of mDia1. Abbreviations: GBD, GTPase binding region; DID, diaphanous inhibitory domain; DD, dimerization domain; CC, coiled coil; FH1, formin homology 1 domain; FH2, formin homology 2 domain; DAD, diaphanous autoinhibitory domain. Nonsense sequence variant p.Gln778* results in a 86.2 kDa truncated protein. (**c**) mRNA levels of *DIAPH1* gene in EBV transformed LCLs from homozygous patients (IV:4, IV:5, IV:6, IV:7), and heterozygous parents (III:1 and III:2) were analyzed using RT-PCR. Three individuals of the same ethnicity and without a sequence variant are used as control (WT). (**d**) Protein blot for mDia1. Lane 1 represents the control subject, lanes 2 and 3 represent the unaffected parents who carry the heterozygous p.Gln778* alteration (III:1 and III:2). Lanes 4, 5, 6, and 7 represent affected individuals with homozygous p.Gln778* alteration (IV:4, IV:5, IV:6, IV:7). The band indicates a molecular weight of ~140 kDa. GAPDH is used as control (lower band).

the highly conserved formin homology (FH2) domain that is responsible for mDia1's ability to generate linear actin filaments and affect microtubule dynamics (Figure 2b and Supplementary Figure 2A and B). The presence of the heterozygous sequence variant in both parents was confirmed via Sanger sequencing. Further analysis of WES data did not identify any other rare, segregating LoF (loss-of-function) homozygous sequence variants (Supplementary Table 6) either within or outside the linkage interval.

We anticipated that the mutant allele would lead to nonsense-mediated decay and evaluated the expression via qRT-PCR in the index family. We found a four-fold decrease in *DIAPH1* expression in probands relative to their parental controls (Figure 2c). Subsequent western blot analysis showed that all affected subjects lacked the predicted 140 kDa mDia1 protein. The heterozygous parents showed protein expression levels approximately one-half of that observed in an unrelated control (Figure 2d).

Further Sanger sequencing of the complete coding and UTR regions of the *DIAPH1* gene in 200 control chromosomes from neurologically normal individuals from Saudi Arabia, a cohort of 136 MCP cases, and 62 consanguineous cases that have homozygosity for the interval surrounding *DIAPH1* and at least one of the following phenotypes, ID, seizure, MCP, lissencephaly, polymicrogyria, cerebellar hypoplasia, and/or autism, failed to identify any additional rare, homozygous LoF alterations. These findings strongly suggest that the homozygous loss-of-function sequence variant identified in *DIAPH1* and the accompanying absence of protein expression is the underlying genetic cause of the disorder in the index family.

mDia1 is localized pericentrosomally in mitotic neural progenitors

To gain insight into the potential functions of mDia1 in brain development, we analyzed *Diap1* mRNA expression in the developing mouse and human forebrain and in hNPCs. *In situ* hybridization of mouse brain at embryonic days (E) 12.5, 14.5, and 17.5 revealed that *Diap1* mRNA was expressed in the ventricular and subventricular zone (VZ and SVZ, respectively) progenitor cells of the dorsal and ventral forebrain and the brainstem (Figures 3a and b). *Diap1* was also observed in neurons of the cortex and hippocampus of later embryonic age (Supplementary Figure 3A). During postnatal development, *Diap1* expression was detected in the cerebral cortex, basal ganglia, hippocampus, thalamus, and external granular layer of the

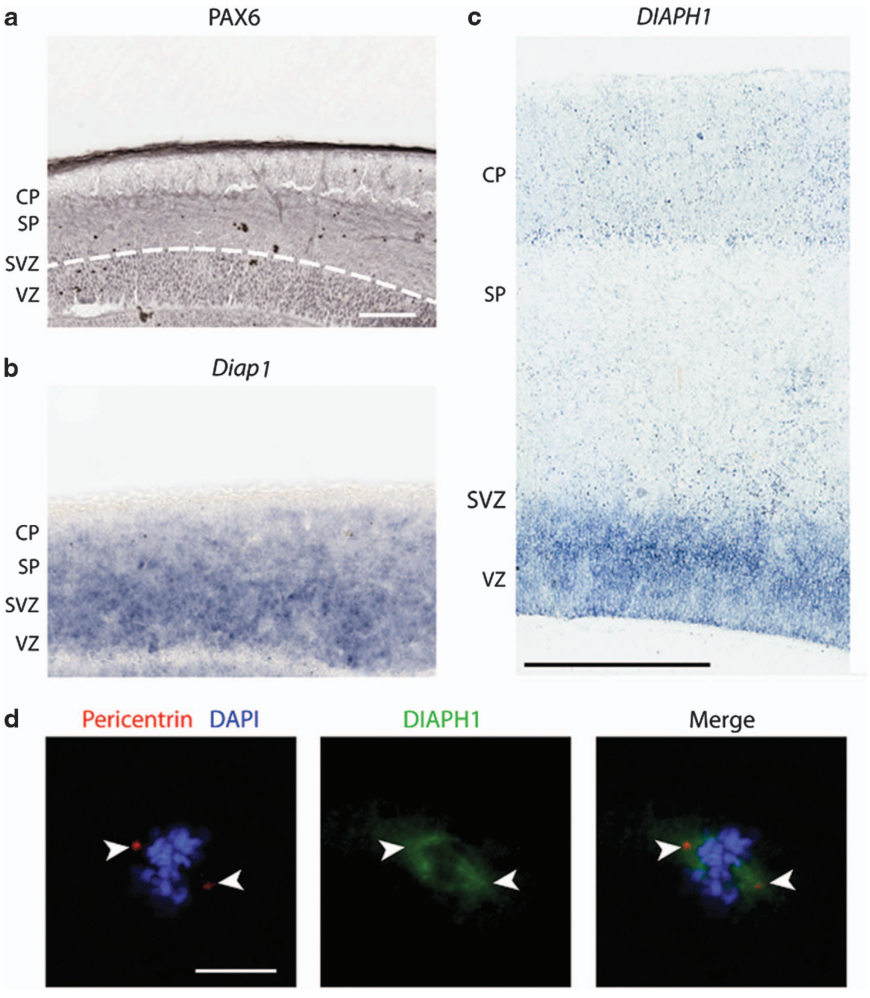

Figure 3 Expression pattern and subcellular localization of DIAPH1 in E14.5-day-old mouse brain (this period of development is equivalent to human brain 12 PCW), fetal human brain, and mitotic human cortical neural progenitor cells. A set of two panels from a developing mouse brain (E14.5) indicates (**a**) PAX6 (under the white line) at VZ, and (**b**) *in situ* hybridization shows *Diap1* expression at VZ and SVZ. (**c**) *In situ* hybridization of early fetal human brain (12 PCW) shows *DIAPH1* expression in the VZ and SVZ. The scale bar indicates 100 μm and 600 μm, respectively. See Supplementary Figure 3 for individual panels. (**d**) Confocal microscopy analysis of fixed human cerebral cortical neural progenitor cells in mitosis (M phase). Cells were co-stained with anti-mDia1 (green), pericentrin (red), and DAPI for DNA (blue). The scale bar indicates 10 μM.

cerebellum (Supplementary Figure 3B). Similar to mouse *Diap1* expression, the human homolog was strongly expressed by VZ and SVZ progenitor cells of the human neocortical wall at 12 weeks post conception (PCW) (Figure 3c).

Given that the majority of MCP genes encode proteins that localize to the centrosome or mitotic spindle, we hypothesized that mDia1 would show similar subcellular localization and tested this in hNPCs using immunofluorescence. Double immunofluorescence staining for mDia1 and pericentrin (PCNT), a centrosomal protein, revealed co-localization with centrosomes and mitotic spindles in mitosis (M phase) (Figure 3d).

To gain insight into function we conducted mRNA co-expression analyses to identify genes with similar spatial and temporal expression pattern to *DIAPH1* and other previously identified MCP genes in the developing human brain. We used exon-array data derived from human brain covering 16 brain regions over 15 periods, ranging from embryonic development to late adulthood.[16] The top 100 most-correlated transcripts of the seed genes, including the seven previously identified MCP genes and *DIAPH1*, were chosen using Pearson's correlation analysis and visualized with Cytoscape. Using this approach among the other MCP genes, we found that *DIAPH1* and *CDK5RAP2* are correlated with each other by sharing eight overlapping genes. To test the significance of this overlapping, we performed a permutation test that showed that the overlapping between *DIAPH1* and *CDK5RAP2* is significant with *P*-value $< 1e-7$, suggesting spatio-temporal expression and potential functional overlap between the two genes in particular (Supplementary Figure 4). Together, these findings indicate that, similar to previously identified MCPH genes, mDia1 is localized pericentrosomally in mitotic neural progenitors and shares a number of functionally related co-expressed genes during human brain development.

Mice lacking mDia1 have enlarged lateral ventricles but not MCP

In order to further evaluate the role of mDia1 in embryonic brain development, we examined *Diap1*-targeted mice (*Diap1*$^{-/-}$) lacking mDia1 protein.[18] We confirmed the absence of mDia1 protein in the developing forebrain using western blot (Supplementary Figure 5). Consistent with the previous study,[19] we found that *Diap1*$^{-/-}$ mice show normal organization of the cerebral cortex using layer-specific markers, CUX1, BCL11B (also known as CTIP2), and FOXP2 at P0 (Figures 4a and b and Supplementary Figure 6). However, our analysis of tissue sections of *Diap1*$^{-/-}$ mice with Nissl staining revealed unilateral dilatation of the ventricles in 60% of mutant mice (9 out of 15) with no blockage of the cerebral aqueduct (Figures 4c and d, Supplementary Figures 7). Previous analyses of mice lacking both *DIAP1* and *DIAP2* (mDia-DKO) had also various degrees of the dilation of the lateral and the third ventricle in all adult mDia-DKO mice and this was due to periventricular dysplasia in the third ventricle resulting in obstruction of CSF circulation.[19]

Given that mDia1 nucleates and processively elongates linear actin filaments[20] and that the actin cytoskeleton has a critical role in the regulation of adherence junctions in the neuroepithelium, we also stained tissue sections of mice using fluorescently labeled phalloidin and immunofluorescence with antibodies against other adherent junction components such as β-catenin (CTNNB1), N-cadherin (CDH2), and E-cadherin (CDH1). We found that actin filaments smoothly lined the apical surface of neuroepithelial progenitor cells in wild-type

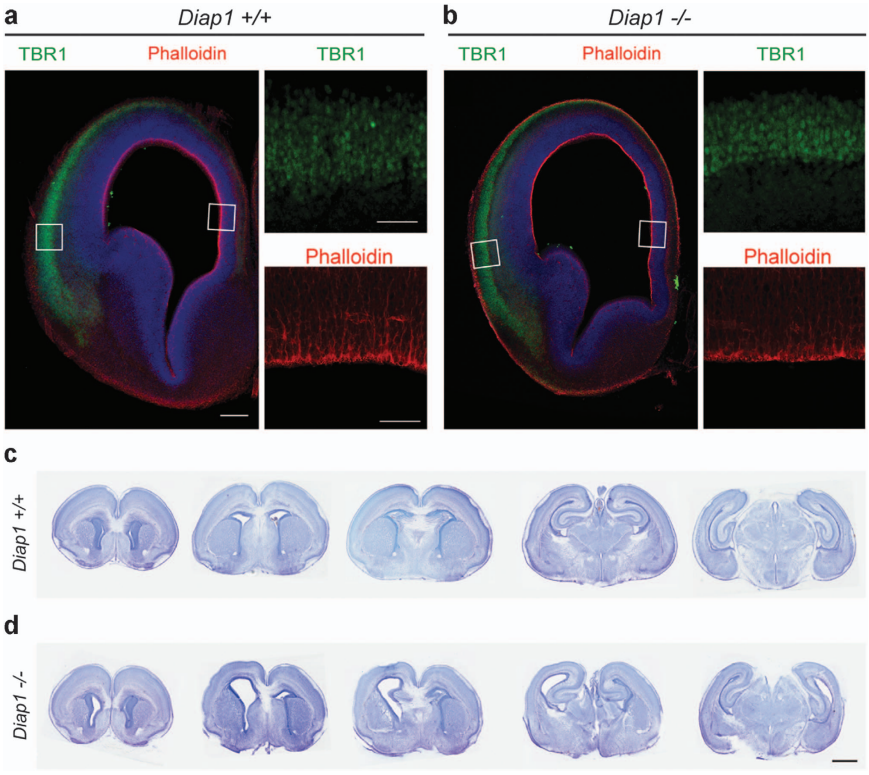

Figure 4 Cortical organization is preserved in the *Diap1*$^{-/-}$ mouse. Image depicts deeper cortical layer marker TBR1, layer VI (green), and phalloidin staining in the coronal sections of the lateral ventricle wall in (**a**) Wt (*Diap1*$^{+/+}$) and (**b**) mutant (*Diap1*$^{-/-}$) mice at E14.5 day. Boxed areas are shown at the higher magnification. Scale bars 200 μm at lower magnification and 50 μm at higher magnification. (**c**–**d**) Nissl staining of coronal brain sections of Diap1-KO and Wt at P0. Note the lateral ventricle dilatation on the Diap1-KO mouse. Scale bar is 1 mm.

and *Diap1*$^{-/-}$ mice (Figure 4b and Supplementary Figure 8, upper panel). Our result showed that, in contrast to the requirement for both mDia1 and mDia3, as revealed by analysis of *Diaph1/2*-DKO, loss of mDia1 alone does not affect the assembly of the apical actin belt at the apical surface of neuroepithelial cells as well as the adhesion molecules in the adherens junctions. mDia1 and other formins also modulate the microtubule (MT) cytoskeleton.[21,22] We investigated α-tubulin and γ-tubulin expression in *Diap1*$^{-/-}$ animals at E14.5. The absence of *DIAPH1* did not grossly alter the organization of tubulin in coronal sections of the lateral vertical wall (Supplementary Figure 8, middle and lower panels).

Prior study of *Diap1/2*-null, but not *Diap1*-null, mice revealed impaired tangential migration of cortical and olfactory interneurons compared with either wild-type or *Diap2*-mutant mice at E16.5.[4] Our analysis of *Diap1*$^{-/-}$ mice did not reveal any obvious alterations in the distribution of GABA-containing interneurons in the cortex (Supplementary Figure 9).

Given that affected human individuals exhibit hypoplasia of the corpus callosum, we analyzed *Diap1*$^{-/-}$ animals for changes in callosal thickness. Comparison between *Diap1*$^{-/-}$ and wild-type littermates at P0 revealed no significant differences in cortical white matter or callosal thickness (Supplementary Figure 10). Together, these findings indicate that loss of function of *Diap1* in mice, unlike in humans, does not lead to MCP or callosal hypoplasia.

DISCUSSION

We have identified a new human MCP gene, *DIAPH1,* in a consanguineous family with syndromic congenital MCP, blindness, seizures, developmental delay, ID, and short stature. mRNA and protein expression studies confirm that, similar to other MCP genes, *DIAPH1* is expressed in neural progenitors where it is associated with the centrosomes and mitotic spindle. Specifically, expression was found within VZ and SVZ zones of the developing cerebral wall. The centrosome is an organelle that has multiple critical roles in the organization of the mitotic spindle and the astral microtubules during mitosis and in neuronal migration to the cortical plate. Similar to most MCP genes that encode proteins that localize to the centrosome or mitotic spindle, the *DIAPH1* gene product mDia1 showed peri-nuclear localization, which was concentrated on mitotic spindles and co-localized with centrosomes in the M phase of hNPCs at mitotic division. Hence, the expression pattern of *DIAPH1* in the brain was consistent with a role in neurogenesis and in regulation of the size of the cerebral cortex. Moreover, previously it has been reported that both *ARP2/3*- and mDia1-dependent actin polymerization is required for centrosome separation in early *Drosophila* embryos[23] and that DIAPH1 localizes to the spindle microtubules of non-neuronal cells during mitotic cell division.[24]

The SAR1008 pedigree described here presented with MCP and growth retardations, sharing features with other syndromal micro-cephalies, including Seckel syndrome (MIM 210600)[25] and MOPD II (MIM 210720).[26] Consistent with our current findings, another centrosomal protein, PCNT, leads to Seckel and MOPD II syndrome and has been shown necessary for efficient recruitment of *Cdk5rap2* to the centrosome in neural progenitors.[27] As noted, our co-expression analysis using known MCP genes identified a link between *DIAPH1* and *CDK5RAP2* that is associated with centrosomal function and mitotic spindle orientation. *CDK5RAP2* localizes at the centrosome during mitosis and interacts directly with microtubule-end binding protein (EB1) to regulate microtubule dynamics and stability.[28] Interestingly, EB1 and adenomatous polyposis coli (APC) interact with the FH1 and FH2 domains of DIAPH1 to stabilize microtubules and promote cell migration.[29] This evidence supports a model in which different MCP genes converge on the same mechanism.

Although animal models have been quite useful for illuminating cellular and molecular mechanisms of MCP, so far few have faithfully and consistently recapitulated the human phenotype. For example, the mouse model for *Aspm*, which was represented by two mouse lines (*Aspm*$^{1-25}$ and *Aspm*$^{1-7}$), identified only mild reductions in brain size in *Aspm*$^{1-7}$, and no significant difference in brain size in *Aspm*$^{1-25}$.[30] Similarly, a single study identified no significant difference between Wt and KO-*Mcph1* mice,[31] whereas a second group showed that *Mcph1* disruption in mice resulted in MCP.[32] In contrast, the mouse model of *Cdk5rap2* shows dramatic reductions in brain size along with other brain phenotypes, including enlarged ventricles.[33]

In this case, *Diap1* knockouts do not recapitulate a small brain phenotype or callosal hypoplasia, but similar to *Cdk5rap2* show enlarged ventricles. These species differences may result from the molecular and cellular differences in how the cerebral cortex develops in the two species. Some of the most prominent features of cortical neurogenesis in humans, when compared with mice, are the prolonged neurogenesis,[34] the elongation of the apical fiber of radial glial cells (RGCs),[35] and the enlargement of the outer SVZ zone and outer RGCs.[36,37] However, the most striking structural finding in *Diap1* KO mice was unilateral ventricular enlargement (9 out of 15). Consistent with these findings, a variable degree of ventricular enlargement has been reported as an MCP-related brain malformation in both mice and humans.[33,38]

Recent published data from mouse KO models or non-neuronal tumor cell lines *in vitro* suggest that mDia1 is a Rho-effector and has a critical role in the maintenance of the adherens junction and polarity of neuroepithelial cells in multiple brain regions and in shaping F-actin dynamics that drives tangential migration of neuronal precursors.[4,19,39] Even though radial migration was promoted and tangential migration was impaired in neuronal precursors from *Diaph1/2*-DKO mice, this migration deficit is more apparent for those migrating a longer distance of tangential migration by regulating movement of F-actin condensation at the leading edge and formation of an F-actin cup at the rear edge of SVZ neural progenitor cells. Hence, the maximum distance between centrosome and nucleus before nuclear translocation was significantly reduced.[4]

Together, our genetic and molecular biological data, coupled with previous findings, provide strong evidence that mDia1 has a crucial role in brain development, leads to MCP in humans and exhibits species differences in its function in the developing nervous system.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)

Supplementary Information

Homozygous loss of *DIAPH1* is a novel cause of microcephaly in humans

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Clinical information about index family

Family Birth Order and Miscarriage:

IV:1 (Miscarriage at 2nd trimester)

IV:2 (normal per family)

IV:3 (died at age of 3 months)

IV:4 (proband)

IV:5 (affected; died at age of 18 years old)

IV:6 (affected)

IV:7 (affected)

IV:8 (affected)

IV: 3 was born full term with congenital heart disease (according to hospital's report) and he was died at age of 3 months in neonatal intensive care unit *(*NICU*)*. No other clinical information is available about him.

Patient IV:4

Age at Interview: 15 years and 11 months

HPI: The parents have difficulty recalling exact dates and events. However, they believe that they first became concerned when the patient started having seizures at three months of age. Over the course of the first two years of her life, they also noticed abnormalities with her development, including delays in language. In addition, they notice that she had significant difficulties with vision.

Currently, they note poor adaptive skills that they attribute partly to her blindness and partly to her decreased intellectual ability. They note that she cannot toilet or dress herself and that she

cannot feed herself. They say, however, that she can walk, sit, and explore objects with her hands. They say that she does not have the intellectual capacity to attend school.

In terms of social functioning, they state that she likes to be around others and will take toys from others and give toys to others. She enjoys affection.

With regards to communication, they say that she understands simple commands and can say simple two-word sentences to make requests, such as, “want rice,” or “go to toilet.” However, she is unable to carry on more complex conversation. She is able to communicate non-verbally using nodding, head-shaking, waving, or taking others by the hand towards objects like the television.

The parents deny significant repetitive behaviors. They say that she will occasionally repeat other’s words but not do so over and over again. She does not repeat her own words. They deny abnormal repetitive movements. They say she likes to play with a broad range of toys, especially those that make a lot of noise. There were not reported sensitivities.

Past/Current Treatment: She is not in school and has not received services.

Past Medical History:

-Microcephaly -- noted since infancy.

Seizures -- Beginning at age 3 months, she began to have occasional seizures. Some were reported as cyanosis around the mouth, others were reported as full body shaking. With medications, she does not have seizures at present.

-Severe bilateral visual impairment – assessed to be central blindness

-History of left lower lobectomy -- status post bronchiectasis.

Medications: Phenobarbital

Social History: She lives at home with her parents and siblings in Saudi Arabia. She is cared for by her parents and a nanny. She does not attend school.

Pregnancy/Birth Issues: The mother had no medical problems during pregnancy. There were no exposures to substances or medications during the pregnancy.

Delivery: Normal vaginal delivery (NVD).

Full Term: Yes

Weight: 2.4kg

Immediate medical issues at birth: she was mildly cyanotic and per parents, she received nasal oxygen but was not intubated. She had jaundice that was treated with light therapy. She was in the hospital for 2 weeks after delivery.

Milestones: The parents had difficulty recalling precise ages but thought she began walking for example, at approximately age 16 months. The parents recall delayed speech. For instance, she started saying two-word sentences at age 3. There was no regression of any skills.

Clinical Observations:

In terms of adaptive behavior she could walk, sit, and explore objects with her hands.

Socially, she seemed to enjoy cuddling with mother. She was upset when parents left the room. She smiled when interacted with. She requested her mother's help with a pop-up toy. She took objects from others and handed objects to others. She verbally requested information about the activity of her sister who was present.

With regards to receptive language, she was able to follow-simple commands – like "give the toy to mother." However, she did not respond to bids at more complex conversation, like "how are you doing?" Her expressive language was limited to simple two-word sentences, like "let's

stop." She could repeat simple words like, "doll." Her prosody was odd. Non-verbally, she used some appropriate gestures and smiled appropriately.

There were no definite stereotypies. She was observed humming to herself and rocking her head back and forth while doing so, but this did not appear to be a stereotypical behavior.

In terms of her intellectual capacity, she could manipulate the levers of a simple pop-up toy, even though she could not see the levers and demonstrated a slight understanding of the cause and effect of the toy. She followed a simple command to give a doll to her mother.

Her appearance was notable for small head size. In addition, she looked pre-pubertal despite her age.

Testing

Hearing Test: Reportedly normal per parents, results not available for review

IQ: Abnormally low per report, no record for review

Neuro-imaging:

CT scan of the Brain: 12/12/95 -- Normal non-contrast CT

Fragile X: Negative

Karyotyping: Normal

Metabolic Screening: Reportedly normal

Other Labs: Electrolytes within normal limits 8/2005

Ophthalmological Examination: Eye anatomy was reported to be within normal limits and a diagnosis of central blindness, "brain blindness," is given.

Anthropomorphic Data: at age of 15 years and 11 months

Height: 127 cm (<2.5 percentile) Saudi Growth Chart (El Mouzan, 2008). This corresponds to CDC Z-Score of -5.5 or -5.5 standard deviations below the mean.

Weight: 20 kg (<2.5 percentile) Saudi Growth Chart (El Mouzan, 2008). This corresponds to CDC z score of -14.94.

Head Circumference: 43.5cm (Greater than 3 standard deviations below the mean for Egyptian girls) (Mean for Egyptian girls age 15 was 53.71cm. SD was 1.37) (Zaki et al., 2008).

National health and nutrition survey (NHANES), CDC/National Center for Health Statistics.

Multi-Axial Diagnosis:

Axis I: Intellectual Disability

Axis III: Microcephaly, Seizures, Bilateral Blindness, Short Stature, Delayed Puberty (Tanner Stage-I according to her breast development), History of left lower lobectomy status post bronchiectasis.

Axis IV: Multiple affected children

Patient IV:5

Age at Interview: 14

Patient IV:5 died when she was 18 years old because of chest infection (according to hospital's report).

HPI: The parents were first concerned medically when she had seizures starting at around age 3 months. The seizures involved jerking of the body, arms, and legs. They also noticed that she did not reach her motor or language milestones on time. They feel that she was about 3 months delayed in all of these.

In terms of social behavior, the parents say that she likes to play with other children. In addition, she seeks and enjoys affection from her mother. She shows her toys to others. She does not play imaginary games.

With regards to receptive language, the parents note that she can understand simple commands. They add that they believe that her receptive language is stronger than her expressive language. She is limited to two-word sentences like, “Want water,” and “Want car.” Her speech is reportedly difficult for others to understand. Her parents say that she can wave goodbye, nod and shake her head, and pull her mother towards the TV when she wants it on.

In terms of adaptive behavior, her parents say that she can feed herself and that she can dress herself in a “messy” way. She cannot fully toilet herself although she does have bowel and bladder control and can request to go to the bathroom. The parents say that she does not have the intellectual capacity to attend school.

The parents do not note any regression of skills or language. There are no reported sensitivities and no reported repetitive behaviors or narrowed interests.

Past/Current Treatment: She is currently receiving rehabilitative services where the focus is on teaching her skills like how to feed herself and how to clothe herself.

Past Medical History:

-Microcephaly

-Seizures. They sound to be tonic-clonic. They started at age 3 months and have been well controlled by medication but do recur occasionally.

-By report, she suffers from blindness bilaterally.

Medications: Phenobarbital.

Social History: She lives at home with her parents and siblings in Saudi Arabia. She is cared for by her parents and a nanny. She does not attend school.

Pregnancy/Birth Issues: The mother had no medical problems during pregnancy. There were no exposures to substances or medications during the pregnancy.

Delivery: NVD

Full Term: Yes

Weight: 2.4kg

Immediate medical issues after birth: She had physiologic jaundice that remitted without treatment. She went home without hospitalization after birth. She fed normally on milk and solids.

Milestones: The mother noted a three month delay in motor development across the board. She says that there was a delay in language in that the patient only spoke a few single words at age two.

Clinical Observations:

In terms of adaptive behavior and intellectual capacity, she could walk, sit, and explore objects with hands. She was able to understand the levers of and manipulate a simple pop-up toy and understood the cause and effect of all the different knobs and levers.

Socially, she was affectionate towards her mother. She smiled when interacted with. She took objects from others and handed objects to others. She asked whether she should close certain parts of the pop-up toy. She engaged in some imaginative play, rolling a car on the ground and making a car sound in imitation of the examiner.

With regards to receptive language, she was able to follow-simple commands – like "give the cars to mother." She could reply to simple questions like, "What is this?" when a doll was put in her hands. She would reply, "doll". However, she did not respond to bids at more complex conversation, like "how are you doing?" Her expressive language was limited to simple two-word sentences, like "Close it?" Non-verbally, she used some appropriate gestures and smiled appropriately.

She exhibited some stereotypical movements such as flapping with both arms and some body rocking.

Her appearance was notable for small head size. In addition, she looked pre-pubertal despite her age.

Testing:

Hearing Test: Normal by parental report

Immunoglobulin levels (IgM, IgA, and IgG) were normal

Metabolic test for amino acid and fatty acid levels in both urine and blood were normal

Neuro-imaging:

> MRI: 5/3/04 Per hospital report, "Bilateral occipital areas show increased signal intensity in T2 with low intensity in T1 mainly in sub-cortical white matter. Prominent cortical sulci and gyri of occipital lobes. Prominent occipital horn of lateral ventricle. Question of vascular like ischemia or congenital metabolic leukodystrophy. No change since June 9, 2002."

Fragile X: Negative

Karyotyping: Normal

Anthropomorphic Data: at age of 14

Height: 116 cm (<2.5 percentile) Saudi Growth Chart. (El Mouzan, 2008). This corresponds to CDC Z score of -6.8 or -6.8 standard deviations from the mean.

Weight: 16.2 kg (<2.5 percentile) Saudi Growth Chart (El Mouzan, 2008). This corresponds to a CDC z score of -11.74.

Head Circumference: 42.5cm (Greater than 3 standard deviations below the mean for Egyptian girls) (Mean for Egyptian girls age 14 was 53.44cm. SD was 1.36) (Zaki et al., 2008).

Multi-Axial Diagnosis:

Axis I: Intellectual Disability

Axis III: Microcephaly, Seizures, Bilateral Blindness, Short Stature, Delayed Puberty (Tanner Stage-I according to her breast development)

Axis IV: Multiple-affected children

Patient IV:6

Age at Interview: 6 years 11 months

HPI: The parents first brought the patient to medical attention when she started having seizures at around age 3 months. They also noticed that she did not reach her motor or language milestones on time.

In terms of social behavior, the parents say that she enjoys interacting with other people. She smiles at them and likes to play with them. In addition, she seeks and enjoys affection from her mother.

With regards to language, the parents note that she can say simple sentences. However, she cannot engage in normal conversation. There was no report of echolalia.

In terms of adaptive behavior, her parents say that she can feed herself, dress herself, toilet herself, open a book, and imitate cooking. They say that she does not have the intellectual capacity to attend school.

The parents do not note any regression of skills or language. There are no reported sensitivities and no reported repetitive behaviors or narrowed interests.

Past/Current Treatment: She has not received services.

Past Medical History:

-Microcephaly

-Seizures. They sound to be tonic-clonic. They started at age 3 months.

-By report, she suffers from severe, bilateral visual impairment

Medications: Phenobarbital.

Social History: She lives at home with her parents and siblings in Saudi Arabia. She is cared for by her parents and a nanny. She does not attend school.

Pregnancy/Birth Issues: The mother had no medical problems during pregnancy. There were no exposures to substances or medications during the pregnancy.

Delivery: Planned Cesarean Section

Full Term: Yes

Weight: Reportedly slightly lower than normal but not on file

Immediate medical issues after birth: the patient required supplemental oxygen at birth but was no intubated. She was in the hospital for 3 weeks after delivery and required blood transfusions. The cause for these is not reported. She went home without hospitalization after birth. She fed normally on milk and solids.

Milestones: The mother noted a three month delay in motor development across the board. She says that there was a delay in language in that the patient did not have words until age 2 years.

Clinical Observations:

In terms of adaptive behavior and intellectual capacity, she could walk, sit, and explore objects with hands. She was able to understand and manipulate a simple pop-up toy but understood the cause and effect of only one of several knobs.

Socially, she was affectionate towards her mother. She smiled when interacted with. She took objects from others and handed objects to others. She asked whether it was ok to put a plastic bottle of medicine into a cardboard box.

With regards to receptive language, she was able to follow-simple commands – like "give me the car." However, she did not respond to bids at more complex conversation, like "how are you doing?" Her expressive language was limited to single words like "open" and "close." Non-verbally, she used some appropriate gestures and expressions, smiling appropriately, for example. At one point she brought a bag over to the examiner and presented it to express that she would like it to be opened.

There were no repetitive behaviors or narrowed interests. Her appearance was notable for small head size.

Testing

Hearing Test: Reportedly normal per parents

IQ: Not tested.

Neuro-imaging:

MRI Brain: April, 2004 Per hospital report, "Normal Axial T1,2"

EEG: Per hospital report, "Bad cortical organization," "encephalopathy" but with epileptiform activity.

Fragile X: Negative

Abnormal laboratory results: 6/16/2007 Liver Function Tests (LFT's): Alk Phos 1296 (0-500), ALT 106 (0-55), AST 121 (5-34).

Ophthalmologist Report: 1/13/08 "Slit lamp exam within normal limits."

Anthropomorphic Data: at age of 6 years 11 months

Height: 100 cm (<2.5 percentile) Saudi Growth Chart. (El Mouzan, 2008). This corresponds to CDC Z score of -4.4 or -4.4 standard deviations.

Weight: 12 kg (<2.5 percentile) Saudi Growth Chart (El Mouzan, 2008). This corresponds to a CDC z-score of -6.24.

Head Circumference: 43cm (Greater than 3 standard deviations below the mean for Egyptian girls) (Mean for Egyptian girls age 7 was 50.57cm. SD was 1.32) (Zaki et al., 2008).

Multi-Axial Diagnosis:

Axis I: Intellectual Disability

Axis III: Microcephaly, Seizures, Severe bilateral visual impairment, Short Stature

Axis IV: Multiple affected children

Patient IV:7

Age at Interview: 2 years and 1 month

HPI: The parents first brought him to medical attention at 3 months of age when he had seizures that were described as his whole body shaking. Upon interview, they were concerned that the patient was not reaching his motor or language milestones on time.

With regards to social behavior, they stated that he generally played by himself and liked toys that made loud noises. In terms of language, the parents reported that he could say “mama,” and “baba.” They said that he did not use non-verbal communication. He did not nod, shake his head, or point, for example. With regards to adaptive skills at the time of the interview at age 2, they said that he could walk but was not able to feed himself. There were no reported repetitive behaviors, regression of skills, or sensitivities.

Past/Current Treatment: He has not received services.

Past Medical History:

-Seizures.

-Microcephaly

-He may suffer from bilateral visual impairment, by report

Medications: Phenobarbital

Social History: He lives at home with her parents and siblings in Saudi Arabia. She is cared for by her parents and a nanny. He does not attend school.

Pregnancy/Birth Issues: The mother had no medical problems during pregnancy. There were no exposures to substances or medications during the pregnancy.

Delivery: Cesarean Section, secondary to prolonged labor.

Full Term: Yes

Weight: 2.56kg

Immediate medical issues after birth: He had physiologic jaundice that lasted only 2-3 days and that was treated with lights.

Milestones: The mother reports that he walked at 1 year and 8 months and that he only has two single words at 2 years of age.

Clinical Observations:

In terms of adaptive behavior and intellectual capacity, he could walk, sit, and hold and explore objects with his hands. He was able hold an object in each hand and to knock them together.

Socially, he smiled when interacted with and appeared to enjoy interacting with other people. He attempted to hand a toy car to his mother and father.

With regards to receptive language, he was able to follow-simple commands – like “give the car to your father.” He responded to his name, but not on the first time it was said. He did not

respond to bids at more complex conversation. His expressive language was limited to several single words like “Mama,” “Baba,” and “No.” He could repeat words, like “car.” His main non-verbal communication was to smile at others and to hand them things.

There were no repetitive behaviors or narrowed interests. His appearance was notable for small head size.

Testing

Hearing Test: None

IQ: None

Neuro-imaging:

MRI: 3/29/2009 Brain. T2 weighted Axial images showed; temporal pole atrophy with normal cortical thickness and increased CSF space in the middle cranial fossa anterior to the temporal pole, dilatation of the lateral ventricles, normal third ventricular size and the atria of the lateral ventricles, small splenium of the corpus callosum, normal appearance of the optic nerves and chiasm. The sylvian fissures appear normal in size and symmetry with normal configuration.

T1 sagittal images confirmed atrophy of the temporal lobe most prominent at the pole and the dilatation of the lateral ventricle. Midline sagittal T1 shows hypoplasia of the rostrum and splenium of the corpus callosum. Aqueduct and fourth ventricle are normal in size.

Coronal FLAIR images confirmed the normal appearance of the chiasm (anterior to the temporal horns of the lateral ventricles).

Ophthalmology: 2-17-09 and 5/1/2009 normal slit lamp and funduscopic examinations.

Fragile X: Negative

Metabolic Screening: National newborn screening HPLC 5/24/09 – Arginine H 406 (16-180), Alanine L 87 (120-720), otherwise normal.

Anthropomorphic Data: at age of 2 years and 1 month

Height: 85 cm (25^{th} percentile) Saudi Growth Chart (El Mouzan, 2008). This corresponds to CDC z score of -0.5 or -0.5 standard deviations.

Weight: 10.2 kg (10^{th} percentile) Saudi Growth Chart (El Mouzan, 2008). This corresponds to a CDC z-score of -2.11.

Head Circumference: 41.5cm (Substantially below the 3^{rd} percentile) Source: Head circumference-for-age percentiles, boys birth to 36 months, CDC growth charts

Multi-Axial Diagnosis:

Axis I: Intellectual Disability

Axis III: Microcephaly, Seizures, Possible visual impairment

Axis IV: Multiple affected children

Patient IV:8

Age at Interview: 3 months and 3 weeks old

HPI: The patient was brought to medical attention at 3 months of age when he had abnormal eye movements his parents thought were seizures.

Past Medical History:

-Microcephaly

-Possible visual impairment, by observation

Medications: Phenobarbital 30 mg a day

Social History: He lives at home with his parents and siblings in Saudi Arabia. He is cared for by his parents.

Pregnancy/Birth Issues: The mother had no medical problems during pregnancy. There were no exposures to substances or medications during the pregnancy.

Delivery: Cesarean Section

Full Term: Yes

Weight: 2,700 grams

Immediate medical issues after birth: He had physiologic jaundice that lasted only one week and that was treated with lights.

Milestones: Delayed milestones

Clinical Observations: He does not appear to see objects in his environment or light.

Ophthalmology: An appointment is pending.

Metabolic Screening: National newborn screening results are pending

Anthropomorphic Data: 3 months and 3 weeks old.

Height: Not available.

Weight: 5 kg (3^{th} percentile) Saudi Growth Chart (El Mouzan, 2008). This corresponds to a CDC z-score of between -2 and -1.5.

Head Circumference: 32 cm. Greater than 3 standard deviations below the mean for Egyptian boys) (Mean for Egyptian boys age 4 months was 41.58cm. SD was 1.14) (Zaki et al., 2008).

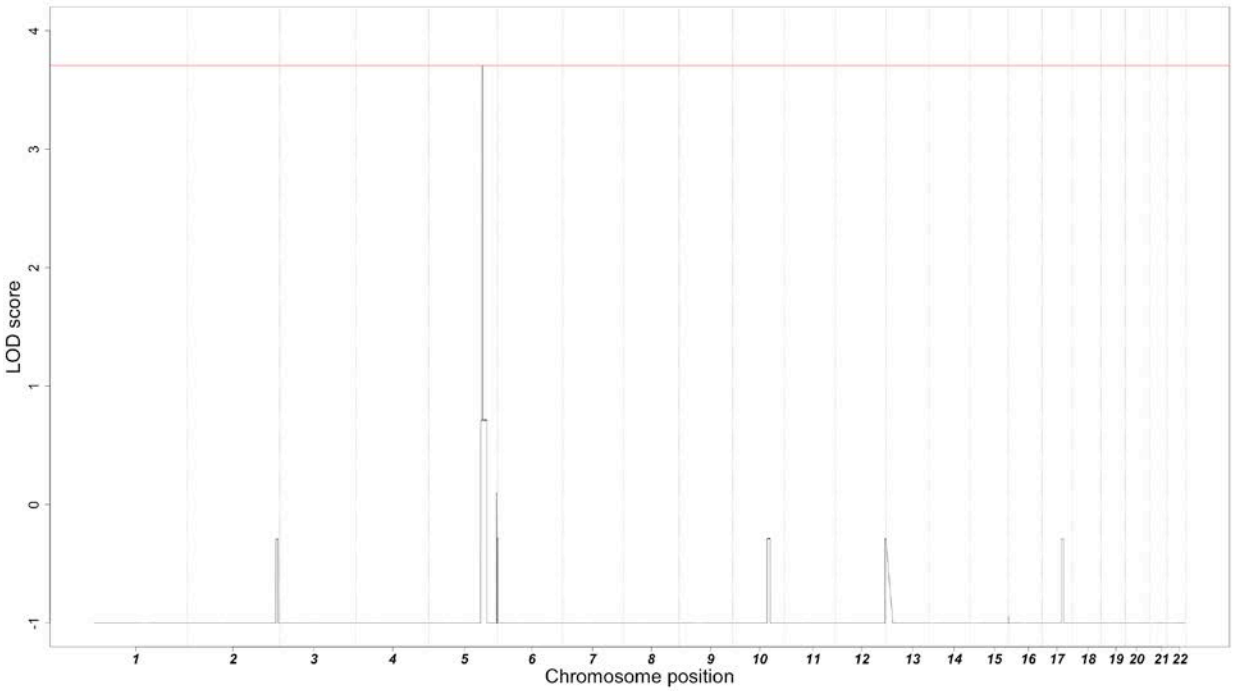
Multi-Axial Diagnosis:

Axis I: Intellectual Disability

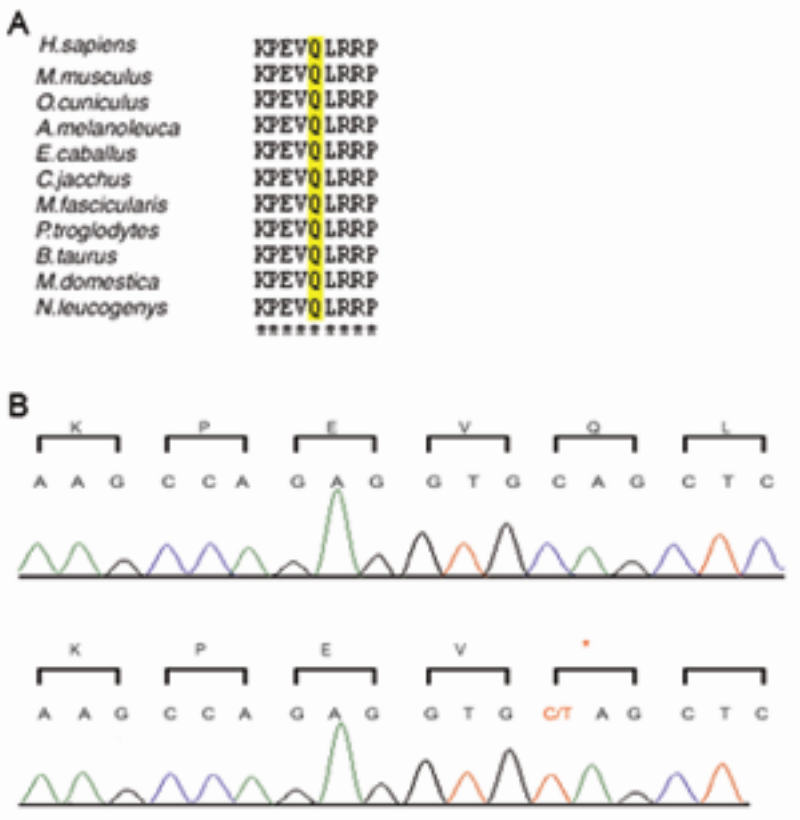
Axis III: Microcephaly, Seizures, Severe bilateral visual impairment, Short Stature

Axis IV: Multiple affected children

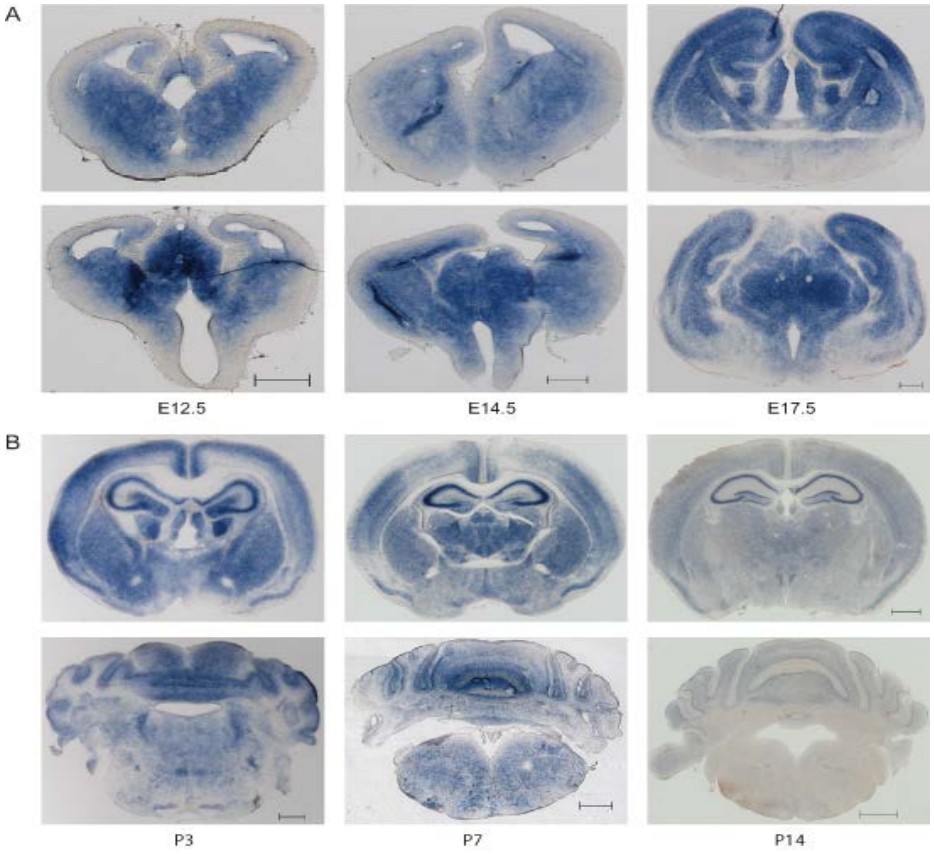
Supplemental Figures



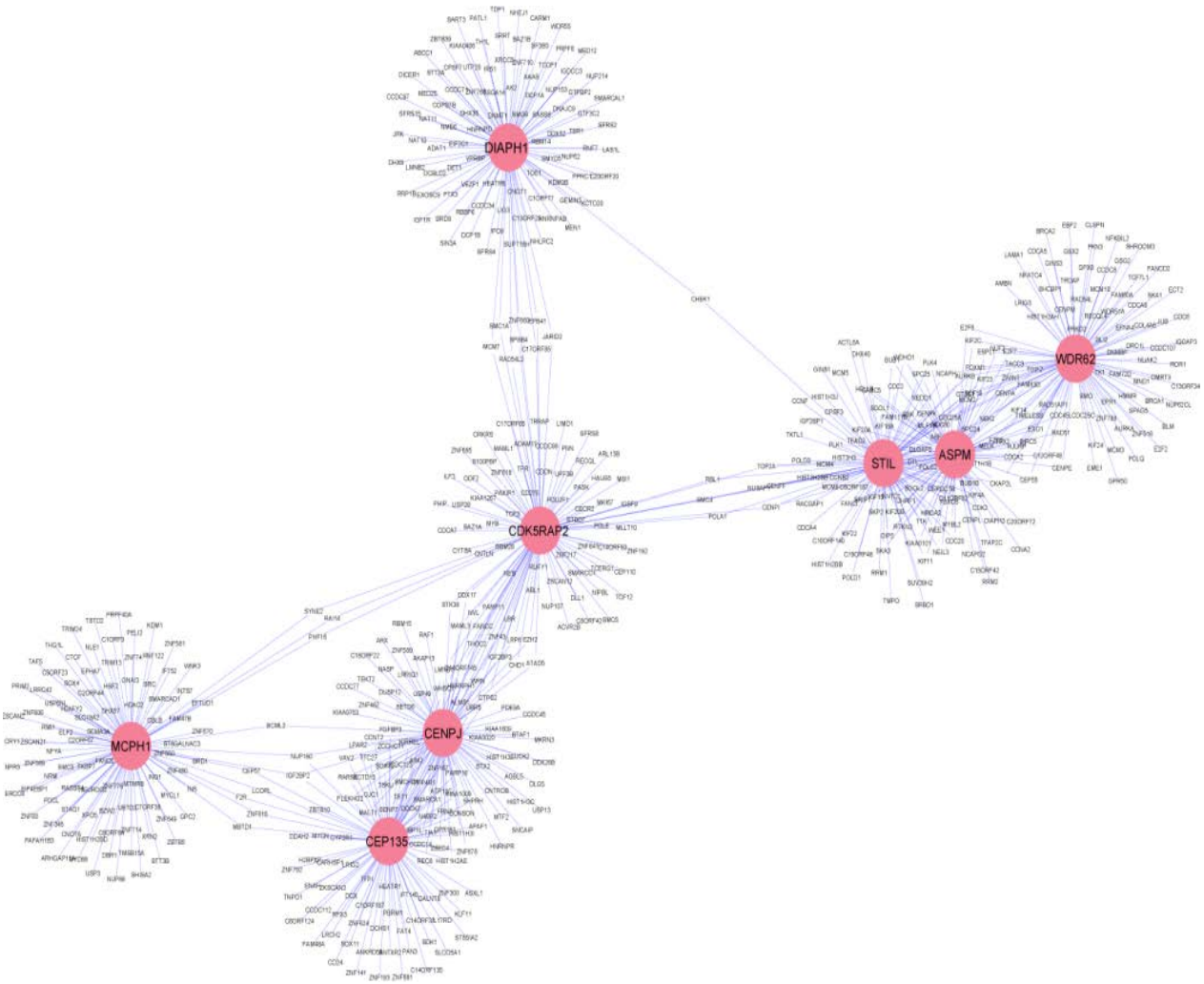
Supplementary Figure 1. Genome-wide parametric linkage analysis for SAR1008 pedigree. Plot shows allele sharing LOD (Logarithm of Odds) on Y axis and chromosome location on X axis. The red horizontal line indicates the maximum theoretical LOD score for this family (3.7). The vertical dashed lines show the boundaries of each chromosome.



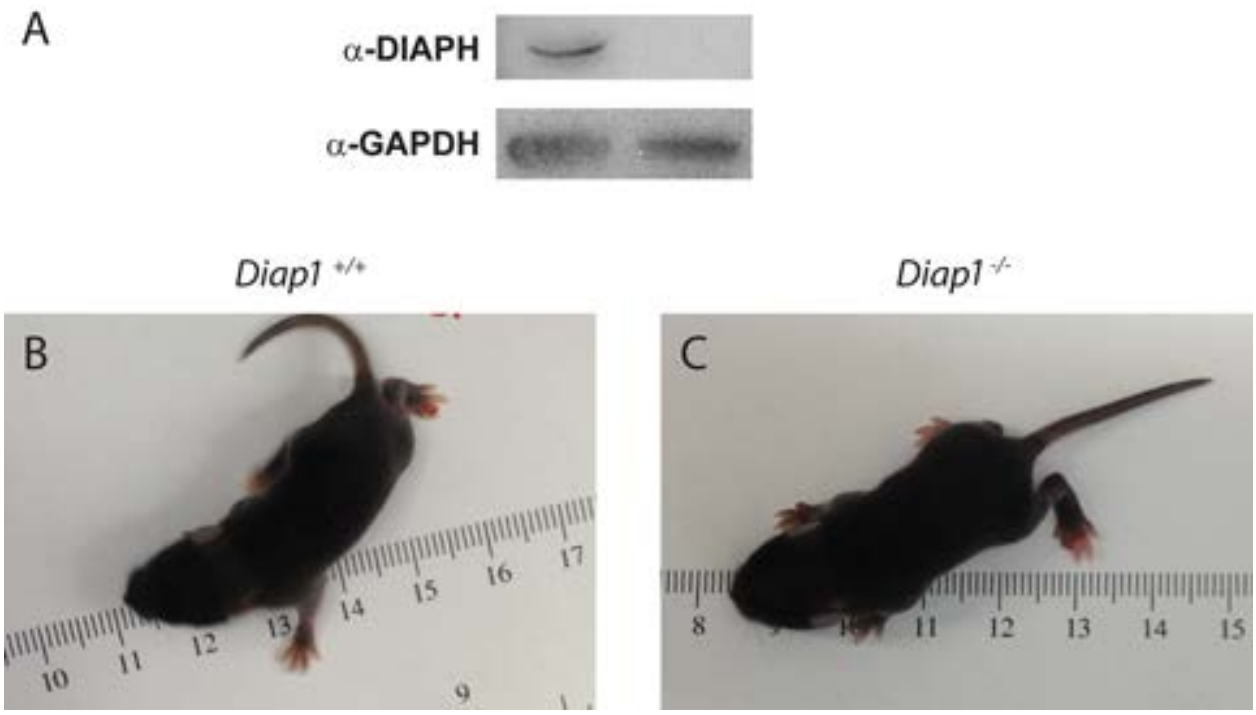
Supplementary Figure 2. The amino acid sequence alignment of DIAPH1 protein across species using ClustalW2 program and nonsense homozygous mutation in *DIAPH1*. (A) The site of p.Gln778* mutation (Q778*) is shaded with yellow. An asterix (*) highlights identical residues. (B) A nonsense mutation in the *DIAPH1* is identified through custom designed target sequencing. Sanger sequencing of cDNA from lymphoblastoid cells confirms this mutation. The altered amino-acid sequence leading to a premature stop-codon (p.Gln778*) is shown in red.



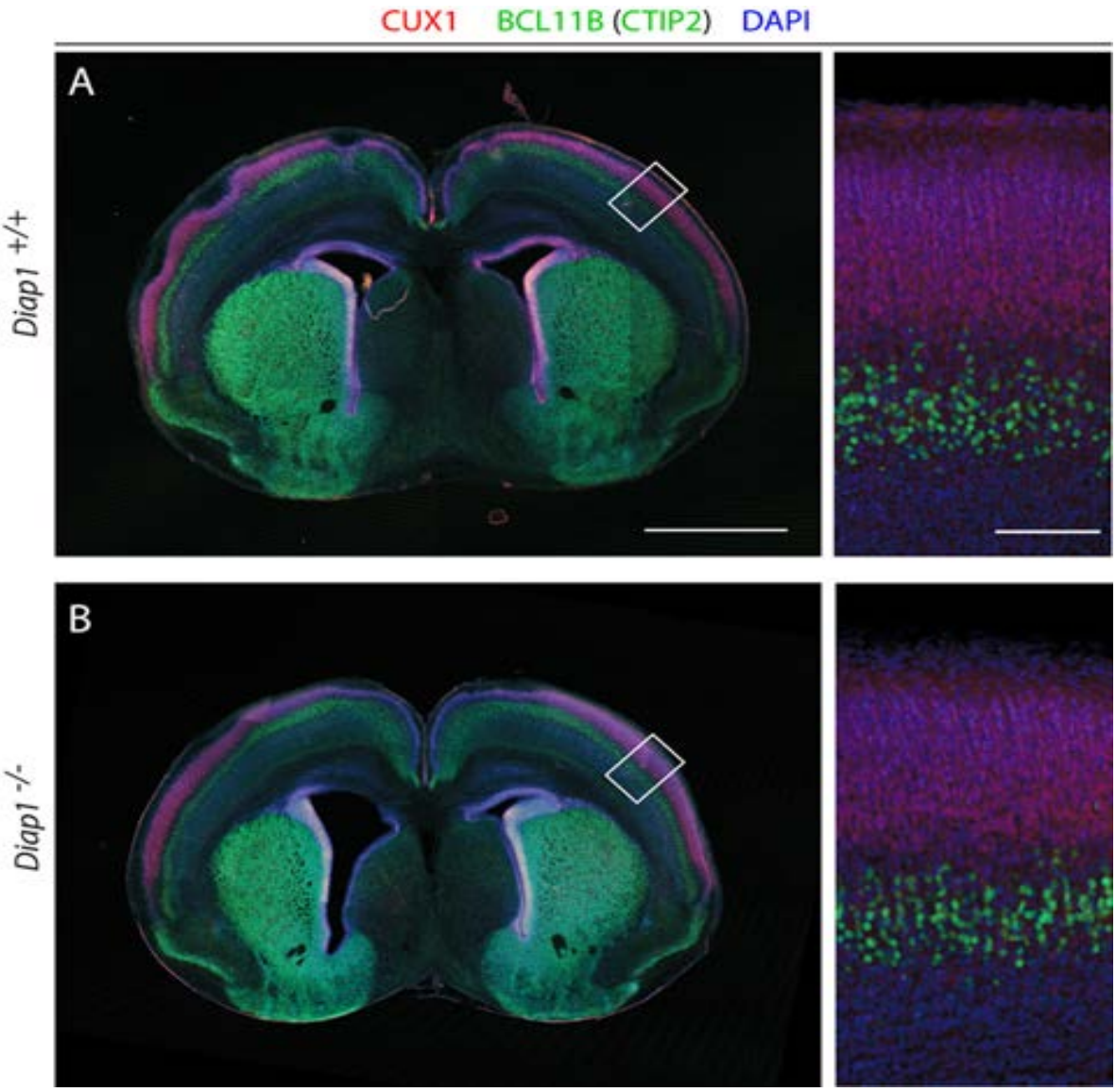
Supplementary Figure 3. *DIAPH1* Expression Pattern in Human and in embryonic and adult mouse brains. (**A**) *In situ* hybridization for *Diap1* mRNA in E12.5, E14.5, and E17.5 mouse brains. Antisense *diap1* riboprobes labeled with digoxigenin-UTP were used to detect *Diap1* mRNA. *Diap1* mRNA was detected across brain regions such as the developing cerebral cortex, ventral forebrain, lateral midbrain, LGE (lateral ganglionic eminences) and MGE (medial ganglionic eminences) in E12.5, E14.5. *In situ* hybridization for *Diap1* mRNA in E17.5 brains showed expression in the cortical plate, the developing hippocampus, and basal ganglia. Scale bars, 500 µm at lower magnification and 100 µm at higher magnification. (**B**) *In situ* hybridization for *Diap1* mRNA during postnatal development on P3, P7, and P14 in mouse brain. *Diap1* mRNA was detected in differentiating neurons cortical plate, hippocampus, thalamus, basal ganglia, and external layer of cerebellum. Scale bars, 500 µm.



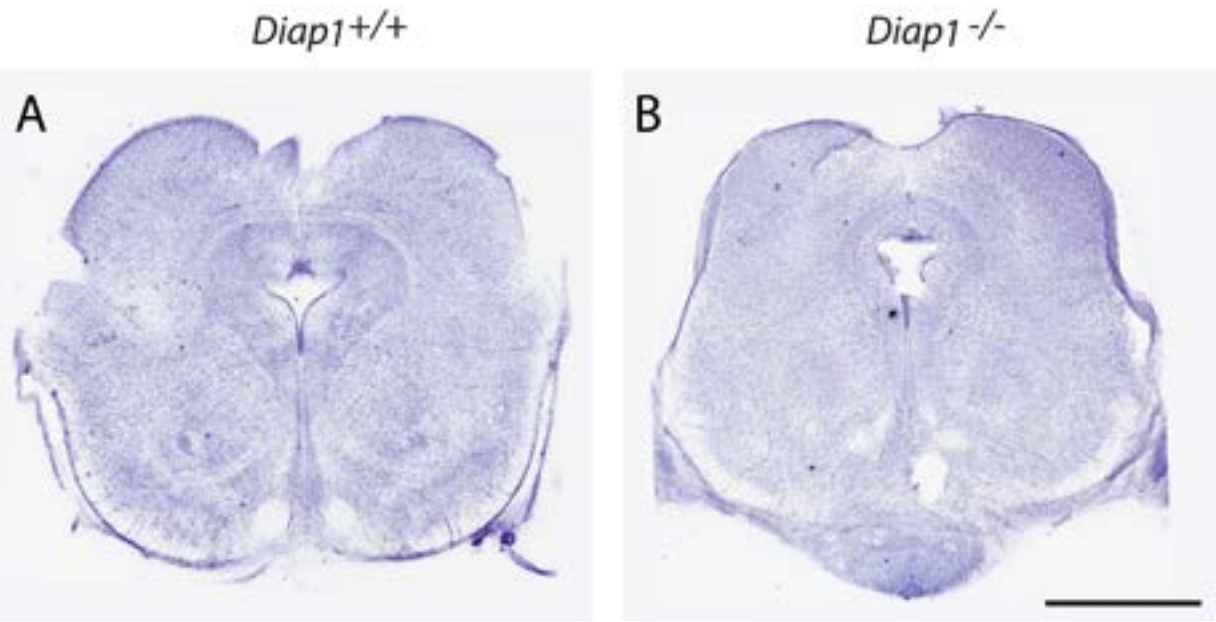
Supplementary Figure 4. **Gene co-expression analysis** revealed co-expressed genes with *DIAPH1* and previously identified microcephaly genes (*MCPH1*, *WDR62*, *CDK5RAP2*, *ASPM*), *CENPJ*, *STIL*, *CEP135*). The software Cytoscape was used to visualize the co-expression network, in which seed microcephaly genes are shown by circles and the correlated genes are connected by lines.



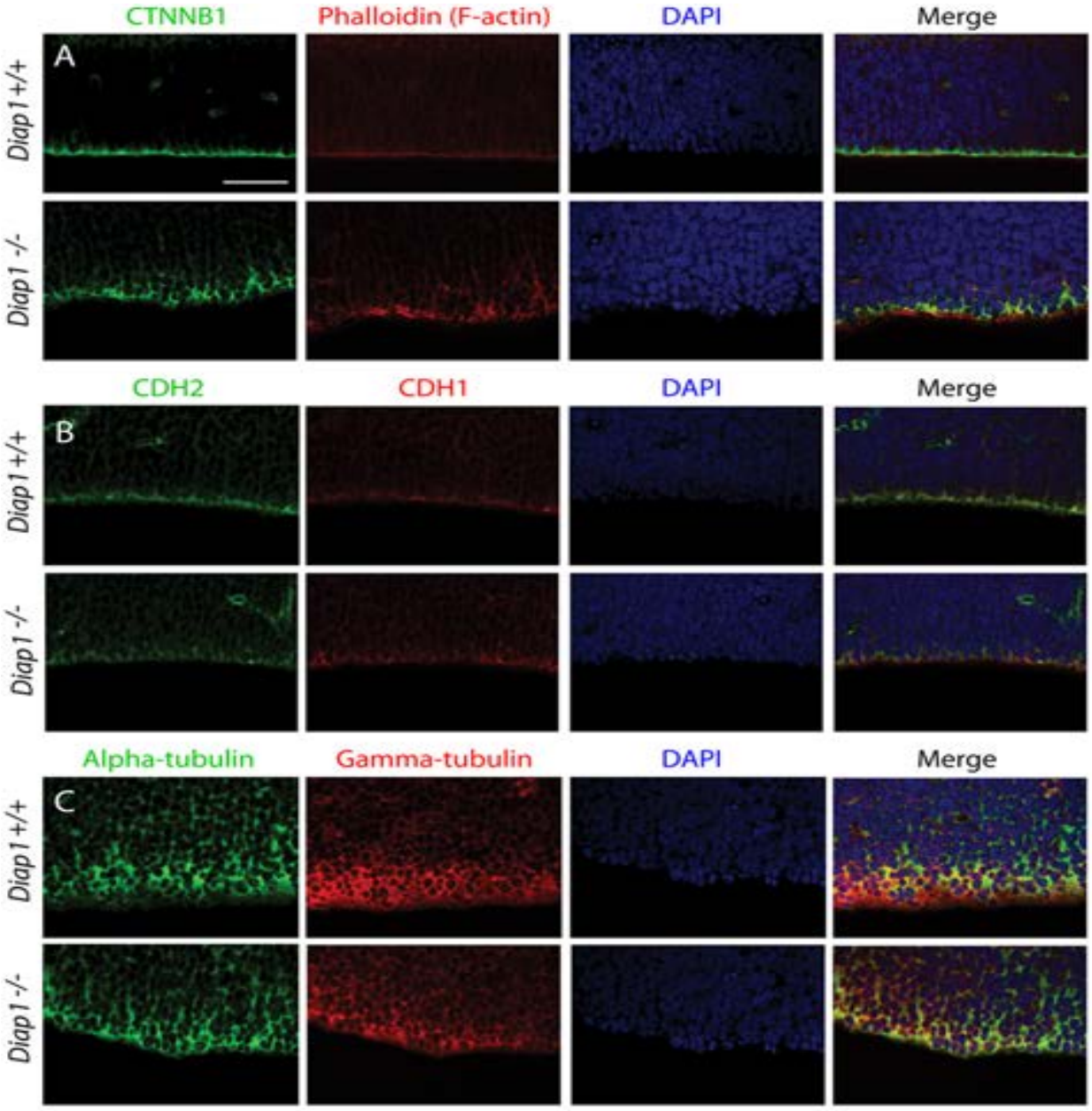
Supplementary Figure 5. mDia1 expression in the whole brain lysates from wild-type (+/+), and homozygous ($^{-/-}$) mice of *Diap1* genotype. GAPDH was used as an internal control. Global appearance of *Diap1*$^{-/-}$ was similar to *Diap1*$^{+/+}$ mouse.



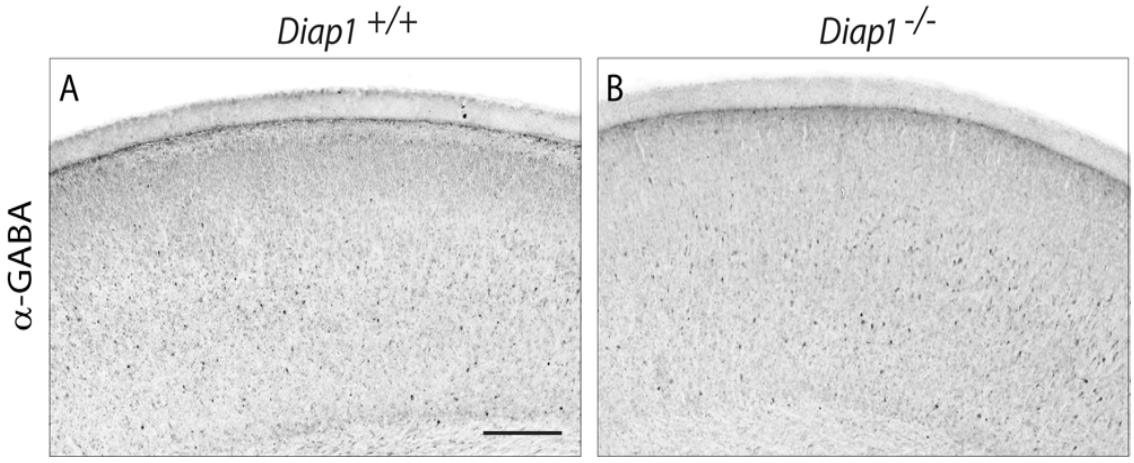
Supplementary Figure 6. mDia1-KO mice show preserved layer organization. Coronal sections of Wt and mDia1-KO mice at P0 were stained with cortical layer specific markers; CTIP2, Layer V (green) and CUX1, Layers II-IV (red). Scale bars 1mm at lower magnification and 50μm at higher magnification.



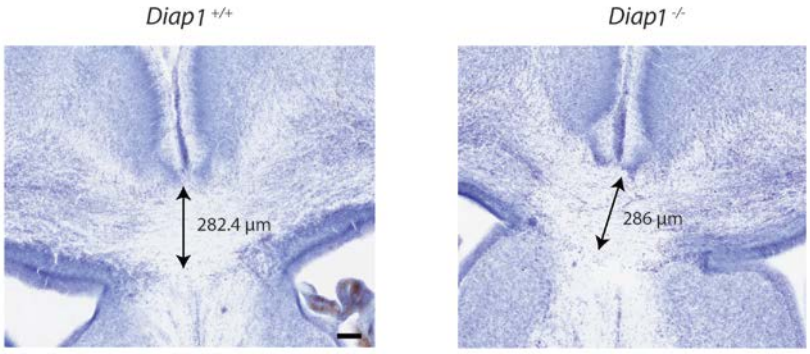
Supplementary Figure 7. Coronal sections of brain from Wt and mDia1-KO mice were stained with Nissl Note that no blockage of cerebral aqueduct of sylvius is detected. Scale bar 1mm.



Supplementary Figure 8. Immunostaining for adherent junction components. β-catenin, phalloidin (Upper panel); N-Cadherin, E-cadherin (Middle panel); and α-tubulin, γ-tubulin (Lower panel) in coronal sections of lateral vertical wall from Wt and mDia1-KO-Mouse at E14.5 day. Nuclei were counterstained with DAPI (blue). All images shown at 50µm magnification.



Supplementary Figure 9. Immunostaining for GABA. Coronal sections of the cerebral cortex from mDia1-KO and Wt at P0 were used. Images shown at 200μm magnification.



Supplementary Figure 10. Corpus callosum measurement. The thickness of the corpus callosum was measured in *Diap1*$^{+/+}$ and *Diap1* $^{-/-}$ at the rostral septal area. Images shown at 100 µm magnification.

Supplementary Table 1. IBD estimates for parents and siblings. To detect familial relationship within the family, pairwised IBD estimate was calculated using the parameters by comparing 40 Arabic ethnicity samples [Independent minor allele frequency is >5%, Hardy Weinberg eq. p=0.0001, 100% call rate, R^2 (SNP correlation coefficient) is 0.23]

IID1[a]	IID2[b]	EZ[c]	Z0[d]	Z1[e]	Z2[f]	PI_HAT[g]	DST[h]	PPC[i]	RATIO[j]
1008-01 (Father)	1008-02 (Mother)	0	0.7379	0.2292	0.0329	0.1475	0.706989	1	2.6079
1008-05 (Affected)	1008-07 (Affected)	0.5	0.1448	0.4704	0.3848	0.62	0.852272	1	16.7247
1008-02 (Mother)	1008-04 (Affected)	0.5	0.0006	0.8667	0.1328	0.5661	0.81598	1	NA
1008-03 (Affected)	1008-05 (Affected)	0.5	0.2143	0.4485	0.3371	0.5614	0.83387	1	10.1389
1008-02 (Mother)	1008-07 (Affected)	0.5	0.001	0.8835	0.1155	0.5573	0.812276	1	1480
1008-01 (Father)	1008-03 (Affected)	0.5	0.0008	0.8967	0.1026	0.5509	0.809552	1	1533.5
1008-01 (Father)	1008-05 (Affected)	0.5	0.0006	0.8995	0.0999	0.5497	0.809013	1	3018
1008-01 (Father)	1008-07 (Affected)	0.5	0.0008	0.903	0.0962	0.5477	0.808213	1	NA
1008-01 (Father)	1008-06 (Normal)	0.5	0.0004	0.9119	0.0877	0.5436	0.806442	1	NA
1008-02 (Mother)	1008-07 (Affected)	0.5	0.0006	0.9127	0.0868	0.5431	0.806226	1	NA
1008-02 (Mother)	1008-05 (Affected)	0.5	0.0014	0.9114	0.0873	0.543	0.806244	1	978.3333
1008-02 (Mother)	1008-03 (Affected)	0.5	0.001	0.9168	0.0822	0.5406	0.805219	1	996.3333
1008-03 (Affected)	1008-07 (Affected)	0.5	0.2373	0.4526	0.3101	0.5364	0.825392	1	9.5311
1008-04 (Affected)	1008-05 (Affected)	0.5	0.2435	0.4436	0.3129	0.5347	0.825249	1	9.7993
1008-03 (Affected)	1008-04 (Affected)	0.5	0.2433	0.4665	0.2901	0.5234	0.820448	1	8.9937
1008-01 (Father)	1008-04 (Affected)	0.5	0.0006	0.9529	0.0465	0.523	0.797695	1	2935
1008-04 (Affected)	1008-06 (Normal)	0.5	0.2457	0.5134	0.2409	0.4976	0.809732	1	7.727
1008-05 (Affected)	1008-06 (Normal)	0.5	0.2629	0.4893	0.2479	0.4925	0.809166	1	7.5627
1008-04 (Affected)	1008-07 (Affected)	0.5	0.258	0.506	0.236	0.489	0.807224	1	9.0489
1008-03 (Affected)	1008-06 (Normal)	0.5	0.2621	0.5189	0.2191	0.4785	0.803143	1	7.2581
1008-06 (Normal)	1008-07 (Affected)	0.5	0.3493	0.4376	0.2131	0.4319	0.791501	1	5.4013

[a]IID1; Individual ID for first individual, [b]IID2; Individual ID for second individual, [c]Expected IBD sharing given PED file, [d]Z0; P(IBD=0,[e]Z1; P(IBD=1),[f]Z2; P(IBD=2), [g]PI_HAT; P(IBD=2)+0.5*P(IBD=1) (proportion IBD), [h]DST; IBS (pairwise identity-by-state (IBS)) distance (IBS2 + 0.5*IBS1)/(N SNP pairs), [i]PPC; IBS binomial test Ratio of het; [j] RATIO; IBS 0 SNPs (expected value is 2)

Supplementary Table 2. Genotyping data from Illumina IMv1 Duo Bead array chip shows the homozygous block across the affected and unaffected members of the pedigree on chromosome 5. Linkage interval is highlighted with yellow.

rs_Number	cM	III:1	III:2	IV:2	IV:4	IV:5	IV:6	IV:7	IV:8
rs10463951	138.18	BB	AA	AB	AB	AB	AB	AB	AB
rs2042243	138.1851	AB	AA	AB	AA	AA	AA	AA	AA
rs6872555	138.2819	AB	AA	AB	AA	AA	AA	AA	AA
rs4270686	138.3226	AB	BB	AB	BB	BB	BB	BB	BB
rs4423993	138.3292	AB	BB	AB	BB	BB	BB	BB	BB
rs2188464	138.4628	BB	BB	BB	BB	BB	BB	BB	BB
rs4976526	138.5062	AB	AA	AB	AA	AA	AA	AA	AA
rs12188192	138.8296	AB	BB	AB	BB	BB	BB	BB	BB
rs2348188	138.844	AB	AB	BB	AA	AB	AA	AA	AA
rs1434660	138.8562	BB	BB	BB	BB	BB	BB	BB	BB
rs2961624	138.8791	BB	BB	BB	BB	BB	BB	BB	BB
rs1229742	138.9029	AA	AA	AA	AA	AA	AA	AA	AA
rs739699	138.9083	AA	AA	AA	AA	AA	AA	AA	AA
rs1528961	138.9186	BB	BB	BB	BB	BB	BB	BB	BB
rs10491336	138.9405	BB	BB	BB	BB	BB	BB	BB	BB
rs2189082	138.9431	BB	BB	BB	BB	BB	BB	BB	BB
rs2057680	138.9516	AB	AA	AB	AA	AA	AA	AA	AA
rs11242395	138.9598	BB	BB	BB	BB	BB	BB	BB	BB
rs2967806	138.96	AA	AB	AB	AA	AB	AA	AA	AA
rs217259	138.96	AA	AA	AA	AA	AA	AA	AA	AA
rs12516561	138.96	BB	BB	BB	BB	BB	BB	BB	BB
rs10515498	138.96	AB	AB	BB	AA	AB	AA	AA	AA
rs10040792	138.96	AB	AB	BB	AA	AB	AA	AA	AA
rs4835696	139.38	AB	BB	AB	BB	BB	BB	BB	BB
rs7379760	139.38	AA	AA	AA	AA	AA	AA	AA	AA
rs4835646	139.38	AA	AA	AA	AA	AA	AA	AA	AA
rs1651031	139.38	AB	AA	AB	AA	AA	AA	AA	AA
rs904612	139.38	BB	AB	AB	BB	AB	BB	BB	BB
rs1800954	139.4812	AA	AA	AA	AA	AA	AA	AA	AA
rs197197	139.5516	AB	AB	BB	AA	AB	AA	AA	AA
rs6580353	139.5935	BB	BB	BB	BB	BB	BB	BB	BB
rs155939	139.6114	AB	BB	AB	BB	BB	BB	BB	BB
rs1623177	139.6271	BB	BB	BB	BB	BB	BB	BB	BB
rs269783	139.6645	AB	AB	AA	BB	AB	BB	BB	BB
rs2436396	139.6768	BB	BB	BB	BB	BB	BB	BB	BB
rs7268	139.7982	AA	AB	AB	AA	AB	AA	AA	AA
rs6872579	139.8412	AB	AA	AB	AA	AA	AA	AA	AA
rs2286394	140.1294	BB	BB	BB	BB	BB	BB	BB	BB
rs3756325	140.5398	BB	BB	BB	BB	BB	BB	BB	BB
rs702390	140.6337	BB	BB	BB	BB	BB	BB	BB	BB
rs2907308	140.64	BB	AB	AB	BB	BB	BB	BB	BB
rs10491311	140.64	AB	BB	AB	BB	BB	BB	BB	BB
rs3749765	140.64	AA	AB	AB	AA	AA	AA	AA	AA
rs2237079	140.64	AB	AA	AB	AA	AA	AA	AA	AA
rs251177	140.64	AB	BB	AB	BB	BB	BB	BB	BB
rs2306339	140.64	BB	BB	BB	BB	BB	BB	BB	BB
rs3763120	140.64	AB	AA	AB	AA	AA	AA	AA	AA
rs349132	140.64	AB	AB	AA	BB	BB	BB	BB	BB

rs32929	140.64	AB	AB	AA	BB	BB	BB	BB	BB
rs10515511	140.64	AA	AA	AA	AA	AA	AA	AA	AA
rs32927	140.64	BB	BB	BB	BB	BB	BB	BB	BB
rs32946	140.64	AB	AB	BB	AA	AA	AA	AA	AA
rs152355	140.64	AB	BB	AB	BB	BB	BB	BB	BB
rs248504	140.64	AA	AA	AA	AA	AA	AA	AA	AA
rs3815355	140.64	AA	AB	AB	AA	AA	AA	AA	AA
rs2974704	140.653	BB	BB	BB	BB	BB	BB	BB	BB
rs10063472	140.7045	BB	BB	BB	BB	BB	BB	BB	BB
rs6888135	140.724	AB	AB	AA	BB	BB	BB	BB	BB
rs351260	140.8706	AB	BB	AB	BB	BB	BB	BB	BB
rs164078	140.9472	AA	AB	AB	AA	AA	AA	AA	AA
rs164495	140.9868	AB	AA	AB	AA	AA	AA	AA	AA
rs6580215	141.097	AA	AA	AA	AA	AA	AA	AA	AA
rs252144	141.2112	AB	AA	AB	AA	AA	AA	AA	AA
rs13185299	141.4377	AA	AA	AA	AA	AA	AA	AA	AA
rs2288819	141.497	AB	BB	AB	BB	BB	BB	BB	BB
rs10072521	141.7078	BB	BB	BB	BB	BB	BB	BB	BB
rs2908528	141.7211	BB	BB	BB	BB	BB	BB	BB	BB
rs11167773	141.8135	BB	BB	BB	BB	BB	BB	BB	BB
rs959662	141.9159	BB	BB	BB	BB	BB	BB	BB	BB
rs254441	142.0658	AA	AA	AA	AA	AA	AA	AA	AA
rs153423	142.1954	AA	AB	AB	AA	AA	AA	AB	AA
rs4912639	142.2689	AB	AB	BB	AA	AA	AA	AB	AA
rs17208817	142.301	AB	AB	AA	BB	BB	BB	AB	BB
rs10491399	142.3418	BB	BB	BB	BB	BB	BB	BB	BB
rs249740	142.4064	AA	AA	AA	AA	AA	AA	AA	AA
rs4244029	142.4553	BB	BB	BB	BB	BB	BB	BB	BB
rs152422	142.5406	BB	BB	BB	BB	BB	BB	BB	BB
rs13179022	142.6088	AB	BB	AB	BB	BB	BB	BB	BB
rs34016	142.6816	AB	BB	AB	BB	BB	BB	BB	BB
rs152528	142.7312	AA	AB	AB	AA	AA	AA	AB	AA
rs2070715	142.748	AB	AB	BB	AA	AA	AA	AB	AA
rs388940	143.1474	AA	AA	AA	AA	AA	AA	AA	AA
rs2436379	143.1953	AA	AA	AA	AA	AA	AA	AA	AA
rs10044036	143.3101	BB	BB	BB	BB	BB	BB	BB	BB
rs3776331	143.8453	BB	AB	AB	BB	BB	BB	AB	BB
rs246599	144.0535	AB	AA	AB	AA	AA	AA	AA	AA
rs258799	144.077	BB	BB	BB	BB	BB	BB	BB	BB
rs258770	144.1599	AB	BB	AB	BB	BB	BB	BB	BB
rs2074637	144.2253	AA	AA	AA	AA	AA	AA	AA	AA
rs3776221	144.2354	AA	AA	AA	AA	AA	AA	AA	AA
rs1438734	144.2522	AB	BB	AB	BB	BB	BB	BB	BB
rs853158	144.2747	AA	AA	AA	AA	AA	AA	AA	AA
rs10482672	144.5042	BB	BB	BB	BB	BB	BB	BB	BB
rs2963156	144.6776	BB	BB	BB	BB	BB	BB	BB	BB
rs7701443	144.7673	AB	AB	BB	AA	AA	AA	AB	AA
rs4912913	144.8348	AA	AB	AB	AA	AA	AA	AB	AA
rs2121152	144.9234	AB	AA	AB	AA	AA	AA	AA	AA
rs4912922	145.0013	AB	AB	AA	BB	BB	BB	AB	BB
rs7712869	145.1302	BB	BB	BB	BB	BB	BB	BB	BB
rs888993	145.1609	AA	AB	AB	AA	AA	AA	AB	AA
rs153516	145.2575	AB	AB	AA	BB	BB	BB	AB	BB
rs12657648	145.377	AB	AB	BB	AA	AA	AA	AB	AA
rs2910256	145.4029	AB	BB	AB	BB	BB	BB	BB	BB
rs1427864	145.4091	AA	AB	AB	AA	AA	AA	AB	AA

rs1366084	145.4305	AA	AA	AA	AA	AA	AA	AA	AA
rs867924	145.5426	BB	BB	BB	BB	BB	BB	BB	BB
rs1024961	145.7954	AB	BB	AB	BB	BB	BB	BB	BB
rs7718152	145.8694	BB	BB	BB	BB	BB	BB	BB	BB
rs1421776	145.9689	AB	AA	AB	AA	AA	AA	AA	AA
rs318373	146.0901	AB	AB	AA	BB	BB	BB	AB	BB
rs4513726	146.2076	AB	BB	AB	BB	BB	BB	BB	BB
rs6580315	146.2661	BB	AB	AB	BB	BB	BB	AB	BB
rs723223	146.363	AB	BB	AB	BB	BB	BB	BB	BB
rs3910188	146.55	BB	BB	BB	BB	BB	BB	BB	BB
rs724382	146.55	AB	BB	AB	BB	BB	BB	BB	BB
rs17102282	146.55	BB	AB	AB	BB	BB	BB	AB	BB
rs2163748	146.56	AB	BB	AB	BB	BB	BB	BB	BB
rs9324976	146.56	BB	AB	AB	BB	BB	BB	AB	BB
rs13359856	146.5861	AA	AA	AA	AA	AA	AA	AA	AA
rs10515549	146.67	AA	AA	AA	AA	AA	AA	AA	AA
rs1363556	146.67	BB	BB	BB	BB	BB	BB	BB	BB
rs1368434	146.862	AA	AA	AA	AA	AA	AA	AA	AA
rs340051	147.1864	AA	AA	AA	AA	AA	AA	AA	AA
rs970456	147.2798	AA	AA	AA	AA	AA	AA	AA	AA
rs886949	147.3661	AB	BB	AB	BB	BB	BB	BB	BB
rs758037	147.4216	AA	AB	AB	AA	AA	AA	AB	AA
rs11435	147.4941	BB	BB	BB	BB	BB	BB	BB	BB
rs6881180	147.7117	BB	BB	BB	BB	BB	BB	BB	BB
rs1201598	147.8936	AA	AA	AA	AA	AA	AA	AA	AA
rs9325005	147.9406	AA	AA	AA	AA	AA	AA	AA	AA
rs7718446	147.9511	AA	AA	AA	AA	AA	AA	AA	AA
rs11747475	148.0037	AA	AB	AB	AA	AA	AA	AB	AA
rs4705334	148.0596	AA	AB	AB	AA	AA	AA	AB	AA
rs9986288	148.1111	AA	AA	AA	AA	AA	AA	AA	AA
rs986061	148.2134	AB	AA	AB	AA	AA	AA	AA	AA
rs322487	148.3359	AB	AB	AA	BB	BB	BB	AB	BB
rs2400228	148.4269	BB	AB	AB	BB	BB	BB	AB	BB
rs10068414	148.7726	BB	BB	BB	BB	BB	BB	BB	BB
rs1480158	148.9792	BB	BB	BB	BB	BB	BB	BB	BB
rs720305	149.0082	AB	AB	BB	AA	AA	AA	AB	AA
rs2053030	149.2827	BB	BB	BB	BB	BB	BB	BB	BB
rs31036	149.3485	AA	AB	AB	AA	AA	AA	AB	AA
rs31039	149.3526	AA	AA	AA	AA	AA	AA	AA	AA
rs11956052	149.4635	AA	AA	AA	AA	AA	AA	AA	AA
rs2400293	149.4997	AA	AA	AA	AA	AA	AA	AA	AA
rs17106769	149.7836	AB	AB	AA	BB	BB	BB	AB	BB
rs2194156	149.949	BB	BB	BB	BB	BB	BB	BB	BB
rs1432830	150.03	AA	AA	AA	AA	AA	AA	AA	AA
rs6895278	150.03	AB	AB	AA	BB	BB	BB	AB	BB
rs994025	150.03	BB	BB	BB	BB	BB	BB	BB	BB
rs1025489	150.03	BB	BB	BB	BB	BB	BB	BB	BB
rs11958481	150.03	AB	AB	AA	BB	BB	BB	AB	BB
rs721570	150.03	AA	AB	AB	AA	AA	AA	AB	AA
rs6580511	150.0575	AA	AA	AA	AA	AA	AA	AA	AA
rs2080085	150.0969	AB	BB	AB	BB	BB	BB	BB	BB
rs2303064	150.2711	BB	BB	BB	BB	BB	BB	BB	BB
rs7723153	150.8526	AA	AA	AA	AA	AA	AA	AA	AA
rs888956	151.2071	AB	BB	AB	BB	BB	BB	BB	BB
rs11742884	151.3001	AA	AA	AA	AA	AA	AA	AA	AA
rs12654778	151.3322	AA	AB	AB	AA	AA	AA	AB	AA

rs3857420	151.3429	BB	BB	BB	BB	BB	BB	BB	BB
rs11957757	151.3475	BB	AB	AB	BB	BB	BB	AB	BB
rs4705286	151.4739	AB	AB	AA	BB	BB	BB	AB	BB
rs4705064	151.4904	BB	AB	AB	BB	BB	BB	AB	BB
rs994446	151.5408	AB	AB	BB	AA	AA	AA	AB	AA
rs36077	151.663	AB	AB	BB	AA	AA	AA	AB	AA
rs4147470	151.8035	BB	BB	BB	BB	BB	BB	BB	BB
rs9325132	151.8326	AB	AB	BB	AA	AA	AA	AB	AA
rs1438692	151.9959	AB	AB	BB	AA	AA	AA	AB	AA
rs1623904	152.0083	AB	AB	BB	AA	AA	AA	AB	AA
rs1659091	152.0186	AB	AA	AB	AA	AA	AA	AA	AA
rs10515626	152.0559	AB	AB	BB	AA	AA	AA	AB	AA
rs352336	152.0672	AB	BB	AB	BB	BB	BB	BB	BB
rs813035	152.0842	AB	AB	AA	BB	BB	BB	AB	BB
rs6887452	152.1773	AA	AA	AA	AA	AA	AA	AA	AA
rs2042249	152.1992	BB	AB	AB	BB	BB	BB	AB	BB
rs476741	152.2233	AB	AB	BB	AA	AA	AA	AB	AA
rs2431718	152.2788	AB	AB	BB	AA	AA	AA	AB	AA
rs241280	152.2907	AB	AB	AA	BB	BB	BB	AB	BB
rs7719315	152.3736	AA	AA	AA	AA	AA	AA	AA	AA
rs1056993	152.5058	AB	AB	AA	BB	BB	BB	AB	BB
rs2279132	152.5134	BB	BB	BB	BB	BB	BB	BB	BB
rs6866304	152.5323	AB	AB	AA	BB	BB	BB	AB	BB
rs6874724	152.5568	BB	BB	BB	BB	BB	BB	BB	BB
rs728837	152.5634	AA	AB	AB	AA	AA	AA	AB	AA
rs2061765	152.5841	BB	BB	BB	BB	BB	BB	BB	BB
rs1422429	152.7369	AB	AA	AB	AA	AA	AA	AA	AA
rs10875551	152.7467	AB	AB	BB	AA	AA	AA	AB	AA
rs30825	152.922	AA	AB	AB	AA	AA	AA	AB	AA
rs173438	152.9869	BB	BB	BB	BB	BB	BB	BB	BB
rs245076	153.0062	AB	AA	AB	AA	AA	AA	AA	AA
rs216128	153.1211	AB	AA	AB	AA	AA	AA	AA	AA
rs216148	153.1464	AB	AA	AB	AA	AA	AA	AA	AA
rs216124	153.1833	BB	BB	BB	BB	BB	BB	BB	BB
rs13362413	153.2544	BB	BB	BB	BB	BB	BB	BB	BB
rs4358508	153.4464	AA	AA	AA	AA	AA	AA	AA	AA
rs980272	153.6918	BB	BB	BB	BB	BB	BB	BB	BB
rs919741	153.7473	BB	BB	BB	BB	BB	BB	BB	BB
rs4958445	153.8478	AA	AB	AB	AA	AA	AA	AB	AA
rs888714	155.1285	AA	AA	AA	AA	AA	AA	AA	AA
rs253296	155.1455	BB	BB	BB	BB	BB	BB	BB	BB
rs1560657	155.2285	BB	AB	AB	BB	BB	BB	AB	BB
rs752922	155.2307	BB	BB	BB	BB	BB	BB	BB	BB
rs253345	155.2817	AA	AA	AA	AA	AA	AA	AA	AA
rs33412	155.3186	BB	BB	BB	BB	BB	BB	BB	BB
rs2054440	156.0642	AA	AA	AA	AA	AA	AA	AA	AA
rs2042235	156.0917	BB	AB	AB	BB	BB	BB	AB	BB
rs3828599	156.0999	BB	BB	BB	BB	BB	BB	BB	BB
rs3792783	156.2143	AA	AA	AA	AA	AA	AA	AA	AA
rs3792780	156.2365	AA	AA	AA	AA	AA	AA	AA	AA
rs999556	156.2524	AB	AB	AA	BB	BB	BB	AB	BB
rs17659044	156.3176	AA	AA	AA	AA	AA	AA	AA	AA
rs1012415	156.324	AB	AB	BB	AA	AA	AA	AB	AA
rs10515644	156.3632	AA	AA	AA	AA	AA	AA	AA	AA
rs1862362	156.3733	AB	AB	AA	BB	BB	BB	AB	BB
rs10515645	156.4182	BB	BB	BB	BB	BB	BB	BB	BB

rs448503	156.4499	BB	BB	BB	BB	BB	BB	BB	BB
rs153470	156.5169	AB	AB	AA	BB	BB	BB	AB	BB
rs1991798	156.5608	BB	BB	BB	BB	BB	BB	BB	BB
rs153445	156.6336	AB	BB	AB	BB	BB	BB	BB	BB
rs7717132	156.6359	BB	BB	BB	BB	BB	BB	BB	BB
rs246498	156.6907	AA	AB	AB	AA	AA	AA	AB	AA
rs2431079	156.7806	BB	BB	BB	BB	BB	BB	BB	BB
rs165359	157.0741	BB	BB	BB	BB	BB	BB	BB	BB
rs2053028	157.1602	BB	BB	BB	BB	BB	BB	BB	BB
rs3097775	157.2276	AB	BB	AB	BB	BB	BB	BB	BB
rs2304054	157.2481	BB	BB	BB	BB	BB	BB	BB	BB
rs7736409	157.2942	AB	BB	AB	BB	BB	BB	BB	BB
rs2278383	157.3102	BB	BB	BB	BB	BB	BB	BB	BB
rs1368368	157.3148	BB	AB	AB	BB	BB	BB	AB	BB
rs2033467	157.4529	AA	AB	AB	AA	AA	AA	AB	AA
rs4958487	157.504	AB	AB	AA	BB	BB	BB	AB	BB
rs890832	157.9423	AA	AA	AA	AA	AA	AA	AA	AA
rs302412	158.0749	AA	AB	AB	AA	AA	AA	AB	AA
rs170020	158.0873	AA	AA	AA	AA	AA	AA	AA	AA
rs10515655	158.1718	AA	AB	AB	AA	AA	AA	AB	AA
rs294958	158.261	AA	AB	AB	AA	AA	AA	AB	AA
rs918462	158.3966	AB	AB	BB	AA	AA	AA	AB	AA
rs6875501	158.4333	BB	BB	BB	BB	BB	BB	BB	BB
rs2961739	158.44	BB	BB	BB	BB	BB	BB	BB	BB
rs7702336	158.44	BB	AB	AB	BB	BB	BB	AB	BB
rs1010100	158.44	AA	AB	AB	AA	AA	AA	AB	AA
rs1438940	158.44	AB	AA	AB	AA	AA	AA	AA	AA
rs10515679	158.44	AB	BB	AB	BB	BB	BB	BB	BB
rs1438946	158.44	AB	AA	AB	AA	AA	AA	AA	AA
rs2973139	158.44	AB	AA	AB	AA	AA	AA	AA	AA
rs1871164	158.4698	BB	AB	AB	BB	BB	BB	AB	AB
rs4958655	158.5243	AA	AB	AB	AA	AA	AA	AB	AB
rs578772	158.6071	AB	BB	AB	BB	BB	BB	BB	BB
rs548294	158.6091	AB	AB	AA	BB	BB	BB	AB	AB
rs12522802	158.7019	AA	AA	AA	AA	AA	AA	AA	AA
rs778821	158.74	BB	AB	AB	BB	BB	BB	AB	AB
rs1493383	158.7644	BB	AB	AB	BB	BB	BB	AB	AB
rs1422884	158.7874	AA	AB	AB	AA	AA	AA	AB	AB
rs729329	158.81	BB	BB	BB	BB	BB	BB	BB	BB
rs11741511	158.8493	BB	AB	AB	BB	BB	BB	AB	AB
rs1461234	159.0875	AB	AB	AA	BB	BB	BB	AB	AB
rs286969	159.1845	AA	AB	AB	AA	AA	AA	AB	AB
rs11744052	159.48	BB	BB	BB	BB	BB	BB	BB	BB
rs889032	159.48	AB	AB	BB	AA	AA	AA	AB	AB
rs3886611	159.5015	AA	AB	AB	AA	AA	AA	AB	AB
rs153411	159.5102	BB	BB	BB	BB	BB	BB	BB	BB
rs6870010	159.5186	AB	AA	AB	AA	AA	AA	AA	AA
rs3172941	159.5413	AB	AA	AB	AA	AA	AA	AA	AA
rs10037652	159.5756	BB	BB	BB	BB	BB	BB	BB	BB
rs283438	159.5937	BB	BB	BB	BB	BB	BB	BB	BB
rs9324786	159.6138	BB	BB	BB	BB	BB	BB	BB	BB
rs12374480	159.6701	AA	AA	AA	AA	AA	AA	AA	AA
rs4958756	159.6969	AA	AB	AB	AA	AA	AA	AB	AB
rs13165424	159.881	AA	AA	AA	AA	AA	AA	AA	AA
rs1319694	159.9439	BB	AA	AB	AB	AB	AB	AB	AB

Supplementary Table 3. Coverage distributions and error rates across the targeted homozygosity interval

		IV:4 (proband)	III:1 (father)
	Number of lanes	1	1
	Read Type	Single read	Single read
	Read length	74	74
Targeted homozygosity Interval	Total number of reads (millions)	19.81	19.65
	% mapped to the genome	96.40	96.89
	Interval size (bp)	998,145	998,145
	% mapped to the interval	71.68	75.42
	Mean Coverage	42.57	47.56
	% of bases covered at least 4X	90.60	91.21
	Mean error rate (%)	0.48	0.48
	2^{nd} base error rate (%)	0.51	0.51
	Last base error rate (%)	0.78	0.91
Whole Exome Sequencing	Total number of reads (millions)	88.3	
	% mapped to the genome	92.26	
	% mapped on target	67.29	
	Mean Coverage	91.7	
	% of bases covered at least 4X	97.54	
	Mean error rate (%)	0.43	
	2nd base error rate (%)	0.49	
	Last base error rate (%)	1.22	

Supplementary Table 4. Variants identified within the homozygous linkage interval on chromosome 5

Start (*hg19*)	Genomic Location	Gene	AA_Change	bp_Change	dbSNP	Minor Allele Frequencies
140724060	g.460A>C	PCDHGA3	p.I154L	c.460A>C	rs11575948	C: 12.335% (392 / 3178)
140725828	g.2228C>T	PCDHGA3	p.A743V	c.2228C>T	rs7736541	C: 47.208% (1040 / 2203)
140730384	g.557G>T	PCDHGB1	p.S186I	c.557G>T	rs62378414	T: 2.466% (54 / 2190)
140731022	g.1195A>G	PCDHGB1	p.K399E	c.1195A>G	rs77250251	G: 2.558% (59 / 2306)
140735215	g.448G>A	PCDHGA4	p.A150T	c.448G>A	rs11575949	A: 12.615% (330 / 2616)
140741738	g.2036G>C	PCDHGB2	p. R679P	c. 2036G>C	rs62378417	C: 11.789% (325.155 / 2758)
140741761	g.2059A>G	PCDHGB2	p.K687E	c.2059A>G	rs57735633	G: 12.489% (274 / 2194)
140750044	g.83T>C	PCDHGB3	p.V28A	c. 83T>C	rs6860609	T: 0.086% (3 / 3476)
140751128	g.1167C>G	PCDHGB3	p.N389K	c.1167C>G	rs2240697	C: 46.002% (1398 / 3039)
140751696	g.1735C>A	PCDHGB3	p.P579T	c.1735C>A	rs62620756	A: 3.105% (68 / 2190)
140763029	g.563A>G	PCDHGA7	p.E188G	c.563A>G	rs2072315	A: 46.040% (2215 / 4811)
140789933	g.2164G>A	PCDHGB6	p.A722T	c.2164G>A	rs3749767	A: 30.564% (1343 / 4394)
140953085	g.45,397C>T	DIAPH1	p.Q778*	c.2332C>T	novel	
141019110	g.1318C>A	RELL2	p.L133I	c.397C>A	rs14251	A: 43.414% (3764 / 8670)
141021096	g.9840C>T	FCHSD1	p.P681L	c.2042C>T	rs32957	T: 3.655% (138 / 3776)
141336264	g.1153C>A	PCDH12	p.H385N	c.1153C>A	rs164075	C: 22.988% (2279 / 9914)

Supplementary Table 5. Primary and secondary antibodies used for immunostaining

Primer Antibodies and dilutions	**Company**
Rabbit anti-Cux1 (1:250)	Santa Cruz-sc13024
Rat anti-Ctip2 (1:250)	Santa Cruz-sc56014
Rabbit anti-TBR1 (1:500)	Abcam-ab31940
Mouse anti beta-cathenin (1:250)	BD Laboratories-610153
Rabbit anti-N-cadherin (1:250)	Santa Cruz-sc7939
Mouse anti E-cadherin (1:250)	BD Laboratories-610405
Alpha-Tubulin FITC-conjugated (1:500)	Sigma-F2168
Rabbit anti-gamma-tubulin (1:250)	Sigma-T5192
Texas Red®-X Phalloidin (1:500)	Invitrogen-T7471
Rabbit anti-GABA (1:5000)	Sigma-A2052
Rabbit anti-Pax6 (1:300)	BD Pharmingen-61462
Secondary Antibodies	
Alexa Fluor® 488 Donkey Anti-Mouse IgG (H+L) (1:1000)	Invitrogen-A21202
Alexa Fluor® 488 Donkey Anti-Rabbit IgG (H+L) (1:1000)	Invitrogen-A21206
Alexa Fluor® 488 Donkey Anti-Rat IgG (H+L) (1:1000)	Invitrogen-A21208
Alexa Fluor® 594 Donkey Anti-Rabbit IgG (H+L) (1:1000)	Invitrogen-A21207
Alexa Fluor® 594 Donkey Anti-Mouse IgG (H+L) (1:1000)	Invitrogen-A21203

Supplemental References

El Mouzan, M. I., Al Salloum, Abdullah A., Al Herbish, Abdullah S., Quarashi, Mansour M., Al Omar., Ahmad A. (2008). Health Profile for Saudi Children and Adolescents (No. AR-20-63) (Riyadh, King Abdulaziz City for Science & Technology), pp. 96.

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