SHORT REPORT

Expansion of the spectrum of *ITGB6*-related disorders to adolescent alopecia, dentogingival abnormalities and intellectual disability

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Alopecia with mental retardation (APMR) is a very rare disorder. In this study, we report on a consanguineous Pakistani family (AP91) with mild-to-moderate intellectual disability, adolescent alopecia and dentogingival abnormalities. Using homozygosity mapping, linkage analysis and exome sequencing, we identified a novel rare missense variant c.898G>A (p.(Glu300Lys)) in *ITGB6*, which co-segregates with the phenotype within the family and is predicted to be deleterious. Structural modeling shows that Glu300 lies in the β -propeller domain, and is surrounded by several residues that are important for heterodimerization with α integrin. Previous studies showed that *ITGB6* variants can cause amelogenesis imperfecta in humans, but patients from family AP91 who are homozygous for the c.898G>A variant present with neurological and dermatological features, indicating a role for *ITGB6* beyond enamel formation. Our study demonstrates that a rare deleterious variant within *ITGB6* causes not only dentogingival anomalies but also intellectual disability and alopecia.

European Journal of Human Genetics (2016) 24, 1223–1227; doi:10.1038/ejhg.2015.260; published online 23 December 2015

INTRODUCTION

Although the nervous system, tooth enamel, mucosal membranes and epidermis are ectodermal in origin, human diseases that affect all four ectodermal derivatives are rare. A few genes have been associated with phenotypes including alopecia and mental retardation (APMR), namely: *DCAF17* (2q31.1, MIM 612515) for Woodhouse–Sakati syndrome (MIM 241080), which includes endocrine disease and hearing impairment;¹ *ALX4* (11p11.2, MIM 605420) for frontonasal dysplasia 2 (MIM 613451) with APMR;² and *MBTPS2* (Xp22.12-p22.11, MIM 300294) for ichthyosis follicularis, atrichia and photophobia with or without intellectual disability or Bresheek syndrome (MIM 308205).³ Four autosomal recessive APMR loci have been mapped, namely APMR1 on 3q26.33–q27.3,⁴ APMR2 on 3q26.2–q26.31,⁵ APMR3 on 18q11.2–q12.2^(ref. 6) and APMR(4) on 1p31.1–p22.3,⁷ but the causal variants within these loci have not been identified. Here we report on a variant in *ITGB6* (MIM 147558), which encodes integrin beta-6, as the cause of autosomal recessive APMR with premature skin aging and dentogingival abnormalities.

MATERIALS AND METHODS

The study was approved by the Institutional Review Boards of Quaid-i-Azam University and Baylor College of Medicine and Affiliated Hospitals. Informed

consent was obtained from participating family members, including permission to publish facial photographs. For family AP91 (Figure 1a), homozygosity mapping was carried out using genome-wide genotypes for ~400 microsatellite markers. Parametric multipoint linkage analysis was performed with MERLIN⁸ using autosomal recessive inheritance with complete penetrance. A DNA sample from affected individual IV-6 was submitted for exome sequencing at the University of Washington Center for Mendelian Genomics. Pathogenicity of identified variants was evaluated using multiple bioinformatics tools (Table 1). For each variant, minor allele frequencies (MAF) were checked in variant databases 1000 Genomes, Exome Variant Server (EVS) and Exome Aggregation Consortium (ExAC) and in 148 in-house exomes from unrelated Pakistani individuals with Mendelian traits that do not overlap with the phenotypes in family AP91. Because of the rarity of the phenotype in family AP91, variants that were homozygous in the in-house exomes were excluded. Rare potentially pathogenic variants were Sanger-sequenced in the rest of family AP91 and the *ITGB6* variant was sequenced in 248 Pakistani control individuals. The *ITGB6* variant was submitted to ClinVar. To check for evolutionary conservation of *ITGB6* amino acid residues, the human sequence was aligned with 51 vertebrate sequences. Phyre2^(ref. 9) was used for molecular modeling. RT-PCR was performed on commercially available human cDNA (Clontech, Mountain View, CA, USA).

RESULTS

Family AP91 is a four-generation consanguineous pedigree (Figure 1a) from the Galiyat region of Pakistan. Affected individuals had normal

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Received 17 July 2015; revised 5 October 2015; accepted 14 October 2015; published online 23 December 2015

Figure 1 Pedigree drawing, clinical information and chromatograms from family AP91. (a) Pedigree of consanguineous family AP91 with intellectual disability, alopecia, progeroid appearance, periodontal disease and tooth abnormalities co-segregating with the *ITGB6* c.898G>A (p.(Glu300Lys)) variant. (b) Photographs of affected individuals IV-2, IV-3 and IV-6 at 40, 39 and 33 years of age, respectively. Note fine sparse scalp hair, sparse eyebrows and eyelashes and deeply set facial wrinkles. Women were veiled because of religious beliefs. Affected male IV-2 had bilateral super-eruption of maxillary lateral incisors, small central incisors and poor alignment of teeth. The affected youngest sister IV-6 also had misaligned mandibular anterior teeth with rough surfaces. Both IV-2 and IV-6 had prominent yellowish-brown tooth stains and severe gingival disease. Individual IV-3 had no remaining teeth. (c) Chromatograms from homozygous affected and heterozygous/wildtype unaffected family members.

thickness of scalp hair at birth although hair was easy to pluck. Progressive hair loss and thinning started at ages 10–12 years. Affected male individual IV-2 (Figure 1b) had obvious scalp hair thinning and lacked moustache and beard hair. All three affected individuals had sparse eyebrows and eyelashes, and early-onset skin aging with deeply set facial wrinkles, giving them a progeroid appearance. Since childhood, the affected individuals had poor speech and memory, were unable to go to school, wandered aimlessly in public places, exhibited aggressive behavior and/or lacked the concept of danger or fear. Based on the Wechsler Adult Intelligence Scale test that was translated to the local dialect, the affected individuals had mild-to-moderate intellectual disability (IQ 53–61). They exhibited dental abnormalities such as plaques, calculi and gingival recession, which may partly be due to poor hygiene but can also be included in the disease spectrum. Individual IV-3 lost all her teeth, while individuals IV-2 and IV-6 had yellowish-brown tooth stains (Figure 1b). The lateral maxillary incisors of individual IV-2 were super-erupted and protruded anteriorly. For individual IV-6, alignment of maxillary teeth was normal, but the mandibular incisors and canines were badly aligned and had rough enamel surfaces. No additional syndromic features such as hearing, visual, cardiopulmonary or endocrine abnormalities were detected.

Both linkage interval and homozygous region identified in family AP91 were flanked by markers D2S1353 and D2S2307, defining a 15.89-Mb locus on chromosome 2q24.1-q31.1. Linkage analysis yielded a maximum multipoint LOD score of 2.65 ($\Theta = 0$) for several markers in the mapped region. Within the homozygous region, exome sequencing revealed 80 homozygous variants, but only one variant, *ITGB6* NM_000888.3:c.898G>A (p.(Glu300Lys)), did not occur in variant databases 1000 Genomes, EVS or dbSNP, but was heterozygous in the ExAC database with an overall MAF of 3.3×10^{-5} . The *ITGB6* variant and three additional rare homozygous exome variants outside the mapped region (Table 1) did not occur in 148 in-house exomes. However Sanger sequencing using DNA samples from the rest of family AP91 revealed co-segregation of only the *ITGB6* (Figures 1a and c) variant with the phenotypes. Additionally the *ITGB6* variant was absent in 496 Pakistani control chromosomes, and is the only rare variant that not only lies within the mapped region and segregates with disease, but also occurs at a highly conserved residue and is predicted to be deleterious by the majority of bioinformatics results (Table 1).

The glutamic acid at position 300 of human *ITGB6* is identical across all species and lies within a highly conserved region from His275 to Ser304 of human *ITGB6* (Figure 2a). The Glu300 residue occurs at the predicted β -propeller domain and lies very close to

Table 1 Rare homozygous exome variants in individual IV-6 from family AP91

Gene	HIVEP3	MED8	PCYOX1	ITGB6
MIM#	606 649	607 956	610 995	147 558
Chr	1	1	2	2
hg19 Coordinate	42 045 460	43 851 838	70 502 099	161 029 103
PhastCons100way	1.00	1.00	1.00	1.00
PhyloP100wayall	5.41	5.996	7.36	5.98
GERP	5.36	5.14	5.40	5.49
dbSNP rsID	NA	148592512	NA	NA
RefSeq accession number	NM_024503.4	NM_052877.4	NM_016297.3	NM_000888.3
cDNA change	c.5009C>T	c.553G>A	c.503G>A	c.898G>A
Amino acid change	p.(Pro1670Leu)	p.(Val185Ile)	p.(Arg168His)	p.(Glu300Lys)
Variant type	Missense	Missense	Missense	Missense
Segregates	No	No	No	Yes
ExAC MAF South Asian	4/14 090 = 0.0003	0/16 512	0/16 508	3/16 506 = 0.0002
ExAC MAF all other	0/104 038	4/104 852 = 0.00004	2/104 868 = 0.00002	1/104 854 = 0.00001
EVS MAF	NA	2/13 004 = 0.0002	NA	0
Scaled CADD score	18.6	24.2	19.5	29.7
fathmm	Tolerated	NA	Tolerated	Damaging
LRT	Damaging	Neutral	Damaging	Neutral
MetaLR	Tolerated	Tolerated	Tolerated	Damaging
MetaSVM	Tolerated	Tolerated	Tolerated	Damaging
MutationAssessor	Non-functional, low	Non-functional, low	Non-functional, low	Non-functional, low
MutationTaster	Disease causing	Disease causing	Disease causing	Disease causing
PolyPhen2_HVAR	Benign	Probably damaging	Probably damaging	Probably damaging
PROVEAN	Deleterious	Neutral	Neutral	Neutral
SIFT	Deleterious	Deleterious	Deleterious	Tolerated

Abbreviations: ExAC, Exome Aggregation Consortium; EVS, Exome Variant Server; MAF, minor allele frequency; NA, not available.

All listed variants passed exome quality control filters and were not identified in 1000 Genomes and in 148 exome sequences from unrelated Pakistani individuals with Mendelian traits that do not overlap with the phenotypes observed in family AP91. Bioinformatics results are from dbNSFP version 2.9.

several amino acids bearing key residues that are essential for the interface between α and β subunits of the integrin heterodimer¹⁰ (Figure 2a). Conversion of the negatively charged side chain of glutamic acid to the positively charged side chain of lysine is predicted to result in a more buried conformation and slight torsion of the hairpin loop between α -helices 4 and 5 (Figure 2b). Thus, it is predicted that the substitution p.Glu300Lys would affect proper contact at the predicted β A domain-propeller interface.¹⁰ Lastly RT-PCR revealed *ITGB6* expression in adult but not fetal brain (Figure 2c).

DISCUSSION

ITGB6 variants were previously shown to cause amelogenesis imperfecta (AI) in young children,^{11,12} and *Itgb6*-knockout mice present with enamel defects,¹³ periodontal disease¹⁴ and juvenile baldness.¹⁵ The occurrence of AI in knockout mice and in children <10 years old was supported by enamel defects seen by electron microscopy.^{11–13} Likewise the three adult affected individuals from family AP91 had tooth loss, yellowish-brown staining, malocclusion, poor alignment and/or rough enamel, all of which may be AI-related. In addition the affected AP91 family members had severe periodontal disease, which was also observed in *Itgb6*^{−/−} mice.¹⁴ Hair loss due to *ITGB6* variants has not been reported in humans, which may be due to the young age of previously ascertained probands.^{11,12} Adolescent alopecia was observed in affected individuals from family AP91, consistent with juvenile baldness on the head and neck of *Itgb6*^{−/−} mice.¹⁵

Although a previously reported AI child who is homozygous for a stop-gain *ITGB6* variant seemed to have average intelligence despite being suspected of having Nance-Horan syndrome¹¹ (MIM 302350), all three adult affected individuals in this report were confirmed to have mild-to-moderate intellectual disability by formal testing. Our

RT-PCR result showed *ITGB6* expression in adult but not fetal brain, suggesting a role for *ITGB6* in maintenance of brain function.

Although *Itgb6*-knockout mice developed gradual, age-related emphysema in adulthood,¹⁶ lung problems were not reported in affected AP91 family members. The two older affected individuals died recently of severe diarrhea. For the living affected female IV-6, it is unknown whether she will eventually develop lung disease due to the *ITGB6* variant.

In response to epithelial injury, *ITGB6* activates latent transforming growth factor beta-1 (TGF β -1) by binding to the arginine-glycine-aspartate motif of its latency-associated peptide, then the activated TGF β -1 enhances healing or fibrosis and downregulates the inflammatory response.¹⁷ Lack of *ITGB6*, therefore, results in enhanced inflammatory response. In knockout mice, degenerating hair follicles were surrounded by foci of mononuclear cells¹⁵ and inflammatory infiltrates were enhanced in gingiva and lung,^{14,16} but not in other epithelial tissues.¹⁷ While TGF β -1 downregulation facilitates faster re-epithelialization post injury and protects against fibrosis,¹⁸ chronic lack of TGF β -1 leads to failure of tissue regeneration resulting in epidermal hypoproliferation, blocked hair follicle growth and neurodegeneration in brain.^{19,20} The phenotypes observed in family AP91 and *Itgb6*^{−/−} mice are therefore consistent with failure of TGF β -1 activation from *ITGB6* deficiency.

In summary, we present *ITGB6* as a gene that is involved in the pathophysiology of APMR, and an *ITGB6* variant that causes a rare disease spectrum including not only dental anomalies but also intellectual disability, periodontal disease and adolescent alopecia, further lending support to the role of TGF β -1 in regeneration and maintenance of ectodermal derivatives. Although children with *ITGB6* variants were reported to have AI only, our findings suggest that they should be followed up over time and examined for additional phenotypes. This study also demonstrates the importance of careful

Figure 2 Protein sequence alignment, molecular modeling and *ITGB6* expression. (a) The glutamic acid at position 300 (E300) of the human sequence is identical across 51 vertebrate species, including 36 mammals, five birds, three reptiles, one amphibian and six fish, and lies within a highly conserved region that includes His275 to Ser304 of human *ITGB6*. (b) Molecular modeling predicts a more buried configuration due to the p.Glu300Lys change and slight torsional adjustments on the hairpin loop within the β -propeller domain. (c) RT-PCR of commercially obtained human cDNA shows *ITGB6* expression in adult brain but not in fetal brain. Control denotes no template. Forward 5'-GAGGGGAAAACCATCATTCA-3' and reverse 5'-GTAGGACAACCCGATGAGA-3' primers for *ITGB6* exon 3 were used. ClinVar accession number for the *ITGB6* variant is SCV000249619.

follow-up phenotyping in families and individuals with congenital disease for other disorders that manifest at a later age.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We thank the family members for their invaluable cooperation and participation in this study. This work was supported by National Institutes of Health grant U54 HG006493 and by the Higher Education Commission (HEC), Islamabad, Pakistan.

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