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ORIGINAL INVESTIGATION

Identification of new *TRIP12* variants and detailed clinical evaluation of individuals with non-syndromic intellectual disability with or without autism

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Abstract The ubiquitin pathway is an enzymatic cascade including activating E1, conjugating E2, and ligating E3 enzymes, which governs protein degradation and sorting. It is crucial for many physiological processes. Compromised function of members of the ubiquitin pathway leads to a wide range of human diseases, such as cancer, neurodegenerative diseases, and neurodevelopmental disorders. Mutations in the *thyroid hormone receptor interactor 12* (*TRIP12*) gene (OMIM 604506), which encodes an E3 ligase in the ubiquitin pathway, have been associated with autism spectrum disorder (ASD). In addition to autistic features, *TRIP12* mutation carriers showed intellectual disability (ID). More recently, *TRIP12* was postulated as a novel candidate gene for intellectual disability in a meta-analysis of published ID cohorts. However, detailed clinical information characterizing the phenotype of these individuals was not provided. In this study, we present seven novel individuals with private *TRIP12* mutations including two splice site mutations, one nonsense mutation, three missense mutations, and one translocation case with a breakpoint in intron 1 of the *TRIP12* gene and clinically review four previously published cases. The *TRIP12* mutation-positive individuals presented with mild to moderate ID (10/11) or learning disability [intelligence quotient (IQ) 76 in one individual], ASD (8/11) and some of them with unspecific craniofacial dysmorphism and other anomalies. In this study, we provide detailed clinical information of 11 *TRIP12* mutation-positive individuals and thereby expand the clinical spectrum of the *TRIP12* gene in non-syndromic intellectual disability with or without ASD.

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Introduction

The ubiquitin pathway is of crucial importance for the proper maintenance of various cellular functions (Rotin and Kumar 2009). It comprises a cascade of enzymatic reactions, which are carried out by the activating E1, conjugating E2, and ligating E3 enzymes and results in the labeling of proteins for degradation, endocytosis, and trafficking of transmembrane proteins. Thus, the ubiquitin pathway determines the fate of cellular proteins and ultimately the proper function of the cell and organism.

It is not surprising that many members of the ubiquitin pathway have been associated with diseases including cancer, such as BRCA1-associated breast and ovarian cancer, neurodegenerative diseases, such as Parkinson and Alzheimer disease, neurodevelopmental phenotypes, and autism spectrum disorder (ASD) (Popovic et al. 2014). More specifically, mutations in HECT (homologous to E6AP C-terminus) type E3 ligases have been shown to be causative for neurodevelopmental disorders, such as *UBE3A* (*ubiquitin-protein ligase E3A*, OMIM 601623) mutations in Angelman syndrome and *HUWE1* (*Hect, Uba, and Wwe Domains-Containing 1*, OMIM 300697) variants in X-linked intellectual disability (ID) (Ambrozkiewicz and Kawabe 2015). Hence, mutations in members of the ubiquitin pathway and especially E3 ubiquitin ligases are important contributors to neurodevelopmental pathophysiology.

The identification of novel genetic causes of ID and ASD has been subject of intensive research. Many research groups utilize whole exome sequencing (WES) as a tool to identify the genetic causes of ID and ASD and to discover novel candidate genes for these entities (Bourgeron 2016; Vissers et al. 2016). The analysis of WES datasets of ID and ASD cohorts is complicated by their mutual comorbidities, as individuals diagnosed with ASD can also show ID and vice versa. The analysis of ASD data sets has revealed novel ASD risk genes including the *thyroid hormone receptor interactor 12* (*TRIP12*) gene, in which de novo mutations were identified in four individuals (Iossifov et al. 2014; O'Roak et al. 2014). All four *TRIP12* mutation carriers showed not only ASD, but also ID. In addition, *TRIP12* mutations were identified in a meta-analysis of 2104 patient–parent trios in four patients with ID, and *TRIP12* was postulated as a novel candidate gene for ID (Lelieveld et al. 2016). However, the three studies did not include detailed clinical information of the *TRIP12* mutation-positive individuals, and the ID study did not include any information on possible autistic features.

The aim of this study is the characterization of the *TRIP12*-associated phenotype. We present the clinical phenotype of 11 individuals with *TRIP12* variants and discuss their clinical findings.

Subjects and methods

Subjects

Trio-based WES was performed on individuals with ID with or without associated clinical findings and their parents. Clinical information was provided by the parents or legal guardians for minors and by the evaluating clinicians. Written informed consent was obtained from the families of the index individuals for participation in this study. The study was performed according to the Declaration of Helsinki protocols and was approved by the local institutional review board [ethical votum 08-3663 for MRNET and 5360/13 for the Technical University Munich; the institutional review board Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen NL36191.091.11; the local ethical review board in Stockholm (2012/2106-31/4)]. DNA from peripheral blood lymphocytes obtained from the affected individuals and their parents was extracted by standard extraction procedures.

Chromosomal analysis

Conventional karyotyping in blood lymphocytes, fragile-X-syndrome diagnostics, and array analyses were normal in Individuals 1–6 and 9–11 [Individual 1: Affymetrix 6.0 Array (Affymetrix, Santa Clara, CA, USA); Individual 2: Illumina HumanOmni1-Quad Array (Illumina, San Diego, CA, USA); Individual 3: Affymetrix CytoScan HD Array (Affymetrix, Santa Clara, CA, USA); Individual 4: Affymetrix 250 k SNP Array (Affymetrix, Santa Clara, CA, USA); Individual 5: Affymetrix CytoScan HD Array (Affymetrix, Santa Clara, CA, USA); Individual 6: 180 K SurePrint G3 Human CGH oligonucleotide microarray (Agilent Technologies, Santa Clara, CA, USA); Individuals 9–11: (Sanders et al. 2015)]. No arrays were performed in individuals 7 and 8.

Conventional karyotyping in Individual 7 identified a de novo translocation [46,X,t(X;2)(p11.3;q36.3)], which was further investigated by whole genome sequencing and breakpoint analysis (see below).

Exome sequencing

For individuals 1 and 2 trio-based WES was performed as described elsewhere (Bramswig et al. 2015). For individuals 3 and 5 WES was performed as explained by de Ligt and colleagues (de Ligt et al. 2012). A MIP (molecular inversion probe) screen was performed for individuals 6 and 8 as described elsewhere (O'Roak et al. 2012). The variants were verified by Sanger sequencing, primer sequences will be provided upon request.

The *TRIP12* frameshift mutation in Individual 4 was previously published by Lelieveld and colleagues [patient ID 24; (Lelieveld et al. 2016)], the *TRIP12* missense mutations in individuals 9 and 10 as well as the *TRIP12* nonsense mutation in Individual 11 were previously reported by O'Roak and colleagues and Iossifov and colleagues [Individual 9: family ID 13290; Individual 10: family ID 12035; Individual 11: family ID 12867; (Iossifov et al. 2014; O'Roak et al. 2014)].

Whole genome sequencing and breakpoint-PCR

We used whole genome paired-end sequencing to pinpoint the exact positions of the chromosome breaks of the de novo translocation in Individual 7 [46,X,t(X;2)(p11.3;q36.3)]. Whole genome sequencing was performed using TruSeq DNA PCR free libraries and sequenced on Illumina HiSeq using high Output mode and v4 chemistry under an Illumina IGN agreement at NGI, Science for Life Laboratory, Solna, Sweden (Illumina Certified Service Provider). Paired-end sequencing in a 2 × 125 bp format yielded 992,571,435 reads from a library with a 357 bp mean insert size. 98.46% reads aligned to the human reference genome hg19 using the Burrows-Wheeler Aligner (BWA; MEM-algorithm, version 0.7.4-r385) (Lee et al. 1995) for a final average autosomal coverage of 39.28X. Structural variations were called using TIDDIT (manuscript in preparation, https://github.com/vezzi/TIDDIT) and CNVnator (Abyzov et al. 2011), and visualized with CIRCOS (Krzywinski et al. 2009) using custom scripts.

Primers flanking the chromosomal junctions of both derivatives were designed located approximately 1 kb away from the breakpoints estimated from the whole genome sequencing data (Supplemental Table 1). The breakpoint-PCR was performed using Phusion High-Fidelity DNA polymerase kit (Thermo Scientific, Pittsburgh, PA, USA) and run out on a 1.5% agarose gel to check product size and specificity. PCR products of expected size, not present in the control samples, were Sanger sequenced according to standard procedures. Obtained sequences were aligned using the BLAT tool (UCSC Genome Browser) (Kent 2002) and later visualized using CodonCode Aligner (CodonCode Corp., Dedham, MA, USA).

cDNA analyses of *TRIP12* splice mutations

To determine the consequences of the potential splice-site mutations in individuals 1 and 2, peripheral blood of the patients was collected into PAXgene Blood RNA tubes (PreAnalytiX), and total RNA was extracted according to the manufacturer's protocol. cDNA was synthesized using the Omniscript RT Kit (Qiagen). The sequences of the primers that were used to amplify and sequence the respective parts of the transcripts are listed in Supplemental Table 1.

Results

Annotation of *TRIP12* variants

Four major isoforms of the human TRIP12 protein are conserved throughout the animal kingdom. Their amino acids (aa) lengths are 2040 aa (isoform a: NP_001271143.1 encoded by NM_001284214.1), 2025 aa (isoform b: NP_001271144.1 encoded by NM_0012824215.1), 1992 aa (isoform c: NP_004229.1 encoded by NM_004238.2) and 1722 aa (isoform d: NP_001271145.1 encoded by NM_001284216.1). All forms use the same translation start (chr2:g.229,880,077-229,880,079) and stop signal (chr2:g.229,767,554-229,767,556), but differ in the usage of internal exons and the lengths of several used exons by *in frame* usage of alternative splice signals. All isoforms share the identical carboxy-terminal HECT domain, but differ in the length of the predicted WWE domain, 68 aa in isoforms b and d, and 41 aa in isoforms a and c. This is due to the usage of the alternative splice signal chr2:g.229,805,884-229,805,886, that results in the 5′-addition of 27 *in frame* codons to the respective exon. A schematic illustration displaying the different isoforms of TRIP12 is provided in Fig. 1A.

By RT-PCR analysis of RNA from peripheral leukocytes and subsequent sequencing of the PCR products, we have determined that *TRIP12* transcripts are a mixture of all possible isoforms, but that the 126-bp exon encoded by chr2:g.229,860,531-229,860,406 (not present in isoforms a, c and d) is predominantly skipped *in frame* in the mature mRNA, and that the predominant transcript NM_001284215.1 contains the longer exon chr2:g.229,805,730-229,805,883 (exon 17 in NM_001284215.1) encoding the longer WWE domain. Therefore, we decided to annotate the variants found in our patients according to transcript NM_001284215.1 encoding TRIP12 isoform b (NP_001271144.1). Numbering of genomic positions is according to GRCh38/hg38. A schematic illustration displaying the localization of the *TRIP12* mutations is provided in Fig. 1B.

Pathogenic *TRIP12* variants in 11 individuals from different cohorts

Individuals 1 and 2 were identified in a cohort of 311 patients with ID (2/311) (Institute of Human Genetics, University Duisburg-Essen and Institute of Human Genetics, University Bonn), and individuals 3, 4 and 5 were identified in a cohort of 2000 patients with ID (3/2000)

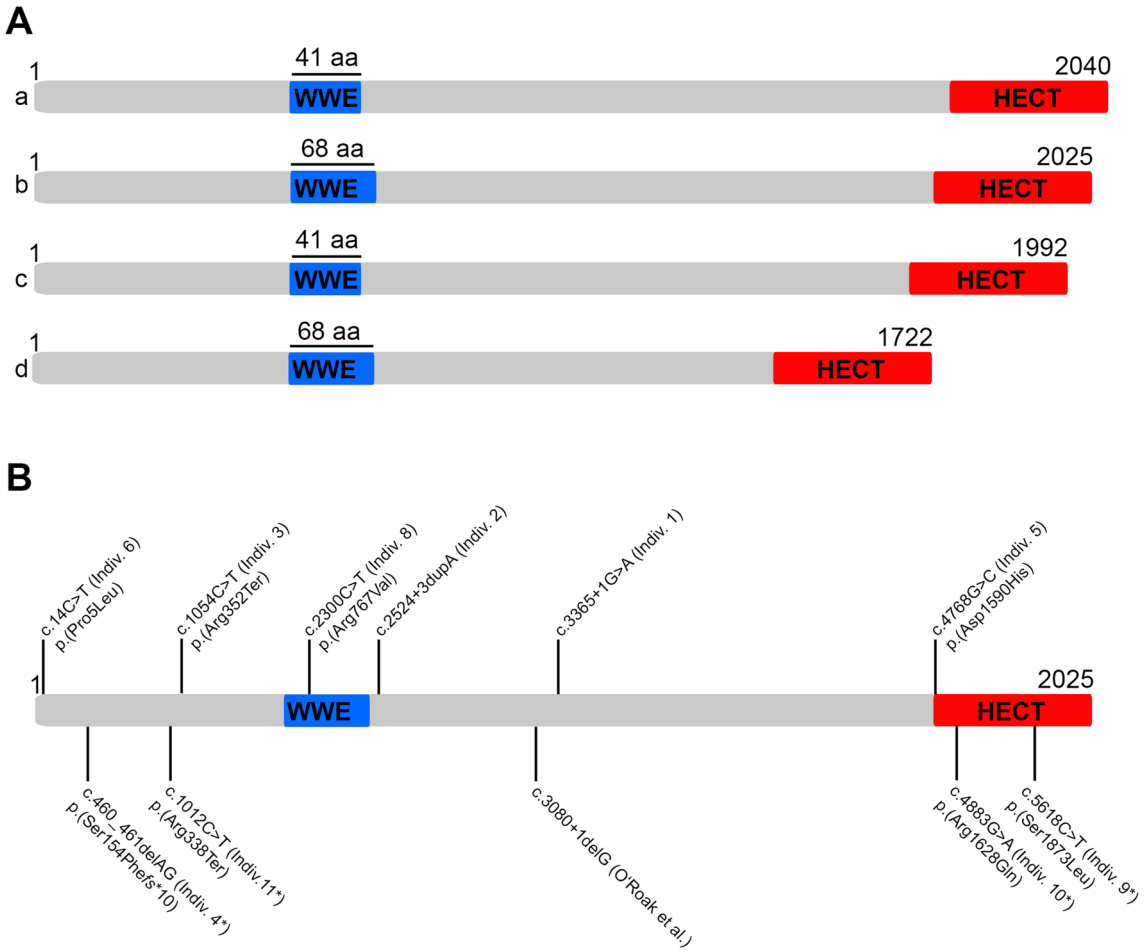

Fig. 1 Schematic illustration of TRIP12 indicating the WWE- and the catalytic HECT domain. The different isoforms of TRIP12 (**A**) and the localization of all *TRIP12* mutations (**B**) are visualized. **A** The numbers above the rectangle depicting the protein indicate the number of amino acids. The amino acids (aa) lengths of the four major TRIP12 isoforms are 2040 aa (isoform a, *first line* NP_001271143.1 encoded by NM_001284214.1), 2025 aa (isoform b, *second line* NP_001271144.1 encoded by NM_0012824215.1), 1992 aa (isoform c, *third line* NP_004229.1 encoded by NM_004238.2) and 1722 aa (isoform d, *fourth line* NP_001271145.1 encoded by NM_001284216.1). All isoforms use the same translation start and stop signal, but differ in the usage of internal exons and the lengths of several used exons by *in frame* usage of alternative splice signals. They share the identical carboxy-terminal HECT domain but differ in the length of the predicted WWE domain. **B** We annotated the variants according to the predominant transcript NM_001284215.1 encoding TRIP12 isoform b (NP_001271144.1). The novel mutations are placed on the top, the previously published mutations on the bottom of the illustration. Individual 4 was previously published by Lelieved et al. (Lelieveld et al. 2016). Individuals 9, 10, and 11 were previously published by Iossifov et al. (2014), O'Roak et al. (2014)

(Radboud University Medical center, diagnostic WES on ID patients). Individual 6 was identified in a cohort of 1500 patients with intellectual disability and/or autism from the Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden. Individual 7 was identified through routine diagnostics for ID. Individual 8 was identified through routine diagnostics for ID, and referred for research participation in another location. Individuals 9–11 were identified in the Simons Simplex Collection cohort of 2600 families, each of which has one child with ASD, and unaffected parents and siblings. The molecular findings of individuals 4, 9, 10, and 11 were published previously (Iossifov et al. 2014; Lelieveld et al. 2016; O'Roak et al. 2014). All clinical information is summarized in Table 1; the prediction interpretation of all missense variants is displayed in Table 2 (Liu et al. 2016); the genomic positions are listed in Supplemental Table 2. Additional variants are listed in Supplemental Table 3.

Individual 1 is the second child of healthy, non-consanguineous parents. The sister of the maternal grandmother had ID of unknown cause. Individual 1 was born at 38^{+6} weeks of gestation with a weight of 3190 g (−0.68 SD), a length of 50 cm (−0.83 SD), and an OFC of 36.5 cm (0.92 SD). He walked at the age of 18 months. His first clinical genetic assessment was at the age of $4^{7}/_{12}$ years when his height was 103 cm (−0.97 SD), his weight was

Table 1 Clinical data of individuals with mutations in *TRIP12* (NM_001284215.1)

Clinical findings	Individual 1	Individual 2	Individual 3	Individual 4 (Lelieveld et al.)	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9 (O'Roak et al., Iossifov et al.)	Individual 10 (O'Roak et al., Iossifov et al.)	Individual 11 (O'Roak et al., Iossifov et al.)	Total
Pathogenic variant	c.3356+1G>A skipping of exon 22, frameshift and premature termination, p.(Ala1061 Glufs*16)	c.2524+3dupA skipping of exon 17, frameshift and premature termination, p.(Ala791 Valfs*16)	c.1054C>T p.(Arg352Ter)	c.460_461delAG p.(Ser154Phe*fs*10)	c.4768G>C p.(Asp1590His)	c.14C>T p.(Pro5Leu) maternal	t(X:2) (p11.3;q36.3)	c.2300C>T p.(Ala767Val)	c.5618C>T p.(Ser1873Leu)	c.4883G>A p.(Arg1628Gln)	c.1012C>T p.(Arg338Ter)	
Gender	m	m	m	f	m	m	f	f	m	m	f	
Country of origin/background	Germany	Germany	Netherlands	Morocco	Netherlands	Iran	Sweden	USA/Caucasian	USA/African American	USA/Hispanic	USA/Caucasian	
Age of mother at birth (years)	33	25	n.a.	31	33	34	24	26	29	22	33	
Age of father at birth (years)	38	30	n.a.	28	41	38	31	34	29	25	40	
Intellectual disability/IQ	(+)/70–80	+/50	+/60	+/49	+/76	+/70	+/50 (estimated)	+/VIQ = 33, NVIQ = 33	+/VIQ = 42, NVIQ = 53	+/VIQ = 81, NVIQ = 55	+/VIQ = 57, NVIQ = 63	11/11
Walked independently (months)	18	21	22	20	17	14	19	23	19	13	20	
First words (months)	18	18	24	20	Not known	16	24	60	10	14	24	
Verbal ability (fluent, phrase speech, single words, no words)	Fluent speech	Fluent speech	Fluent speech	Fluent speech, simple sentences	Phrase speech	Fluent speech	Single words	Phrase speech	Single words	Phrase speech	Fluent speech	
Hypotonia	+	+	−	−	+	−	+	−	−	−	−	4/11
Autism	+	+	−	+	−	+	−	+	+	+	+	8/11
Seizures (years)	−	+ Twice [4 years], abnormal EEG	−	−	−	+ [1 year]	+ Once [7 years during varicella infection]	−	−	−	Abnormal EEG	3/11
Vision problem	−	Astigmatism, strabism, hyperopia, corrected with glasses	−	−	−	−	Strabism in childhood	−	−	−	+ (Corrected with glasses)	2/11
Hearing loss	−	−	−	−	−	−	−	−	−	−	−	0/11

Table 1 continued

Clinical findings	Individual 1	Individual 2	Individual 3	Individual 4 (Lelieveld et al.)	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9 (O'Roak et al., Iossifov et al.)	Individual 10 (O'Roak et al., Iossifov et al.)	Individual 11 (O'Roak et al., Iossifov et al.)	Total
Frequent infections	−	+	−	−	−	−	−	+ (Otitis media, strep) resulting in tonsillectomy and adenoids	−	−	−	2/11
Behavioral anomalies	Stereotypic behavior	Stereotypic and aggressive behavior	−	+	Possible ADHD	Mildly autistic	ADHD, friendly, happy, some stereotypic behavior	Hyperactivity, impulsivity	Regression (word loss)	−	−	8/11
Birth (weeks)	38 + 6	37 + 1	39 + 5	39	35 + 6	39	39 + 6	38	36 + 3	40	37	
Weight (g)/(SD)	3190/−0.68	3140/0.02	3280/−0.8	3000/−1.1	3000/0.3	3350/−0.31	2400/−2.25	3005/−0.72	2834/−1.12	3543/0.38	3147/−0.18	
Length (cm)/(SD)	50/−0.83	50/−0.14	n.a.	n.a.	n.a.	51/−0.39	48/−1.68	49.53/−0.19	52.1/1.13	52.1/1.88	53.3/2.25	
OFC (cm)/(SD)	36.5/0.92	35.5/0.69	n.a.	n.a.	n.a.	35/−0.23	Normal	n.a. (normal)	n.a. (normal)	n.a. (normal)	n.a. (normal)	
Age at examination [years]	11 4/12	11 3/12	7 6/12	14 8/12	8 6/12	15	26 4/12	10 10/12	6	5 8/12	16 8/12	
Weight [kg]/[SD]	36/0.01	43/0.57	20.9/−1.07	64/1.2	32/0.61	60/0	78/0.52	59.6/1.59	20.2/−0.21	29.5/2.09	58.9/0.38	
Height [cm]/[SD]	145.5/0.04	144.5/−0.41	117/−1.38	168/0.7	136/0.13	160/−2	161/−0.6	148.9/−0.19	117/0.21	119/1.05	157.5/−0.79	
OFC [cm]/[SD]	54.3/−0.01	54.5/0.25	52/−0.61	58.8/2.2	55/1.5		Normal	54.6/1.05	53.1/0.6	52.0/−0.04	56.1/1.11	
Craniofacial anomalies												
Hypertelorism	+	−	−	−	−	−	−	−	n.a.	n.a.	n.a.	1/8
Epicanthic folds	−	−	−	−	+	−	−	−	n.a.	n.a.	n.a.	1/8
Fullness of upper eyelid	+	−	−	−	−	−	−	−	n.a.	n.a.	n.a.	1/8
Depressed nasal bridge	−	−	−	−	+	−	−	−	n.a.	n.a.	n.a.	1/8
Short nose	+	+	−	− (Large nose)	−	−	−	−	n.a.	n.a.	n.a.	2/8
Anteverted nares	+	−	−	−	−	−	−	−	n.a.	n.a.	n.a.	1/8
Broad nasal tip	+	−	−	−	−	−	−	+	n.a.	n.a.	n.a.	1/8
Low columella	−	−	−	+	−	+	+		n.a.	n.a.	n.a.	3/8
Long philtrum	+	+	−	−	−	−	−	−	n.a.	n.a.	n.a.	2/8

Table 1 continued

Clinical findings	Individual 1	Individual 2	Individual 3	Individual 4 (Lelieveld et al.)	Individual 5	Individual 6	Individual 7	Individual 8	Individual 9 (O'Roak et al., Iossifov et al.)	Individual 10 (O'Roak et al., Iossifov et al.)	Individual 11 (O'Roak et al., Iossifov et al.)	Total
Smooth philtrum	+	+	−	−	−	−	−	−	n.a.	n.a.	n.a.	2/8
Wide mouth	+	−	−	+	−	−	−	+	n.a.	n.a.	n.a.	3/8
Thin upper lip vermillion	+	+	−	− (Full lips)	−	−	−	−	n.a.	n.a.	n.a.	2/8
Exaggerated Cupid´s bow	+	+	−	−	+	−	+	−	n.a.	n.a.	n.a.	4/8
High palate	n.a.	−	n.a.	−	−	+	+	+	n.a.	n.a.	n.a.	3/6
Small, posteriorly rotated ears	+	−	+	−	+	−	−	−	n.a.	n.a.	n.a.	3/8
Low-set ears	+	−	−	−	−	−	+	−	n.a.	n.a.	n.a.	2/8
Large ear lobe		+	−	−	+	−	+	−	n.a.	n.a.	n.a.	3/8
Hand anomalies	−	−	−	Extra transverse crease dig IV left, clinodactyly dig V both sides	−	−	−	−	n.a.	n.a.	n.a.	
Feet anomalies	"Underriding" third toe	−	−	Sandal gap, clinodactyly dig IV and V both sides	−	−	High valve, small, fleshy feet size 35	Short, wide toes with slight sandal gap and minimal 2–3 syndactyly	n.a.	n.a.	n.a.	
Other anomalies	−	Intermittent diarrhea, meteorism lactose intolerance	−	−	Intentional tremor (also in father)	−	−	−	−	−	−	
Obvious internal anomalies	−	−	−	−	−	−	−	−	−	−	−	0/11
Brain anomalies	−	−	−	−	−	−	Not investigated	−	−	−	−	

NVIQ nonverbal IQ, *VIQ* verbal IQ, *ADHD* attention deficit hyperactivity disorder, *n.a.* not available

Table 2 Predicted interpretations of the reported *TRIP12* missense variants (individuals 5, 6, 8, 9, and 10)

Prediction tool	CADD	Polyphen2	SIFT	Provean	Mutation taster
Individual 5: chr2:g.229,787,606C>G	29.4	Probably damaging	Damaging	Damaging	Disease causing
Individual 6 (maternal): chr2:g.229,880,066G>A	29.6	Probably damaging	Damaging Tolerated	Neutral Damaging	Disease causing
Individual 8: chr2:g.229,807,778G>A	21.4	Probably damaging Possibly damaging	Tolerated	Neutral	Disease causing
Individual 9: chr2:g.229,771,583G>A	35	Probably damaging	Damaging	Damaging	Disease causing
Individual 10: chr2:g.229,785,842C>T	35	Possibly damaging Probably damaging	Damaging	Damaging	Disease causing

As some prediction tools use multiple transcript/protein information there might be more than one interpretation. Numbering of genomic positions is according to GRCh38/hg38. The database dbNSFP v3.0 was utilized to determine the functional predictions (Liu et al. 2016)

13 kg [− 0.94 SD, Body mass index (BMI): 14.61 kg/m^2], and his occipital frontal circumference (OFC) was 52.5 cm (+0.95 SD). He had muscular hypotonia and did not show any obvious internal anomalies or seizures. First he presented with mild ID (IQ 70) and showed a friendly demeanor. Facial dysmorphisms included hypertelorism, a deep nasal bridge, a short nose with a broad nasal tip and hypoplastic alae nasi, a long philtrum, a wide mouth with a thin upper vermillion, an exaggerated Cupid's bow of the upper, thin lip and slightly low set, small and posteriorly rotated ears. He showed "underriding" third toes. His second assessment was at the age of $11^4/_{12}$ years when his height was 145.5 cm (+0.04 SD), his weight was 36 kg (+0.01 SD, BMI: 17.12 kg/m^2), and his OFC was 54.3 cm (-0.01 SD) with similar facial dysmorphism (Fig. 2A, B). He had developed autistic features and was diagnosed with ASD. His development had been tested again in a known environment, which resulted in an IQ score of 80, his parents noted that test results are very dependent on his varying daily performance. They reported that it is very important for him to follow routines and that he has trouble calming down in the evening and falling asleep. He speaks fluently, but adheres to his subject of interest. WES revealed a heterozygous de novo splice site mutation in the *TRIP12* gene [chr2:g.229,798,874C>T [GRCh38/hg38]; NM_001284215.1:c.3356+1G>A], which leads to skipping of exon 22, to a shift of the open reading frame, and premature translation termination [p.(Ala1061Glu*fs**16)].

Individual 2 is the first son of healthy, non-consanguineous German parents. His mother and his brother have been diagnosed with congenital von Willebrand disease. The sister of the unaffected father has ID of unknown cause, and her son shows developmental delay at the age of 2 years. Individual 2 was born by Caesarean *s*ection at 37^{+1} weeks of gestation with a weight of 3140 g (+0.02 SD), a length of 50 cm (−0.14 SD), and an OFC of 35.5 cm (+0.69 SD). At the age of 3 months, he showed a life-threatening episode of apnea and unconsciousness (apparent life-threatening event/ALTE). During childhood, recurrent infections occurred. Motor developmental delay was obvious at the age of 6 months. He walked at the age of 21 months. Expressive and receptive speech development was also delayed. He spoke first words at the age of 18 months and two-word sentences at the age of 2 years. At the age of 4 years, seizures were observed twice, but no anticonvulsant therapy was necessary. Electroencephalograms (EEGs), magnetic resonance imaging (MRI) scan, and metabolic investigations gave normal results. ASD was diagnosed at the age of 4 years. His first clinical genetic assessment was at the age of $4^8/_{12}$ years, when his height was 108 cm (−0.45 SD), his weight was 21 kg (+0.80 SD, BMI: 18 kg/m^2), and his OFC was 50 cm (−1.22 SD). He had muscular hypotonia and did not show any obvious internal anomalies. He presented with moderate ID [Snijders-Oomen non-verbal intelligence test (SON-R) at 4 years: IQ 50] and showed behavioral problems with aggressive behavior, restlessness, distractibility and stereotypic movements. His speech and language development was delayed with multiple dyslalia, dysgrammatism, and receptive language deficiency. He wore glasses because of astigmatism and hyperopia. Facial dysmorphisms included narrow palpebral fissures, mild epicanthic folds, a smooth and long philtrum, a thin upper lip with an exaggerated Cupid's bow, a small mandible, retrognathia, and fleshy ear lobes. His second clinical genetic assessment was at the age of $11^3/_{12}$ years. His height was 144.5 cm (−0.41 SD), his weight was 43 kg (+0.57 SD, BMI: 20.6 kg/m^2), and his OFC was 54.5 cm (+0.25 SD). He showed orofacial hypotonia with hypersalivation. Behavioral problems had improved. The parents reported on intermittent diarrhea and meteorism, lactose intolerance was diagnosed. He spoke fluently in multi-word sentences with slurred articulation, but only about topics of his interest. Recently performed EEGs gave abnormal results (two independent parietal spikes on both sides, low background activity), but no seizures occurred. WES revealed a heterozygous de novo splice site mutation in the *TRIP12* gene [chr2:g.229,805,727dupT [GRCh38/hg38]; NM_001284215.1:c.2524+3dupA], which leads to skipping of exon 17, to a shift of the open

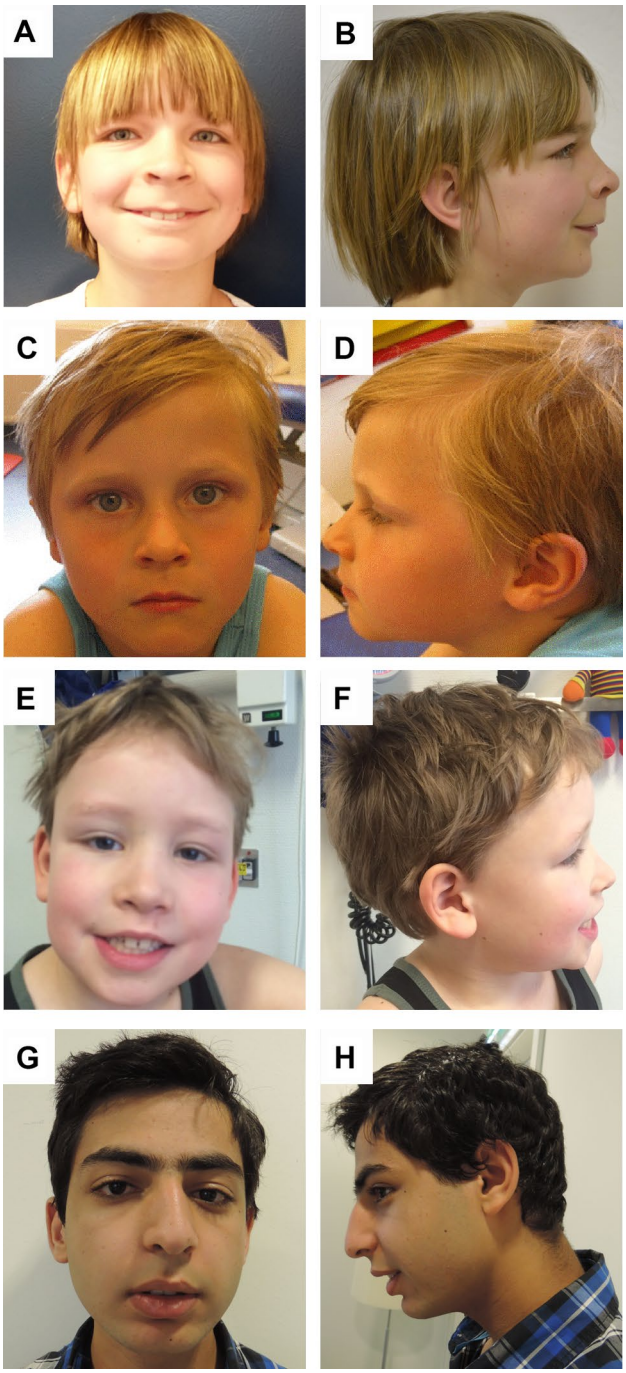

Fig. 2 Clinical photographs of patients with mutations in *TRIP12*. **A**, **B** Individual 1 at the age of 11 4/12 years. He presents with hypertelorism, fullness of the upper eyelid, hypoplastic alae nasi, long and smooth philtrum and a wide mouth with a Cupid's bow of the upper lip and a thin upper lip vermillion. **C**, **D** Individual 3 at the age of 7 6/12 years showing hypoplastic alae nasi and a U-shaped, thin upper lip. **E**, **F** Individual 5 at the age of 86/12 years displaying epicanthic folds, depressed nasal bridge, hypoplastic ale nasi, Cupid's bow of the thin upper lip and large ear lobes. The ears of individuals 1, 3 and 5 (**B**, **D**, **F**) are small and posteriorly rotated. **G**, **H** Individual 6 at the age of 15 years. He shows a synophrys and a low columella

reading frame, and premature translation termination [p.(Ala791Val*fs**16)].

Individual 3 was referred at the age of 7 years to the multidisciplinary clinic for diagnostic evaluation of his developmental delay. He was born at term after an uneventful pregnancy with a secondary Caesarian section because of a deteriorating cardiotocography (CTG). His birth weight was 3280 g (−0.8 SD), Apgar score 8/9. The neonatal period was unremarkable with the exception of some distress when feeding. Because of a position preference he received physiotherapy. He started walking independently at the age of 22 months and had a delayed language and speech development, for which he received language therapy from 24 months. At the age of 4 years, he had a tested IQ (TIQ) 67 [verbal IQ (VIQ) 72, performance IQ (PIQ) 70; Wechsler Preschool and Primary Scale of Intelligence (WPPSI)] and at 5 years of age TIQ 60 [performance scale (PS) 66, reasoning scale (RS) 71 (SON-R 2.5−7)]. He attended special education from the age of 6 years. He showed fluent speech. Physical examination at the age of 7 years showed a height of 117 cm (−2.2 SD), weight of 20.9 kg (0 SD), and OFC of 52 cm (−0.1 SD). He showed hypoplastic alae nasi, an exaggerated Cupid's bow, thin upper lip, and small, posteriorly rotated ears (Fig. 2C, D). Brain MRI, EEG, and metabolic screening showed no abnormalities. By WES, he was found to have a heterozygous truncating mutation in the *TRIP12* gene [chr2:g.229,836,938G>A [GRCh38/hg38]; NM_001284215.1:c.1054C>T; p.(Arg352Ter)].

Individual 4 (Lelieveld et al. 2016) was born at term after an uneventful pregnancy with a birth weight of ~3000 g (−1.1 SD). Family history showed speech delay in a paternal aunt and maternal cousin. Her non-consanguineous parents also had one unaffected son. The neonatal period was unremarkable. She sat and crawled at a normal age, but started walking independently at the age of 20 months and also spoke her first words at the age of 20 months. At 14 years, she had a TIQ of 49 (VIQ = 54 and PIQ = 49), and attended special education. Because of signs of ADHD, treatment with Ritalin was started at the age of 8 years, but this was terminated at the age of 12 years because of limited effect. She was diagnosed with ASD [("Autisme-en Verwante stoornissenschaal-Z Revisie", 1999 (AVZ-R)]. She had no sleeping difficulties and normal vision and hearing. She speaks fluently, but uses simple sentences. Physical examination at the age of 16 years showed a height of 168 cm (+0.7 SD), weight of 64 kg (+1.2 SD) and OFC of 58.8 cm (+2.2 SD). Facial features comprised full eyebrows, prominent nose, long columella, short philtrum, full lips, and slightly low-set ears with overfolded helices. Hands showed tapering fingers with short distal phalanges,

clinodactyly of the 5th digit, and an extra transverse crease at the left 4th digit. She had a sandal gap and clinodactyly of the 4th and 5th digits bilaterally. Brain MRI, EEG, and metabolic screening showed no abnormalities. She had a de novo mutation in the *TRIP12* gene [chr2:g.229,859,212_229,859,213delCT [GRCh38/hg38]; NM_001284215.1: c.460_461delAG; p.(Ser154Phe*fs**10)].

Individual 5 was born as the second child of non-consanguineous Dutch parents. The first child was a boy with achondroplasia. The patient was born prematurely at gestation date 35^{+6} because of premature rupture of membranes. He was born, after a normal delivery, with a weight of approximately 3000 g (+0.3 SD), and Apgar score 9/10. Because of elevated billirubin he had UV therapy for a few days. He started walking at age of 18 months. He showed delayed speech development. He was hypotonic and had an intentional tremor like his father. His brain MRI and metabolic tests were unremarkable. In school he presented with learning difficulties. He did not speak fluently, but used simple sentences. His assessment was at the age of 8 years and 6 months. He showed a friendly and sociable behavior, and presented with an epicanthus, a wide sunken nasal bridge, a Cupid's bow of the thin upper lip, and small, posteriorly rotated ears with large earlobes (Fig. 2E, F). He showed slight joint hypermobility. WES revealed a heterozygous, de novo mutation in the *TRIP12* gene [chr2:g.229,787,606C>G [GRCh38/hg38]; NM_001284215.1: c.4768G>C; p.(Asp1590His)].

Individual 6 is the first child born to healthy, non-related parents. He is now 16 years old and he has a younger healthy sister. No comparable cases were reported in the extended family. He was born after an uneventful pregnancy and the first year of life was without remarks. Psychomotor development was mildly delayed. Subsequent assessment resulted in a diagnosis of mild intellectual disability with an even profile including disabilities in motor function, cognition and language. He attended regular school until 12 years of age, and after that he was enrolled in a special tutoring class. Neuropsychiatric investigation resulted in a diagnosis of atypical autism and attention deficit disorder. Epilepsy has been present since the age of 1 year. He had generalized tonic–clonic seizures and absence seizures, which were successfully treated with anti-epileptic drugs. He has been seizure-free for many years. Brain imaging (computed tomography) gave normal results. He presented with a synophrys and a low columella (Fig. 2G, H). MIP screening showed a missense variant in *TRIP12* [chr2:g.229,880,066G>A [GRCh38/hg38]; NM_001284215.1: c.14C>T, p.(Pro5Leu)]. Subsequent testing of the parents showed that the variant was inherited from the mother. The variant was not listed in the ExAC or 1000Genomes.

Individual 7 is the first child of non-consanguineous parents of Swedish ancestry. She was born after an uneventful pregnancy with normal delivery at 39^{+6} weeks of gestation with a birth weight of 2400 g (−2.25 SD), a birth length of 48 cm (−1.68 SD), and normal OFC. The neonatal period was normal, she was breastfed for 7 months. She had slight hypotonia and started to walk at 1 year and 7 months. At the age of 2 years, there was no speech development. At 7 years, she had one episode of seizures during a varicella infection. She has been very healthy and has not suffered from frequent infections. She has no malformations and no hearing or visual impairment. Currently, at the age of 26 years, she has profound ID, has only learned to use some simple sign language, and she is able to speak a few single words. Her estimated IQ is 50 (Zhang et al. 2005). She is very friendly and outgoing, and has some stereotypic behavior. She has attention deficit hyperactivity disorder (ADHD) and poor fine motor skills. She has been overweight since early childhood. At age 26 she has a height of 161 cm (-0.61 SD), a weight of 78 kg (0.52 SD, BMI 30.1 kg/m^2). She is shorter and more overweight compared to her mother and siblings and has smaller feet (size 35). She has no obvious dysmorphic features but a somewhat narrow face, a high palate, slightly low set, large ears and ear lobes, columella below alae nasi, and a frontal hair upsweep. She was investigated by karyotyping and a de novo translocation between chromosome 2 and chromosome X, 46,X,t(X;2)(p11.3;q36.3) was identified. The exact GRCh38/hg38 genomic coordinates for the breakpoints were chr2:g.229,914,177 (+strand) and chrX:g.42,494,675 (+strand) (Fig. 3). No genes were disrupted on chromosome X and the 2q breakpoint was located in intron 1 of the *TRIP12* gene.

Individual 8 is the second child of healthy, non-consanguineous parents. She is an 11-year-old Caucasian female and has a 13-year-old sister with cardio-facio-cutaneous syndrome. There is no ID in relatives. Individual 8 was born vaginally at 38 weeks of gestation with a weight of 3005 g (−0.72 SD), a length of 49.5 cm (−0.19 SD), and an unknown, but reportedly within normal limits, OFC. Labor was induced via Pitocin due to failure to progress. She experienced hyperbilirubinemia at birth, which did not require treatment. Individual 8 showed developmental delays in all areas by 12 months. Her language was particularly delayed, and she did not use single words until 60 months and phrases until 72 months. She first walked at 23 months and has a somewhat unusual gait. She has had a history of constipation since infancy, and a history of problems with incontinence beginning at 7 years of age. She has no history of seizures or regression, and had a normal MRI at 10 years of age. Her assessment at the age of 10 $^{10}/_{12}$ years indicated a height of 149 cm (−0.19 SD), a weight of 60 kg (+1.59 SD), and OFC of 54.6 cm (+1.05 SD). She met strict diagnostic criteria for ASD, confirmed with Autism Diagnostic Interview (ADI), Autism Diagnostic

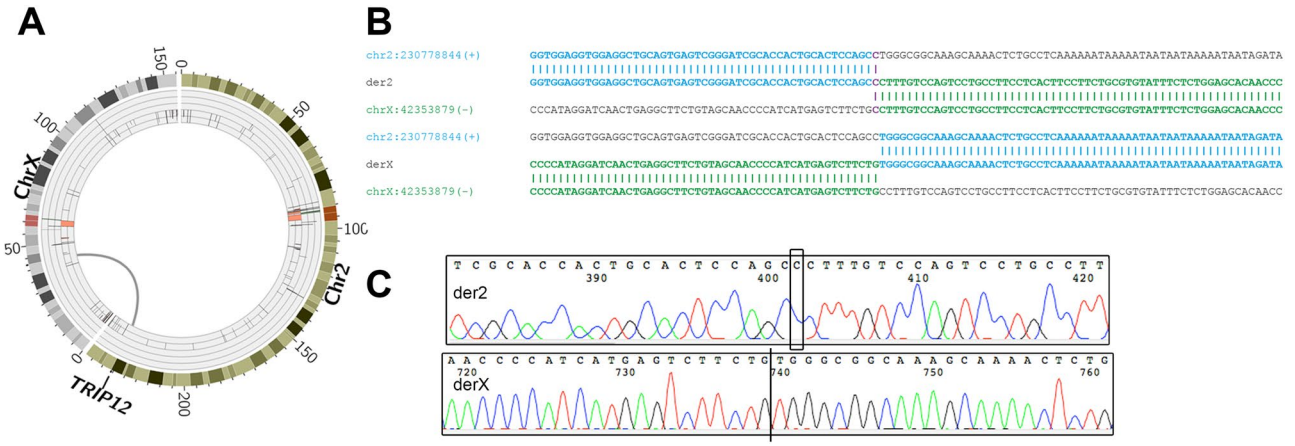

Fig. 3 Whole genome sequencing and breakpoint-PCR results in Individual 7 with a balanced reciprocal translocation, 46,X,t(X;2)(p11.3;q36.3). **A** Whole genome sequencing results are visualized using a Circos plot. The genomic breakpoint on chromosome 2 is located within *TRIP12* and no genes were disrupted on chromosome X. **B**, **C** Breakpoint-PCR and Sanger sequencing show a one nucleotide microhomology on derivative chromosome 2 and a clean fusion without microhomology or any gain/loss of genetic material on derivative chromosome X

Observation Schedule (ADOS), and expert clinical judgment using the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). She was also diagnosed with moderate ID (based on diagnostic history, parent report of symptoms, and clinical judgment) and ADHD-combined (based on parent report of symptoms and clinical judgment). Her cognitive and adaptive functioning abilities were very impaired (VIQ = 33; NVIQ = 33, Adaptive Skill Composite = 53). She spoke in short phrases with extremely low receptive [Peabody Picture Vocabulary Test (PPVT) Standard Score = 41] and expressive vocabulary skills [expressive vocabulary test (EVT) Standard Score = 58]. She shows autism-related impairments in social communication including loud, jerky speech, little reciprocal conversation, use of unusual phrases, no use of descriptive gestures, limited showing, limited directed facial expressions to others, and unusual social overtures. She exhibits unusual preoccupations (wanting to know where things were purchased), demonstrates repetitive behaviors (stacking, lining things up), and unusual sensory interests (picking at food on shirt, shoe soles have to be removed). Parent report about Individual 8's social responsiveness on the social responsiveness scale (SRS) suggests severe impairment in social awareness, social cognition, social communication, and restricted interests and repetitive behavior and moderate impairment in social motivation. Her parent endorses some concerns with externalizing emotional problems (aggression towards her family), attention deficit/hyperactivity problems, and oppositional defiant problems. She has extremely low fine motor coordination (Purdue Pegboard *T*-scores <10 in all tasks) and gross motor skills (Movement ABC-2 Total Test Standard Score = 1). Facial features include arched eyebrows, somewhat heart-shaped hairline, wide nose, deep philtrum, wide mouth with downturned corners, widely spaced teeth, and high palate. She has short, wide toes with slight sandal gap and minimal 2-3 syndactyly. MIP screening revealed a heterozygous de novo non-synonymous mutation in the *TRIP12* gene [chr2:g.229,807,778G>A [GRCh38/hg38]; NM_001284215.1: c.2300C>T; p.(Ala767Val)].

Individual 9 (Iossifov et al. 2014; O'Roak et al. 2014) is the second child of healthy, non-consanguineous parents. He is a 6-year-old African American male and has two siblings. Individual 9 was born at 36^{+3} weeks of gestation with a weight of 2834 g (−1.12 SD), a length of 52 cm (+1.13 SD), and an unknown, but reportedly within normal limits, OFC. Labor was induced via Pitocin for an unspecified reason. After birth, patient had difficulties regulating temperature. Abnormalities were first noted in his development at 18 months of age. He first used single words at 10 months and short phrases at 57 months, but parents report a loss of language skills in the first few years of life. He walked at the age of 19 months. Medical history is largely unremarkable. No gastrointestinal or neurological disturbances were reported. He has a history of sleep problems including difficulties falling asleep, difficulty waking in the morning, and difficulty breathing at night. Individual 9 had an MRI and EEG at 2 years; both showed normal results. At the time of his assessment, at the age of 6 years, his height was 117 cm (+0.21 SD), his weight was 20 kg (−0.21 SD) and his OFC was 53.1 cm (+0.6 SD). He met strict diagnostic criteria for ASD, confirmed with ADI, ADOS, and expert clinical judgment using the DSM-5. He presented with moderate ID, and adaptive and cognitive abilities were impaired (VIQ = 42, NVIQ = 53, Adaptive Behavior Composite = 63). He spoke using

predominantly single words and had extremely low receptive vocabulary abilities (PPVT Standard Score = 51). Individual 9 has a history of elevated attention, internalizing, externalizing, and affective problems. Parent report about his social responsiveness on the SRS suggests severe impairment in social awareness, social motivation, social cognition, social communication, and autistic mannerisms. Information regarding dysmorphology is unavailable. WES revealed a heterozygous de novo missense mutation in the *TRIP12* gene [chr2:g.229,771,583G>A [GRCh38/hg38]; NM_001284215.1: c.5618C>T; p.(Ser1873Leu)].

Individual 10 (Iossifov et al. 2014; O'Roak et al. 2014) is the only child of healthy, non-consanguineous parents. He is a 5-year-old Hispanic male. Individual 10 was born at 40 weeks of gestation by Caesarean section with a weight of 3543 g (+0.38 SD), a length of 52 cm (+1.88 SD), and an unknown, but reportedly within normal limits, OFC. Labor was induced via Pitocin for unspecified reasons and an emergent Caesarean section was performed. After birth, patient experienced hyperbilirubinemia. Individual 10 first used single words at 14 months and phrases at 33 months. He walked at the age of 13 months. Abnormalities were first noted in his development at 18 months. Individual 10 has an unremarkable medical history and no gastrointestinal or neurological disturbances were reported. He had an EEG at 3 years of age, which was normal. He has a history of sleep problems including sleep disordered breathing and incontinence at night. At the time of his assessment, at the age of $5^{8}/_{12}$ years, his height was 119 cm (+1.05 SD), his weight was 29 kg (+2.09 SD), and his OFC was 52.0 cm (-0.04 SD). He met strict diagnostic criteria for ASD, confirmed with ADI, ADOS, and expert clinical judgment using the DSM-5. The cognitive abilities of Individual 10 fall in the low range with some variability (VIQ = 81, NVIQ = 55). Adaptive abilities fall within low range (Adaptive Composite = 70). He spoke using phrase speech and had low receptive vocabulary abilities (PPVT Standard Score = 77). He has a history of elevated attention and externalizing problems. Parent report about his social responsiveness on the SRS suggests severe impairment in social awareness, social motivation, social cognition, social communication, and autistic mannerisms. Information regarding dysmorphology is unavailable. WES revealed a heterozygous de novo missense mutation in the *TRIP12* gene [chr2:g.229,785,842C>T [GRCh38/hg38]; NM_001284215.1: c.4883G>A; p.(Arg1628Gln)].

Individual 11 (Iossifov et al. 2014; O'Roak et al. 2014) is the second child of healthy, non-consanguineous parents. She is a 16-year-old Caucasian female with one sibling. Individual 11 was born vaginally at 37 weeks of gestation with a weight of 3147 g (−0.18 SD), a length of 53 cm (+2.25 SD), and an unknown, but reportedly within normal limits, OFC. Mother reports that Individual 11 had poor suck and feeding difficulties, and was irritable and inconsolable as an infant. Abnormalities were first noted in her development at 6 months of age. She first used single words at 24 months and first used phrases at 48 months of age. She walked at the age of 20 months. Individual 11 has a history of chronic constipation, chicken pox, and otitis media. She had an EEG done at age 4, which showed unspecified abnormal results. Individual 11 has a history of sleep problems including sleepwalking and difficulties falling asleep. At the time of her assessment at the age of $16^{8}/_{12}$ years her height was 157 cm (−0.79 SD), her weight was 20 kg (−0.21 SD), and her OFC was 56.1 cm (+1.11 SD). She met strict diagnostic criteria for ASD, confirmed with ADI, ADOS, and expert clinical judgment using the DSM-5 and mild ID (confirmed with cognitive and adaptive testing). Her cognitive abilities (VIQ = 57, NVIQ = 63) and adaptive skills (Adaptive Composite = 64) were impaired. She spoke using fluent language, but had moderately low receptive vocabulary abilities (PPVT Standard Score = 78). Individual 11 also had a history of elevated attention problems. Parent report about her social responsiveness on the SRS suggested moderate impairment in social awareness, social motivation, social cognition, social communication, and autistic mannerisms. Information regarding dysmorphology is unavailable. WES revealed a heterozygous de novo nonsense mutation in the *TRIP12* gene [chr2:g.229,836,980G>A [GRCh38/hg38]; NM_001284215.1: c.1012C>T; p.(Arg338Ter)].

Discussion

The introduction of next-generation sequencing (NGS)-based methods has significantly contributed to the identification of more than 700 genes linked to isolated ID and syndromic ID (Vissers et al. 2016) and the discovery of more than 700 ASD-associated genes (Bourgeron 2016). These numbers are continuously rising, a more up-to-date number of associated genes can be found in the SysID database (Kochinke et al. 2016). The mutual comorbidities of ID and ASD complicate the interpretation of these results and thus call for detailed clinical analysis.

TRIP12 (OMIM 604506) was first identified as a thyroid hormone receptor interacting protein (Lee et al. 1995) and more recently shown to function as a human HECT type E3 ubiquitin-protein ligase (Park et al. 2008, 2009). It directly catalyzes substrate ubiquitination and thereby labels cellular proteins for proteasomal degradation. TRIP12 is involved in the ubiquitin fusion degradation pathway (Park et al. 2009) and the regulation of DNA-damage induced chromatin ubiquitination (Gudjonsson et al. 2012). The TRIP12 protein has a HECT, a WWE (tryptophan–tryptophan) protein–protein interaction motif, and an

ARM domain (armadillo/β-catenin-like repeats) (Hanoun et al. 2014). Northern blot analysis detected expression of TRIP12 in 16 human tissues with high expression in skeletal muscle and low expression in the brain (Nomura et al. 1994).

The identification of mutations in *TRIP12* in individuals from ASD cohorts with ASD and ID (Iossifov et al. 2014; O'Roak et al. 2014) raised the question whether *TRIP12* can be validated as a gene associated with ASD and ID, and whether this is also applicable to individuals with ID/developmental delay without ASD. Recently, data from a large meta-analysis lead to the identification of *TRIP12* as a candidate gene for ID, but information on the possible presence of autistic features in the *TRIP12* mutation-positive individuals was not provided (Lelieveld et al. 2016). The identification of variants in novel candidate genes for ASD and ID calls for validation in other cohorts and the detailed clinical evaluation of mutation-positive individuals. We discuss the phenotypic findings in 11 *TRIP12* mutation-positive individuals below.

TRIP12-associated phenotype

To assess the *TRIP12*-associated phenotype, we compared the clinical information (Table 1) and available photographs (Fig. 2A–H) of the 11 *TRIP12* mutation-positive individuals.

All individuals presented with ID (10/11) or learning disability (IQ 76 in Individual 5; 1/11). There was a discrepancy between VIQ and NVIQ with VIQ scores being higher than NVIQ scores. Autism was diagnosed in 8/11 *TRIP12* mutation carriers; behavioral anomalies including stereotypic behavior were noted in 8/11 individuals. Fluent speech was acquired by 6/11, phrase speech by 3/11, and single words by 2/11 individuals. Most *TRIP12* mutation-positive individuals (8/11) did not show seizures with the exception of Individual 2 (twice at the age of 4 years), Individual 6 (seizures since the age of 1 year) and Individual 7 (once at the age of 7 years during a varicella infection) and individuals 2 and 11 had an abnormal EEG. Individuals with a *TRIP12* mutation did not present with any obvious internal anomalies (0/11; Table 1), had normal growth parameters and did not display any common hand, feet or other anomalies. Comparison of the facial phenotype demonstrated that the mouth region with an exaggerated Cupid's bow is similar in individuals 1, 2, and 5 (Fig. 2A, C, E) and individuals 1, 3, and 5 (Fig. 2B, D, F) present with small, posteriorly rotated ears. It was postulated that mutations in *TRIP12* lead to specific clinical ID phenotypes (Lelieveld et al. 2016), but our detailed analysis of the clinical data of 11 *TRIP12* mutation-positive individuals leads us to the conclusion that *TRIP12* mutations do not lead to a recognizable syndrome.

Genotype–phenotype correlation

In this study we identified two novel splice site (individuals 1 and 2), one novel nonsense (Individual 3), and three novel missense *TRIP12* mutations [individuals 5, 6 (inherited maternally) and 8] and one individual with a novel translocation between one chromosome 2 and one X chromosome with one breakpoint within the intron 1 of *TRIP12* (Fig. 3). The other *TRIP12* mutations (individuals 4, 9–11) were previously published (Iossifov et al. 2014; Lelieveld et al. 2016; O'Roak et al. 2014). All mutations are depicted in a schematic illustration in Fig. 1B. At this point we speculate that haploinsufficieny of TRIP12 is the major pathogenic mechanism.

To assess whether there is a correlation between the type of *TRIP12* mutation and the presence of ASD, we grouped the *TRIP12* mutation carriers by their type of mutation. Nonsense, frameshift, and splice mutations leading to a frameshift and premature termination were detected in five individuals (individuals 1, 2, 3, 4, and 11). Four of these five individuals were diagnosed with ASD, Individual 3 had no autistic features. Individual 7 with the translocation and breakpoint in *TRIP12* did not show any autistic features. Four of the five individuals with *TRIP12* missense mutations (5, 6, 8, 9, and 10) were diagnosed with ASD. The missense variant in Individual 6 was also detected in his healthy mother. The CADD score, PolyPhen2, and Mutation Taster rate this variant as probably damaging and disease causing, while the SIFT and Provean tools rate it both as damaging and tolerated/neutral (Table 2). The variant was not listed in the ExAC or 1000Genomes. While there is the possibility that there may be reduced penetrance in the carrier mother, we cannot be certain about this variant's pathogenicity. In summary, our preliminary data on a small number of *TRIP12* mutation carriers suggest that the type of *TRIP12* mutations is not correlated with the absence or presence of autistic features.

Analysis of a larger number of individuals with *TRIP12* mutations is needed to further evaluate a possible genotype–phenotype correlation. In order to further delineate the clinical spectrum associated with de novo mutations in the *TRIP12* gene, we have established the website http://www.trip12gene.com to collect detailed clinical information of additional patients that will be identified in the coming years.

In summary, we describe the *TRIP12*-associated phenotype, we show that *TRIP12* is a risk gene for non-syndromic ID with and without ASD and that *TRIP12* mutation carriers present with a broad phenotypic range within the neurodevelopmental phenotypes.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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