The genetic landscape of familial congenital hydrocephalus

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ABSTRACT

Objective: Congenital hydrocephalus is an important birth defect, the genetics of which remains incompletely understood. To date, only four genes are known to cause Mendelian diseases in which congenital hydrocephalus is the main or sole clinical feature, two X-linked (*L1CAM* and *AP1S2*) and two autosomal recessive (*CCDC88C* and *MPDZ*). In this study, we aimed to determine the genetic etiology of familial congenital hydrocephalus with the assumption that these cases represent Mendelian forms of the disease.

Methods: Exome sequencing combined, where applicable, with positional mapping.

Results: We identified a likely causal mutation in the majority of these families (21/27, 78%), spanning 16 genes, none of which is X-linked. Ciliopathies and dystroglycanopathies were the most common etiologies of congenital hydrocephalus in our cohort (19% and 26%, respectively). In one family with four affected members, we identified a homozygous truncating variant in *EML1*, which we propose as a novel cause of congenital hydrocephalus in addition to its suggested role in cortical malformation. Similarly, we show that recessive mutations in *WDR81*, previously linked to cerebellar ataxia, mental retardation, and dysequilibrium syndrome 2, cause severe congenital hydrocephalus. Furthermore, we confirm the previously reported candidacy of *MPDZ* by presenting a phenotypic spectrum of congenital hydrocephalus associated with five recessive alleles.

Interpretation: Our study highlights the importance of recessive mutations in familial congenital hydrocephalus and expands the locus heterogeneity of this condition.

INTRODUCTION

Hydrocephalus is defined as an active distension of the ventricular system of the brain due to inadequate passage of cerebrospinal fluid from its point of production within the cerebral ventricles to its point of absorption into the systemic circulation [1]. Hydrocephalus is a highly morbid condition if not managed appropriately to relieve the central nervous system from the building pressure caused by a distended ventricular system. Environmental and genetic factors are known to play a role in the etiology of hydrocephalus although their relative contribution varies with age. For example, whereas infections are the leading cause of hydrocephalus in children, single gene mutations are most commonly associated with congenital (prenatal) hydrocephalus [2].

Congenital hydrocephalus is a severe birth defect affecting 4.65 per 10,000 births and is associated with high morbidity and mortality [3, 4]. Although many syndromes can be associated with congenital hydrocephalus, there are surprisingly very few genes that are known to cause the disease as the sole or primary clinical feature [5]. *L1CAM* (MIM 308840) is the best known disease gene in congenital hydrocephalus with an estimated contribution to as many as 30% of suspected X-linked cases [6]. This gene has been linked to a number of neurological phenotypes with or without congenital hydrocephalus, in addition to non-syndromic congenital hydrocephalus [7]. *AP1S2* (MIM 300629) is another X-linked gene that has been found to be mutated in patients with congenital hydrocephalus, including those with phenotypes once proposed to be allelic to *L1CAM*-related disorders [8, 9]. Much less is known about the autosomal contribution to congenital hydrocephalus. In 2010, Ekici et al reported a homozygous truncating mutation in *CCDC88C* in a consanguineous multiplex family, a finding that was independently confirmed by a subsequent report [10, 11]. In 2013, we reported the identification of a novel locus

in two families with autosomal recessive congenital hydrocephalus [12]. A founder homozygous truncating variant in *MPDZ* was identified in both families. No independent confirmation has been published to date to confirm the candidacy of *MPDZ*.

Despite the identification of these four genes, the genetic landscape of congenital hydrocephalus remains largely unexplored [13]. Previous studies have mostly targeted *L1CAM* sequencing in cohorts of patients with congenital hydrocephalus [6, 14, 15]. Pursuing Mendelian causes of congenital hydrocephalus is important because they can expand our knowledge of the molecular underpinning of the disease. It is also a pre-requisite for the proper interpretation of genome sequencing in patients with congenital hydrocephalus with the attendant benefit of precise recurrence risk estimate. In this study, we report on the genomic analysis of a relatively large cohort of families with recurrence of congenital hydrocephalus. Our results reveal marked genetic heterogeneity, which we further expand by proposing *EML1* and *WDR81* as bone fide disease genes in congenital hydrocephalus.

MATERIALS AND METHODS

Human Subjects

The cohort described in the study comprises families with at least two children who were diagnosed with congenital hydrocephalus. Informed consent was obtained from all study participants who were enrolled under an IRB-approved research protocol (KFSHRC RAC# 2080 006 and 2121 053). Additionally, we include three previously unpublished patients each with a biallelic mutation in *MPDZ* diagnosed by clinical exome sequencing. Informed consent was

obtained to publish their clinical data by their respective institutions (Cincinnati Children's Hospital and The Hospital for Sick Children in Toronto).

Genomic Analysis

All patients recruited through KFSHRC underwent autozygosity mapping irrespective of the given history of consanguinity to investigate potentially unrecognized relatedness given the high degree of inbreeding in Saudi Arabia. DNA samples from the all the affected patients were genotyped using Axiom SNP chip array following the manufacturer's instructions. This was followed by genomewide autozygome analysis using runs of homozygosity >2Mb as surrogates of autozygosity, as determined by AutoSNPa[16]. In parallel, the previously described "Mendeliome assay" was applied to search for likely causal variants in previously reported ciliopathy genes [17]. When negative, exome sequencing was pursued as follows. Exome capture was performed using TruSeq Exome Enrichment kit (Illumina) following the manufacturer's protocol. Samples were prepared as an Illumina sequencing library, and in the second step, the sequencing libraries were enriched for the desired target using the Illumina Exome Enrichment protocol. The captured libraries were sequenced using Illumina HiSeq 2000 Sequencer. The reads were mapped against UCSC hg19 (http://genome.ucsc.edu/) by BWA (http://bio-bwa.sourceforge.net/). The SNPs and Indels were detected by SAMTOOLS (http://samtools.sourceforge.net/). Variants from WES were filtered such that only novel (or very low frequency <0.1%), coding/splicing, homozygous variants that are within the autozygome of the affected individual and are predicted to be pathogenic were considered as likely causal variants [18, 19]. Frequency of variants was determined using publically available variant databases (1000 Genomes, Exome Variant Server, ExAC and gnomAD) as well as a database of 4,577 in-house ethnically-matched exomes and gene panels. Pathogenicity was

likely if the mutation is loss-of-function (splicing/truncating) or, in the case of missense/in-frame indels, replaces a highly conserved amino acid and is predicted to be pathogenic by the three in-silico prediction modules PolyPhen, SIFT and CADD. All reported variants in this study have been Sanger validated and their segregation with the disease confirmed in the respective families.

RESULTS

Congenital Hydrocephalus is Genetically Heterogeneous

A total of 27 families with recurrent congenital hydrocephalus were recruited (see Table S2 for clinical details and Figure S1 for pedigrees). Genomic analysis revealed a likely causal variant in the majority (21/27, 78%) of cases (Table S1). No mutations in *L1CAM* or *AP1S2* were identified even though six families only had affected males (see Figure S1, and Figure 2 for all family pedigrees). Dystroglycanopathies was the most common disease category accounting for 26% of cases (7 families, 7 mutations in 6 genes). As shown in Table S2, most of these cases were suspected clinically by the finding of elevated serum creatinine kinase (CK). Ciliopathies was the second most commonly diagnosed category accounting for 19% of cases (5 families, 5 mutations in 5 genes). The ciliopathy cases met the diagnostic criteria of Meckel-Gruber syndrome (n=4) or Acrocallosal syndrome (n=1).

Less known causes of congenital hydrocephalus identified included the recently identified *KIAA1109*-related Alkuraya-Reymond syndrome in families 12 and 19 with congenital hydrocephalus and severe arthrogryposis (revision in preparation), and *PLAT*-related hydranencephaly and diaphragmatic hernia[18].

Mutations in *EML1* and *WDR81* Cause Congenital Hydrocephalus

A homozygous truncating variant was identified in *EM1L* in family 22 IV:7 (Figure 1A). This is a 2yr old girl with congenital hydrocephalus, profound global developmental delay, intractable epilepsy, and positive family history (Figure 1 and Table S2). Brain MRI showed extensive polymicrogyria, band heterotopia, agenesis of corpus callosum, dilated lateral ventricles, compressed tentorium cerebelli and herniated cerebellar tonsil (Figure 1B-G). Exome sequencing and homozygosity mapping revealed a nonsense mutation in *EML1* (NM_004434.2:c.1567C>T, p. (Arg523*) (Figure 1H). The variant introduces a premature stop codon in the two known NCBI Reference Sequence protein-coding transcripts (NM_004434.2 and NM_001008707.1) and has a CADD score of 37. It is absent in 4,577 ethnically matched exomes and panels and ExAC as well as gnomAD database. Sanger sequencing confirmed the segregation of the variant in all the unaffected siblings in the family. The variant is located at the end of the N-terminal ß-propeller domain of the protein and is predicted to cause loss of the largest part of C-terminal ß-propeller (Figure 1 H).

WDR81 was found to be mutated in two families with severe congenital hydrocephalus (Figure 2 A). Family 13 consists of a consanguineous couple who lost two pregnancies with severe hydrocephalus and cerebellar hypoplasia (Figure 2 B and C). A homozygous truncating mutation was identified in *WDR81* NM_001163809.1: c.3286C>T, p. (Gln1096*) (Figure 2F). Family 26 also consists of a consanguineous couple with history of a stillbirth diagnosed antenatally with massive hydrocephalus and absent cerebellum. Similar findings were detected on antenatal ultrasound of the subsequent pregnancy that resulted in a male neonate with severe hydrocephalus and Dandy-Walker malformation (Figure 2D and E). A homozygous missense variant in *WDR81* was identified NM_001163809.1:c.845G>A, p. (Gly282Glu) (Figure 2F) that

is completely absent in ExAC, gnomAD and 4,577 Saudis with high pathogenicity scores (SIFT: deleterious (0), PolyPhen: probably damaging (1), CADD (26)).

Confirming *MPDZ* as a bona fide Congenital Hydrocephalus Disease Gene

Three families, in our cohort harbored the founder *MPDZ* truncating variant NM_003829.3:c.628C>T, p. (Gln210*) (Table S1). We estimated the carrier frequency of this mutation at 0.00044 (2 among 4,575 screened), which probably explains why this was the single most common founder mutation in our cohort. Representative clinical images of the affected members in families 4 and 24 with *MPDZ* mutation are shown in Figure 3A and 3B. Given the lack of a second mutation in *MPDZ* in our cohort, we initiated an international collaboration to seek additional pathogenic alleles and determine their clinical consequences. This collaboration uncovered three previously unpublished cases that are described below:

Family 1 (The Hospital for Sick Children in Toronto (SICK F1)): The proband is a 2.5 year old girl born to a consanguineous Palestinian couple. Antenatal U/S revealed mild hydrocephalus, polyhydramnios, and a two-vessel cord. Postnatal MRI showed dilatation of the lateral ventricles and the third ventricle, and bilateral subependymal grey-matter heterotopia. There was a soft tissue structure obliterating the cerebral aqueduct (Figure 3 C and D). Past-medical history is also notable for a moderate ASD, right sided congenital diaphragmatic hernia and macular hypoplasia. Fine motor skills and language are nearly within the normal range, but she had delayed motor development. Dysmorphic features include frontal bossing, facial asymmetry, a bulbous nose, persistent fetal pads and striking creases over her knees. Clinical exome sequencing revealed a novel homozygous one base pair deletion in *MPDZ* with resulting frameshift and premature truncation NM_001330637.1: c.4469delA, p. (Gln1490Argfs*19). The parents were confirmed heterozygous carriers of the mutation.

Family 2 (The Hospital for Sick Children in Toronto (SICK F2))): An 8 year old male was referred to clinical genetics for multiple congenital anomalies (foveal dysplasia, portal vein thrombosis, lung hypoplasia, aberrant subclavian artery, malrotation of the gut, and sensorineural hearing loss). Parents are non-consanguineous of Scottish/Irish/Dutch descent with negative family history. CT of chest at age 4m showed absence of the right upper lobe bronchus and small unilobar right lung. Eye evaluation at age 3yrs showed foveal dysplasia, thin inner retina, and bilateral lacrimal ducts stenosis. On examination at age 5.5 years, the following were noted: two posterior hair whorls, downslanting palpebral fissures, posteriorly rotated ears, microretrognathia, small teeth, joint hypermobility and normal neurological examination. MRI brain showed small olfactory bulbs, minimal dilatation of the lateral brain ventricles which have squared frontal horns and dilated occipital horns, multiple bilateral foci of subependymal nodular grey matter heterotopia, an enlarged massa intermedia and possibly accessory commissures in the third ventricle (Figure 3E and F). Clinical exome sequencing revealed two novel heterozygous variants in *MPDZ* that confirmed to be inherited in trans: (NM_001330637.1:c.2230C>T, p. (Arg744*) and c.3211C>T, p. (Arg1071*).

Family 3 (Cincinnati Children's (CIN F1)): a 15 months old male referred from Kuwait for liver transplant evaluation. He was a product of full term pregnancy but was born small for gestational age (birth weight 2450 grams). He had prolonged NICU course due to cholestatic jaundice and liver biopsy was consistent with progressive familial intrahepatic cholestasis. He was also found to have a small left multicystic dysplastic kidney. His echocardiography was normal. Parents are first cousins and he has a healthy sister. On initial physical examination (15 months old) he was noted to have relative macrocephaly and a large full anterior fontanelle. He also had bilateral iris coloboma, prominent optic nerve, hypotonia and reduced reflexes. Brain

MRI revealed enlarged third and lateral ventricles and extra-axial spaces, and increased signal intensity within the central tegmental tracts and frontal horns which may represent areas of localized hypomyelination or gliosis (Figure 3G and H). Although NGS panel revealed a likely cause of his cholestasis (homozygous *TJP2* NM_001170416.1: c.570_574dup, p. (Leu192Profs*3)), we hypothesized that he has another distinct neurological disorder and proceeded with clinical exome sequencing, which revealed, in addition to the pathogenic variant in *TJP2*, a novel homozygous variant in *MPDZ* gene (NM_001330637.1: c.5278 G>A, p.(Ala1760Thr)). The variant is absent in ExAC and predicted to be pathogenic (PolyPhen: probably damaging (0.948), SIFT: deleterious (0.01) and CADD 34).

DISCUSSION

Although congenital hydrocephalus is known to be enriched for genetic causes compared to other forms of pediatric hydrocephalus, little is known about the magnitude of this genetic contribution. Small family size in the studied populations greatly limits recurrence even in Mendelian forms of the disease. Furthermore, until recently only *L1CAM* has been known to cause non-syndromic congenital hydrocephalus so studies on the genetics of the disease have been largely limited to investigating sequence variants in this gene [20]. To our knowledge, our study is the first to take a comprehensive genomic approach in the investigation of a cohort of congenital hydrocephalus selected only on the basis of recurrence to enrich for Mendelian causes.

Consistent with our prior experience in the study of genetics of recessive diseases [21-26], we found a high hit rate of exome sequencing in the study cohort. Although recurrence in our cohort was

not selected on the basis of autosomal recessive inheritance (some families had male-only affected members), our analysis did not reveal any X-linked mutations. This is surprising when one considers the reports of up to 74% of congenital hydrocephalus in families with male recurrence caused by *L1CAM* mutations [6]. This may be explained by the significant contribution of autosomal recessive mutations to the etiology of recurrent congenital hydrocephalus in our cohort. In addition, we note that the strictly familial nature of our cohort limits the contribution of de novo *L1CAM* mutations, although these account for a very small percentage of previously reported mutations [15].

EML1 encodes a microtubule-binding protein that influences planar polarity. It has been shown to determine the mitotic plate angle of neuroprogenitors in relation to the periventricular germinal zone protein, and that its deficiency causes abnormal migration pattern with resulting band heterotopia in mouse [27]. In that same study, two missense, likely hypomorphic variants in *EML1* were identified in two patients with band heterotopia [27]. The family in this study has, in addition to band heterotopia, severe congenital hydrocephalus in several affected members. We posit that the severe, potentially null, nature of the mutation resulted in a much more severe migration disorder and abnormal posterior fossa configuration with resulting "distal" hydrocephalus. Thus, not only does our study provide the first independent confirmation of *EML1* involvement in band heterotopia in humans, but it also expands the phenotype to also include severe congenital hydrocephalus.

Similarly, our study expands the phenotypes associated with *WDR81* to also include severe congenital hydrocephalus with cerebellar hypoplasia. In 2011, Gulsuner and colleagues reported a single consanguineous family with quadrupedal locomotion, mental retardation, and cerebro-cerebellar hypoplasia (CAMRQ) in which they identified a homozygous missense variant in

WDR81 [28]. Although this association has not been independently confirmed by follow up reports in humans, this finding helped in the mapping of an N-ethyl-N-nitrosourea-induced mutant mouse with Purkinje fiber degeneration to a missense variant in *WDR81* [29]. Unlike the mutation reported in the context of CAMRQ (P856L) and the mouse mutation (L1349P), we report the first truncating mutation Gln45*, which may explain the much more severe phenotype we observed in this family. Surprisingly, the same severe phenotype was observed in the other family we report with a homozygous missense variant, which suggests that this variant is an equally severe loss of function mutation. The independent identification of likely causal mutations in *WDR81* in two families with a strikingly similar phenotype comprising a lethal form of congenital hydrocephalus with cerebellar hypoplasia strongly support the notion that *WDR81* is a bone fide autosomal recessive disease gene for this phenotype.

We have originally reported *MPDZ* in two families (one with long term survival to adulthood) with a remarkably consistent phenotype comprising severe congenital hydrocephalus with variable penetrance of heterotopia, coloboma and ASD [12]. Lack of subsequent reports and the suggestion that *MPDZ* mutation may result in an eye-limited phenotype cast doubt on the original link to congenital hydrocephalus [30]. However, we show in this study that the founder mutation in *MPDZ* is the most commonly encountered mutation in familial congenital hydrocephalus, consistent with its relatively high carrier frequency. More importantly, we report three additional patients each with a novel biallelic variant in *MPDZ* that is likely deleterious and they all share the core feature of hydrocephalus, albeit of variable severity. Patient 1 also features coloboma, strabismus and ASD, and Patient 2 features coloboma that we reported at variable penetrance in the original families. In addition, all additional families appear to share

some degree of heterotopia, which suggests that this is an important clinical consequence of *MPDZ* mutation in humans.

Our study is not designed to be generalizable to all forms of congenital hydrocephalus. However, it seems reasonable to recommend that a prenatally diagnosed hydrocephalus should be aggressively investigated for the possibility of a Mendelian cause. Exome sequencing of these cases is warranted given the broad differential diagnosis and the high yield of this diagnostic tool in the setting of Mendelian forms of the disease. While others have recommended *L1CAM* sequencing in all cases of congenital hydrocephalus, we believe the decreasing cost of exome sequencing and the genetic heterogeneity of the condition warrant considering exome sequencing as the first-tier test in these patients. The unsolved cases in our series may represent non-Mendelian familial aggregation [31]. Alternatively, they may harbor challenging classes of Mendelian mutations that evaded detection using our standard filtering strategy and these will be subject to deeper scrutiny in the near future.

In summary, our results show a remarkably high contribution of recessive mutations to familial congenital hydrocephalus that clearly surpass that of *L1CAM*, at least in our study population. We provide independent confirmation of *MPDZ* as a bona fide congenital hydrocephalus disease gene, and expand the phenotype of *EML1* and *WDR81* to also include congenital hydrocephalus in severe cases. We encourage the use of exome sequencing to investigate an underlying genetic cause of congenital hydrocephalus, especially in familial cases.

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AUTHOR CONTRIBUTION

RS and FSA Contributed to the conception and design of the study; RS, MAS, NP, NE, WK, IA, SS, EA, MZS, AA, SM, NI, FA, MH, TA, RA, EA, BS, BA, WA, NA, HL, RJH, RD, RML, HA, GY, EF and FSA contributed to the acquisition and analysis of data; RS, MAS, NE, HL, RJH, RD, RML, HA, GY, EF and FSA contributed to drafting the text and preparing the figures.

POTENTIAL CONFLICT OF INTEREST

None to declare

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FIGURE LEGENDS

Figure 1: A) Pedigree of family 22 showing the consanguinity between the parents. Red asterisks indicate individuals from whom DNA was available for testing mutation segregation. B) Facial picture to the index in family 22 with mutation in *EML1* showing the macrocephaly, and plagiocephaly. C-G) MRI images of the index in family 22 showing band heterotopia (white arrows), polymicrogyria (red arrows) and hydrocephalus (blue arrows). H) Upper panel: Diagram of *EML1* and sequence chromatogram of the nonsense mutation with the parent tracing shown for comparison (sequence differences are indicated by black asterisks, red arrows indicate the sites of the mutations). Lower panel: schematic of EML1 protein showing the position of the early stop codon (yellow triangle) upstream the large part of the C-terminal ß-propeller domain (Protein domains and positions was based on PMID:24706829).

Figure 2: A) Pedigree of families 13 and 26 showing their consanguineous nature. Red asterisks indicate individuals from whom DNA was available for segregation analysis. B and C) Ultrasound images for the affected member in family13 with the nonsense mutation in *WDR81* showing massive ventriculomegaly (B) and dilated posterior fossa (C). D and E) Ultrasound and CT scan images for the affected member in family 26 with missense mutation in *WDR81* showing hydrocephalus (blue arrows), absent cerebellum and abnormal brain tissue. F) Upper panel: Diagram of *WDR81* and sequence chromatogram of the two mutations in this gene with the control tracing for comparison (sequence differences are indicated by black asterisks; red arrows indicate the sites of the mutations). Lower panel: schematic of WDR81 protein showing the position of the early stop codon (yellow triangle) upstream the seven WRD repeat domains of this protein (protein domains and positions were based on the database uniprot.org).

Figure 3: A-H representative clinical images to the affected members with mutation in *MPDZ* A) T2 Axial MRI image for the affected member in family 4 showing massively enlarged lateral ventricles (red arrow) and simplified gyration pattern (white arrow). B) Ultrasound image for the affected member in family 24 showing the severe ventriculomegaly (red arrow) and signs of holoprosencephaly. C and D) are Axial FLAIR and sagittal T1 of the brain MRI images for patient in SICK F1 showing moderate dilatation of the lateral ventricles with extensive nodular gray matter heterotopia (red arrow) along the ependyma of the lateral ventricles bilaterally which is most confluent at the trigones and occipital horns. There is no midline shift or Chiari malformation. There is some fusion of the forniceal columns and a prominent massa intermedia with some fusion of the thalami also suspected. E and F) Brain MRI images for patient in SICK F2 showing dilatation of the lateral ventricles with extensive nodular gray matter heterotopia (red arrow) noted along the ependyma of the lateral ventricles bilaterally which is most confluent at the trigones and occipital horns. A thin linear band in the midline appears to extend from the prominent mass intermediate to the proximal aqueduct (blue arrow). G and H) Brain MRI images for patient in CIN F1 showing enlarged lateral ventricles. I): Upper panel: Diagram of MPDZ where red arrows indicate the sites of the five with five recessive alleles. Lower panel: schematic of MPDZ protein showing the showing the location of the mutations (yellow triangle) (protein domains and positions were based on the database uniprot.org).

Table S1: Genomic data for the Saudi families included in this study.

Table S2: Clinical summaries for the Saudi families included in this study that the likely causative mutation has been identified and their diagnosis were established.

Table S3: Pedigree of the Saudi families included in this study showing the consanguinity of the parents. Red asterisks indicate individuals from whom DNA was available for testing mutation segregation.

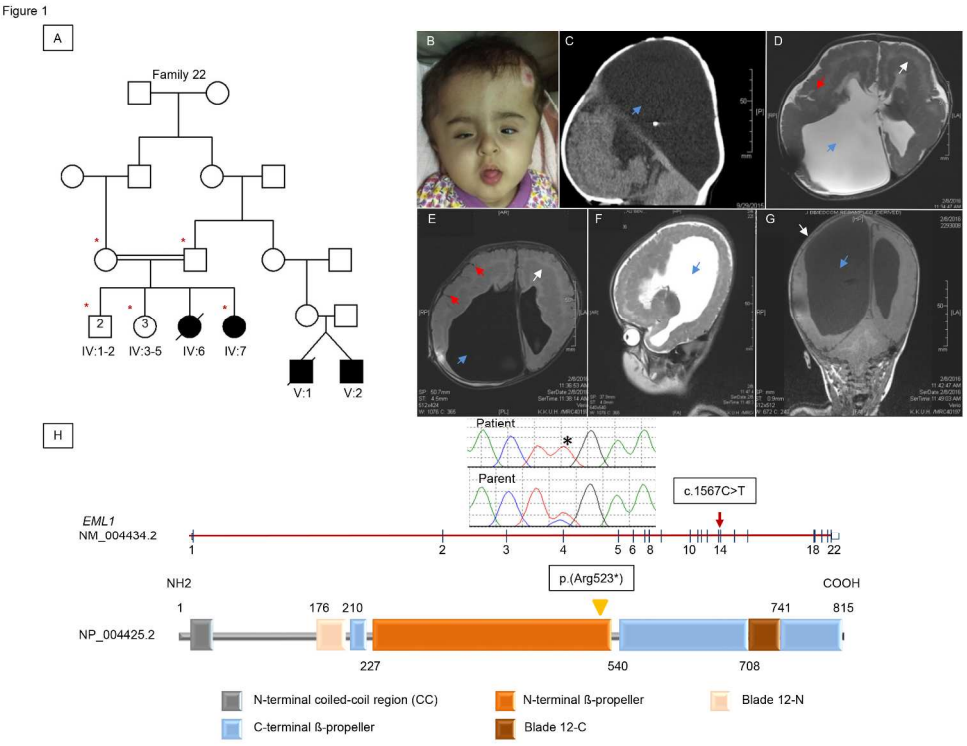

Figure 1

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Figure 2

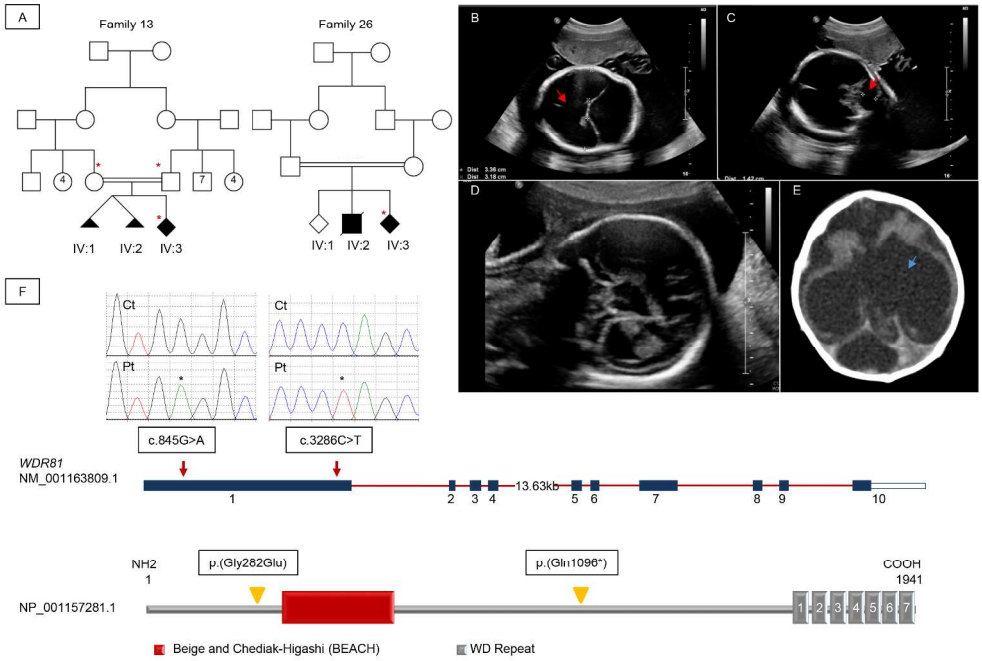

Figure 2

254x190mm (300 x 300 DPI)

Figure 3

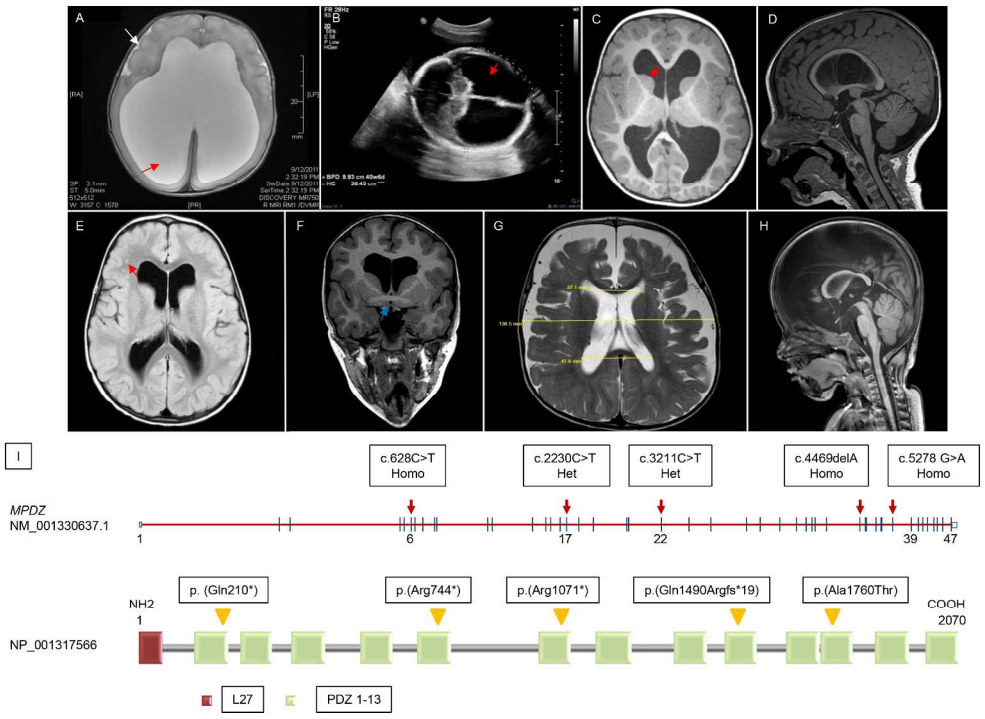

Figure 3

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