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ORIGINAL INVESTIGATION

Haploinsufficiency of the E3 ubiquitin-protein ligase gene *TRIP12* causes intellectual disability with or without autism spectrum disorders, speech delay, and dysmorphic features

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Abstract Impairment of ubiquitin–proteasome system activity involving ubiquitin ligase genes *UBE3A*, *UBE3B*, and *HUWE1* and deubiquitinating enzyme genes *USP7* and *USP9X* has been reported in patients with neurodevelopmental delays. To date, only a handful of single-nucleotide variants (SNVs) and copy-number variants (CNVs) involving *TRIP12*, encoding a member of the HECT domain E3 ubiquitin ligases family on chromosome 2q36.3 have been reported. Using chromosomal microarray analysis and whole-exome sequencing (WES), we have identified, respectively, five deletion CNVs and four inactivating SNVs (two frameshifts, one missense, and one splicing) in *TRIP12*. Seven of these variants were found to be de novo; parental studies could not be completed in two families. Quantitative PCR analyses of the splicing mutation showed a dramatically decreased level of *TRIP12* mRNA in the proband compared to the family controls, indicating a loss-of-function mechanism. The shared clinical features include intellectual disability with or without autistic spectrum disorders, speech delay, and facial dysmorphism. Our findings demonstrate that

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E3 ubiquitin ligase TRIP12 plays an important role in nervous system development and function. The nine presented pathogenic variants further document that *TRIP12* haploinsufficiency causes a childhood-onset neurodevelopmental disorder. Finally, our data enable expansion of the phenotypic spectrum of ubiquitin–proteasome dependent disorders.

Introduction

Copy-number variants involving chromosome 2q36.3 are rare. To date, only two interstitial deletion CNVs involving *TRIP12* [MIM 604506], encoding a member of the HECT domain E3 ubiquitin ligases family, have been reported in the literature: a de novo ~5.4 Mb CNV deletion on 2q36.2q36.3 has been identified in an individual with severe intellectual disability (ID), multiple renal cysts, and dysmorphic features (Doco-Fenzy et al. 2008) and a de novo ~60 kb deletion involving *FBXO36* and the first non-coding exon of *TRIP12* has been described in a subject with an autistic spectrum disorder (Pinto et al. 2014). An ~180 kb CNV duplication encompassing *FBXO36* and the 5′ portion of *TRIP12* has been reported in an individual with macrocephaly (Fig. 1a) (Oikonomakis et al. 2016). Recently, four *TRIP12 de novo* single-nucleotide variants have been identified amongst 64 autism spectrum disorders (ASDs) candidate genes resequenced in 5979 individuals; two missense and two truncating variants were detected in individuals with ASDs and ID, ranging from mild to moderate severity (O'Roak et al. 2014). Most recently, Lelieveld et al. (2016) have reported two additional truncating mutations in *TRIP12* among 2637 de novo mutations across 1990 genes identified in a meta-analysis of 2104 trios involving individuals with sporadic ID (Fig. 1b, c). While this study was under review, Bramswig et al. (2017) reported a cohort of 11 individuals with variants in *TRIP12,* including the three patients reported by O'Roak et al. (2014). In addition, Subject 8 in our study was also enrolled in Bramswig et al.'s cohort (individual 8). We compare the clinical features in our patient cohort with all reported patients and discuss the *TRIP12*-associated phenotypic spectrum.

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Methods

Study cohort

The study cohort consists of nine unrelated patients. Five patients carrying deletion CNVs in chromosome 2q36.3 were identified in our clinical database of 75,795 subjects referred for clinical chromosomal microarray analysis. Three patients carrying SNVs were found through our database of 9056 individuals referred for clinical whole-exome sequencing. These three patients were chosen through filtering for potentially LoF variants in previously unsolved cases with overlapping neurological phenotypes. The ninth subject was identified from a cohort of 207 individuals through CHU Angers, France, who had global developmental delay and was referred for WES trio analysis. To further investigate these novel variants, we have enrolled all nine individuals (age range from 1.8 to 15 years) in research studies approved by the Institutional Review Boards of Baylor College of Medicine and CHU Angers in France. Written informed consents were obtained from the families. Consents for publication of the photographs were attained from the parents/legal guardians of subjects 5, 7, 8, 9, and subject Suppl 1.

Microarray and molecular analyses

The five CNV deletions were detected using customized exon-targeted oligo arrays (OLIGO V8, V9, and V10) designed at Baylor Genetics (Boone et al. 2010; Wiszniewska et al. 2014), which cover more than 4800 known or candidate disease genes with exon level resolution. The WES-targeted regions cover >23,000 genes for capture design (VCRome by NimbleGen®). The mean coverage of target bases was >140X, and >96% target bases were covered at >20X (Yang et al. 2014). WES trio analysis was performed using SureSelectXT Target Enrichment Kit capture by Agilent Technologies, Santa Clara, CA, USA, with the mean coverage of 52X at the genomics platform of Nantes (Biogenouest Genomics). PCR amplification and Sanger sequencing to verify all candidate variants were done in the proband and the parents when available, according to standard procedures, and candidate variants were annotated using the *TRIP12* RefSeq transcript NM_004238. Quantitative PCR was performed to determine *TRIP12* mRNA expression in the blood samples of the proband and controls by Applied Biosystems TaqMan Assays system based on the manufacturer's instruction. The primers TRIP12_Forward (5′-GGATGCTGTGAGCAGAGAGA-3′) and TRIP12_Reverse (5′-CATCTGATCCATCGTCATCG-3′) were used in the qPCR experiments.
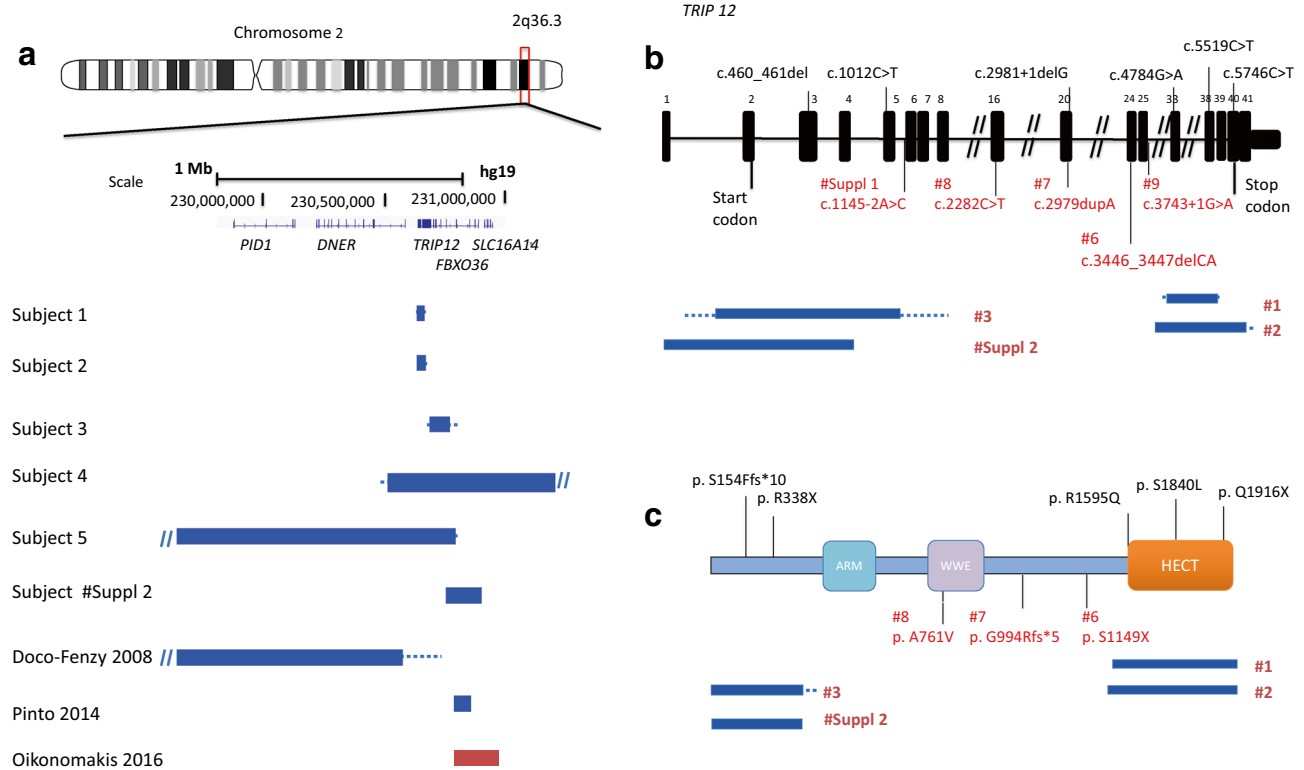
Fig. 1 a Schematic representation of 2q36.3 CNV deletions involving *TRIP12* identified by exon-targeted CMA in six subjects from this study and three CNV subjects reported previously. Deletions are indicated in blue and duplications in *red*. The *dotted lines* depict the maximum deletion regions. **b** *TRIP12* gene exonic structure. Three *TRIP12* exonic deletion CNVs identified in subjects 1–3 are indicated under the diagram (*blue bars* indicate the minimum deletion region and the *dotted lines* depict the maximum deletion regions). The variants from this study are indicated in *red* and the previously reported variants are indicated in *black*. Reported variants include two missense c.5519C>T (p.S1840L) and c.4784G>A (p.R1595Q) and two truncating c.2981+1delG and c.1012C>T (p.R338X) changes from ASDs cohort studies (O'Roak et al. 2014) and c.5746C>T (p.Q1916X) and c.460_461del (p.S154Ffs*10) from 2104 trio studies (Lelieveld et al. 2016). **c** Functional domains of TRIP12 include ARM domain (*turquoise*), WWE domain (*purple*), and HECT (E6AP-type E3 ubiquitin-protein ligase) (*orange*) (color figure online)

Results

Study cohort

In our cohort, all nine individuals presented with moderate-to-severe DD/ID and six of them manifested autistic behaviors (see Table 1 for detailed information). Similarly, Bramswig et al. also reported that all their 11 patients had DD/ID and 8 out of 11 manifested some autistic behaviors. However, our patients showed more severe verbal defects. Out of eight patients with detailed clinical information, only one patient achieved fluent speech (12.5%), six patients had phrase or single words (75%), and one patient had no speech at 4 year of age (12.5%). In the Bramswig et al.'s cohort, 6 out of 11 patients could speak fluently (54.5%); the other patients spoke either phrases or single words (45.5%). Behavioral abnormalities in our cohort vary substantially, ranging from poor social interaction to extremely impulsive and aggressive behaviors, which were also reported in Bramswig et al.'s patients. Dysmorphic facial features are also variable; however, narrow up-slanting palpebral fissures (seen in four out of our seven subjects, 57.1%) and a distinct mouth with downturned corners (four out of our eight subjects in main cohort plus one supplementary patient, 55.6%) appear to be the most commonly shared facial feature in our cohort (Fig. 2); these two features were not discussed in Bramswig et al.'s cohort. Of note, obesity was documented in four of our seven children (57.1%), with one progressing to morbid obesity. Interestingly, although the weight of subject 2 was 23.3 kg at 7 years of age (~52 centile), his calculated BMI was ~91 centile. Two subjects were also reported as obese in Bramswig's cohort. Combined together, 6 out of 18 individuals were documented as obese (33.3%). Microcephaly

Table 1 Summary of clinical presentations, and CMA and molecular findings in nine individuals with *TRIP12* mutations

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Summary from this study	Summary from Bramswig et al.)
Gender	Male	Male	Male	Female	Female	Male	Male	Female	Male	6 males, 3 females	7 males, 4 females
Age at examination (years)	15	7	15	4	1.8	4	10	12.57		1.8–15	5.7–26.3
TRIP12 variant (NM_004238) genomic coordinates (min/max, hg19)	Deletion of 9 exons (30–38), chr2:230,634,390/230,636,077–230,654,539/230,655,748	Deletion of 17 exons (25–41), chr2: 230,631,958/230,632,049–230,660,565/230,661,210	Deletion of 4 exons (2–5), chr2:230,679,862/698,794–230,781,114/230,801,002	Deletion of *TRIP12* and adjacent 7 genes, chr2:230,478/230,513,445–231,457,431/231,508,839	Deletion of *TRIP12* and 3 adjacent genes, chr2:229,076,749/229,152,599–230,801,061/230,811,273	Frameshift c.3446_3447delCA (p.S1149*X), Chr2: 230,661,450	Frameshift, c.2979dupA (p.G994Rfs*5), Chr2: 230,666,969	Missense c.2282C>T (p.A761V), Chr2: 230,672,494	Splicing, c.3743+1G>A, Chr2: 230,659,894	5 CNVs (microdeletions), 3 SNVs/indels (2 truncating, 1 missense)	1 translocation, 10 SNVs/indels (5 truncating, 5 missense)
Ethnicity	Mexican	NA	NA	Caucasian	NA	Saudi Arabia	United Arab Emirates (second cousins parents)	North European/Caucasian	Caucasian		
Inheritance	De novo	Unknown	Unknown	De novo	De novo	De novo	De novo	De novo	De novo	7 *de novo*, 2 parents NA	9 *de novo*, 2 parents NA
Maternal age at birth (years)	45	NA	NA	NA	NA	25	25	26	29	25–45	22–34
Paternal age at birth (years)	47	NA	NA	NA	NA	35	27	34	48	27–48	25–41
DD/ID	+	+	+	+	+	+	+	+	+	9/9	11/11
Speech delay	+	+	ND	+	+	+.	+	+	+	8/8	10/11, 1 ND
Autistic behaviors	+Poor social interaction	-	ND	+Stereotypic behaviors	+	+	+	+	-	6/8	8/11
Other behavioral anomalies		Hyper anxiety. Untypical behaviors	ND		Repetitive, aggressive behaviors/sensory issues	Hyperactive and destructive behavior	ND	Extremely impulsive and aggressive behaviors.	-	6/7	8/11
First words (months)	36	60	ND	24	ND	ND	ND	24	42		
Verbal ability	Fluent speech	<100 words at 7 years	ND	30 words	5–6 words	No words	Single words	Phrase speech	Few phrase speech	1/8 fluent speech, 6/8 phrase or single words, 1/8 no words	6/11 fluent speech, 5/11 phrase or single words
Motor delay	+	-	ND	+	+	+	+	+	+	7/8	7/11
Walking independently (months)	15	ND	ND	20	ND	ND	22	23	29	15–29	13–23

Table 1 continued

	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Summary from this study	Summary from Bramswig et al.
Seizures	–	–	ND	–	+	–	–	–	–	1/8	3/11
Microcephaly/OFC (cm) (percentile)	+52.2 (2–10%)	ND	ND	+49 (25%)	–	ND	–	–	–	2/6	0/11, 1 ND
Obesity/weight (kg, percentile)	+86 (morbid obesity)	−23.3 (BMI 91%)	ND	+	−65%	ND	+97%	+90%	−50 to 75%	4/7	2/11
Height (cm)	174 (75–90%)	113.5 (6%)	ND	103 (50%)	89 (46%)	ND	ND	ND	123 (75%)	Variable	Variable
Dysmorphic features											
Head shape	Brachycephaly, dolichocephaly		ND			Sloping forehead	–	–	–	2/8	ND
Narrow palpebral fissures	+		ND	–	+	–	+	+	ND	4/7	ND
Downturned mouth corners	+		ND	–	+	–	+	+	–	4/8	ND
Wide mouth	+		ND	–	–	–	+	+	–	3/8	3/8
Hypertelorism	+		ND	–	–	–	–	–	–	1/8	1/8
Epicanthic folds	–		ND	+	+	–	+	–	–	3/8	1/8
Depressed nasal bridge	–		ND	–	–	– (high nasal bridge)	–	–	–	0/8	1/8
Short nose	– (long nose)		ND	–	–	–	–	–	–	0/8	2/8
Anteverted nares	–		ND	+	–	–	–	–	–	1/8	1/8
Low hanging columella	+		ND	–	–	–	–	–	–	1/8	3/8
Long philtrum	–		ND	–	–	–	–	– (short philtrum)	–	0/8	2/8
Smooth philtrum	–		ND	–	–	–	–	–	–	0/8	2/8
Thin upper lip vermilion	– (full lips)		ND	–	–	–	+	–	–	1/8	2/8
Exaggerated Cupid's bow	–		ND	–	–	–	–	–	–	0/8	4/8
Pointed chin	–		ND	–	–	+	–	+	–	2/8	ND
Large ear lobe	+		ND	–	–	+	–	–	+	3/8	3/8
Low set ears	–		ND	–	+	–	–	–	–	1/8	2/8
Hand anomalies	–		–	–	–	–	Spindle shaped fingers	–	–		
Hearing loss	–		–	+Unilateral	–	–	–	–	–	1/9	0/11
Brain anomalies	–		–	–	–	–	–	–	–		

NA not available, *ND* not determined because of non-availability or non-applicability, *OFC* occipitofrontal circumference

Subject 5   Subject 7

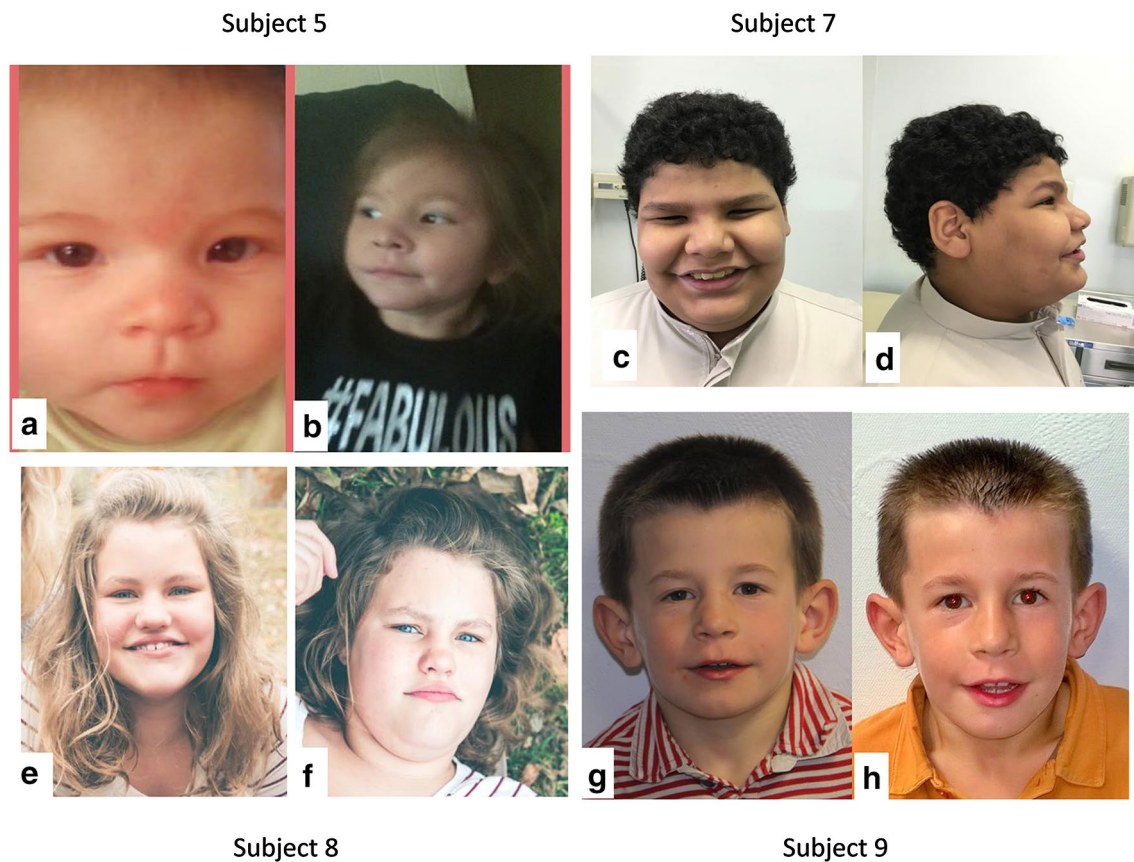Subject 8   Subject 9

Fig. 2 Photographs of subjects 5, 7, 8, and 9 are shown. Note narrow up-slanting palpebral fissures and a distinct mouth with downturned corners. Informed consent for publication of these photographs was obtained

was observed in two our subjects and was not seen in Bramswig et al.'s cohort, and epilepsy was seen in one our individual but in three individuals in Bramswig et al.'s cohort. These data suggest that microcephaly (12.5%) and seizure (21%) are only minor features of *TRIP12* associated syndrome. Among the other facial features, the most commonly shared features between two cohorts include wide months (37.5%) and large ear lobe (37.5%).

Microarray and molecular analysis

The five deletion CNVs involving *TRIP12* range in size from 20 kb to 1.7 Mb (Fig. 1a). CNV deletions in subjects 1–3 removed 9 exons (exon 30–38), 17 exons (exon 25–41), and 4 exons (exon 2–5) of *TRIP12,* respectively, whereas the deletions in subjects 4 and 5 spanned the entire *TRIP12* gene and the adjacent genes (Fig. 1a). In subject 5, the 2q36.3 imbalance resulted from a translocation t(1;2)(p31.2;q36.3). Parental studies showed that three of the five CNVs arose de novo (subjects 1, 4, and 5); the parental samples were unavailable to evaluate inheritance for two deletion CNVs (subjects 2 and 3).

No additional clinically relevant CNVs were identified in these five individuals.

WES revealed two mutations, predicted to be truncating, c.3446_3447delCA (p.S1149X) in subject 6 and c.2979dupA (p.G994Rfs*5) in subject 7, one missense variant c.2282C>T (p.A761V) in subject 8, and splice-site truncating mutation (c.3743+1G>A) in subject 9 (Fig. 3a–c, f). With the exception of *TRIP12* rare variants, no additional deleterious/disease-causing mutations in other known disease genes were identified in these four probands (Subject 6–9).

The catalytic HECT ubiquitin ligase domain is located between amino acids 1590-1990; hence, c.3446_3447delCA (p.S1149X) in subject 6 and c.2979dupA (p.G994Rfs*5) in subject 7 both result in loss of this domain (Fig. 1c), and are predicted to cause loss of normal protein function either through nonsense mediated mRNA decay mechanism or loss of ligase function activity. Both truncating variants were confirmed by Sanger sequencing to be *de novo* events (Fig. 3a, b).

The missense mutation in codon 761 (p.A761V) in subject 8, which was also confirmed to arose de novo by Sanger sequencing, is located in a highly conserved
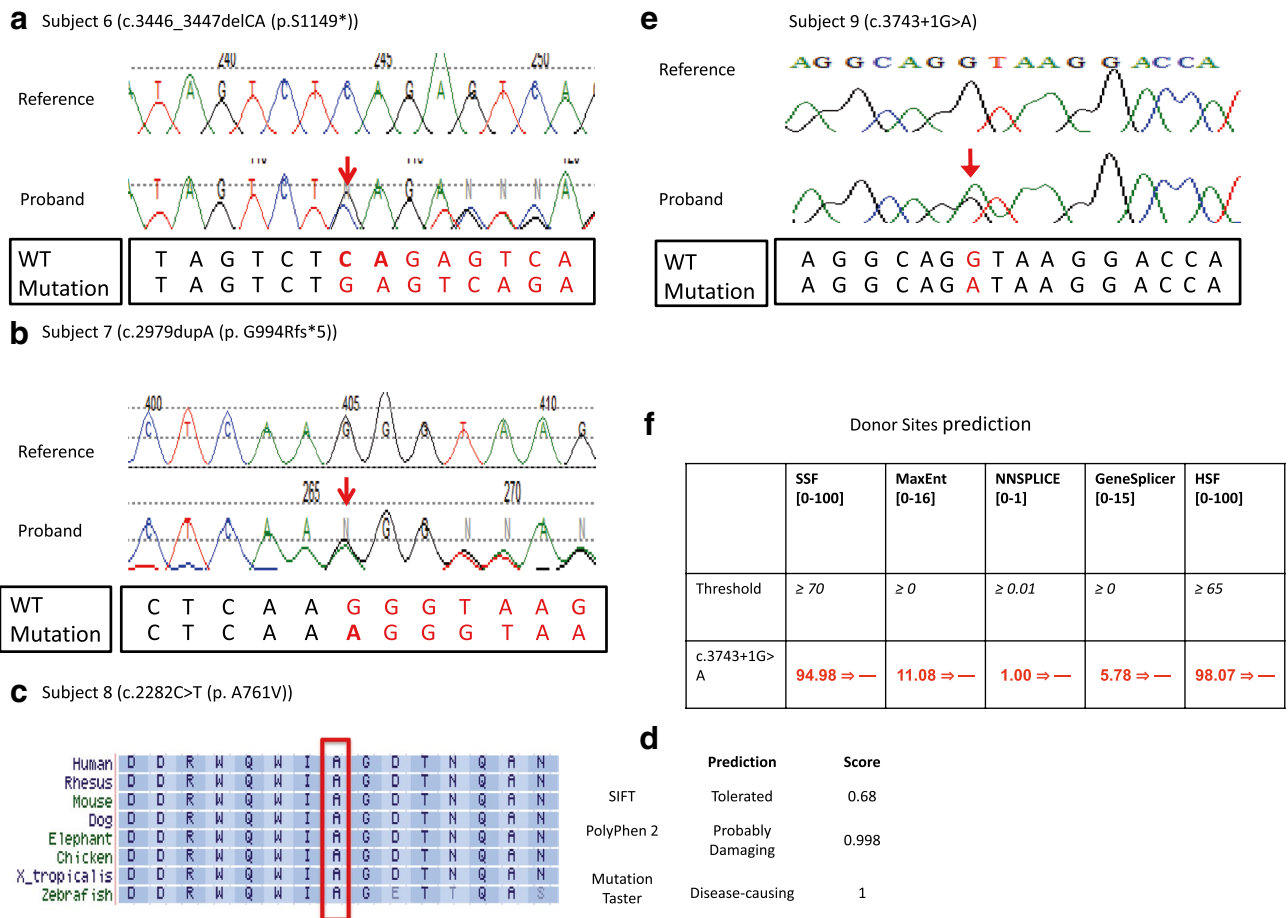
Fig. 3 a, **b** Sanger sequencing traces from subject 6 and subject 7 are presented. The WT and mutation sequences are shown separately below the Sanger traces. **c** Alignment of the TRIP12 protein sequence from multiple species around amino-acid position 761 (NM_004238) that was altered from alanine to valine in subject 8. This alanine at position 761 was well conserved from human to zebrafish, and this missense change was predicted to be deleterious/disease-causing by MutationTaster and Polyphen-2 (**d**). **e** Chromatogram of de novo variant c.3743+1G>A and **f** results of in silico analysis of the 3743+1G>A variant found in subject 9 using five splicing prediction tools included in Alamut v2.7. The *numbers in brackets* indicate the value range generated by each predication tool. The threshold used by each prediction tool indicates that above this threshold, the positions are predicted to be true splice-sites. The *numbers in red* are the actual value calculated by each prediction algorithm. All five prediction tools suggested that the donor splice site was abolished at the variant position (color figure online)

amino acid (Fig. 3d) within the protein–protein interaction-related WWE domain (Aravind 2001). A non-polar (alanine) to polar (threonine) change likely affected the interactions of TRIP12 with its binding partners. Two in silico prediction tools, PolyPhen-2 and MutationTaster, predicted deleterious/damaging and potentially disease-causing effects of this mutation (Fig. 3e).

The c.3743+1G>A de novo mutation observed in subject 9 is predicted to result in a complete loss of an invariant splice site by at least five donor site prediction tools incorporated in the Alamut software: SSF, MaxEnt, NNSPLICE, GeneSplicer, and HSF (Fig. 3g). To further characterize the identified *TRIP12* variant, we performed quantitative PCR on this splicing change c.3743+1G>A

in the blood samples of subject 9, his both parents, and an unaffected sister. qPCR data showed dramatic reduction of *TRIP12* mRNA level in the proband compared to three unaffected family member controls (Supplementary Figure S2), indicating that this splicing mutation led to loss of *TRIP12* function through the non-sense mediated decay.

Together, our results implicate that all nine variants identified in subjects 1–9 represent pathogenic or likely pathogenic alleles. None of these rare *TRIP12* variants, except the shared subject 8 (c.2282C>T), were observed in the common SNPs (dbSNP build 148), the 1000 Genomes, the Exome Variant Server (ESP6500), the Exome Aggregation Consortium (ExAC), or the

Atherosclerosis Risk in Communities (ARIC; exome data of ~6000 subjects) databases (1000 Genomes Project; Atherosclerosis Risk in Communities Study; ExAC Brower. Exome Aggregation Consortium; NHLBI Exome Sequencing Project (ESP). Exome Variant Server). The c.2282C>T (p.A761V) change was recently seen three times in the ExAC database (Jan 31st, 2017) and four times in the gnomAD database (Jan 31st, 2017). Based on this very recent genotypic information from ExAC and gnomAD database, we re-classified this missense change as variant of unknown significance (VUS). Of note, the pLI score (Lek et al. 2016) of *TRIP12* reported in ExAC database is 1.00. ExAC database uses the observed and expected variants counts to determine the probability whether a given gene is intolerant for loss-of-function variation (pLI score). The closer pLI is to one, the more LoF intolerant the gene appears to be. We consider pLI ≥ 0.9 as an extremely LoF intolerant. No LoF variants in *TRIP12* were reported in ExAC as of January 22, 2017, whereas 76.9 variants were expected, suggesting that this gene is intolerant to LoF SNVs. The information of the four SNP variants CADD scores can be found in Supplementary Table 2.

Discussion

TRIP12, an evolutionarily conserved 1992 amino-acid protein that belongs to the HECT domain E3 ubiquitin ligases family, is widely expressed in various tissues. TRIP12 contains a WWE motif, predicted to mediate specific protein–protein interactions in ubiquitin and the ADP-ribose conjugation system (Aravind et al. 2001), a HECT domain, functioning as E6AP-type E3 ubiquitin-protein ligase containing a conserved cysteine residue that is necessary for the tagging of target proteins with ubiquitin, and an ARM (armadillo/β-catenin-like repeats) domain (Fig. 1c) (Rotin and Kumar 2009).

Recent studies have revealed a few potential binding partners for TRIP12, including amyloid precursor protein-binding protein (APP-BP1) (Park et al. 2008); ADP-ribosylation factor 1 (ARF) (Chen et al. 2013; Velimezi et al. 2013); pancreas transcription factor 1a (PTF1a) (Hanoun et al. 2014); SRY (Sex determining region Y)-Box 6 (SOX6) HECT, UBA, and WWE domain containing 1, E3 ubiquitin-protein ligase (HUWE1) (Poulsen et al. 2012); and ring finger protein 168 (RNF168) (Gudjonsson et al. 2012), suggesting that TRIP12 plays an important role in a broad range of physiological processes. TRIP12 has been shown also to regulate DNA repair, cell growth, and ARF-P53 pathway-related oncogenic stress (Chen et al. 2010, 2013; Gudjonsson et al. 2012). TRIP12 was also found to be required for the

proper mouse brain development and neuronal cell proliferation, differentiation, and apoptosis (Kajiro et al. 2011). Moreover, a recent study using RNAi cell knockdown demonstrated that TRIP12 likely functions together with HUWE1, encoded by an X-linked syndromic ID gene (MIM: 300706) in the ubiquitin fusion degradation (UFD) pathway. TRIP12 was also found to interact with APP-BP1, an important cell cycle checkpoint protein, and regulate its level of expression (Chen et al. 2000).

During this study, we also ascertained an individual with a splicing variant c.1145-2A>C in *TRIP12* that was not present in his mother (see subject Suppl 1 in Supplementary Table S1 and Supplementary Figure S3–S6 for the detailed clinical and molecular data). The father was not available for the study. The c.1145-2A>C variant in the canonical mRNA isoform NM_004238 of *TRIP12* is predicted to result in a complete loss of an invariant splice site by four acceptor site prediction tools incorporated in the Alamut software: MaxEnt, NNSPLICE, GeneSplicer, and HSF (Supplementary Figure S4b). There are four verified RefSeq isoforms (NM_001284214, NM_001284215, NM_001284216, and NM_004238 used as reference isoform in this study) in *TRIP12*. In contrast to the c.3743+1G>A splicing mutation in subject 9, which affects all four isoforms and showed a much decreased expression level in quantitative PCR experiment (Supplementary Figure S2), the c.1145-2A>C splicing variant in subject supplementary 1, which affects only one isoform (NM_004238) and is predicted to result in synonymous changes in the other three isoforms by conceptual translation (Supplementary Figure S5), showed a similar *TRIP12* RNA expression comparing to an unaffected control (Supplementary Figure S6a). These molecular findings are also consistent with the clinical features of the subject supplementary 1 with much milder neurocognitive phenotype than subject 9. Considering that the isoform-specific expression pattern in brain tissue is unknown, the pathogenicity of this splicing variant cannot be determined at this stage and thus has been classified as a variant of unknown significance.

In addition, by exploring the DECIPHER database, we have identified four individuals (DECIPHER IDs: 250590, 252476, 301556, and 281305) with CNV deletions involving *TRIP12* and smaller than 1.5 Mb in size. Three of these deletions were reported as *de novo* events, and the inheritance of the fourth CNV (281305) has not been determined. This fourth deletion was the smallest in size (144 kb), involved only *TRIP12* and *FBXO36,* and was detected in a 31-month-old male with psychomotor delay and non-specific asymmetric white matter (leukoencephalopathy). Multicystic renal dysplasia and renal insufficiency observed in this DECIPHER subject were consistent with the previous reports on *TRIP12* CNV deletions (see Supplementary

Table S1 for detailed clinical information) (Doco-Fenzy et al. 2008).

Compliance with ethical standards

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