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Diagnosis of *TBC1D32*-associated conditions: Expanding the phenotypic spectrum of a complex ciliopathy

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- Study conception and design: Vora NL, Davis EE
- Data collection: Gilmore, KL, Harris SC, Davis EE
- Recruitment and evaluation of study subjects: Vora, NL, Gilmore KL, Chong K, Chitayat D, Jorge AAL, Freire, BL, Lerario, A, Shannon P, Cope H, Gallentine WB, Le Guyader G, Bilan F, Letard P, Davis EE
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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abstract

Exome sequencing is a powerful tool in prenatal and postnatal genetics and can help identify novel candidate genes critical to human development. We describe seven unpublished probands with rare likely pathogenic variants or variants of uncertain significance that segregate with recessive disease in *TBC1D32*, including four fetal probands in three unrelated pedigrees and three pediatric probands in unrelated pedigrees. We also report clinical comparison with seven previously published patients. Index probands were identified through an ongoing prenatal exome sequencing study and through an online data sharing platform (Gene Matcher™). A literature review was also completed. TBC1D32 is involved in the development and function of cilia and is expressed in the developing hypothalamus and pituitary gland. We provide additional data to expand the phenotype correlated with *TBC1D32* variants, including a severe prenatal phenotype associated with life-limiting congenital anomalies.

Keywords: Ciliopathy, *TBC1D32,* prenatal phenotype, exome sequencing

Introduction

Ciliopathies describe a heterogenous group of disorders resulting from abnormal ciliary function or structure. Oro-Facial-Digital (OFD) syndromes are defined by abnormalities of the face, oral cavity, and extremities. Multiple OFD subtypes have been defined based on additional features, such as kidney disease, brain malformations and dysfunction, and retinal abnormalities.[1] Adly et al.[2] reported *TBC1D32,* encoding TBC1 domain family member 32, as a candidate causal gene in a simplex patient associated with a severe ciliopathy phenotype. Based on the observation of facial cleft, microphthalmia and coloboma, and polydactyly, the authors characterized the phenotype within the OFD spectrum (type IX).

Exome sequencing can be a powerful tool to uncover novel candidate genes. However, one of the challenges encountered with whole exome and genome sequencing is the limited ability to define genotype-phenotype correlations for rare genetic conditions. This is especially true in prenatal diagnosis as the fetal phenotype is often not well described. Absent functional information, imperfect ultrasound and MRI resolution and absent detailed fetal autopsy contribute to the challenge of interpreting rare variants identified through fetal exome sequencing.[3] Clinicians often rely on literature reviews and collaboration with other genetics professionals to classify variants. Additionally, there is currently no publicly available prenatal database with genotype-phenotype information.

To date, variants in *TBC1D32* have been reported in seven individuals;[2, 4-6] however, *TBC1D32* has no associated phenotype cataloged in Online Mendelian Inheritance in Man (OMIM). We present 7 additional probands, including 4 with severe prenatal phenotypes, associated with *TBC1D32* gene variants to provide additional data on the spectrum of phenotypes associated with this complex ciliopathy.

Materials and Methods

Index probands (Proband 5 and 6) were identified through the Fetal Exome Sequencing (FES) Study at the University of North Carolina at Chapel Hill. Anomalous fetuses with normal microarray were identified prospectively and retrospectively and exome sequencing and variant filtering was completed on the fetus-mother-father trios, as previously described.[14] The UNC Hospitals Clinical Molecular Genetics Laboratory performed Sanger DNA sequencing analysis to confirm the presence of the genetic variant and appropriate segregation with disease in the trio. This study was approval from the University of North Carolina at Chapel Hill Institutional Review Board (IRB) (13-4084) before patient consent and enrollment. Participants had pretest counseling about exome sequencing and possible results and were consented to participate in the research study.

Gene Matcher™[,7] was queried to identify unpublished affected individuals with *TBC1D32* gene variants. Researchers were contacted via email and asked to contribute patient information for

inclusion in this series. A literature review was completed using PubMed to identify all previously published patients.

Results

Proband 1

A 3-month-old female was referred for evaluation for microcephaly (Table 1). She was the product of a full-term uncomplicated pregnancy with no known maternal exposures. Consanguinity was denied and both parents were reportedly healthy and of European descent. Significant physical exam findings included microcephaly, brachycephaly, deep set eyes, mild prominent nose, long philtrum, and thin upper lip, and mild 2-3 toe syndactyly. Increased muscle tone, mild restriction of joints, and developmental delay were reported.

Head CT imaging showed ventriculomegaly involving the lateral ventricles bilaterally as well as the third ventricle and sulcal prominence. Increased extra-axial space overlying the convexities of the frontal lobes bilaterally was also present. A small cerebellum was noted, and multiple skull deformities were identified, including asymmetric flattening of the right posterior parietal bone and hypoplastic frontal bones bilaterally. There was no evidence of craniosynostosis. Brain and spinal cord MRI confirmed the head CT scan findings and also showed enlargement of the posterior fossa with hypoplasia of the inferior cerebellar vermis.

Genetics
Karyotype and microarray were normal. Trio exome sequencing revealed compound heterozygous missense variants in *TBC1D32* GenBank ID: NM_152730.6: c.3182G>C p.(Gly1061Ala) and c.2134A>G p.(Asn712Asp). Both changes are rare (gnomAD frequency: 0.0006684 and 0.000004047, respectively), and neither is present in homozygosity in gnomAD (www.gnomad.broadinstitute.org). They are both considered variants of uncertain significance. (Figure 1 and 2; Supplementary Table 1).

Proband 2

A 3-month-old female was referred for clinical genetics assessment due to micromelia and brachydactyly (Table 1). She was the first child of unrelated and healthy parents. During pregnancy, mild short long bones and borderline ventriculomegaly were identified. On exam, the patient was noted to have strabismus, a single median incisor, choanal stenosis, short limbs, and relative macrocephaly. She was noted to have a large forehead, prominent forehead, epicanthus inversus, narrow palpebral fissures, ptosis, and midface hypoplasia. Obstructive sleep apnea, and global developmental delay were also diagnosed.

MRI of brain suspected an ectopic pituitary gland in relation with Growth Hormone (GH) and Thyroid Stimulating Hormone (TSH) deficiency.

Genetics
Karyotype was not performed. Sequencing of recurrent *FGFR3* mutations was normal. Microarray revealed 17p12 microdeletion (including *PMP22*) unrelated to the patient phenotype. Exome sequencing identified compound heterozygous variants in *TBC1D32* GenBank ID: NM_152730.6: c.2481+4A>T, considered to be a pathogenic splicing variant by performing a splicing reporter minigene assay (exon 21 skipping) and c.3572A>G (p.Tyr1191Cys), considered to be a missense likely pathogenic variant. Both changes are rare (gnomAD frequency: 0.00002685 and 0.000008025, respectively), and neither is present in homozygosity in gnomAD. (Figure 1 and 2; Supplementary Table 1).

Proband 3

A 26-year-old primigravid of Sri Lankan descent was referred for targeted ultrasound at 20 weeks gestation for multiple fetal anomalies. The patient and her partner were healthy and consanguineous. No significant family history was reported. Ultrasound findings were significant for hydrocephalus, possible lobar holoprosencephaly, bilateral microphthalmia/anophthalmia, proboscis and severe midface hyperplasia, congenital heart defect, possible esophageal atresia, wrists in flexed position, rocker bottom feet and dorsiflexed large toes (Table 1).

The patient underwent induction of labor for pregnancy termination at 20 weeks gestation and autopsy was completed Findings included abnormal facies with enlarged cranium, microphthalmia, patent partially fused nares superior to orbits, long philtrum, and micrognathia (Figure 3). The sella turcica was absent and there was no identifiable pituitary gland. The adrenal glands were hypoplastic. Lobar holoprosencephaly with fused a fused optic nerve containing dysplastic retinal elements, severely dysplastic hypothalamus, fused thalami, aqueductal atresia, brainstem disorganization with absent corticospinal tracts and a poorly demarcated cerebellar vermis were observed (Figures 4 and 5). The heart demonstrated an incomplete, balanced atrioventricular septal defect.

Genetics
Karyotype and microarray were normal. A homozygous variant of uncertain significance was identified in *TBC1D32*, GenBank ID: NM_152730.6:c.2650C>T p.(Arg884Trp). Sequence analysis confirmed that both parents were carriers of the variant. This variant is rare in gnomAD (minor allele frequency 0.0000441) and is not present in homozygosity in healthy populations. In silico analysis supports that this missense variant has a deleterious effect on protein structure and function. (Figure 1 and 2; Supplementary Table 1).

Proband 4

A 33-year-old primigravid woman of Sri Lankan descent was referred for targeted ultrasound at 19 weeks following abnormal maternal serum screening. Maternal history was significant for poorly controlled epilepsy treated with levetiracetam and perampanel. Consanguinity was

reported. There was no significant family history. Ultrasound showed fetal growth restriction, severe ventriculomegaly, severe midface hypoplasia, possible proboscis, and abnormal posturing of the upper extremities (Table 1).

The patient underwent induction of labor for pregnancy termination at 22 weeks gestation and an autopsy was completed. On autopsy there was severe midface hypoplasia with high positioned nose, low set and posteriorly rotated ear, microphthalmia, patent partially fused nares superior to the orbits, long philtrum, and micrognathia (Figure 3). The sella turcica was shallow and dysplastic and the adrenal glands hypoplastic. The brain demonstrated lobar holoprosencephaly with fused a fused optic nerve containing dysplastic retinal elements, severely dysplastic hypothalamus, fused thalami, aqueductal atresia, brainstem disorganization with absent corticospinal tracts and a poorly demarcated cerebellar vermis (Figures 4 and 5). Additionally, axillary pterygia, short digits, renal hypoplasia, and a micropenis were noted. Radiology demonstrated short, curved ulnae bilaterally.

Genetics
Microarray reported normal male. Holoprosencephaly Panel at Gene Dx was negative for *TGIF, SHH, SIX3, and ZIC2.* A homozygous variant of uncertain significance was identified in *TBC1D32*, GenBank ID: NM_152730.6:c.2650C>T p.(Arg884Trp). Sequence analysis confirmed that both parents were heterozygous carriers of the variant. This variant is rare in gnomAD (minor allele frequency 0.0000441) and is not present in homozygosity in healthy populations. In silico analysis supports that this missense variant has a deleterious effect on protein structure and function. (Figure 1 and 2; Supplementary Table 1).

Proband 5 and Proband 6

A consanguineous Pakistani couple with two affected pregnancies was identified through an ongoing fetal exome sequencing study. In the couple's first affected pregnancy (Proband 5; Table 1), an abnormal maternal serum screen prompted a detailed anatomy ultrasound. Multiple anomalies were identified, including suspected holoprosencephaly, proboscis, suspected anophthalmia, situs inversus, bilateral echogenic kidneys, omphalocele, ambiguous genitalia, and shortened long bones.

The patient underwent an induction of labor for pregnancy termination at 16 weeks gestation and autopsy was completed. Significant findings included, hypoplastic midface, anophthalmia, low-set posteriorly rotated ears, absent nose, bilateral submental pterygia, a small omphalocele, partial syndactyly of toes 2-5 on the right, bilateral sandal gap, complete situs inversus with dextrocardia absent sella turcica, no pituitary tissue identified grossly, possible absent corpus callosum, absence of olfactory tracts and bulbs, rectus gyri, and optic nerves. Small/hypoplastic adrenal glands were also noted.

In the couple's second affected pregnancy (Proband 6; Table 1), ultrasound showed multiple fetal anomalies including, suspected anencephaly, midline facial cleft, small omphalocele,

polydactyly, clubbed feet, and intrauterine growth restriction. Intrauterine fetal death was diagnosed at 21 weeks gestation followed by a spontaneous vaginal delivery. A fetal autopsy was not completed. Per the delivery note, the fetus was female with anencephaly, facial cleft, small omphalocele, clubbed feet, left foot postaxial polydactyly and left-hand postaxial polydactyly.

Karyotype and microarray were normal in both affected fetuses. Exome sequencing data identified homozygous loss of function variants in *TBC1D32*, GenBank ID: NM_152730.6 c.3325_3326del (p.Ser1109Leufs*4) (Figure 1). Sanger sequence analysis confirmed that both the mother and the father are heterozygous carriers of the frameshifting variant. This change is absent from the genome aggregation database and considered likely pathogenic according to ACMG variant classification criteria. (Figure 1 and 2; Supplementary Table 1).

Proband 7

A 10-year-old Brazilian female with short stature and intellectual disability was referred for clinical genetics assessment (Table 1). Consanguinity was reported. The patient was noted to have proportional postnatal onset short stature. Mild facial dysmorphism including coarse face, deep set eyes, pectus excavatum, mild ptosis and full lips. She had moderate to severe intellectual disability. Additional findings included microcephaly and blue sclera.

A skeletal survey showed nonspecific shortening of the long bones and absence of the distal phalanges of the fifth toe. She had a negative screen for mucopolysaccharidosis type 1 and type IV and normal IGF-1 and GH peak. She had a normal brain MRI.

Genetics
Karyotype and microarray were normal. Exome sequencing identified a homozygous missense variant in *TBC1D32* GenBank ID: NM_152730.6:c.3527A>G p.(His1176Arg), considered to be a variant of uncertain significance (Figure 1). This change is rare in gnomAD (MAF 0.00000803) and is not present in homozygosity in healthy populations (Table 3) The change is also rare in ABraOM (https://abraom.ib.usp.br), a Brazilian public genomic database, (MAF 0.001708) and only present in the heterozygous state. (Figure 1 and 2; Supplementary Table 1).

Discussion

In this study, we report seven new probands in six unrelated pedigrees with biallelic *TBC1D32* variants and seven previously reported patients associated with a complex ciliopathy phenotype (Tables 1; Supplementary Table 1). Common findings include ocular differences in the majority of individuals (10 of 14), including anophthalmia/microphthalmia (5 of 14), absent or abnormal nose formation (10 of 14), and facial clefting (5 of 14). Digital differences, including polydactyly and syndactyly, were noted in 10 individuals. CNS abnormalities were identified in all probands including, anencephaly, hydrocephalus, holoprosencephaly, microcephaly, and a

poorly demarcated vermis with cerebellar hypoplaisa. Five of the fourteen reported probands had absence/hypoplasia of the pituitary gland. Additional findings include, congenital heart disease, intestinal malrotation, omphalocele, and *situs inversus* (Table 1).

Ciliopathies comprise a heterogenous group of disorders caused by dysfunction of the primary cilium, a microtubule-based organelle found in almost every mammalian cell that is critical for integrating and transducing extracellular signals[8,9] *TBC1D32* was first associated with a severe ciliopathy phenotype by Adly et al.[2] In their report, an affected male infant born to consanguineous parents was noted to have multiple facial anomalies, including left anophthalmia, right microphthalmia, midline facial cleft, and choanal stenosis. Polydactyly and congenital heart defects were also noted. MRI was significant for absent pituitary gland and agenesis of the corpus callosum. Exome sequencing identified a homozygous splice junction variant in *TBC1D32I*, c.1372+1G>T p.(Arg411_Gly458del). The authors concluded that this phenotypic constellation most closely matched Orofacial Digital syndrome (OFD) Type IX, given the prominent eye involvement.[2] Tongue differences, including bifid and hamartomatous tongues have also been reported with OFD Type IX.[10] Notably, there is a lack of classic tongue findings in individuals with *TBC1D32* variants (1 of 14; Table 1).

TCB1D32 has been identified as a primary ciliary protein through cilia proteomic studies.[11] In mice and zebrafish models, *TCB1D32* has been shown to be involved in the development and function of cilia. It has been hypothesized that TCB1D32 interacts with cell cycle-related kinase (CCRK) to allow for the correct assembly of the cilia axoneme and membrane, which is required for the sonic hedgehog (Shh) signaling pathway.[11,12] In the *bromi* mouse model, mutants harbor a deletion of the *TBC1D32* homologue that prevents the correct development of cilia and interrupts Shh signaling. *Bromi* mutants have exencephaly, poorly developed eyes, and preaxial polydactyly.[12] Similar findings are noted in the individuals with severe fetal presentation in this series, including anencephaly and holoprosencephaly, anophthalmia, and polydactyly (Table 1).

Human gene expression analyses have identified *TCB1D32* in developing hypothalamus and pituitary gland and in adult cDNA libraries.[5] It has been suggested that *TCB1D32* is activated during the early stages of hypothalamo-pituitary formation and may play a role in other parts of the developing brain.[5] Differences in the CNS were observed in all of the reported probands harboring *TBC1D32* variants (Table 1).

The probands reported in this study further define the phenotype associated with *TBC1D32* variants. However, consistent with the notorious phenotypic variability of the ciliopathies, clearly defined genotype-phenotype are difficult to establish. This is exemplified by the spectrum of phenotypes displayed among the four individuals harboring the recurrent c.1372+1G>T, p.(Arg411Gly458del) variant (Tables 1). Second-site modifiers, potentially impacting other ciliary proteins, may explain the heterogeneity of clinical presentation in this small cohort as described for other ciliopathies.[13]

Fetal phenotypes often vary significantly from the well-defined pediatric or adult phenotype and accurate and thorough documentation is imperative to assist with diagnosis and

classification of variants.[14] Prenatal ultrasound findings were noted in 10 of the 14 reported probands, including a severe phenotype with multiple life-limiting anomalies in seven individuals. Two of the pediatric patients had minor ultrasound findings reported, including short longs bones, ventriculomegaly, and polyhydramnios. We also present an affected individual diagnosed with an absent pituitary gland by fetal autopsy, a finding reported in four of the pediatric patients. Although prenatal diagnosis continues to improve with advances in the field of ultrasound, fetal autopsy can aid in the diagnosis of additional anomalies and improve diagnostic yield.[15] Deep phenotyping with additional postnatal evaluation is critical for defining the fetal phenotype for rare genetic conditions.

Conclusion

We describe a total of 14 affected individuals with *TBC1D32* variants, expanding the phenotype of this complex ciliopathy. Prior functional studies and the probands presented provide support that *TBC1D32* plays a critical role in the development of the pituitary gland, eyes, and brain.

This report also highlights the importance of data sharing for rare genetic diseases. It is essential for clinicians and researchers to collaborate through online platforms to accurately define rare variants. Additionally, detailed documentation of prenatal ultrasound findings and autopsy results are essential for defining the prenatal phenotype of rare genetic conditions.

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References

1. Bruel AL, Franco B, Duffourd Y, Thevenon J, Jego L, Lopez E, Deleuze JF, Doummar D, Giles RH, Johnson CA, Huynen MA, Chevrier V, Burglen L, Morleo M, Desguerres I, Pierquin G, Doray B, Gilbert-Dussardier B, Reversade B, Steichen-Gersdorf E, Baumann C, Panigrahi I, Fargeot-Espaliat A, Dieux A, David A, Goldenberg A, Bongers E, Gaillard D, Argente J, Aral B, Gigot N, St-Onge J, Birnbaum D, Phadke SR, Cormier-Daire V, Eguether T, Pazour GJ, Herranz-Pérez V, Goldstein JS, Pasquier L, Loget P, Saunier S, Mégarbané A, Rosnet O, Leroux MR, Wallingford JB, Blacque OE, Nachury MV, Attie-Bitach T, Rivière JB, Faivre L, Thauvin-Robinet C (2017). Fifteen years of research on oral-facial-digital

syndromes: from 1 to 16 causal genes. J Med Genet. 54(6):371-380. doi: 10.1136/jmedgenet-2016-104436.

2. Adly N, Alhashem A, Ammari A, Alkuraya FS (2014). Ciliary genes TBC1D32/C6orf170 and SCLT1 are mutated in patients with OFD type IX. Hum Mutat. 35(1):36-40. doi: 10.1002/humu.22477.

3. Vora NL, Gilmore K, Brandt A, Gustafson C, Strande N, Ramkissoon L, Hardisty E, Foreman AKM, Wilhelmsen K, Owen P, Weck KE, Berg JS, Powell CM, Powell BC (2020). An approach to integrating exome sequencing for fetal structural anomalies into clinical practice. Genet Med. 22(5):954-961. doi: 10.1038/s41436-020-0750-4.

4. Monies D, Abouelhoda M, Assoum M, Moghrabi N, Rafiullah R, Almontashiri N, Alowain M, Alzaidan H, Alsayed M, Subhani S, Cupler E, Faden M, Alhashem A, Qari A, Chedrawi A, Aldhalaan H, Kurdi W, Khan S, Rahbeeni Z, Alotaibi M, Goljan E, Elbardisy H, ElKalioby M, Shah Z, Alruwaili H, Jaafar A, Albar R, Akilan A, Tayeb H, Tahir A, Fawzy M, Nasr M, Makki S, Alfaifi A, Akleh H, Yamani S, Bubshait D, Mahnashi M, Basha T, Alsagheir A, Abu Khaled M, Alsaleem K, Almugbel M, Badawi M, Bashiri F, Bohlega S, Sulaiman R, Tous E, Ahmed S, Algoufi T, Al-Mousa H, Alaki E, Alhumaidi S, Alghamdi H, Alghamdi M, Sahly A, Nahrir S, Al-Ahmari A, Alkuraya H, Almehaidib A, Abanemai M, Alsohaibaini F, Alsaud B, Arnaout R, Abdel-Salam GMH, Aldhekri H, AlKhater S, Alqadi K, Alsabban E, Alshareef T, Awartani K, Banjar H, Alsahan N, Abosoudah I, Alashwal A, Aldekhail W, Alhajjar S, Al-Mayouf S, Alsemari A, Alshuaibi W, Altala S, Altalhi A, Baz S, Hamad M, Abalkhail T, Alenazi B, Alkaff A, Almohareb F, Al Mutairi F, Alsaleh M, Alsonbul A, Alzelaye S, Bahzad S, Manee AB, Jarrad O, Meriki N, Albeirouti B, Alqasmi A, AlBalwi M, Makhseed N, Hassan S, Salih I, Salih MA, Shaheen M, Sermin S, Shahrukh S, Hashmi S, Shawli A, Tajuddin A, Tamim A, Alnahari A, Ghemlas I, Hussein M, Wali S, Murad H, Meyer BF, Alkuraya FS (2019). Lessons Learned from Large-Scale, First-Tier Clinical Exome Sequencing in a Highly Consanguineous Population. Am J Hum Genet. 2019 Jun 6;104(6):1182-1201. doi: 10.1016/j.ajhg.2019.04.011.

5. Hietamäki J, Gregory LC, Ayoub S, Iivonen AP, Vaaralahti K, Liu X, Brandstack N, Buckton AJ, Laine T, Känsäkoski J, Hero M, Miettinen PJ, Varjosalo M, Wakeling E, Dattani MT, Raivio T (2020). Loss-of-Function Variants in TBC1D32 Underlie Syndromic Hypopituitarism. J Clin Endocrinol Metab. 105(6):1748–58. doi: 10.1210/clinem/dgaa078.

6. Alsahan N, Alkuraya FS (2020). Confirming TBC1D32-related ciliopathy in humans. Am J Med Genet A. 182(8):1985-1987. doi: 10.1002/ajmg.a.61717.
7. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015 Oct;36(10):928-30. doi: 10.1002/humu.22844.

8. Satir P, Christensen ST (2007). Overview of structure and function of mammalian cilia. Annu Rev Physiol. 69:377-400. doi: 10.1146/annurev.physiol.69.040705.141236.

9. Shamseldin HE, Shaheen R, Ewida N, Bubshait DK, Alkuraya H, Almardawi E, Howaidi A, Sabr Y, Abdalla EM, Alfaifi AY, Alghamdi JM, Alsagheir A, Alfares A, Morsy H, Hussein MH, Al-Muhaizea MA, Shagrani M, Al Sabban E, Salih MA, Meriki N, Khan R, Almugbel M, Qari A, Tulba M, Mahnashi M, Alhazmi K, Alsalamah AK, Nowilaty SR, Alhashem A, Hashem M, Abdulwahab F, Ibrahim N, Alshidi T, AlObeid E, Alenazi MM, Alzaidan H, Rahbeeni Z, Al-Owain M, Sogaty S, Seidahmed MZ, Alkuraya FS (2020). The morbid genome of ciliopathies: an update. Genet Med. 22(6):1051-1060. doi: 10.1038/s41436-020-0761-1.

10. Gurrieri F, Franco B, Toriello H, Neri G. Oral-facial-digital syndromes: review and diagnostic guidelines. Am J Med Genet A. 2007; 143A(24):3314-23. doi: 10.1002/ajmg.a.32032.

11. Ishikawa H, Thompson J, Yates JR 3rd, Marshall WF (2012). Proteomic analysis of mammalian primary cilia. Curr Biol. 22(5):414-9. doi: 10.1016/j.cub.2012.01.031.

12. Ko HW, Norman RX, Tran J, Fuller KP, Fukuda M, Eggenschwiler JT (2010). Broad-minded links cell cycle-related kinase to cilia assembly and hedgehog signal transduction. Dev Cell. 18(2):237-47. doi: 10.1016/j.devcel.2009.12.014.

13. Kousi M, Söylemez O, Ozanturk A, Mourtzi N, Akle S, Jungreis I, Muller J, Cassa CA, Brand H, Mokry JA, Wolf MY, Sadeghpour A, McFadden K, Lewis RA, Talkowski ME, Dollfus H, Kellis M, Davis EE, Sunyaev SR, Katsanis N (2020). Evidence for secondary-variant genetic burden and non-random distribution across biological modules in a recessive ciliopathy. Nat Genet. 52(11):1145-1150. doi: 10.1038/s41588-020-0707-1.

14. Gray KJ, Wilkins-Haug LE, Herrig NJ, Vora NL. Fetal phenotypes emerge as genetic technologies become robust (2019). Prenat Diagn. 39(9):811-817. doi: 10.1002/pd.5532.

15. Nayak SS, Shukla A, Lewis L, Kadavigere R, Mathew M, Adiga PK, Vasudeva A, Kumar P, Shetty J, Shah H, Girisha KM (2015). Clinical utility of fetal autopsy and its impact on genetic counseling. Prenat Diagn. 35(7):685-91. doi: 10.1002/pd.4592.

Table 1: Summary of clinical and genetic findings in pedigrees with recessive *TBC1D32* variants

	Hietamäki J, et al. (2020)	Hietamäki J, et al. (2020)	Hietamäki J, et al. (2020)	Monies D, et al. (2019)	Aldy, N et al. (2013)	Hietamäki J, et al. (2020)	Proband 1	Proband 2	Proband 3	Proband 4	Proband 5	Proband 6	Proband 7	Alsahan et al. (2020)
***TBC1D32* variants**	c.1165_1166dup and c.2151del	c.1165_1166dup and c.2151del	c.1372+1G>T homo-zygous	c.1372+1G>T homo-zygous	c.1372+1G>T homo-zygous	c.1372+1G>T homo-zygous	c.2134A>G and c3182G>C	c.2481+4A>T and c.3572A>G	c.2650 C>T homo-zygous	c.2650 C>T homo-zygous	c.3325_3326delAG homo-zygous	c.3325_3326delAG homo-zygous	c.3527A>G homo-zygous	C3724C>T homo-zygous
Oral/Facial														
Lip/Palate	-	-	High arched palate	Cleft lip	Midline facial cleft	Midline facial cleft	-	-	-	-	-	Midline facial cleft	-	-
Nose	Upturned nose	Upturned nose	Upturned nose, choanal atresia	-	Choanal atresia	-	Prominent	Choanal atresia	Single fused nostril	High position, partially fused nares	Absent	-	-	Single nostril
Ears	Low set	Low set	Low set	-	-	-	-	-	-	Low set	Low set	-	-	-
Tongue	-	-	Bifid	-	-	-	-	-	-	-	-	-	-	-
Dentition	-	Abnormal	-	-	-	-	-	Median central incisor	-	-	-	-	-	-
Facial	Hyper-telorism, prominent forehead	Hyper-telorism, prominent forehead	Hyper-telorism, prominent forehead	-	Midface hypoplasia	-	Deep set eyes, long philtrum, thin upper lip	Midface hypoplsia	Long philtrum, micro-gnathia	Midface hypo-plasia	Midface hypo-plasia	-	Hyper-telorism, deep set eyes, coarse facies	-
Eye														
Anophthalmia	-	-	-	-	-	-	-	-	-	-	Bilateral	-	-	Bilateral
Microphthalmia	-	-	-	-	Bilateral	-	-	-	Bilateral	Bilateral	-	-	-	-
Cyclopia	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Coloboma	-	-	-	-	Bilateral	-	-	-	-	-	-	-	-	-
Vision	Astig-matism	Retinal dystrophy	Reduced visual acuity	-	-	-	-	Strabismus, hetero-chromia iridis	Retinal dysplasia	Retinal dysplasia	-	-	-	-
CNS														
Size	-	-	-	-	Macro-cephaly	-	Micro-cephaly	Macro-cephaly	Enlarged cranium	-	Enlarged cranium	Anen-cephaly	-	Enlarged cranium

Holoprosencephaly	-	-	-	+	-	-	-	-	Lobar	Lobar	-	-	-	-
Ventriculomegaly	+	-	-	-	-	-	+	+	+	+	-	-	-	+
Pituitary gland	Absent	Absent	Hypoplastic	-	Absent	-	-	Ectopic	Absent sella turcica	Dysplastic sella turcica	Absent sella turcica	-	-	-
Optic nerve	-	-	Septo-optic dysplasia	-	-	-	-	-	Fused	Fused	Absent	-	-	-
Thalamus	-	-	-	-	-	-	-	-	Fused thalami, dysplastic hypo-thalami	Fused thalami, dysplastic hypo-thalami	-	-	-	-
Cerebellum	-	-	-	-	Vermis hypoplasia	-	Dandy Walker	-	Abnormal vermis	Abnormal vermis	-	-	-	-
Corpus callosum	-	-	Agenesis	Agenesis	Agenesis	-	-	-	-		-	-	-	-
Limb														
Polydactyly	-	-	Postaxial	-	+	-	-	-	-	-	-	Unilateral postaxial	-	-
Syndactyly	-	2,3 toes	-	-	-	-	2, 3 toes	-	-	-	2-5 toes	-	-	-
Digits	-	Sandal gap	-	Sandal gap	-	-	-	Brachy-dactyly	-	Short digits	Sandal gap	Sandal gap	Absent 5th toe phal-ange	-
Other	-	Hyper-mobile joints	Rhizomelic shortening	Club foot	-	Short long bones	Mild restriction of joints	-	Flexed wrists, rocker bottom feet	Short, curved ulnea		-	Short long bones	Short long bones
Cardiac														
Congenital heart defect	-	-	-	VSD	PDA, ASD	-	-	-	AVSD	-	Dextro-cardia	-	-	-
Gastrointestinal														
Omphalocele	-	-	-	-	-	-	-	-	-	-	+	+	-	-
Malrotation	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Situs	-	-	-	-	-	-	-	-	-	-	Situs inversus	-	-	-
Genitourinary														
Renal	-	-	-	-	-	-	-	-	-	Hypo-plasia	-	-	-	-
Adrenal	-	-	-	-	-	-	-	-	Hypoplasia	Hypo-plasia	-	-	-	-
Genitalia	Micro-penis,	-	-	-	Ambiguous	-	-	-	-	Micro-penis	-	-	-	-

	Crypt-orchidism													
Developmental														
Delays	Global	Motor	Global	-	-	-	Global	Global	-	-	-	-	ID	-
Seizures	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Prenatal presentation														
	-	-	Short long bones, midline cystic brain changes, poly-hydramnios	Multiple anomalies	Cleft lip, abnormal hands, small head	Midline facial cleft, short femur length	-	Short long bones, ventriculo-megaly	Multiple anomalies	Multiple anomalies fetal growth restriction	Multiple anomalies	Multiple anomalies, fetal growth restriction	-	Multiple anomalie

Abbreviations: VSD, ventricular septal defect; PDA, patent ductus arteriosus; ASD, atrial septal defect; AVSD, atrioventricular septal defect; ID, intellectual disability

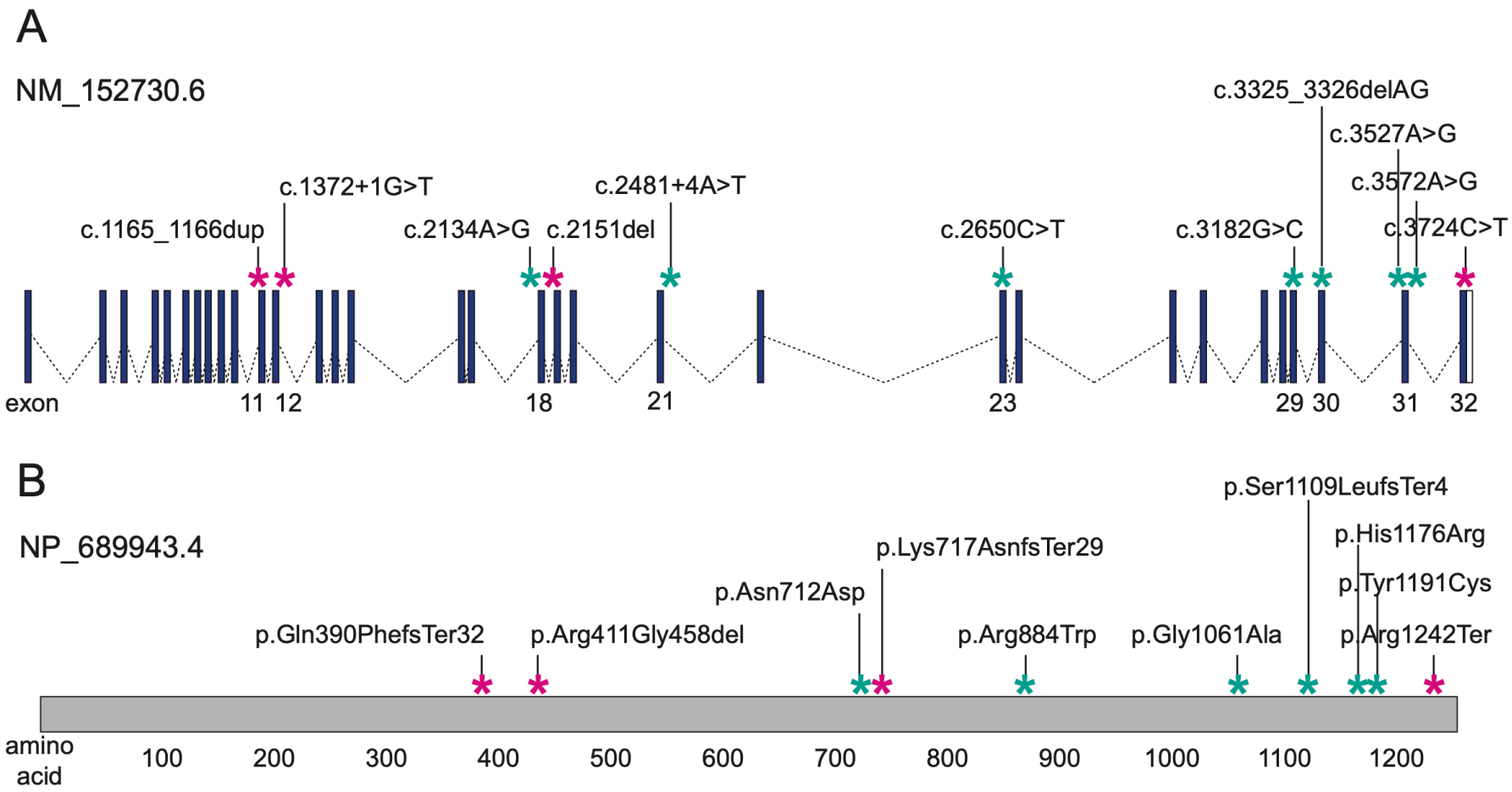
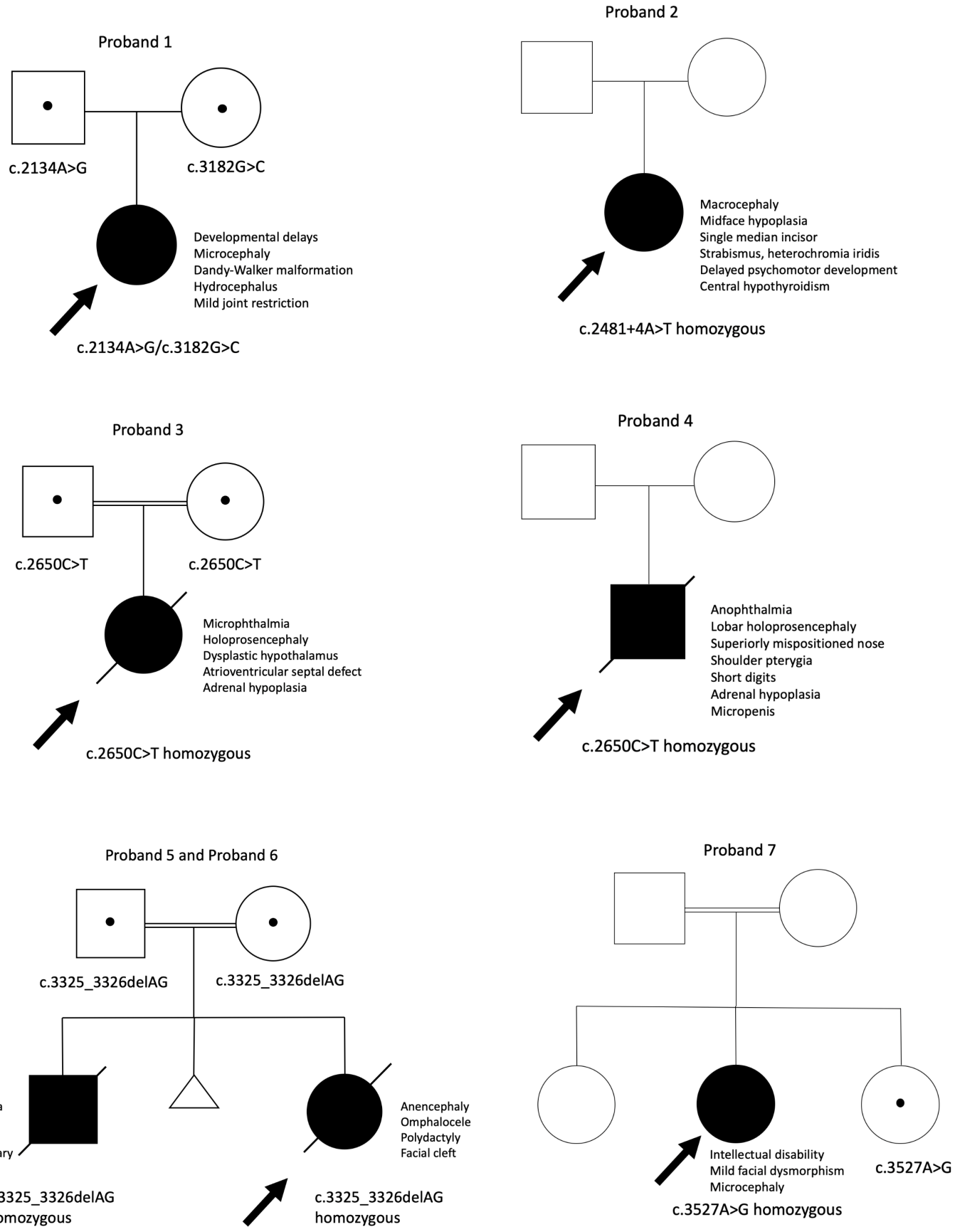


Figure 1. TBC1D32 Schematic. A. Genomic locus on chr6:121,079,494-121,334,480 (GRCh38, reverse strand). Blue boxes, exons; white boxes, untranslated regions; dashed lines, introns; exons harboring case-associated variants are labeled at bottom. B. Location of variants on a linear protein schematic; amino acids are labeled at bottom. For both panels: teal asterisks, hitherto unreported case-associated variants; magenta asterisks, previously published pathogenic variants.

Figure 2. Pedigrees of previously unpublished *TBC1D32* variants that segregate with disease



Note: Variants are heterozygous unless indicated otherwise in each pedigree

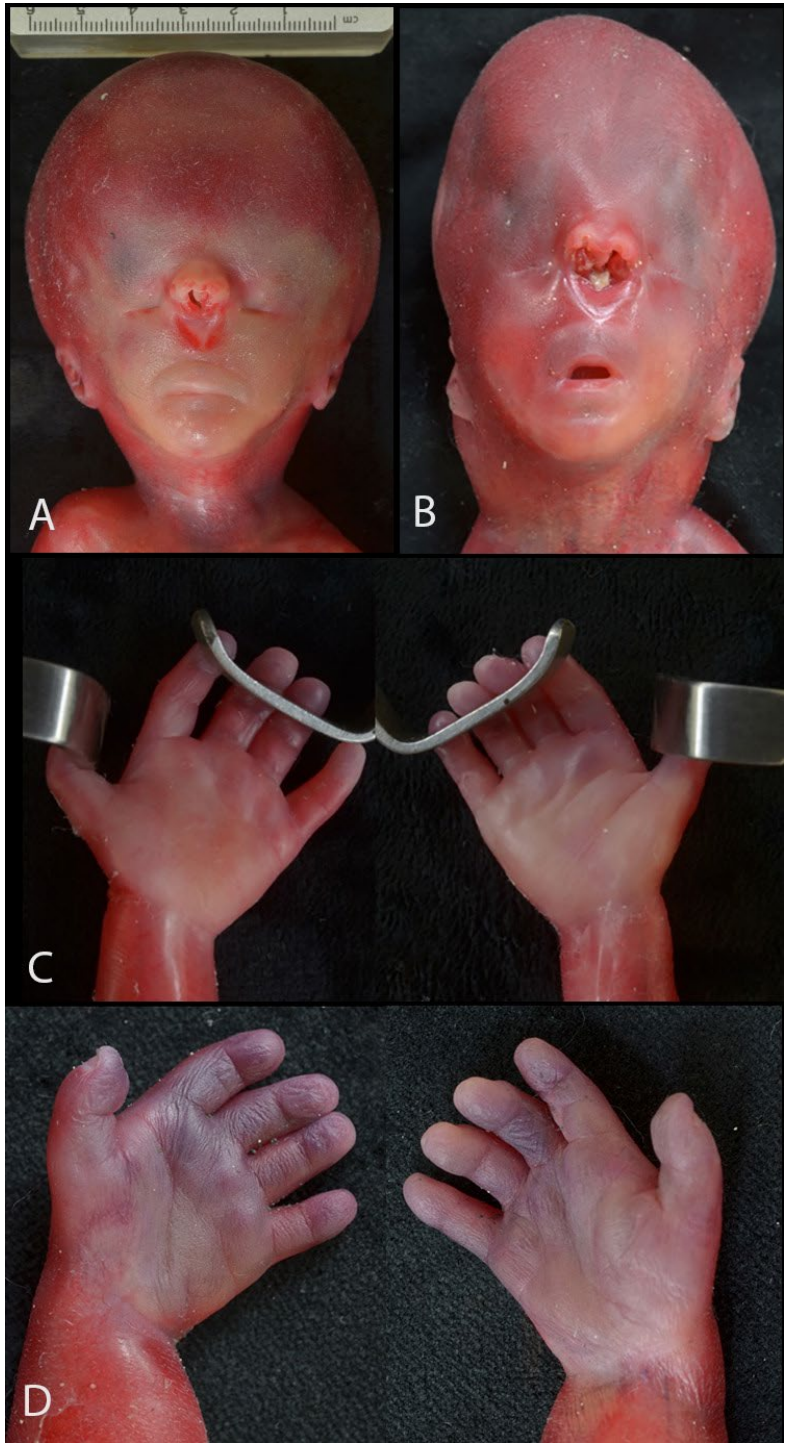


Figure 3. Facies

Comparison of similar facies of Proband 3 (A) and Proband 4 (B). Proband 3 (A) with an enlarged head with microphthalmia and hypotelorism. The columella is absent, resulting in a fused single nostril although the nasal septum is present internally. The philtrum is long with a wedge-shaped depression below the fused nostril, and there is micrognathia.

Comparison of hands of Proband 3 (C) and Proband 4 (D). Proband 4 (D) with hypoplastic, poorly demarcated middle phalanges on digits 2-5, with short, thick fingers.

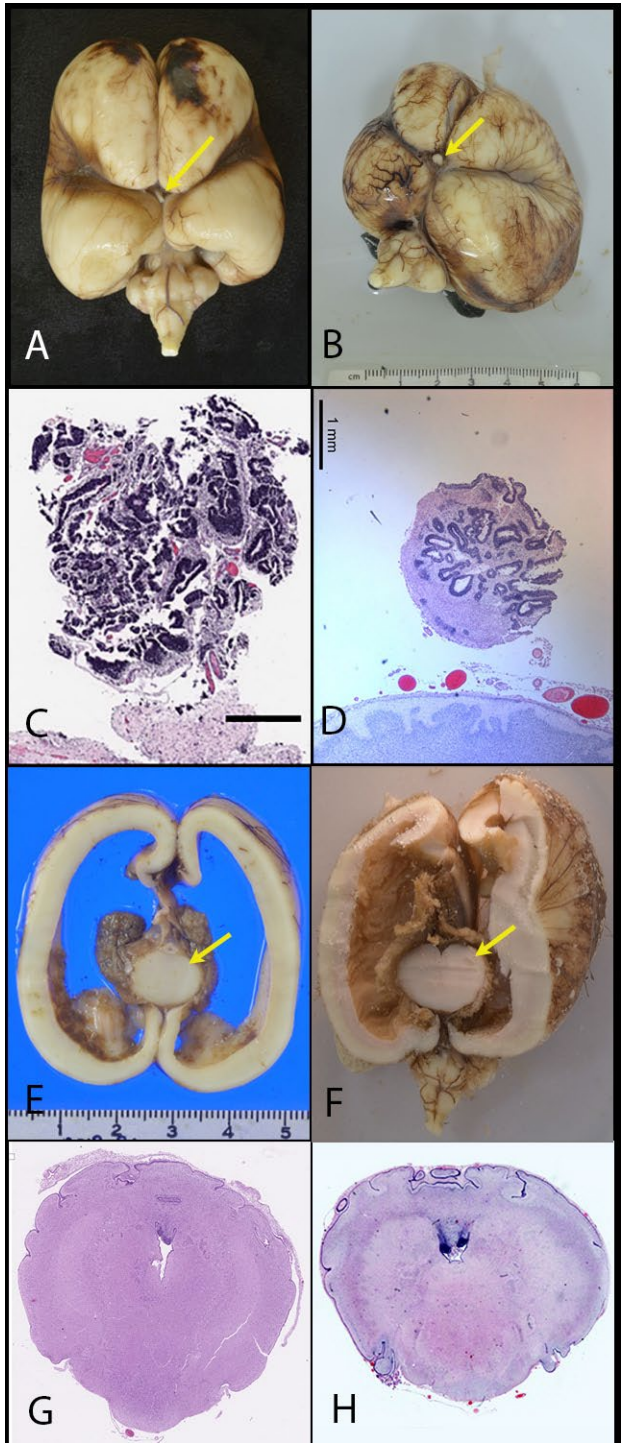


Figure 4. Central nervous system.
Inferior surface of brain in Proband 3 (A) and Proband 4 (B) demonstrating well marked interhemispheric fissure with absent olfactory bulbs and tracts, absent infundibulum, and single, thick fused optic nerve (arrow).
Section though optic nerve in Proband 3 (C) and Proband 4 (D) demonstrating rosettes of ciliated neuroepithelial elements resembling fetal retina.
Coronal sections in Proband 3 (E) and Proband 4 (F) demonstrating small fused thalamus (arrow) with massive ventriculomegaly and thinning of the cortical mantle. In both cases the midbrain was small and the aqueduct stenotic:
Cross sections of the cerebellum and pons in Proband 3 (G) and Proband 4 (H) demonstrate a minute 4th ventricle, a poorly demarcated cerebellar vermis and absent corticospinal tracts.

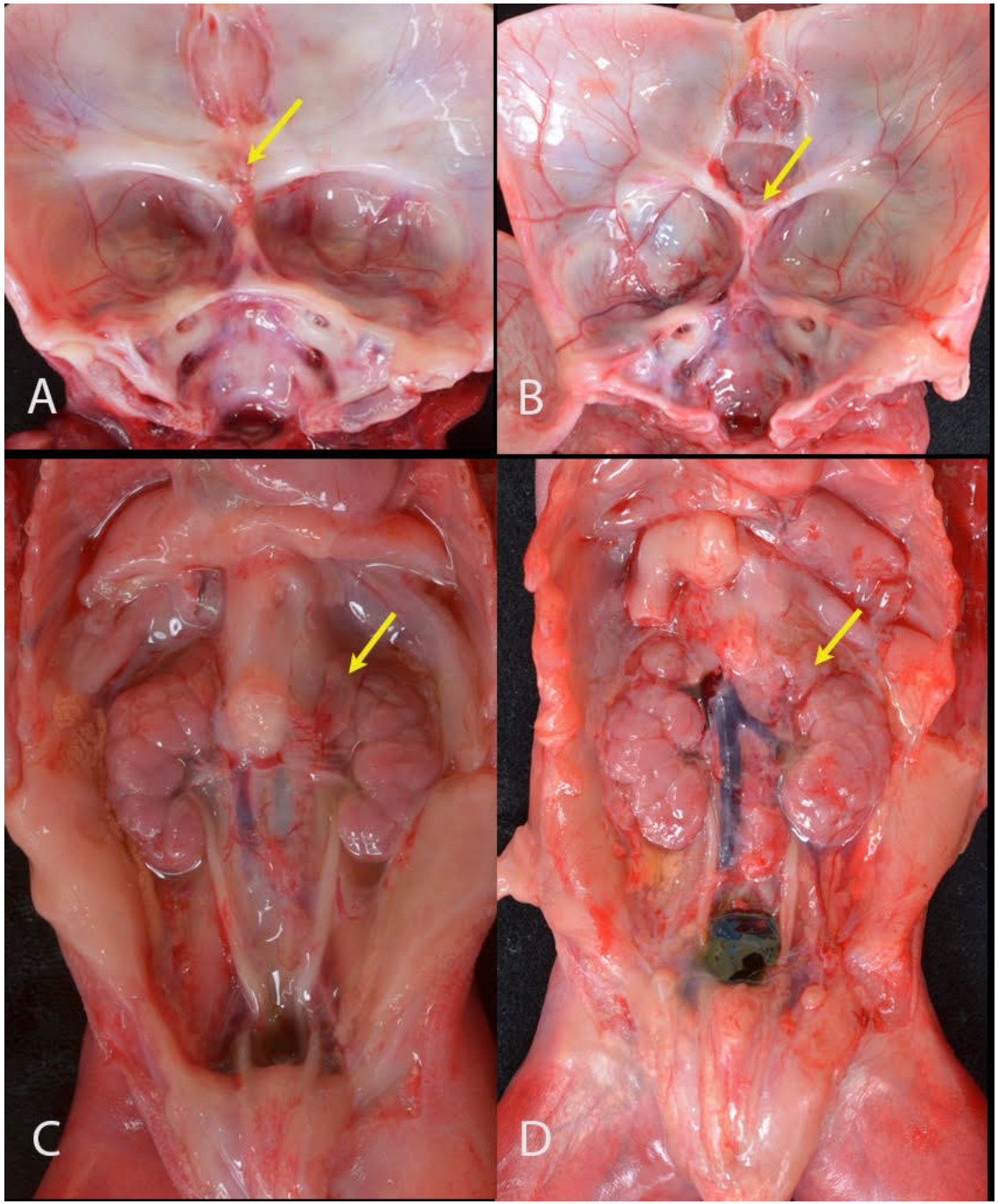


Figure 5. Internal Structures
Comparison of skull bases of Proband 3 (A) and Proband 4 (B). Proband 3 (A) with absent sella turcica. Arrow indicates fused optic nerve. No pituitary was present, even on subserial sectioning of the sphenoid. Proband 4 (B) demonstrating wide, shallow sella anterior to (arrow) fused optic nerve. A small pituitary was present. In both fetuses the hypothalamus was disorganized and no infundibulum was present. Retroperitoneum demonstrating small adrenal glands (arrows)in Proband 3 (C) and Proband 4 (D)

Table 1: Summary of clinical and genetic findings in pedigrees with recessive *TBC1D32* variants

	Hietamäki J, et al. (2020)	Hietamäki J, et al. (2020)	Hietamäki J, et al. (2020)	Monies D, et al. (2019)	Aldy, N et al. (2013)	Hietamäki J, et al. (2020)	Proband 1	Proband 2	Proband 3	Proband 4	Proband 5	Proband 6	Proband 7	Alsahan et al. (2020)	**Total**
***TBC1D32* variants**	c.1165_ 1166dup and c.2151del	c.1165_ 1166dup and c.2151del	c.1372+1 G>T homo-zygous	c.1372+1 G>T homo-zygous	c.1372 +1G>T homo-zygous	c.1372+1 G>T homo-zygous	c.2134 A>G and c3182G>C	c.2481+4 A>T and c.3572A>G	c.2650 C>T homo-zygous	c.2650 C>T homo-zygous	c.3325_ 3326delAG homo-zygous	c.3325_ 3326delAG homo-zygous	c.3527 A>G homo-zygous	C3724C>T homo-zygous	-
Oral/Facial															
Lip/Palate	-	-	High arched palate	Cleft lip	Midline facial cleft	Midline facial cleft	-	-	-	-	-	Midline facial cleft	-	-	**5/14**
Nose	Upturned nose	Upturned nose	Upturned nose, choanal atresia	-	Choanal atresia	-	Prominent	Choanal atresia	Single fused nostril	High position, partially fused nares	Absent	-	-	Single nostril	**10/14**
Ears	Low set	Low set	Low set	-	-	-	-	-	-	Low set	Low set	-	-	-	**5/14**
Tongue	-	-	Bifid	-	-	-	-	-	-	-	-	-	-	-	**1/14**
Dentition	-	Abnormal	-	-	-	-	-	Median central incisor	-	-	-	-	-	-	**2/14**
Facial	Hyper-telorism, prominent forehead	Hyper-telorism, prominent forehead	Hyper-telorism, prominent forehead	-	Midface hypoplasia	-	Deep set eyes, long philtrum, thin upper lip	Midface hypoplsia	Long philtrum, micro-gnathia	Midface hypo-plasia	Midface hypo-plasia	-	Hyper-telorism, deep set eyes, coarse facies	-	**10/14**
Eye															
Anophthalmia	-	-	-	-	-	-	-	-	-	-	Bilateral	-	-	Bilateral	**2/14**
Microphthalmia	-	-	-	-	Bilateral	-	-	-	Bilateral	Bilateral	-	-	-	-	**3/14**
Cyclopia	-	-	-	+	-	-	-	-	-	-	-	-	-	-	**1/14**
Coloboma	-	-	-	-	Bilateral	-	-	-	-	-	-	-	-	-	**1/14**
Vision	Astig-matism	Retinal dystrophy	Reduced visual acuity	-	-	-	-	Strabismus, hetero-chromia iridis	Retinal dysplasia	Retinal dysplasia	-	-	-	-	**6/14**
CNS															
Size	-	-	-	-	Macro-cephaly	-	Micro-cephaly	Macro-cephaly	Enlarged cranium	-	Enlarged cranium	Anen-cephaly	-	Enlarged cranium	**7/14**
Holoprosencephaly	-	-	-	+	-	-	-	-	Lobar	Lobar	-	-	-	-	**3/14**
Ventriculomegaly	+	-	-	-	-	-	+	+	+	+	-	-	-	+	**6/14**
Pituitary gland	Absent	Absent	Hypoplastic	-	Absent	-	-	Ectopic	Absent sella turcica	Dysplastic sella turcica	Absent sella turcica	-	-	-	**8/14**
Optic nerve	-	-	Septo-optic dysplasia	-	-	-	-	-	Fused	Fused	Absent	-	-	-	**4/14**

Thalamus	-	-	-	-	-	-	-	-	Fused thalami, dysplastic hypo-thalami	Fused thalami, dysplastic hypo-thalami	-	-	-	-	**2/14**
Cerebellum	-	-	-	-	Vermis hypoplasia	-	Dandy Walker	-	Abnormal vermis	Abnormal vermis	-	-	-	-	**4/14**
Corpus callosum	-	-	Agenesis	Agenesis	Agenesis	-	-	-	-		-	-	-	-	**3/14**
Limb															
Polydactyly	-	-	Postaxial	-	+	-	-	-	-	-	-	Unilateral postaxial	-	-	**3/14**
Syndactyly	-	2,3 toes	-	-	-	-	2, 3 toes	-	-	-	2-5 toes	-	-	-	**3/14**
Digits	-	Sandal gap	-	Sandal gap	-	-	-	Brachy-dactyly	-	Short digits	Sandal gap	Sandal gap	Absent 5th toe phal-ange	-	**7/14**
Other	-	Hyper-mobile joints	Rhizomelic shortening	Club foot	-	Short long bones	Mild restriction of joints	-	Flexed wrists, rocker bottom feet	Short, curved ulnea		-	Short long bones	Short long bones	**9/14**
Cardiac															
Congenital heart defect	-	-	-	VSD	PDA, ASD	-	-	-	AVSD	-	Dextro-cardia	-	-	-	**4/14**
Gastrointestinal															
Omphalocele	-	-	-	-	-	-	-	-	-	-	+	+	-	-	**2/14**
Malrotation	-	-	-	-	-	+	-	-	-	-	-	-	-	-	**1/14**
Situs	-	-	-	-	-	-	-	-	-	-	Situs inversus	-	-	-	**1/14**
Genitourinary															
Renal	-	-	-	-	-	-	-	-	-	Hypo-plasia	-	-	-	-	**1/14**
Adrenal	-	-	-	-	-	-	-	-	Hypoplasia	Hypo-plasia	-	-	-	-	**2/14**
Genitalia	Micro-penis, Crypt-orchidism	-	-	-	Ambiguous	-	-	-	-	Micro-penis	-	-	-	-	**3/14**
Developmental															
Delays	Global	Motor	Global	-	-	-	Global	Global	-	-	-	-	ID	-	**6/14**
Seizures	-	-	-	-	+	-	-	-	-	-	-	-	-	-	**1/14**
Prenatal presentation															
	-	-	Short long bones, midline cystic brain changes, poly-hydramnios	Multiple anomalies	Cleft lip, abnormal hands, small head	Midline facial cleft, short femur length	-	Short long bones, ventriculo-megaly	Multiple anomalies	Multiple anomalies fetal growth restriction	Multiple anomalies	Multiple anomalies, fetal growth restriction	-	Multiple anomalies	**10/14**

Abbreviations: VSD, ventricular septal defect; PDA, patent ductus arteriosus; ASD, atrial septal defect; AVSD, atrioventricular septal defect; ID, intellectual disability