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Homozygous loss-of-function variants of *TASP1*, a gene encoding an activator of the histone methyltransferases KMT2A and KMT2D, cause a syndrome of developmental delay, happy demeanor, distinctive facial features, and congenital anomalies

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/humu.23844.

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Abstract

We report four unrelated children with homozygous loss-of-function variants in *TASP1* and an overlapping phenotype comprising developmental delay with hypotonia and microcephaly, feeding difficulties with failure-to-thrive, recurrent respiratory infections, cardiovascular malformations, cryptorchidism, happy demeanor, and distinctive facial features. Two children had a homozygous founder deletion encompassing exons 5-11 of *TASP1*, the third had a homozygous missense variant, c.701C>T (p.Thr234Met), affecting the active site of the encoded enzyme, and the fourth had a homozygous nonsense variant, c.199C>T (p.Arg67*). *TASP1* encodes taspase 1 (TASP1) which is responsible for cleaving, thus activating, the lysine methyltransferases KMT2A (MLL1) and KMT2D (MLL2), which are essential for histone methylation and transcription regulation. The consistency of the phenotype, the critical biological function of TASP1, the deleterious nature of the *TASP1* variants, and the overlapping features with Wiedemann-Steiner and Kabuki syndromes respectively caused by pathogenic variants in *KMT2A* and *KMT2D* all support that *TASP1* is a disease-related gene.

Keywords: TASP1, histone methylation, novel gene, novel syndrome, whole exome sequencing, chromosomal microarray

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The marked contrast between the static genome and the highly variable transcriptome and proteome of a given organism is a testimony to the complexity of gene expression control. Key to the dynamic spatial and temporal control of gene expression is the chromatin state (packaging of DNA around histone proteins), which in turn dictates DNA accessibility to the transcriptional machinery (Greer & Shi, 2012). There are numerous post-translational modifications of histones e.g. phosphorylation, acetylation, ubiquitylation, and methylation that net effect of which determines the level of gene expression (Tan et al., 2011). One of the best known forms of histone methylation involves histone 3 (H3) lysine residues, which can be monomethylated, dimethylated, or trimethylated (Greer & Shi, 2012). The state of methylation of a number of key lysine residues represents an important component of the epigenetic mark which correlates with gene expression states, and its dynamicity is achieved by the opposing action of methyltransferases and demethylases (Young, Dimaggio, & Garcia, 2010).

While histone methylation has been extensively studied in the fields of cancer, there is a growing appreciation that abnormal histone methylation plays an important etiological role in intellectual disability whether in isolation or as part of multiple congenital anomalies syndromes (Mastrototaro, Zaghi, & Sessa, 2017). Some of these monogenic forms of intellectual disability are well established in the literature such as Sotos syndromes, which is caused by mutations in *NSD1* encoding a histone methyltransferase (Kurotaki et al., 2002). The list of intellectual disability syndromes caused by mutations in genes encoding various players in histone methylation has been expanding quickly due to growing use of genome sequencing. A few notable examples include Wiedemann-Steiner syndrome and Kabuki syndrome 1, which are caused by

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mutations in *KMT2A* (lysine methyltransferase 2A, also known as *MLL1*) and *KMT2D* (lysine methyltransferase 2D, also known as *MLL2*), respectively (Jones et al., 2012; Ng et al., 2010).

Factors that control the activity of methyltransferases and demethylases represent an additional layer of complexity in the dynamic fine-tuning of the epigenetic mark. In view of the established role of histone methylation in intellectual disability syndromes, genes which encode these factors are attractive candidates in the search for novel candidate genes (Larizza & Finelli, 2019). In this report, we suggest one such gene is *TASP1* (MIM# 608270). This gene encodes the proenzyme taspase 1 (TASP1) which is autocatalytically cleaved into alpha and beta subunits resulting in a mature heterodimeric enzyme. This enzyme is an endopeptidase that utilizes the N-terminal threonine of its mature beta subunit as the active-site nucleophile to proteolyze polypeptide substrates following aspartate, hence called threonine aspartase (taspase). The active TASP1 is responsible for cleaving, thus activating, a number of broad-acting nuclear factors which play important roles in transcription regulation (Hsieh, Cheng, & Korsmeyer, 2003; Niizuma, Cheng, & Hsieh, 2015). TASP1 activates the lysine methyltransferase KMT2A and KMT2D which subsequently induce *HOX* and cyclin genes that are crucial for cell fate determination and cell cycle progression, respectively (Hsieh et al., 2003; Takeda et al., 2006).

We present here four unrelated children with an autosomal recessive syndrome caused by homozygous loss-of-function variants in *TASP1*. The first two were diagnosed in a single institution in the Arabian Peninsula. The third was diagnosed in another institution in the Arabian Peninsula. The fourth was diagnosed in Germany and recruited

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through GeneMatcher (Sobreira, Schiettecatte, Valle, & Hamosh, 2015). The common clinical features among these unrelated children and the deleterious nature of the variants suggest that *TASP1* is a disease-associated gene responsible for a consistent, previously unrecognized syndrome. The study was approved by Al-Ain Medical District Human Research Ethics Committee, Ethics Committee of the Technical University of Munich, and the Ethics Committee of the King Faisal Specialist Hospital and Research Centre.

The first child was a four-year-old boy with global developmental delay, hypotonia, microcephaly, feeding difficulties, drooling, failure-to-thrive, recurrent respiratory infections, cryptorchidism, cardiovascular malformations (atrial septal defect (ASD) and ventricular septal defect (VSD)), hearing impairment in the right ear, clinodactyly, left single palmer crease, brachydactyly, excessive hair on trunk and extremities, a happy demeanor with frequent laughing and clapping, and distinctive facial features **(Figure 1A; Supp. Clinical Data).** Chromosomal microarray (CMA), performed at Baylor Genetics Laboratory, Houston, Texas, USA as previously described (Gambin et al., 2017), revealed a homozygous deletion at 20p12.1 corresponding to a minimum deletion boundary of chr20:13,463,860-13,532,560 (hg19) **(Supp. Figure S1A).** The minimum deleted region (69 kb) only includes exons 9-11 of *TASP1* (NM_017714.2). Whole exome sequencing (WES) did not reveal any relevant pathogenic variants in known disease-causing genes potentially responsible for the phenotype. Furthermore, no sequence coverage was obtained by WES for part of the *TASP1* gene, which is consistent with the homozygous deletion affecting this gene.

The second child was a one-year old girl with global developmental delay, hypotonia, microcephaly, feeding difficulties, failure-to-thrive, recurrent respiratory

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infections, cardiovascular malformation (patent foramen ovale (PFO) and VSD), left hydronephrosis, left preauricular skin tag, right hand preaxial polydactyly, excessive hair on trunk and extremities, bilateral single palmar creases, congenital dermal melanocytosis and a tuft of hair in the sacral region, bilateral hyperopia, happy demeanor with frequent laughing, and distinctive facial features **(Figure 1B; Supp. Clinical Data).** CMA performed at PreventionGenetics, Marshfield, Wisconsin, USA as previously described (Alabdullatif, Al Dhaibani, Khassawneh, & El-Hattab, 2017) revealed a homozygous deletion at 20p12.1, corresponding to a minimum deletion boundary of chr20:13,466,774-13,593,390 (hg19) **(Supp. Figure S1B).** The minimum deleted region (126 kb) only includes exons 5-10 of *TASP1* (NM_017714.2). Both parents were found to be heterozygous for this deletion and none of the 5 healthy siblings was found to have a homozygous deletion in this region **(Figure 1B).** WES did not reveal any relevant pathogenic variants. Furthermore, no coverage was obtained by WES for a part of *TASP1*, which is consistent with the homozygous deletion affecting this gene. This child was previously reported at an earlier age (Suleiman, Mundt, Sampath, & El-Hattab, 2018).

Breakpoint mapping for the first and second child was performed using PCR strategy where custom primers were designed to sequence across the deletion breakpoints. Breakpoint mapping revealed that both children had the exact same deletion which was larger than the minimum deletions predicted by CMA. The *TASP1* deletion has a size of 149.4 kb spanning chr20:13,448,380-13,597,783 (hg19) and included the entirety of exons 5-11 **(Figure 1E; Supp. Figure 2S).** This deletion includes the active site of TASP1 and is predicted to lead to a frameshift

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[NC_000020.10:g.13448380_13597783del (p.Ser329Valfs*15)], supporting the loss-of function nature of this variant. Breakpoint mapping also provided an insight about the possible mechanism of this deletion. Reviewing the sequence around breakpoints revealed regions of microhomology nucleotides TG **(Supp. Figure 2S).** These TG nucleotides at both 5' and 3' breakpoints possibly cause the microhomology-mediated deletion (Hastings, Ira, & Lupski, 2009). The two children were from different countries of the Arabian Peninsula. To investigate whether this deletion is a founder or recurrent, we performed autozygome analysis for their families as previously described (Alkuraya, 2016). They were found to have the same haplotype indicating that this deletion is a founder Arab variant **(Supp. Figure S3).**

The third child was a three-year-old boy with global developmental delay, hypotonia, microcephaly, drooling, feeding difficulties, failure-to-thrive, short stature, cryptorchidism, an episode of generalized tonic clonic seizure, happy demeanor, and distinctive facial features **(Figure 1C; Supp. Clinical Data).** CMA did not reveal any pathogenic copy number variants (CNVs). WES, performed at Medical Diagnostic Laboratory, Riyadh, Saudi Arabia as previously described (Monies et al., 2017), revealed a homozygous missense variant in *TASP1* (NM_017714.2): c.701C>T (p.Thr234Met) (NC_000020.10:g.13514763G>A). Both parents were heterozygous carriers and the variant was confirmed by Sanger sequencing **(Figure 1C, Supp. Figure S1C, Figure 1E).** This variant was not found in over 140,000 individuals of the Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/). The amino acid residue p.Thr234 of TASP1 is highly conserved during evolution and the p.Thr234Met missense variant was found consistently to be disease causing or damaging by multiple *in silico* prediction

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tools. Furthermore, the p.Thr234 residue is the active-site nucleophile to proteolyze polypeptide substrates (Takeda et al., 2015). In addition, substitution of p.Thr234 with Alanine has already been shown *in vitro* to render TASP1 inactive (Hsieh et al., 2003). Given the novelty, the conservation, the location at the active site of TASP1, the consistent *in silico* prediction of the variant, and the strikingly overlapping phenotype to other affected children, the missense variant c.701C>T (p.Thr234Met) is likely to be disease-related.

The fourth child was a four-year-old boy with global developmental delay, hypotonia, microcephaly, seizures, feeding difficulties, recurrent respiratory infections, VSD, cryptorchidism, inguinal hernias, pale optic discs, convergent strabismus, amblyopia of left eye, periauricular skin tag on the left tragus, bilateral single palmar crease, and distinctive facial features **(Figure 1D; Supp. Clinical Data).** CMA did not reveal any pathogenic CNVs. WES, performed at the Institute of Human Genetics, Klinikum rechts der Isar, Technical University of Munich, Munich, Germany as previously described (Wortmann et al., 2015), revealed a homozygous nonsense variant in *TASP1* (NM_017714.2): c.199C>T (p.Arg67*) (NC_000020.10:g.13605846G>A). This variant was confirmed by Sanger sequencing and both parents were confirmed to be heterozygous carriers **(Figure 1D, Supp. Figure S1D, Figure 1E)**. This variant was reported once in a heterozygous state in gnomAD with allele frequency of $4*10^{-6}$ indicating that it is an extremely rare variant. The pathogenicity of this nonsense variant is supported by the expected outcome which is a severely truncated protein or nonsense-mediated decay most likely leading to a complete loss of function.

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The four unrelated children presented here shared common features including developmental delay with hypotonia and microcephaly, feeding difficulties with failure-to-thrive, recurrent respiratory infections, cardiovascular malformations, cryptorchidism, happy demeanor, and the following distinctive facial features: excessive forehead hair, arched and thick eyebrows with synophrys, epicanthus, hypertelorism, thick eyelids with periorbital fullness, broad nasal bridge, long and smooth philtrum, thin upper lip, and low-set prominent ears **(Table 1).** All had homozygous loss-of-function variants in *TASP1* **(Figure 1E)**. The consistency of the phenotype observed in multiple unrelated individuals, the family co-segregation studies, critical biological function of TASP1, and the deleterious nature of the *TASP1* variants support that *TASP1* is a disease-related gene and TASP1 deficiency leads to a distinctive syndrome characterized by developmental delay, respiratory infections, cryptorchidism, cardiac anomalies, happy demeanor, and distinctive facial features. Interestingly, the reported children shared unique neurobehavioral features characterized by severe expressive language impairment with better receptive skills and happy affect with frequent laughing that is reminiscent of Angelman syndrome. Such characteristic neurobehavioral features along with the common distinctive facial appearance can allow the clinical recognition of this syndrome.

Of note, the phenotype we observe in the children reported here overlaps with Wiedemann-Steiner and Kabuki syndrome 1 syndromes, which are caused by loss-of-function monoallelic pathogenic variants in *KMT2A* and *KMT2D*, respectively. Wiedemann-Steiner syndrome is characterized by developmental delay, distinctive facial features (long eyelashes, thick or arched eyebrows, synophrys, broad nasal bridge, downslanted and vertically narrow palpebral fissures, hypertelorism, epicanthus, low-set

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ears, thin upper lip, exaggerated Cupid's bow), hypertrichosis cubiti, feeding difficulties and growth failure (short stature, microcephaly, and failure-to-thrive), skeletal malformations (hands and feet anomalies, scoliosis, rib anomalies, sacral dimple), and cardiac malformations (ASD and PDA) (Jones et al., 2012). Kabuki syndrome is characterized developmental delay, typical facial features (long palpebral fissures, thick and arched eyebrows, short columella with depressed nasal tip, and large prominent ears), skeletal anomalies (joint hyperlaxity, kyphoscoliosis, brachydactyly, and clinodactyly), persistence of fetal fingertip pads, recurrent infections, feeding problems, growth failure, seizures, congenital heart defects, genitourinary and gastrointestinal anomalies, cleft lip and palate, ptosis, strabismus, hirsutism, hypodontia, and hearing loss (Adam, Hudgins, & Hannibal, 1993). The homozygous *TASP1* loss-of-function variants in the reported children here are expected to result in impaired activation of the TASP1 downstream targets KMT2A and KMT2D explaining the overlap between the features of Wiedemann-Steiner and Kabuki syndromes and the phenotype of these children. This observation further supports that *TASP1* is a novel disease-related gene which is associated with a syndrome overlapping with Wiedemann-Steiner and Kabuki syndromes as these syndromes are related to abnormal histone modification.

Furthermore, taspase 1-null mice have been generated and found to share some features with the reported children with the identified homozygous *TASP1* loss-of function variants. Taspase $1^{-/-}$ mice were found to be smaller in size compared with wild-type and heterozygous knock-out mice. This phenotype commences *in uteri* with embryos being significantly smaller and the animals who survived the newborn period were smaller through adulthood. Taspase $1^{-/-}$ mouse embryonic fibroblasts exhibit

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impaired proliferation with down-regulation of the cyclin genes supporting that the small size is due to deregulation of signaling pathways resulting in impaired cell proliferation (Takeda et al., 2006). Similar to taspase1-null mice, children with *TASP1* homozygous loss-of-function variants demonstrated growth impairment with microcephaly and failure-to-thrive. Taspase 1 deficient mice also demonstrated skeletal abnormalities of vertebra, ribs, and sternum (Takeda et al., 2006). Skeletal abnormalities were not a major feature in the reported children, however, digital deformities including polydactyly, brachydactyly, and clinodactyly have been observed each in one child. As taspase 1 is essential for the cleavage and activation of KMT2A and KMT2D, taspase 1 deficient cells demonstrated markedly decreased histone H3 methylation (Takeda et al., 2006). Defects of TASP1 downstream transcription factors can potentially contribute to the observed phenotype. During mouse head formation, TASP1-mediated cleavage of the general transcription factor TFIIA (GTFIIA) regulates cell proliferation and morphogenesis by maintaining limited transcription of the negative cell cycle regulators. TASP1 deficiency in mice has been shown to led to multiple craniofacial malformations associated with inadequate cell proliferation. Evaluation of mice expressing noncleavable TASP1 targets revealed that TFIIA is the principal TASP1 substrate that orchestrates craniofacial morphogenesis (Niizuma et al., 2015; Takeda et al., 2015). Therefore, defective GTFIIA may play a role in the observed distinctive facial features in individuals with TASP1 deficiency. KMT2A, which in important in neuronal differentiation (Lim et al., 2009), may play a role in the neurodevelopmental phenotype.

In conclusion, we show that the presence of homozygous loss-of-function variants in *TASP1* is associated with a recognizable syndrome. The phenotypic overlap with

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Wiedemann-Steiner and Kabuki syndromes and the established role of TASP1 in the activation of KMT2A and KMT2D strongly hint at the potential pathogenesis which is likely related to abnormal histone modification. The identification of more cases is needed to further delineate the phenotypic spectrum of this newly described syndrome.

Acknowledgments: we thank the participating families.

Competing interests: SS, TJ, MM, and LW are employees of PreventionGenetics, LLC. BW is an employee of Baylor Genetics.

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

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

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Figure

Figure 1 A: Pedigrees and photos of the first child showing the following distinctive facial features: prominent glabella, excessive forehead hair, thick and arched eye brows with synophrys, epicanthus, downslanted palpebral fissures, hypertelorism, periorbital fullness with thick eyelids, broad nasal bridge, long and smooth philtrum, wide mouth with thin upper lip and thick lower lip, microretrognathia, prominent low-set ears, and a webbed neck.

B: Pedigrees and photos of the second child showing the following distinctive facial features: excessive forehead hair, arched and thick eyebrows with synophrys, epicanthus, downslanted palpebral fissures, hypertelorism, periorbital fullness with thick eyelids, broad nasal bridge, long and smooth philtrum, thin upper lip, downturned corners of mouth, microretrognathia, prominent low-set ears with overfolded right ear helix, and a webbed neck.

C: Pedigrees and photos of the third child showing the following distinctive facial features: prominent glabella, thick and arched eyebrows with synophrys, epicanthus, hypertelorism, thick eyelids with periorbital fullness, broad nasal bridge, long and smooth philtrum, wide mouth with thin upper lip and thick lower lip, and low-set prominent ears.

D: Pedigree of the fourth child. Parents did not give permission to publish photographs of this child. The child had the following distinctive facial features: excessive forehead hair, thick and arched eyebrows with synophrys, epicanthus, hypertelorism, periorbital fullness, broad nasal bridge, microretrognathia, high-arched palate, and low-set and prominent ears with overfolded ear helix.

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E: Diagram presenting the *TASP1* gene, TASP1 protein, and the reported variants. The 14 exons of *TASP1* (NM_017714.2) are presented as black rectangles that are numbered from 1-14. Note that exon 1 of *TASP1* is non-coding and the start Met is in exon 2. Corresponding chromosomal position of *TASP1*: chr20: 13,368,036 - 13,621,583 (hg19). TASP1 protein (NP_060184.2) is presented as blue and green rectangle. The blue/light part represent the alpha subunit (amino acids 1 to 233) and the green/dark part is the beta subunit (amino acid 234 to 420). Note that the p.Thr234 is the first amino acid of the beta subunit and the active-site nucleophile to proteolyze polypeptide substrates (Hsieh et al., 2003; Khan, Dunn, & Tong, 2005). The red/dark rectangle on the top represent the deletion of exons 5 – 11; chromosomal position: chr20: 13,448,380 - 13,597,783 (hg19). The location of nonsense variant c.199C>T (p.Arg67*) and the missense variant c.701C>T (p.Thr234Met) are marked at the bottom of the diagram. Note that the amino acid residue p.Thr234 of TASP1 is highly conserved during evolution with a 5.7899 score in GERP (http://mendel.stanford.edu/SidowLab/downloads/gerp), a 0.953 mammalian score in phyloP20way and a 9.362 vertebrate score in phyloP100way (https://omictools.com/phylop-tool), a 0.984 mammalian score in phastCons20way and 1 vertebrate score in phastCons100way (http://compgen.cshl.edu/phast/), and a 4.4549 score in MutationAssessor (http://mutationassessor.org/r3/). Furthermore, the p.Thr234Met missense variant was found consistently to be disease causing or damaging by multiple *in silico* prediction tools including: SIFT (http://sift.bii.a-star.edu.sg/) (score 0; converted rankscore 0.9122), MutationTaster (http://www.mutationtaster.org/) (accuracy 1; converted rankscore 0.8103), DANN (https://cbcl.ics.uci.edu/public_data/DANN/) (score 0.9992), FATHMM

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(http://fathmm.biocompute.org.uk/) (score -3.5; converted rankscore 0.9466), MetaSVM (https://omictools.com/meta-svm-tool) (score 1.0907; rankscore 0.9927), and Provean (http://provean.jcvi.org/index.php) (score -5.49; converted rankscore 0.8573). Variants have been submitted to Global Variome shared LOVD (http://www.lovd.nl).

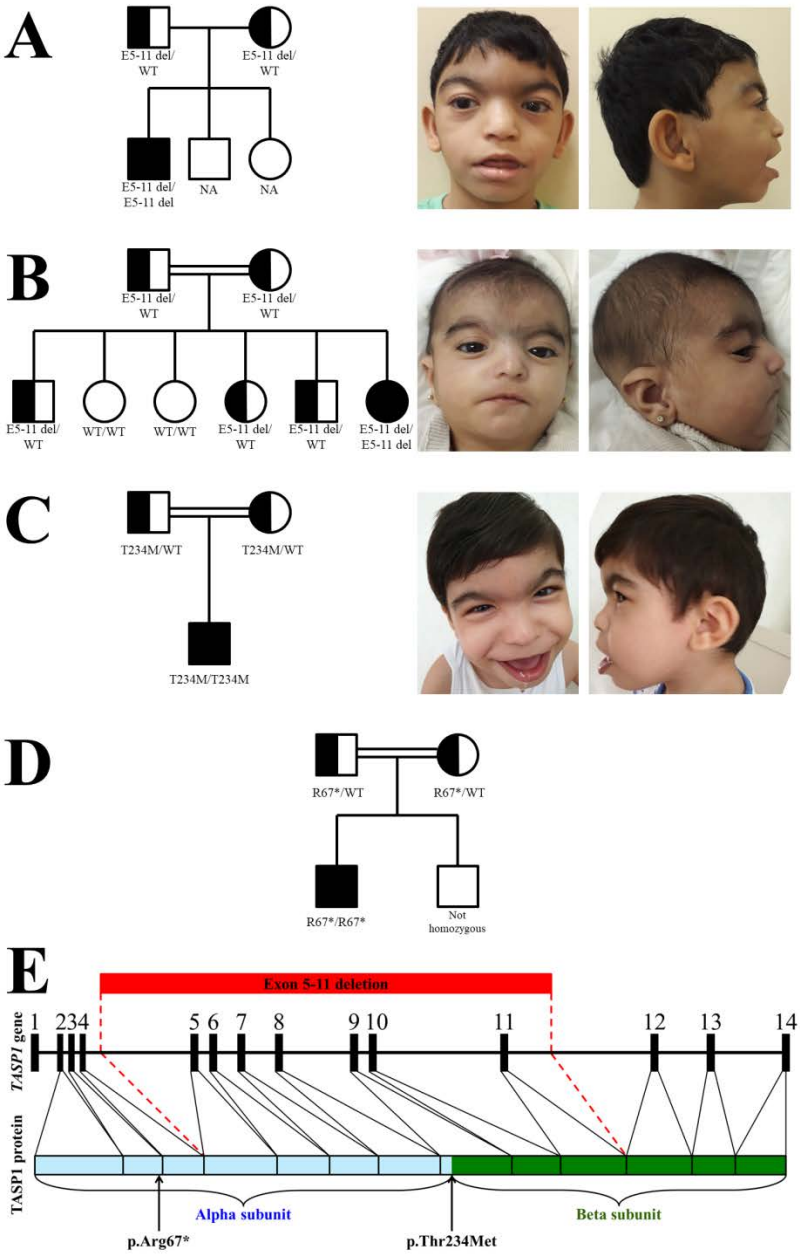

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Table 1. Clinical features of the reported children

	1	**2**	**3**	**4**	
Age* and gender	4 years boy	1 year girl	3 years boy	4 years boy	
TASP1	Hom ex 5-11 del	Hom ex 5-11 del	Hom c.701C>T (p.T234M)	Hom c.199C>T (p.R67*)	
Neurodevelopment					
Developmental delay	+	+	+	+	**4/4**
Microcephaly	+	+	+	+	**4/4**
Hypotonia	+	+	+	+	**4/4**
Feeding and growth					
Feeing difficulties	+	+	+	+	**4/4**
Failure to thrive	+	+	+	-	**3/4**
Happy demeanor	+	+	+	-	**3/4**
Recurrent respiratory infections	+	+	-	+	**3/4**
Cardiovascular malformation	VSD/ASD	PFO/VSD	-	VSD	**3/4**

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Cryptorchidism	+	NA	+	+	**3/3**
Single palmar crease	+	+	-	+	**3/4**
Distinctive facial features					
Prominent glabella	+	-	+	-	**2/4**
Excessive forehead hair	+	+	-	+	**3/4**
Thick eyebrows	+	+	+	+	**4/4**
Arched eyebrows	+	+	+	+	**4/4**
Synophrys	+	+	+	+	**4/4**
Epicanthus	+	+	+	+	**4/4**
Downslanted palpebral fissures	+	+	-	-	**2/4**
Hypertelorism	+	+	+	+	**4/4**
Thick eyelids	+	+	+	-	**3/4**
Periorbital fullness	+	+	+	+	**4/4**
Broad nasal bridge	+	+	+	+	**4/4**
Long and smooth philtrum	+	+	+	-	**3/4**
Wide mouth	+	-	+	-	**2/4**

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Thin upper lip	+	+	+	-	**3/4**
Thick lower lip	+	-	+	-	**2/4**
Downturned corners of mouth	-	+	-	-	**1/4**
Microretrognathia	+	+	-	-	**2/4**
High arched palate	-	-	-	+	**1/4**
Low-set ears	+	+	+	+	**4/4**
Prominent ears	+	+	+	+	**4/4**
Overfolded ear helix	-	+	-	+	**2/4**
Webbed neck	+	+	-	-	**2/4**
Less common manifestations					
Dermatological					
Hirsutism	+	+	-	-	**2/4**
Preauricular skin tag	-	+	-	+	**2/4**
Congenital dermal melanocytosis	-	+	-	-	**1/4**
Skeletal					
Polydactyly	-	+	-	-	**1/4**

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Brachydactyly	+	-	-	-	**1/4**
Clinodactyly	+	-	-	-	**1/4**
Short stature	-	-	+	-	**1/4**
Abdominal/Gastrointestinal					
Inguinal hernia	-	-	-	+	**1/4**
Drooling	+	-	+	-	**2/4**
Renal					
Hydronephrosis	-	+	-	-	**1/4**
Neurological					
Seizures	-	-	+	+	**2/4**
Hearing impairment	+	-	-	-	**1/4**
Ophthalmologic					
Hyperopia	-	+	-	-	**1/4**
Strabismus	-	-	-	+	**1/4**
Amblyopia	-	-	-	+	**1/4**
Pale optic disc	-	-	-	+	**1/4**

* Age at last examination.

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(Hom: homozygous; ex: exon; del: deletion; VSD: ventricular septal defect; ASD: atrial septal defect; PFO: patent foramen ovale; NA: not applicable)

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