An Amish founder variant consolidates disruption of CEP55 as a cause of hydranencephaly and renal dysplasia

Lettie E Rawlins[1,2], Hannah Jones[1], Olivia Wenger[3], Myat Aye[1], James Fasham[1,2], Gaurav V Harlalka[1], Barry A Chioza[1], Alexander Miron[4], Sian Ellard[1], Matthew Wakeling[1], Andrew H Crosby[1] and Emma L Baple[1,2].

1. Medical Research, RILD Wellcome Wolfson Centre, University of Exeter Medical School, Royal Devon & Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW, UK
2. Peninsula Clinical Genetics Service, Royal Devon & Exeter Hospital (Heavitree), Gladstone Road, Exeter, EX1 2ED, UK
3. New Leaf Center, Clinic for Special Children, PO Box 336, 15988B East Chestnut Street, Mount Eaton, Ohio 44659, US
4. PlexSeq Diagnostics, 11000 Cedar Avenue, Suite 290, Cleveland, OH 44106, US

Correspondence:

Emma Baple E.Baple@exeter.ac.uk

Clinical Senior Lecturer (Genomic Medicine)

Consultant in Clinical Genetics

Medical Research (Level 4),

RILD Wellcome Wolfson Centre,

Royal Devon & Exeter NHS Foundation Trust,

Barrack Road, Exeter, EX2 5DW, UK

And

Andrew Crosby A.H.Crosby@exeter.ac.uk

Professor of Human Genetics

Medical Research (Level 4),

RILD Wellcome Wolfson Centre,

Royal Devon & Exeter NHS Foundation Trust,

Barrack Road, Exeter, EX2 5DW, UK

Running Title: CEP55 causing hydranencephaly and renal dysplasia

Abstract

The centrosomal protein-55 kDa (CEP55 [OMIM 610000]) plays a fundamental role in cell cycle regulation and cytokinesis. However, the precise role of CEP55 in human embryonic growth and development is yet to be fully defined. Here we identified a novel homozygous founder frameshift variant in *CEP55*, present at low frequency in the Amish community, in two siblings presenting with a lethal fetal disorder. The features of the condition are reminiscent of a Meckel-like syndrome comprising of Potter sequence, hydranencephaly and cystic dysplastic kidneys. These findings, considered alongside two recent studies of single families reporting loss of function candidate variants in *CEP55*, confirm disruption of CEP55 function as a cause of this clinical spectrum and enable us to delineate the cardinal clinical features of this disorder, providing important new insights into early human development.

Key words: CEP55; hydranencephaly; renal dysplasia; Potter sequence; Meckel syndrome; Meckel-like; whole exome sequencing

Introduction

CEP55 is a centrosome- and midbody-associated protein that has been shown to play a central role in cell cycle regulation and is recognised as a key protein involved in the abscission process, the final stage of cytokinesis. (1-3). CEP55 facilitates abscission through the recruitment of two ESCRT (endosomal sorting complex required for transport)-I subunit associated proteins to the midbody: tumor susceptibility gene 101 (TSG101) and apoptosis-linked gene 2 interacting protein X (ALIX) (4). The transcribed CEP55 centrosomal protein has three central coiled-coil domains and is expressed at the perinuclear membrane, cytoplasm, and nucleus (2, 5).

Recently candidate homozygous nonsense variants in *CEP55* have been identified in five fetuses from two families in association with a lethal disorder, the features of which include dysplastic kidneys and complex brain malformations (6, 7). Homozygous *cep55l* knockout mutant zebrafish display a significant reduction in the size of brain structures, and a decreased number of renal tubules, consistent with the human phenotype (6).

Here we describe a novel Amish homozygous founder frameshift *CEP55* variant in two affected Amish fetuses presenting with hydranencephaly and Potter sequence secondary to cystic renal dysplasia and anhydramnios.

Materials and methods

Samples were taken with informed consent (University of Arizona protocol 10-0050-01) for DNA extraction. SNP genotyping was performed using the HumanCytoSNP-12 v2.1 beadchip array (Illumina). Whole exome sequencing (WES) analysis (NextSeq500: Illumina) involved: Agilent Sureselect Whole Exome v6 targeting, read alignment (BWA-MEM (v0.7.12), mate-pairs fixed and duplicate removal (Picard v1.129), InDel realignment / base quality recalibration (GATK v3.4-46), SNV/ InDel detection (GATK HaplotypeCaller),

annotation (Alamut v1.4.4) and read depth (GATK DepthOfCoverage). Dideoxy sequencing was undertaken using standard techniques. The *CEP55* variant (NM_018131.4: c.514dup; p.(Ile172Asnfs*17) and associated phenotype data was submitted to ClinVar (www.ncbi.nlm.nih.gov/clinvar, accession SCV000808984).

Results

Subjects

An Ohio Amish couple, distantly related through a 4th generation common ancestor (Figure 1B), presented in their second pregnancy with dichorionic diamniotic twins, one male (twin A) and one female (twin B). Antenatal ultrasound scanning (USS) undertaken at 21+2 weeks gestation revealed twin B to have hydranencephaly, a multicystic dysplastic right kidney (the left kidney was not visualised), shortened bowed long bones and anhydramnios. Twin A was found to have bilateral renal pelvis dilatation, but no further abnormalities were detected. A subsequent USS undertaken at 28 weeks gestation to further examine twin B revealed intrauterine growth restriction (IUGR) and pericardial effusion, the bladder and stomach were not identified. The twins were born following an uncomplicated spontaneous vaginal delivery at 42 weeks gestation. Twin B survived for 90 minutes after birth; the clinical features of Potter Sequence with cutaneous syndactyly were documented (Figure 1C). The couple had previously experienced a stillbirth of a male fetus at 42 weeks gestation. The fetus presented with multiple fetal anomalies at 19 weeks gestation. Anomalies included: IUGR, anhydramnios, hydranencephaly, described as ‘hydrocephalus seen throughout the cranium’, and bilateral hydronephrosis. The fetal birth weight was 3lb 14oz and features of Potter sequence, bilateral lower limb bowing, talipes and syndactyly were identified. (Figure 1C). Karyotyping of placental samples was normal but post-mortem (PM) examination was declined for both fetuses. Assuming that a founder variant was responsible for the condition,

we used a combination of autozygosity mapping and whole exome sequencing to study this novel syndrome and identify the underlying molecular cause.

Genetic studies

Whole genome SNP genotyping of both twins identified a number of homozygous genomic regions particular to the affected individual, the largest of which was a 20Mb region of 10q22.3-q24.1 (rs1769756- rs7081796, chr10:g.79164647-99204526 [hg38]). Subsequent whole exome sequencing of DNA from the affected twin excluded previously described Amish founder variants associated with cystic kidney disease, including *NPHP3* (8). Rare variants predicted to have a functional consequence were cross-referenced with SNP genotyping data, identifying a single candidate homozygous variant of relevance to the phenotype. This variant (NM_018131.4: c.514dup; p.(Ile172Asnfs*17), chr10:g.93507042dup, [hg38]) in *CEP55,* predicted to result in a premature stop, is within the chromosome 10 locus. It is present in a single heterozygote in gnomAD and not listed in ClinVar, NCBI or HGMDpro databases. Dideoxy sequencing confirmed the presence and cosegregation of the variant (Figure 1B). 179 samples from healthy Amish adults were analysed and seven heterozygous carriers were identified, corresponding to an estimated allele frequency of 0.02 in this population.

Discussion

This is the third reported family with likely homozygous loss of function variants in *CEP55* identified in association with a lethal fetal disorder (comparison of cases; Table 1). Frosk *et al.* reported a family with Dutch-German Mennonite ancestry and three affected fetuses homozygous for a *CEP55* nonsense variant (NM_018131.4) c.1274C>A; p.Ser425* presenting with dysplastic kidneys hydraencephaly, cerebellar hypoplasia and multinucleated

neurones at PM (6). The authors termed this disorder MARCH syndrome (multinucleated neurons, anhydramnios, renal dysplasia, cerebellar hypoplasia and hydranencephaly syndrome [OMIM 236500]) and highlighted an additional nine cases in the literature with phenotypic overlap including features of hydranencephaly, renal dysplasia and syndactyly (9-13). However, as far as we are aware, *CEP55* genetic analysis has not been undertaken in these individuals. Interestingly in two cases neuropathological PM findings identified multinucleated neurons (9, 10). Bondeson *et al.* reported a Swedish couple with two affected fetuses homozygous for *CEP55* c.256C>T; p.Arg86* (NM_018131.4) with features in one including: hydranencephaly, enlarged cystic kidneys, oligohydramnios and cystic hygroma (7). The second fetus had a slightly different phenotype comprising occipital encephalocele, cerebral cyst and cystic hygroma, the kidneys were severely degraded due to fetal autolysis. PM examination was only possible for one fetus and, although neither were identified to have polydactyly or liver abnormalities, the authors classified the combination of clinical features as a Meckel-like syndrome.

All seven cases described with biallelic *CEP55* variants display phenotypical similarity, with renal dysplasia or cysts resulting in oligohydramnios and Potter sequence, and central nervous system (CNS) abnormalities comprising hydranencephaly or cerebral cysts, as the cardinal clinical features (Table 1). Interestingly, as noted by Bondeson *et al*., the presence of these two congenital anomalies fulfils two of the characteristic Meckel syndrome (MKS) clinical triad (7). However, in the absence of polydactyly, none of the seven cases described to date fulfil the triad. Although PM examination was undertaken in only three cases, hepatic ductal plate malformation, a frequent finding in classical MKS, was not identified. Occipital encephalocele, the most frequent CNS abnormality in MKS (83.8% of cases) (14), was documented in one fetus (7). A spectrum of other CNS abnormalities have been described in MKS cases, including cerebral cysts and hydrocephalus, of which

hydranencephaly can be considered a severe form. Several other features reported in MKS were identified in this patient cohort, including syndactyly, hydronephrosis, and short bowed limbs. In view of the wide variability seen in MKS, the lack of diagnostic criteria, and the phenotypic overlap with CEP55 patients, this disorder should be considered a MKS-like condition (7).

The Amish siblings described here enable us to more precisely delineate the clinical consequences of CEP55 loss-of-function, with hydranencephaly and cystic renal dysplasia as the predominant features. The identification of seven additional Amish control samples heterozygous for the *CEP55* frameshift variant highlights the importance of testing when an Amish affected fetus presents with Potter sequence or an MKS-like phenotype. Antenatal USS and PM investigations following stillbirth or neonatal death are infrequently undertaken by the Amish, and despite no previous reports, the allele frequency suggests that the condition is under recognised.

Unfortunately, while no tissue was available for cerebral histological analysis from the Amish and Bondeson cases (7), all three Frosk cases (6) displayed multinucleated neurons in cerebral tissue, and in one case multinucleated hepatocytes. Disruption of the CEP55 binding site for TSG101 and ALIX likely results from both the p.Arg86* and p.(Ile172Asnfs*17) variants (Figure 1A), which may prevent the cytokinesis abscission process resulting in incomplete cell division, providing a plausible explanation for the multinucleated neurons. The c.256C>T; p.Arg86* variant, located in exon 3 would be predicted to result in nonsense mediated mRNA decay, however studies on heterozygous parents identified equal levels of wild type and truncated transcript (7). The p.Ser425* variant is predicted to delete the terminal 40 amino acids critical for localisation during cytokinesis. Consistent with this, subcellular localisation studies showed the variant disrupts localisation to the midbody during cell division (6).

The lack of development of cerebral structures suggests that loss of CEP55 function may also play a role in cell migration during embryogenesis and development. Impaired cytokinesis may contribute to impaired neuronal migration in cells with aberrant cellular division, potentially explaining the MKS-like phenotype as both impaired cilia function and cell division processes may cause abnormal neuronal migration. However further studies of CEP55 function are required to fully determine the precise pathomolecular basis of this lethal multisystem congenital anomaly disorder. Taken together, our findings consolidate CEP55 as a molecule fundamental to normal human development, with homozygous loss of function associated with a Meckel-like lethal fetal disorder profoundly affecting brain and kidney development.

Acknowledgements

We are grateful to the parents of the affected children for taking part in this study and to the Amish community and colleagues at the New Leaf Centre: Clinic for Special Children, Ohio for their continuing support of our work. We would like to acknowledge Genetic Technologists, Katie Guegan for technical assistance with DNA extraction from fetal tissue and Anna-Marie Bussell and Jessica Settle for their technical assistance with whole exome sequencing undertaken at the Royal Devon & Exeter NHS Foundation Trust, UK. The work was supported by MRC Clinical Research Training fellowship G1001931 (to E.L. Baple), MRC grant G1002279 (to A.H. Crosby), MRC Proximity to Discover and Confidence in Concept (UEMS) (to E.L. Baple and A.H. Crosby), the Newlife Foundation for disabled children (to L.E. Rawlins, E.L. Baple, and A.H. Crosby).

Conflict of Interest

The authors have no conflicts of interest to declare.

References

1. Zhao WM, Seki A, Fang G *et al*. Cep55, a microtubule-bundling protein, associates with centralspindlin to control the midbody integrity and cell abscission during cytokinesis. Mol Biol Cell. 2006;17(9):3881-96.

2. Fabbro M, Zhou BB, Takahashi M, *et al*. Cdk1/Erk2- and Plk1-dependent phosphorylation of a centrosome protein, Cep55, is required for its recruitment to midbody and cytokinesis. Dev Cell. 2005;9(4):477-88.

3. Xu ZY, Ma XS, Qi ST, *et al*. Cep55 regulates spindle organization and cell cycle progression in meiotic oocyte. Sci Rep. 2015;5:16978.

4. Lee HH, Elia N, Ghirlando R, *et al*. Midbody targeting of the ESCRT machinery by a noncanonical coiled coil in CEP55. Science. 2008;322(5901):576-80.

5. Chen CH, Lu PJ, Chen YC, *et al*. FLJ10540-elicited cell transformation is through the activation of PI3-kinase/AKT pathway. Oncogene. 2007;26(29):4272-83.

6. Frosk P, Arts HH, Philippe J, Gunn *et al*. A truncating mutation in CEP55 is the likely cause of MARCH, a novel syndrome affecting neuronal mitosis. J Med Genet. 2017;54(7):490-501.

7. Bondeson ML, Ericson K, Gudmundsson S, *et al*. A nonsense mutation in CEP55 defines a new locus for a Meckel-like syndrome, an autosomal recessive lethal fetal ciliopathy. Clinical genetics. 2017;92(5):510-516.

8. Simpson MA, Cross HE, Cross L, *et al*. Lethal cystic kidney disease in Amish neonates associated with homozygous nonsense mutation of NPHP3. Am J Kidney Dis. 2009;53(5):790-5.

9. Bendon RW, Siddiqi T, de Courten-Myers G, *et al*. Recurrent developmental anomalies: 1. Syndrome of hydranencephaly with renal aplastic dysplasia; 2. Polyvalvular developmental heart defect. Am J Med Genet Suppl. 1987;3:357-65.

10. Gschwendtner A, Mairinger T, Soelder E, *et al*. Hydranencephaly with renal dysgenesis: a coincidental finding? Case report with review of the literature. Gynecol Obstet Invest. 1997;44(3):206-10.

11. Hamby WB, Krauss RF, Beswick WF. Hydranencephaly; clinical diagnosis; presentation of 7 cases. Pediatrics. 1950;6(3):371-83.

12. Strauss S, Bouzouki M, Goldfarb H, *et al*. Antenatal ultrasound diagnosis of an unusual case of hydranencephaly. J Clin Ultrasound. 1984;12(7):420-2.

13. Chu G, Miller W, Norton M, *et al*. Hydranencephaly with binucleate neurons-renal dysplasia-syndactyly syndrome in three siblings J Neuropathol Exp Neurol. 1998;54:483.

14. Barisic I, Boban L, Loane M, *et al*. Meckel-Gruber Syndrome: a population-based study on prevalence, prenatal diagnosis, clinical features, and survival in Europe. Eur J Hum Genet. 2015;23(6):746-52.

15. Curry CJ, Jensen K, Holland J, *et al*. The Potter sequence: a clinical analysis of 80 cases. American Journal of Medical Genetics 1984; 19: 679-702.

Figure Legends

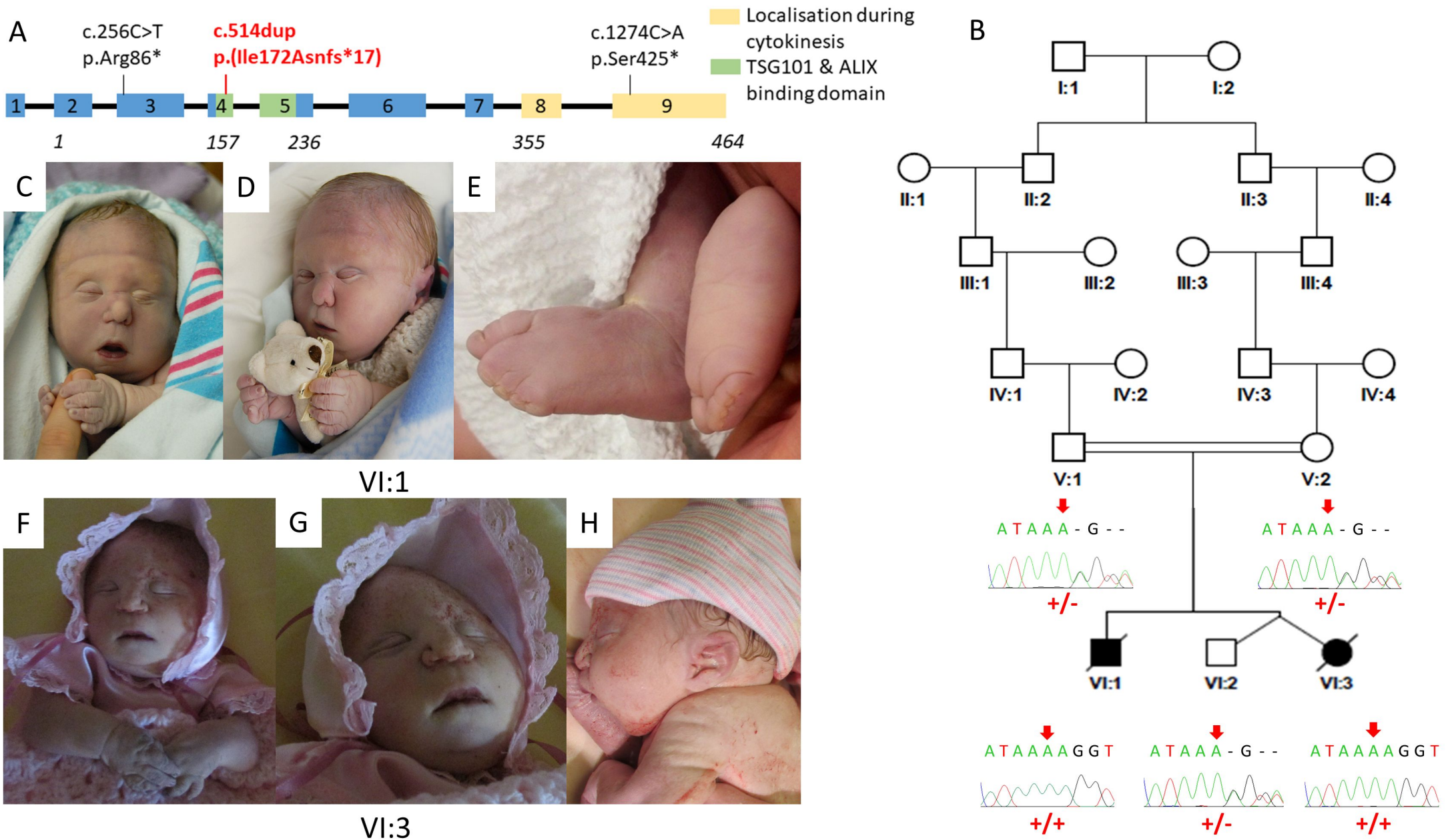
FIGURE 1: *CEP55* variants, family pedigree, genotype and images of affected individuals

(A) Schematic representation indicating the position of the disease-associated *CEP55* gene variants in relation to genomic organisation with the *CEP55* c.514dup; p.(Ile172Asnfs*17) variant identified in the current study highlighted by red text (exons; coloured boxes, introns; black lines). The correlation between coding regions and CEP55 polypeptide functional domains is also indicated; tumor suppressor gene 101 (TSG101) and apoptosis-linked gene 2 interacting protein X (ALIX) binding domains (green), and terminal region of the protein involved in localisation during cytokinesis (yellow). (B) Simplified pedigree of the Amish family investigated, with electropherograms showing the DNA sequence at the position of the *CEP55* variant (c.514dup) confirmed as homozygous in affected twin B and the deceased male sibling, and heterozygous in both parents and unaffected twin A. *CEP55* genotype is shown in red under electropherograms in generations V and VI (+, c.514dup; –, WT) (C-H) Clinical features of individuals homozygous for the *CEP55* variant (c.514dup). (C-E) VI:1 showing features of Potter sequence (also known as oligohydramnios sequence and used to describe a combination of distinctive facial and other associated phenotypic features that are a result of too little amniotic fluid. Features include epicanthic folds, retrognathia, a flattened nose, low-set ears, pulmonary hypoplasia and limb contractures including talipes) (15). Additional features observed included bilateral 2-5 toe syndactyly with a widened first wen space and a bulbous nasal tip. (F-H) VI:3 (Twin B) showing features of Potter sequence. The features of both affected fetuses that can be attributed as secondary to oligohydramnios include redundant skin folds, short neck, flattened face, short pinched nose, retrognathia, small palpebral fissures and low set ears, brachydactyly, tapering fingers, and short 5^{th} fingers with clinodactyly.

Table Legends

TABLE 1: Comparison of cases with biallelic *CEP55* variants

Abbreviations: TOP, termination of pregnancy; IUFD, intrauterine fetal death; SB, stillbirth; ND, neonatal death; CNS, central nervous system. (+), indicates presence of a feature in an affected subject; (-), indicates absence of a feature in an affected subject); n/k, not known; n/a, not available.


A
c.256C>T
p.Arg86*
c.514dup
p.(Ile172Asnfs*17)
c.1274C>A
p.Ser425*
Localisation during cytokinesis
TSG101 & ALIX binding domain
1
2
3
4
5
6
7
8
9
1
157
236
355
464
B
I:1
I:2
II:1
II:2
II:3
II:4
III:1
III:2
III:3
III:4
IV:1
IV:2
IV:3
IV:4
V:1
V:2
A T A A A - G - -
+/-
A T A A A - G - -
+/-
VI:1
VI:2
VI:3
A T A A A A G G T
+/+
A T A A A - G - -
+/-
A T A A A A G G T
+/+
C
D
E
VI:1
F
G
H
VI:3

CASE	Bondesen et al.	Bondesen et al.	Frosk et al.	Frosk et al.	Frosk et al.	Individual	Individual
Patient	II:2	II:3	303	305	306	VI:1	VI:3
CEP55 (NM_018131.4) Genotype	c.256C>T p.Arg86*	c.256C>T p.Arg86*	c.1274C>A p.S425*	c.1274C>A p.S425*	c.1274C>A p.S425*	c.514dup p.(Ile172Asnfs*17)	c.514dup p.(Ile172Asnfs*17)
Sex	M	M	M	M	M	M	F
Pregnancy outcome (gestation)	TOP (20)	IUFD (14+6)	SB (30)	ND (35)	SB (32)	SB (42)	ND (42)
Gestation anomalies identified	19 + 4	10 + 4	20	n/k	n/k	19	21 + 2
POTTER SEQUENCE	n/a	n/a	+	+	+	+	+
RENAL FEATURES							
Renal aplasia/dysplasia	+	n/k	+	+	+	n/k	+
Ureteral agenesis	n/k	n/k	+	+	+	n/k	n/k
Renal cysts	+	n/k	-	-	-	n/k	+
Bilateral hydronephrosis	+/-	n/k	-	-	-	+	-
Oligohydramnios	+	n/a	+	+	+	+	+
Pulmonary hypoplasia	n/k	n/k	+	+	+	n/k	n/k
Contractures	-	n/a	+	+	+	-	+
Talipes	+	n/a	+	+	+	+	+
CNS FEATURES							
Hydranencephaly	+	-	+	+	+	+	+
Cerebral cysts	-	+	-	-	-	-	-
Cerebellar hypoplasia	+	n/k	+	+	+	n/k	n/k
Encephalocele	-	+	-	-	-	-	-
Multinucleated neurons	n/k	n/k	+	+	+	n/k	n/k
Skull asymmetry	+	-	-	-	-	-	-
GROWTH							
IUGR	+	n/k	n/a	n/a	n/a	+	+
CARDIAC FEATURES							
Dysplastic aortic valve	n/k	n/k	+	n/k	+	n/k	n/k
Dilated left ventricle	n/k	n/k	+	n/k	+	n/k	n/k
Pericardial effusion	n/k	n/k	-	-	-	-	+
SKELETAL FEATURES							
Shortened/bowed long bones	n/k	n/a	-	-	-	+	+
Vertebral abnormalities	n/k	n/a	+	+	-	n/k	n/k
OTHER FEATURES							
Cystic hygroma	+	+	+	+	+	-	-
Redundant neck skin	n/a	n/a	+	+	+	+	+
Bulbous nasal tip	n/k	n/a	+	+	+	+	+
Syndactyly	n/a	n/a	+	+	+	+	+
Brachydactyly	n/a	n/a	+	+	+	+	+
Widened first web space	n/a	n/a	+	+	+	+	+
Single umbilical artery	+	n/a	-	-	-	-	+