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Case Report

Two novel mutations of *ITGB4* associated with pyloric atresia with epidermolysis bullosa

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Abstract: Pyloric atresia (PA) associated with epidermolysis bullosa (EB) is a rare autosomal recessive genetic disease with a poorly understood etiology. The integrin beta-4 (*ITGB4*) gene is one of the major obligated genes responsible for PA-EB. In this study, we reported a patient who was diagnosed with PA on day 9 of life and then diagnosed with EB at 25 days old. We sequenced the *ITGB4* gene of the patient and his parents and identified three variants, c.425T>C (p.M142T) in exon 5, c.1888C>T (p.R630C) in exon 16, and c.5302A>G (p.T1768A) in exon 39. The first and the last variants were not found in 1000 Genome and Exome Aggregation Consortium (ExAC) databases, while two heterozygote carriers of p.R630C were found in ExAC. Despite the fact that PA-EB patients usually die in infancy, the case presented here has survived more than 2 years after gastroduodenostomy. Our study adds to the phenotypic heterogeneity that represents PA-EB and expands the variant spectrum known for the *ITGB4* gene.

Keywords: Pyloric atresia, epidermolysis bullosa, neonatal, *ITGB4* gene, genetic diseases

Introduction

Pyloric atresia (PA) is a rare disease of the digestive tract that constitutes less than 1% of all gastrointestinal (GI) atresia cases, and is estimated to have an incidence of 1 in 100,000 live births [1]. There are three recognized anatomic variants of PA, type 1, in which the mucosal membrane occludes the lumen; type 2, otherwise known as longitudinal segmental atresia; and type 3, known as gap aplasia [2]. This condition is rarely coinherited with epidermolysis bullosa (EB), which is an skin disorder characterized by blister formation at the epidermal basement membrane zone in response to minor mechanical trauma [3].

There are four main types of EB, EB simplex, junctional EB, dystrophic EB, and Kindler syndrome [4]. The first three types have been reported in association with PA, while junctional EB is most frequently reported. The association between PA and EB is rare, but the outcome of such an association is universally fatal.

Integrin beta-4 (*ITGB4*) mutations are responsible for the majority of PA-EB cases [5]. *ITGB4* is a receptor for laminin and plays a critical structural role in the hemidesmosome of epithelial cells. Furthermore, *ITGB4* is required for the regulation of keratinocyte polarity and motility [6]. In the literature, most of the prenatal diagnoses have been reported in cases with a positive family history of PA-EB. In this paper, we reported the clinical characteristics and candidate gene sequencing analysis in PA-EB neonates and their's parents.

Case report

A male infant was born by cesarean section at full-term and he was the first child born to healthy unrelated parents from China. No family history of skin, gastro-intestinal disorders and antenatal problems were noted. The boy had jaundice and non-bilious vomiting on the second day of life. An abdominal X-ray examination (**Figure 1A**) revealed that the stomach was hugely distended and filled with air, with a lack of air distally. An upper gastrointestinal radiography revealed complete obstruction of the gastric outlet (**Figure 1B**). Laparotomy done on day 10 of life showed a hugely distended stomach with type 1 atresia at the pylorus (**Figure 1C**).

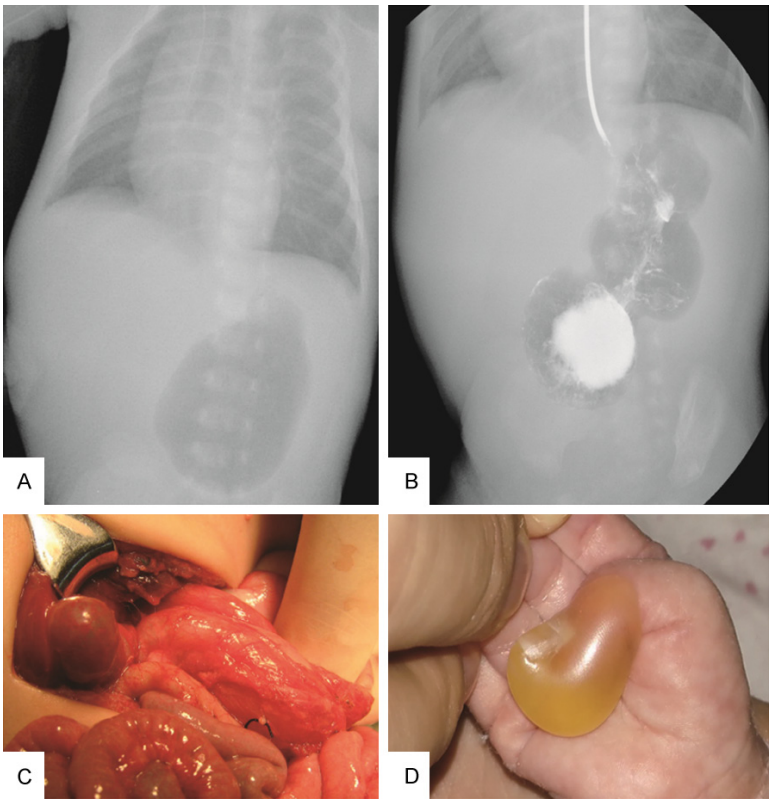

Figure 1. Clinical presentation of EB-PA patient. A. A plain abdominal X-ray showing a single large gastric gas bubble, with no air distal to pylorus. B. Upper GI series with Gastrografin show a 'single bubble sign' representing pyloric obstruction. C. Intra-operative image of fibrous pyloric cord. D. A blister that appeared on the infant's hand.

The membrane was excised, and a Heinicke Miculicz pyloroplasty was performed, and blisters appeared on his face, neck and hands at 19 days old (**Figure 1D**). A clinical diagnosis of PA-EB was made. 17 days after the procedure, we removed the jejunal feeding tube and he tolerated oral feeding well. He had now survived for more than 2 years.

The neonate and their parents' blood samples were collected, and genomic DNA was extracted with QIAamp DNA Blood Mini kit (Qiagen). WES (Whole Exome Sequencing) was performed on the genomic DNA using HiSeq 2000 platform (Illumina). Variant calls from the WES data were obtained with the use of GATK. The detected variants were confirmed using PCR and PCR-amplified DNA products were subjected to direct automated sequencing (3500XL Genetic Analyzer, Applied Biosystems).

The WES data consisted of 113,736,517 effective bases reads, with the average sequencing depth of ×113.13 on target. Of the target region, 99.6% was covered, of which 97.6% was covered at ×10 and 95.5% was covered at ×20. In this data, we observed a total of 102,425 genetic variants. After annotation and filtration, 18 essential variants were retained in a total of 15 genes (**Table 1**).

On the basis of phenotype and the clinical data listed in Online Mendelian Inheritance in Man (OMIM), the neonate which our analytic pipeline quickly picked out three variants in the *ITGB4* gene: c.425T>C (p.M142T) in exon 5, c.1888C>T (p.R630C) in exon 16 and c.5302A>G (p.T1768A) in exon 39 (**Table 2**). Analysis of the parental DNA showed that the patient's father was a carrier of the c.425T>C (p.M142T) variant and that the patient's mother had the c.1888C>T (p.R630C) and c.5302A>G (p.T1768A) variants. These variants were confirmed by Sanger sequencing in the family (**Figure 2**).

These variants were not found in the 1000 Genome and ExAC databases except for p.R630C, which had two heterozygote records in ExAC.

Discussion

PA-EB is a group of genetic alterations affecting the skin and mucous membranes, which appear fragile and blister easily [7]. Some researchers have proposed that PA in this syndrome could be explained by hemidesmosomal defects causing junctional blistering of the mucosa with subsequent peptic digestion and an inflammatory scarring reaction [8]. While PA-EB is characterized by skin and mucous membrane fragility, indicated by blistering with little or no trauma, patients also sometimes present with ureteral and renal anomalies. PA-EB is severe and mostly lethal in the neonatal

Table 1. Eighteen essential variants retained in 15 genes

Gene	Report_location	Report_Variant	Zygo	Report_OMIM	Report_Inherit
ATR	chr3: 142268353	NM_001184: exon 15: c.3139G>A (p.D1047N)	Het	Seckel syndrome 1, [MIM:210600]	AD/AR
BMP15	chrX: 50659026	NM_005448: exon 2: c.598C>T (p.H200Y)	Hemi	Ovarian dysgenesis 2, [MIM:300510]; Premature ovarian failure 4, [MIM:300510]	X-linked
CCDC88C	chr14: 91779470	NM_001080414: exon 15: c.2690C>A (p.T897N)	Het	Hydrocephalus, nonsyndromic, autosomal recessive, [MIM:236600]	AD/AR
CSRP3	chr11: 19207879	NM_003476: exon 5: c.298C>T (p.R100C)	Het	Cardiomyopathy, hypertrophic, 12, [MIM:612124]	AD
DEPDC5	chr22: 32232976	NM_001242896: exon 26: c.2189C>T (p.P730L)	Het	Epilepsy, familial focal, with variable foci, [MIM:604364]	AD
DMD	chrX: 32456414	NM_004006: exon 29: c.4015C>A (p.L1339I)	Hemi	Becker muscular dystrophy, [MIM:300376]; Cardiomyopathy, dilated, 3B, [MIM:302045]; Duchenne muscular dystrophy, [MIM:310200]	X-linked
FREM1	chr9: 14756383	NM_144966: exon 30: c.5396A>G (p.Q1799R)	Het	Bifid nose with or without anorectal and renal anomalies, [MIM:608980]; Manitoba oculotrichoanal syndrome, [MIM:248450]; Trigonocephaly 2, [MIM:614485]	AD/AR
HERC1	chr15: 63970323	NM_003922: exon 37: c.6791G>A (p.R2264Q)	Het	Macrocephaly, dysmorphic facies, and psychomotor retardation, [MIM:617011]	AR
HERC1	chr15: 63970454	NM_003922: exon 37: c.6660C>G (p.I2220M)	Het	Macrocephaly, dysmorphic facies, and psychomotor retardation, [MIM:617011]	AR
ITGB4	chr17: 73723892	NM_000213: exon 5: c.425T>C (p.M142T)	Het	Epidermolysis bullosa of hands and feet, [MIM:131800]; Epidermolysis bullosa, junctional, non-Herlitz type, [MIM:226650]; Epidermolysis bullosa, junctional, with pyloric atresia, [MIM:226730]	AD/AR
ITGB4	chr17: 73732673	NM_000213: exon 16: c.1888C>T (p.R630C)	Het	Epidermolysis bullosa of hands and feet, [MIM:131800]; Epidermolysis bullosa, junctional, non-Herlitz type, [MIM:226650]; Epidermolysis bullosa, junctional, with pyloric atresia, [MIM:226730]	AD/AR
ITGB4	chr17: 73753364	NM_000213: exon 39: c.5302A>G (p.T1768A)	Het	Epidermolysis bullosa of hands and feet, [MIM:131800]; Epidermolysis bullosa, junctional, non-Herlitz type, [MIM:226650]; Epidermolysis bullosa, junctional, with pyloric atresia, [MIM:226730]	AD/AR
PTGIS	chr20: 48130832	NM_000961: exon 7: c.956_956delA	Het	Hypertension, essential, [MIM:145500]	AD
SCN1A	chr2: 166892601	NM_001165963: exon 16: c.3386C>T (p.T1129M)	Het	Dravet syndrome, [MIM:607208]; Epilepsy, generalized, with febrile seizures plus, type 2, [MIM:604403]; Febrile seizures, familial, 3A, [MIM:604403]; Migraine, familial hemiplegic, 3, [MIM:609634]	AD
SFTPC	chr8: 22020086	NM_003018: exon 2: c.43-1G>A	Het	Surfactant metabolism dysfunction, pulmonary, 2, [MIM:610913]	AD/AR
SIK1	chr21: 44839794	NM_173354: exon 9: c.1064G>A (p.R355H)	Het	Epileptic encephalopathy, early infantile, 30, [MIM:616341]	AD
TBX5	chr12: 114793737	NM_000192: exon 9: c.1157C>A (p.P386H)	Het	Holt-Oram syndrome, [MIM:142900]	AD
ZIC2	chr13: 100634714	NM_007129: exon 1: c.396C>A (p.G132G)	Het	Holoprosencephaly 5, [MIM:609637]	AD

Table 2. Two novel mutations in *ITGB4* found in the family

Gene	Chr	Position	Variatn	ExAC	1000_ genome	SIFT_ score	SIFT_ prediction	Polyphen2_ score	Polyphen2_ prediction	MutationTaster_ score	MutationTaster_ prediction
ITGB4	17	73723892	NM_000213: exon 5: c.T425C: p.M142T	0	0	0	D	1	D	1	D
ITGB4	17	73753364	NM_000213: exon 39: c.A5302G: p.T1768A	0	0	0	D	0.067	B	1	D

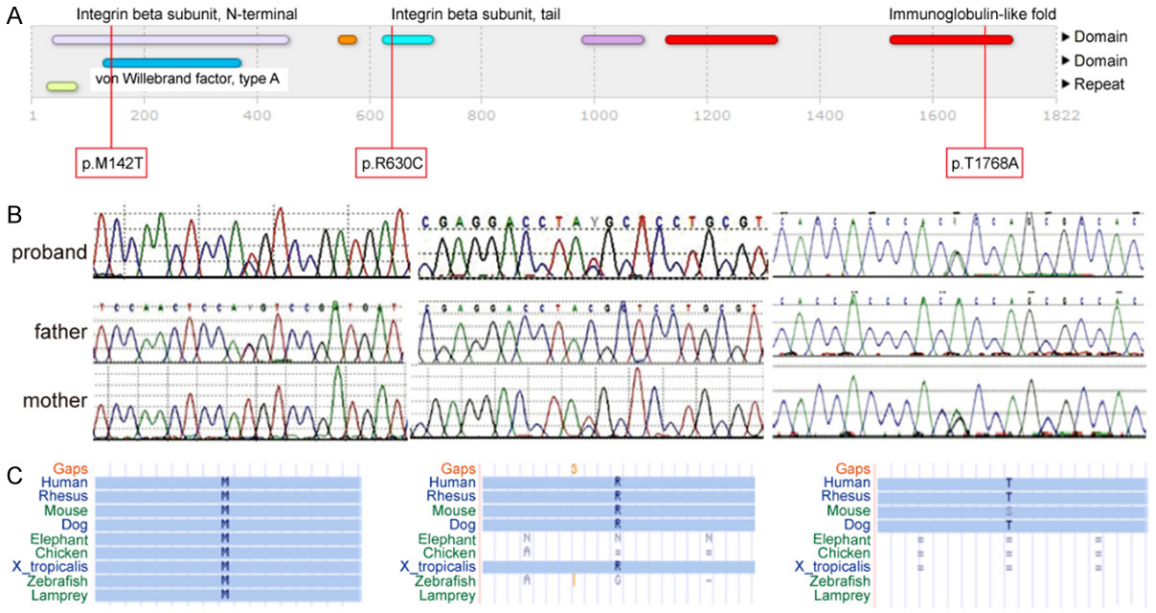

Figure 2. Analysis of *ITGB4* gene mutations in patient. A. Schematic view of the *ITGB4* structure. The red vertical lines indicate the three mutations. B. Sanger sequencing results confirmed the three mutations. C. The three mutations were conserved in human *ITGB4* polypeptide chain in comparison to other species.

period. Lucky, the patient reported in this article has survived for more than 2 years thus far.

As an autosomal recessive disorder, mutations in three genes are known to cause PA-EB: *ITGB4* in 80% of cases, *ITGA6* in 5% of cases, and *PLEC* in 15% of cases [9]. The alpha 6 beta 4 (α6β4) integrin heterodimer subunit of hemidesmosomes attaches keratinocytes to the basal membrane and is encoded by *ITGB4* and *ITGA6* [10, 11]. This integrin is also expressed in the epithelial lining of the stomach. α6β4 integrin levels have been shown to be reduced or completely absent in the skin of several patients with EB-PA [12]. Besides the genes encoding α6β4 integrin subunit polypeptides have been shown to harbor a large number of mutations in patients with EB-PA, the majority of them residing in *ITGB4* [12]. A study by Pulkkinen *et al.* described several *ITGB4* mutations in PA-EB patients. These mutations included a homozygous 2-bp deletion in exon 34 (4501delTC), compound heterozygosity for a 2-bp deletion in exon 3 and a cysteine substitution in exon 7, and a nonsense mutation within exon 4 (Q73X) [13]. And Natsuga *et al.* demonstrated that a recurrent c.1938delC mutation in *ITGB4* is a founder mutation in PA-EB patients [14].

In this study, genetic testing in 1 Chinese PA-EB patient and his parents identified two novel variants that were single basepair substitutions in *ITGB4*, c.425T>C (p.M142T) and c.5302A>G (p.T1768A).

In summary, we detected two novel mutations in the *ITGB4* gene in PA-EB patient. These mutations show genotype-phenotype correlations that contribute to the molecular understanding of this disease, an important issue for prognosis and for the development of novel evidence-based therapeutic options for PA-EB management. This study adds to the understanding of this disease because it revealed novel mutations that add to the mutation spectrum underlying PA-EB.

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Disclosure of conflict of interest

None.

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