Published in final edited form as:

Am J Med Genet A. 2021 September ; 185(9): 2756–2765. doi:10.1002/ajmg.a.62186.

HHAT-related multiple congenital anomalies: Report of an additional family and delineation of the syndrome

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Abstract

Previous reports of four affected individuals from two unrelated families with *HHAT* (hedgehog acyl-transferase) related multiple congenital anomaly syndrome. Microcephaly, small cerebellar vermis, holoprosencephaly, agenesis of corpus callosum, intellectual disability, short stature, skeletal dysplasia, microphthalmia-anophthalmia and sex reversal constitute the phenotypic spectrum of this condition with variable expression. We report an additional family with three affected conceptuses: two abortuses and one living proband. We did proband-parents trio exome sequencing and identified a biallelic in-frame deletion c.365_367del; (p.Thr122del) in exon 5 of *HHAT*. With this report, we delineate the phenotype and allelic heterogeneity of the *HHAT*-related multiple congenital anomaly syndrome.

Keywords

HHAT; multiple congenital anomaly syndrome; Nivelon-Nivelon-Mabille syndrome (NNMS); holoprosencephaly; microphthalmia; sex reversal; skeletal dysplasia

INTRODUCTION

"Chondrodysplasia-pseudo-hermaphroditism syndrome" (MIM# 600092) described by Nivelon et al. is the first clinical report of a family with an affected female with short stature, skeletal dysplasia, microcephaly, deep-set eyes, small iris, everted upper lip and sex reversal with a karyotype of 46, XY. The couple terminated their second pregnancy at 21 weeks of gestation in view of intra uterine growth retardation, microcephaly and skeletal dysplasia.

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AUTHOR CONTRIBUTION

KMG performed the clinical evaluation, collected family details, established the diagnosis, and designed concept for the study. SP and RP performed analysis of exome. SP performed the segregation analysis for the variants identified in the study. NS performed antenatal sonography and provided the images for the affected pregnancies in the present family. SP wrote the first draft of the manuscript. AS participated in the clinical evaluation, exome analysis and writing the manuscript. All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

We declare that the authors do not have any conflict of interest. All the authors have read and approved the final manuscript.

The karyotype of the fetus was 46, XX. (Nivelon et al., 1992; Thauvin-Robinet et al., 2005). Later, exome sequencing was performed for the living proband and a novel homozygous missense variant, c.449G>T; p.(Gly150Val) (NM_001170564.1) (equivalent to the long isoform [c.860G>T; p (Gly287Val); NM_001122834.3]) was identified in *HHAT*. The Gly287 residue lies in the highly conserved MBOAT (Membrane bound acyl transferase) domain (Callier et al., 2014).

Subsequently, Abdel-Salam et al., described a second family with two affected female siblings with microcephaly, small cerebellar vermis, seizures and skeletal dysplasia. In this family, they identified a biallelic missense variant, c.770C>T, p.(Leu257Pro) in exon 7 of *HHAT* (NM_001122834.3) as the likely cause of the multiple malformation syndrome (Abdel-Salam et al., 2019). The Leu257 residue also lies in the MBOAT domain. The parents were healthy and found to be carriers of this sequence variant while it was absent in the unaffected elder female sibling.

HHAT (Hedgehog acyl-transferase) mediates the post-translational modification of the downstream hedgehog proteins, Sonic (SHH), Indian (IHH), and Desert (DHH) which are the vital components of the hedgehog signalling pathway. These genes have a role in embryonic development of the central nervous system, face, musculo-skeletal system and genitourinary system (Abramyan, 2019; Dennis et al., 2012; Jeong, Mao, Tenzen, Kottmann, & McMahon, 2004; Konitsiotis et al., 2015). *HHAT* knockout mice showed that the loss of function of HHAT can cause severe malformations that include holoprosencephaly, acrania, agnathia, dwarfism, skeletal defects, osteochondrogenic defects, and variation of sexual characteristics (Callier et al., 2014; Dennis et al., 2012).

In this report, we describe the third family with multiple malformations in three pregnancies with a novel biallelic in-frame deletion, c.365_367del; (p.Thr122del) in exon 5 of *HHAT* in the living proband. We hereby delineate the phenotypes of *HHAT*-related multiple congenital anomaly syndrome and postulate the mechanisms underlying the variable manifestations of this condition.

CLINICAL REPORT

We ascertained a consanguineously married healthy couple with three pregnancies with multiple congenital anomalies. Their first pregnancy resulted in fetal loss at 27 weeks of gestation with intrauterine growth retardation, microcephaly, small chest with pectus excavatum, short fingers and long bones, large and low set ears, hypoplastic nasal bone and midface retrusion. The third pregnancy was medically terminated at 22 weeks of gestation. The antenatal ultrasonography scan at 22 weeks of gestation had showed mono-ventricle and alobar holoprosencephaly in the fetus. The fetus also had microcephaly, situs inversus, narrow and bell-shaped thorax, micromelia and brachydactyly. The 3D ultrasound reconstruction showed the presence of a single nostril (Figure 4).

The proband is second-born, currently, a seven-year-old female (Figure 1). Antenatal ultrasonography at 28-29 weeks of gestation had shown a small left eye, small cerebellum and bilateral renal pyelectasis in the fetus. There is no history of maternal exposure to

teratogens/medicines or infections during the antenatal period. Proband was born at term via normal spontaneous vaginal delivery. Her birth weight was 2.5 kg (− 2.2 SD) and she cried immediately at the time of birth. There was no history of seizures nor feeding difficulties. A cyst like structure in the right orbit at the time of birth was noted in her (Figure 2). At one month of age, her head circumference was noted to be 33 cm (− 4.2 SD), total length was 47 cm (− 4.6 SD) and weight was 2.78 kg (− 3.1 SD). Mild delay was noted in her early developmental milestones. She achieved good neck stability by four to five months-of-age, cooing and babbling by one and a half months, rolling over by nine months, sitting with support by one year of age, independent walking by one year eight months and monosyllables by nine months. Currently, she is seven-years-old, can speak in full sentences, she is toilet trained and goes to a special school.

Her height was 105 cm (− 3 SD), head circumference was 43 cm (− 8 SD) and weight was 14 kg (− 5.2 SD) at seven years of age. She has short stature, microcephaly, severe microphthalmia, closed palpebral fissures and mid-face retrusion with hypoplasia of the upper half of face, broad nasal bridge, widened ala nasi, long philtrum, short columella with a groove at the base, everted lower lip, large and prominent ears. She also had overcrowding of teeth with an erupting permanent single upper central incisor. She has fifth finger clinodactyly and brachydactyly of hands and feet (Figure 2). A sacral dimple was also noted. She could hardly perceive light. Her cholesterol levels were normal and creatinine phosphokinase (CPK) levels were 922.0 U/L (normal levels: 20-180 U/L). Her skeletal survey at seven years of age revealed mild scoliosis with normal vertebrae, narrow, bell shaped thorax, thin ribs (Supplementary figure 1), short and angel shaped epiphyses of phalanges of hands and feet (Figure 3). She underwent brain magnetic resonance imaging at seven years of age which was unremarkable (Supplementary figure 2). Echocardiography at the time of birth showed mildly dilated right atrium and ventricles and mild pulmonary arterial hypertension. However, at seven-years-old echocardiography was unremarkable. Her FSH and LH levels were age appropriate. Abdominal and pelvic ultrasonography revealed normal internal organs including uterus and ovaries.

MOLECULAR TESTING AND RESULTS

Informed consent and consent for medical photography with clearance from the institutional ethics committee were obtained from the family for publication of images and genetic data. The proband and her parents underwent karyotyping from peripheral blood. The genomic DNA was extracted using the standard phenol-chloroform method. Single nucleotide polymorphism (SNP) array was carried out using the Illumina HumanCytoSNP-12 BeadChip (San Diego, California, United States). For analysis, we used the Illumina KaryoStudio 1.4.3.0 Build 37 (CNV Plugin V3.0.7.0) software (San Diego, California, United States). Proband-parents trio exome sequencing was performed. The exonic and flanking genomic regions were captured using Agilent SureSelect Clinical Research Exome V2 (CREv2) capture kit (Agilent technologies Inc, Santa Clara, California, United States). Exome sequencing had an average coverage of 100 to 130X, 95% of bases covered at a minimum of 20X with 90% sensitivity (Girisha et al., 2019). Raw data was retrieved in FASTQ format and aligned to GRCh37 assembly using Burrows-Wheeler Aligner (v0.7.15) and our in-house pipeline based on Genome Analysis Toolkit Best Practices (Supplementary

material). This data was annotated by ANNOVAR and our in-house scripts (Wang, Li, & Hakonarson, 2010). The filtered variants were analyzed using variant prioritization and filtering strategy as outlined in table S1 (Supplementary material). The rare homozygous variants observed in the proband are listed in table S2 (Supplementary material). Validation of the disease causing variant and segregation analysis in the family were done by Sanger sequencing. Multiple sequence alignment checking for conservation of the amino acid residue across the species was performed using Clustal Omega (EMBL-EBI Hinxton). We did not analyze the consequences of this variant *in vivo* further.

The proband had a karyotype of 46, XY while parental karyotypes were normal. Chromosomal microarray also confirmed the karyotype (46, XY) without any clinically significant copy number variants. Trio exome sequencing in the family identified a homozygous in-frame deletion, c.365_367del; p.(Thr122del) in exon 5 of *HHAT* (NM_001122834.3) as the likely cause of her phenotype in view of previous two publications (Abdel-Salam et al., 2019; Callier et al., 2014) and the mouse phenotype (Dennis et al., 2012). Sanger sequencing confirmed the carrier status of the parents. This variant is not observed in population databases like gnomAD and in our in house database of 960 exomes which is derived from sequencing the affected subjects and their unaffected families with a suspected monogenic disorder at our centre. *In silico* analysis tools (MutationTaster, Sorting intolerant from tolerant indel) predicted the sequence variant was is damaging to the HHAT protein function (Supporting information). The multiple sequence alignment using Clustal Omega showed that the Thr122 residue is conserved across mammals (Supplementary figure 3). Using American College of Medical Genetics and Genomics variant classification, this variant would currently be classified as a variant of uncertain significance.

DISCUSSION

We describe a family with the proband demonstrating severe microphthalmia, microcephaly, skeletal dysplasia (narrow bell shaped thorax, short and angel shaped epiphyses of hands and feet) and midface retrusion, short columella with a groove at the base, prominent ears, long philtrum, depressed nasal bridge, everted lower lip, and a single central incisor. She also has complete sex reversal. Exome sequencing in the living proband revealed a novel biallelic variant, c.365_367del; (p.Thr122del) in exon 5 of *HHAT* (NM_001122834.3) as the likely cause of this multiple congenital anomaly syndrome. Two more pregnancies were affected with one of them demonstrating alobar holoprosencephaly. The products of pregnancy (III-3 in Fig 1a) were not tested molecularly. However, a constellation of holoprosencephaly, intrauterine growth retardation, microcephaly and skeletal dysplasia suggests this pregnancy is also affected with HHAT-related multiple congenital anomaly syndrome.

We summarize the clinical and molecular findings in all the three families (present and previous two reports) and seven affected individuals in table 1. The overlapping phenotypes in the previous two families and the present family include microcephaly, short stature, elevated creatinine phosphokinase levels and skeletal dysplasia. Small cerebellar vermis was reported in the previous two families (Abdel-Salam et al., 2019; Callier et al., 2014). Alobar holoprosencephaly and a single median central incisor is reported only in the present family

and no other signs of holoprosencephaly were noted previously in individuals with variants in *HHAT*. Sex reversal with a karyotype of 46, XY in the female proband was previously reported by Nivelon et al (Nivelon et al., 1992) and this is seen in the present family as well. Ophthalmic defects like microphthalmia were also observed in the family reported by Nivelon et al (Nivelon et al., 1992) and in the present family.

With this report, three families with sequence variants in *HHAT* are described with a multiple congenital anomaly syndrome. In the first family reported by Nivelon et al.,(Callier et al., 2014; Nivelon et al., 1992; Thauvin-Robinet et al., 2005), a homozygous missense variant, c.860G>T; p.(Gly287Val); NM_001122834.3 in exon 8 of *HHAT* was identified. In the second family by Abdel-Salem et al., (Abdel-Salam et al., 2019), a homozygous missense variation, c.770C>T, p.(Leu257Pro) in exon 7 of *HHAT* (NM_001122834.3) was identified. Both Gly287 and Leu257 residue lie in the highly conserved MBOAT domain (Figure 5). In the present family, a homozygous in frame deletion, c.365_367del; p.(Thr122del) in exon 5 of *HHAT* (NM_001122834.3) was identified as the likely cause of the condition which lies just proximal to the MBOAT domain. These variants are likely to cause complete loss of function of HHAT leading to the defective downstream signalling by disrupting the ability of HHAT to palmitoylate Hh proteins such as SHH, IHH and DHH (Callier et al., 2014) leading to further phenotypic consequences as seen in all the affected families.

We also note report of a family with HHAT variants (Agha et al., 2014). The two affected siblings had profound intellectual disability. Brain malformations, genital abnormalities or facial dysmorphism were absent. Exome sequencing detected a heterozygous missense mutation, c.1158G>C, p.(Trp386Cys) in *HHAT* (NM_001122834.3) in them. This variant was not seen in their parents and a healthy sibling raising the possibility of germline mosaicism in one of the healthy parents. However, in the present and previously two reported families, the carrier parents are healthy (Abdel-Salam et al., 2019; Callier et al., 2014). Thus, it is not clear if a heterozygous variant in *HHAT* can truly lead to an autosomal dominant form of intellectual disability.

Cell to cell communication mediated via several signalling pathways plays a pivotal role during intrauterine development. The hedgehog signalling pathway mediates cell patterning, differentiation and determination of cell fate (Matus, Magie, Pang, Martindale, & Thomsen, 2008). The original hedgehog gene was first discovered in *Drosophila*, governing cell fate and differentiation of gut, wings, eye and muscle while its paralogues have a role in development of almost all vital systems including nervous system, face, genitourinary system and connective tissue (Hooper & Scott, 2005). The Hh family has three important protein components, Sonic (SHH), Indian (IHH), and Desert (DHH). These downstream proteins involved in the pathway are implicated in a wide range of functioning including limb patterning, maintenance of antero-posterior and left-right symmetry, development of limbs, somites, nervous system, eyes, bones, cartilage and muscles. Pathogenic variations in genes encoding Hh related and the downstream signalling proteins have been associated with several developmental defects in *Drosophila* and other vertebrate model organisms. Developmental defects of forebrain and hindbrain including holoprosencephaly, corpus callosal dysgenesis, cerebellum and vermis hypoplasia; musculo-skeletal defects, limb

defects, genito-urinary defects are attributed to disturbed hedgehog pathway in humans as well (Abramyan, 2019; Callier et al., 2014; Dennis et al., 2012; Li et al., 2020; Roessler & Muenke, 2003).

SHH is one of the key regulators of forebrain and hindbrain development during as it has a role in mediating the differentiation of axial mesoderm into neural ectoderm (Sagai, Amano, Maeno, Ajima, & Shiroishi, 2019). *SHH* is also expressed in the zone of polarizing activity of the developing limb bud, thereby mediating antero-posterior limb and finger patterning (Riddle, Johnson, Laufer, & Tabin, 1993; Tickle & Towers, 2017). The defective *SHH* functioning is known to cause severe facial defects, holoprosencephaly, cerebellum and vermis defects; and limb defects (De Luca et al., 2016; Sagai et al., 2019). The OMIM phenotypes associated with *SHH* are holoprosencephaly type 3 (MIM# 142945), microphthalmia with coloboma 5 (MIM# 611638), schizencephaly (MIM# 269160) and a single median maxillary central incisor (MIM# 147250). *IHH* is found to have a role in chondrocyte differentiation and maturation of the skeletal elements and defects in *IHH* function is reported to cause skeletal defects like acro-capito-femoral dysplasia (MIM# 607778) and short stature (Laurie, Kokubo, Nakamura, Saga, & Funato, 2016; Vasques et al., 2018). *DHH* is mainly expressed in the Sertoli cells of the developing testis and defective *DHH* function is associated with 46XY gonadal dysgenesis with minifascicular neuropathy (MIM# 607080) and 46XY sex reversal 7 (MIM# 233420) (Baldinotti et al., 2018; Paris et al., 2017).

Holoprosencephaly is a genetically and phenotypically heterogeneous malformation and is characterized by incomplete cleavage of the forebrain and originates from failed midline delineation during early development. The severity of this malformation ranges from cyclopia, alobar, semi-lobar or lobar forms of holoprosencephaly to milder forms including median facial defects like hypotelorism, clefts of lip or palate or median single central incisor (Dubourg et al., 2018; Roessler & Muenke, 2003). Pathogenic variants in at least thirteen genes (*SIX3, GLI2, HPE6, SHH, PTCH1, CDON, ZIC2, HPE8, CNOT1, TGIF1, HPE1* and *STAG2)* have been identified till date in patients with holoprosencephaly (Amberger, Bocchini, Schiettecatte, Scott, & Hamosh, 2015). Apart from these, copy number variations including multi exon deletions, contiguous gene deletions or large chromosomal imbalances like trisomy 13 are also reported to cause holoprosencephaly (Pineda-Alvarez, Dubourg, David, Roessler, & Muenke, 2010). In the present family, the proband has a single median central incisor with no evidence of any other brain malformation on magnetic imaging resonance of brain. In the third pregnancy of her parents, the fetus had a mono-ventricle suggestive of alobar holoprosencephaly and a single nostril (Figure 4). There was no evidence of holoprosencephaly in either of the previously reported two families by Nivelon et al and Abdel-Salam et al. HHAT is expressed during the early embryonic life in the neural crest cells (Dennis et al., 2012). Knockout of HHAT in mice showed disturbances in the hedgehog signalling during embryogenesis and resulted in defects with a variable severity that included holoprosencephaly, agnathia, acrania, eye defects, skeletal abnormalities and osteochondrogenic defects; disorders of sexual development (Callier et al., 2014; Dennis et al., 2012). These features are similar to those observed with the loss of function of other Hh genes like *SHH, DHH* and *IHH* observed in the knockout mice (Chen, Li, Kawakami, Xu, & Chuang, 2004; Dennis et al.,

2012). These findings strongly support that holoprosencephaly is a part of the phenotype of HHAT-related multiple congenital anomaly syndrome.

Small cerebellar vermis is reported previously with HHAT-related multiple congenital anomalies syndrome (Abdel-Salam et al., 2019; Nivelon et al., 1992; Thauvin-Robinet et al., 2005). Role of SHH in cerebellar development and patterning has been reported (De Luca et al., 2016). These findings suggests that cerebellar vermis hypoplasia is one of the clinical finding associated with HHAT related multiple congenital anomaly syndrome, though it was not observed in the present family.

In the present family, severe microphthalmia-anophthalmia with closed palpebral fissure was noted in the living proband. Microphthalmia was also noted in the proband reported by Nivelon et al., (Nivelon et al., 1992). Microphthalmia-anophthalmia either in isolation or associated with holoprosencephaly due to pathogenic variants in *SHH* are already known (Nanni et al., 1999; Schimmenti et al., 2003). These suggest that defective downstream hedgehog signalling can lead to eye defects as seen in the previously reported patient (Nivelon et al., 1992) and in the present family.

In the present family, the proband had normal internal and external female genital organs and her karyotype was 46, XY. Similarly, in the first family reported by Nivelon et al., (Callier et al., 2014; Nivelon et al., 1992), the elder female proband had a karyotype of 46, XY. Previous studies suggest that *HHAT* is expressed in all the cell lineages during embryonic life at the time of sex determination whereas later it is particularly expressed in the testicular Sertoli cells and Leydig cells (Callier et al., 2014). The transfection study and the HHAT *in vitro* activity assay performed by Callier et al., confirmed that p.Gly287Val identified in their family, results in non-functional HHAT protein activity and lacks the ability to palmitoylate the other vital hedgehog proteins (Callier et al., 2014; Das, Sanghavi, Gawde, Idicula-Thomas, & Vasudevan, 2011). These findings convince us that sex reversal is a feature of this condition where chromosomal sex is male.

Short stature, micromelia, brachydactyly, trapezoidal vertebrae, thin ribs, were the findings noted in all the three families including the present one suggesting an acromelic skeletal dysplasia is a part of this syndrome (Abdel-Salam et al., 2019; Nivelon et al., 1992). *IHH*, an important component of the hedgehog signalling pathway and is expressed in the chondrocytes and has a major role in osteo-chondro differentiation of the skeletal elements (Vasques et al., 2018). Brachydactyly type A1 (MIM# 112500) and acrocapitofemoral dysplasia (MIM# 607778) are the two phenotypes associated with *IHH* as of today (Amberger et al., 2015). This might be a rare form of skeletal dysplasia characterized by short stature, scoliosis, narrow bell-shaped thorax, thin ribs, micromelia, brachydactyly, angel shaped epiphysis of phalanges and short middle phalanges of hands and feet with some resemblance to short rib thoracic dysplasia (a ciliopathy with skeletal manifestations, a possible connection is the role of SHH in ciliary biogenesis). While in the previous two reports, the authors have defined the skeletal phenotype as chondrodysplasia.

The creatinine phosphokinase levels were noted to be highly elevated in the affected individuals from all the three families (Abdel-Salam et al., 2019; Nivelon et al., 1992)

indicative of a possible muscular dystrophy. Thus, elevated creatinine phosphokinase is an important finding in HHAT-related multiple congenital anomaly syndrome, though we are unable to explain this finding currently. A follow up or muscle biopsy in affected individuals might reveal the full phenotype.

To summarize, we conclude that the biallelic variants in *HHAT* underlie an autosomal recessive clinical entity (multiple malformation syndrome) characterized by microcephaly, small cerebellar vermis, holoprosencephaly, skeletal dysplasia and sex reversal, previously described as chondrodysplasia-pseudo-hermaphroditism syndrome. We list the possible mechanisms that give rise to some of the phenotypic features observed in this condition. Further reports of patients and studies on impact of mutations on the downstream signalling cascade are likely to improve our current understanding of the *HHAT*-related multiple congenital anomaly syndrome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

We would like to dedicate this work to Dr John M Graham Jr, for his contributions to dysmorphology education in India. We wish to thank the family members of the patient for their participation in this study. We also thank the National Institutes of Health, USA for funding the project titled ‘Genetic Diagnosis of Neurodevelopmental Disorders in India’ (1R01HD093570-01A1). We would also like to acknowledge the Indian Council of Medical Research for awarding the Nurturing Clinical Scientist fellowship to SP for pursuing her research studies (HRD/Head NCS-2019-03).

DATA AVAILABILITY STATEMENT

The data providing the evidence of the study is available from the corresponding author upon reasonable request.

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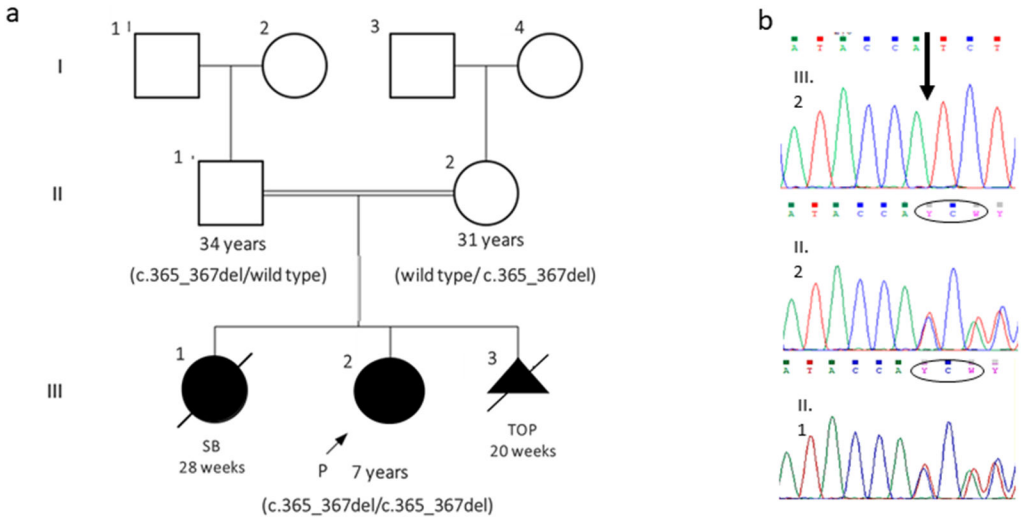

Figure 1.

a. Pedigree of the family. b. Sanger chromatograms of the bi-allelic variant, c.365_367del in *HHAT* in the living proband and its segregation in her parents. III-3 was not tested molecularly.

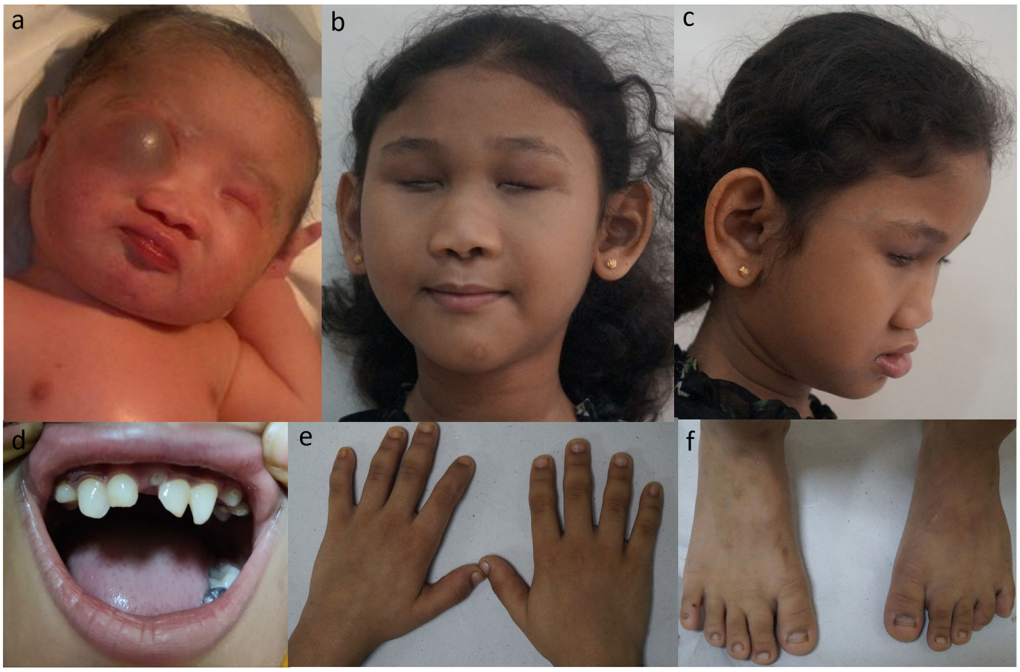

Figure 2.

Clinical photographs of the proband at birth (a) and at seven years of age (b, c, d, e and f). She had bilateral microphthalmia with a cyst like structure in the right orbit at birth (a). She has depressed nasal bridge and widened ala nasi, long philtrum, short columella and large and prominent ears (b). She also has mid face hypoplasia and everted lower lip (c). Her single deciduous central incisor is being replaced by a newly appearing permanent tooth (d). She has brachydactyly and fifth finger clinodactyly in hands (e) and brachydactyly of toes (f).

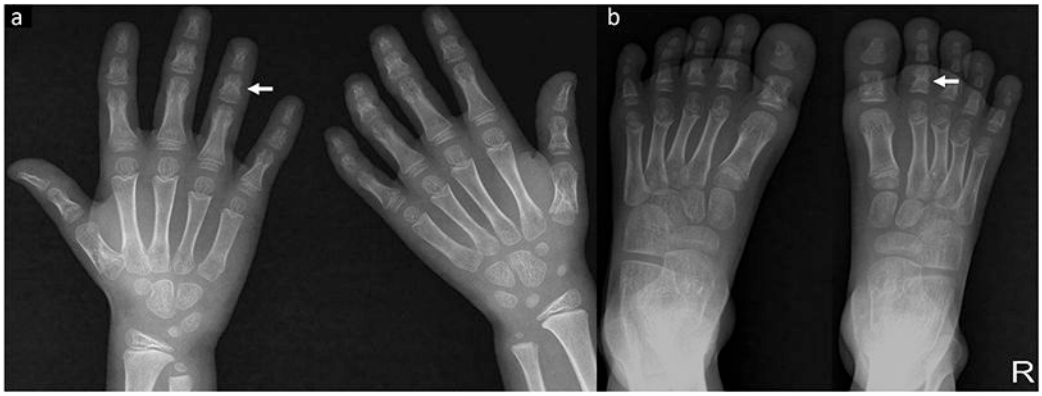

Figure 3.

Radiographs of the proband at 7 years of age show short ulna in both hands (a) short phalanges (a and b) and angel shaped middle phalanges in hands and feet (arrow) (b).

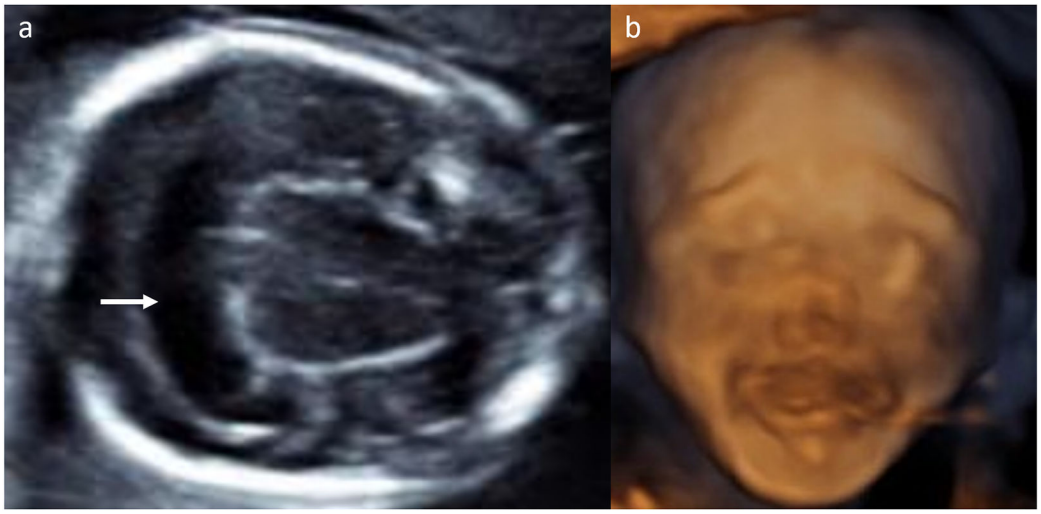

Figure 4.
Ultrasonographic image of III.3 at 22 weeks of gestation shows monovetricle (arrow) and alobar holoprosencephaly (a). 3D reconstruction of images suggests single nostril in the fetus (b).

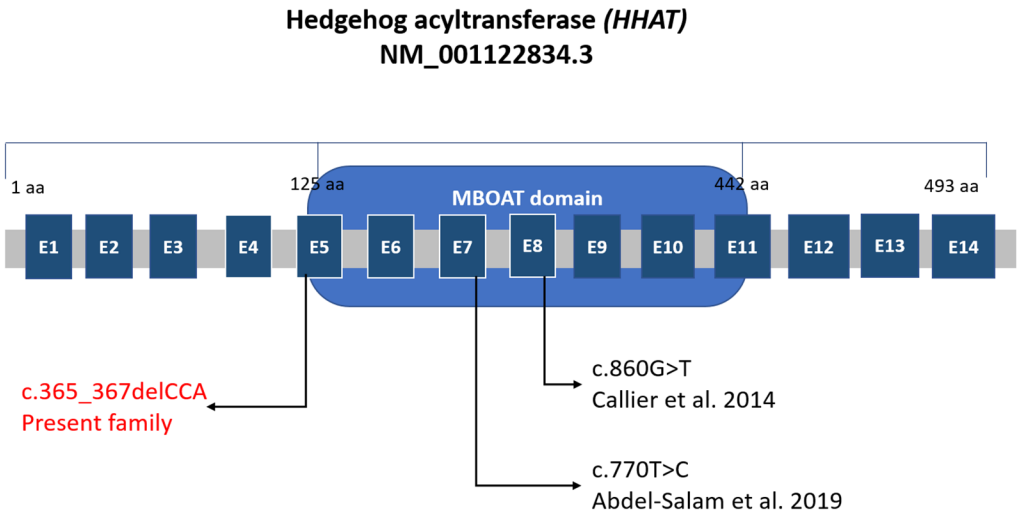

Figure 5.

Cartoon of the *HHAT* depicting the structure, MBOAT (Membrane Bound O-Acyltransferase) domain and disease-causing variants.

Table 1:

Demographic, clinical and mutation profiles of patients with *HHAT*-related multiple congenital anomaly synrome

	Family 1		**Family 2**		**Family 3**		
Reference	Callier et al., 2014		Abdel-Salam et al., 2019		Present family		
Country of origin	France		Egypt		India		
Consanguinity	−		+		+		
Gender	Female	Female	Female	Female	Female	Female	ND
Age	NA	Fetus with gestational age 17 weeks	6 years	3 years	Fetus with gestational age 28 weeks	7 years	Fetus with gestational age 20 weeks
Anthropometry							
Head circumference	42.5 (−7.5 SD)	ND	44 cm (−6 SD)	41 cm (−6 SD)	ND	43 cm (−8 SD)	ND
Height	110.5 (−9 SD)	ND	111 cm (−1 SD)	89 cm (−1 SD)	ND	103 cm (−3.1 SD)	ND
Weight	ND	ND	19 kg (−0.5 SD)	12 kg (−1 SD)	ND	14 kg (−1.9 SD)	ND
Intrauterine growth retardation	−	+	−	−	+	+	+
Facial dysmorphism							
Upslanting palpebral fissures	+	ND	+	+	ND	−	ND
Puffy eyelids	+	ND	−	−	ND	−	ND
Hypotelorism	−	ND	−	−	ND	−	+
Large, low set ears	ND	ND	ND	ND	+	+	ND
Hypoplastic nasal bone	ND	ND	ND	ND	+	+	+
Macrognathia	+	ND	−	−	ND	−	ND
Everted lower lip	−	ND	−	−	ND	+	ND
Mid-face retrusion	+	ND	−	−	+	+	+
Prognathism	+	ND	−	−	ND	−	ND
Single central incisor	ND	NA	ND	ND	NA	+	NA
Single nostril	−	−	−	−	ND	−	+
Central nervous system							
Intellectual disability (IQ)	+ (67)	NA	− (70)	− (77)	NA	−	NA
Microcephaly	+	ND	+	+	+	+	+
Holoprosencephaly	−	ND	−	−	ND	+	+
Cerebellar vermis hypoplasia	+	ND	+	+	−	−	−
Thinning of corpus callosum	−	ND	−	+	−	−	−
Dysarthria	−	NA	−	−	NA	−	NA

	Family 1		Family 2		Family 3		
Seizures	−	NA	+ (generalised tonic-clonic seizures)	+ (hemi-convulsions)	NA	−	NA
Eyes							
Hypoplastic irides	+	ND	−	−	ND	−	ND
Coloboma of both optic discs	+	ND	−	−	ND	−	ND
Microphthalmia/ anopthalmia	+	ND	−	−	ND	+	ND
Myopia	+	ND	−	+	NA	−	NA
Orbital hypoplasia	−	−	−	−	ND	+	+
Optic atrophy	−	ND	−	−	ND	−	ND
Musculo-skeletal findings							
Scoliosis	+	ND	−	−	ND	+	ND
Narrow, bell-shaped thorax	+	ND	−	−	+	+	+
Trapezoidal vertebrae	+	+	+	+	ND	−	ND
Chondrodysplasia	+	+	−	−	ND	−	ND
Abnormal clavicles	+	+	+	+	ND	ND	ND
Thin ribs	+	+	−	−	ND	+	ND
Narrow iliac wings	+	+	+	+	ND	−	ND
Micromelia	+	ND	−	−	+	+	+
Brachydactyly	+	ND	+	−	+	+	+
Enlarged epiphysis and metaphysis	−	ND	+	+	ND	−	ND
Angel shapped epiphysis of phalanges	ND	ND	ND	ND	ND	+	ND
Short middle phalanges of hands and feet	ND	ND	ND	ND	ND	+	ND
Muscular hypertrophy	+	ND	+	+	ND	−	−
Hypoplastic nails	+	+	+	+	ND	−	ND
Genitourinary system							
External genitalia	Normal female	Normal female	Normal female	Normal female	Normal female	Normal female	ND
Pubertal development	Normal	NA	Normal	Normal	NA	NA	NA
Menstrual status	Normal	NA	NA	NA	NA	NA	NA
Sex reversal	+	−	−	−	ND	+	ND
Cholesterol levels	Increased	NA	Normal	Normal	NA	Normal	NA
Creatinine phosphokinase levels	Elevated	NA	Elevated	Slightly elevated	ND	Elevated	ND

	Family 1		Family 2		Family 3		
Genetic/Genomic testing							
Karyotype	46, XY	46, XX	46, XX	46, XX	ND	46, XY	ND
Trancript ID	NM_001122834.3	NA	NM_001122834.3	NM_001122834.3	NA	NM_001122834.3	NA
DNA change	c.860G>T/ c.860G>T	NA	c.770T>C/ c.770T>C	c.770T>C/ c.770T>C	NA	c.365_367del/ c.365_367del	NA
Protein change	p.(Gly287Val)	NA	p. (Leu257Pro)	p. (Leu257Pro)	NA	p.(Thr122del)	NA

(+) present; (−) absent; (NA) not applicable; (ND) not documented