Novel missense p.R252L mutation of ITGB4 compounded with known 3793+1G>A mutation associated with nonlethal epidermolysis bullosa-pyloric atresia with obstructive uropathy

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Key words: epidermolysis bullosa; genetic diseases/mechanisms; skin signs of systemic disease; vesicoureteric disorders.

INTRODUCTION

Epidermolysis bullosa-pyloric atresia (EB-PA) is an autosomal recessive genodermatosis most commonly caused by mutations in the *ITGB4* gene, which encodes a subunit of the hemidesmosomal $\alpha 6\beta 4$ integrin. Patients with EB-PA present as infants with combinations of skin blistering and fragility, pyloric atresia, and ureteral and renal abnormalities. Ureteral and renal abnormalities are not required for diagnosis, but may include dysplastic kidneys, obstructive uropathy, bladder agenesis, or collection system duplications. Although many cases are lethal, reports of nonlethal forms with variable degrees of cutaneous fragility and multisystem complications emphasize significant phenotypic variability due to genetic heterogeneity and high degrees of pleotropism.

While the majority (80%) of EB-PA mutations are localized to the *ITGB4* gene, 15% are found in *PLEC1* and 5% in *ITGA6*.[1] Diagnosis is confirmed with molecular genetic testing to identify pathogenetic subtypes. As new variants continue to be discovered, it is important to delineate the many genotype-phenotype relationships and their effect on prognosis. Herein we report the case of a heterozygous *ITGB4* mutation containing a novel R252L variant compounded with the known 3793+1G>A mutation, which caused mild cutaneous EB with pyloric atresia and severe obstructive uropathy (EB-PA-OU), and we review similar cases reported in the literature (Fig 1).

Abbreviations used:

EB:	epidermolysis bullosa
EB-PA:	epidermolysis bullosa-pyloric atresia
EB-PA-OU:	epidermolysis bullosa-pyloric atresia with obstructive uropathy
JEB:	junctional epidermolysis bullosa
PTC:	premature termination codon

CASE DESCRIPTION

A 6-year-old Black boy with a clinical diagnosis of EB-PA and no evidence of consanguinity or family history of related disease presented with 4 years of recurrent dysuria and hematuria, thought to be due to sloughing of the urothelium secondary to EB. At birth, the patient had skin fragility and blistering as well as PA, which was treated surgically. Aside from minimal blistering of the inguinal area and feet, the patient's cutaneous symptoms had resolved during infancy.

Physical exam was notable for constitutional growth delay, Fitzpatrick skin type V, a flaccid inguinal bulla (induced during examination), linear shallow erosions of the distal urethra, and hyperpigmented macules on the legs, representing post-inflammatory pigment alteration. No evidence of

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Funding sources: None.

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JAAD Case Reports 2021;11:63-8.
2352-5126

https://doi.org/10.1016/j.jdcr.2021.03.016

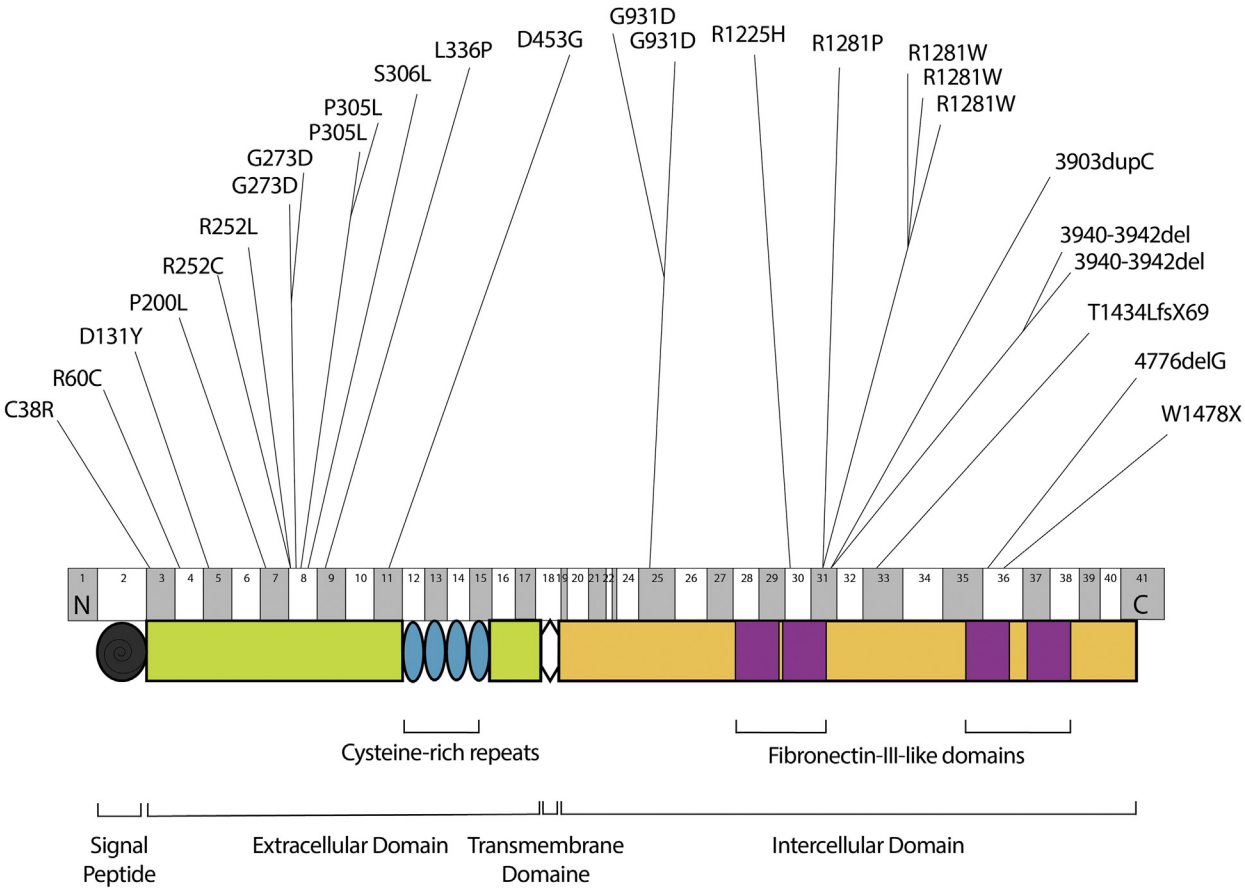

Fig 1. Gene Map of EB-OU ITGB4 Mutations: Diagram of the ITGB4 gene and its respective cellular domains. Exons are labeled above in white and grey boxes with corresponding branches of EB-OU ITGB4 mutations to their approximate exonic location. Higher densities of mutations occur between exons 7-9 and 30-36. 81.3% (n = 26) of EB-OU mutations are exonic in nature with 76.9% of the mutations involving exons 7-9 (n = 9) and 30-36 (n = 11). Exons 8 (n = 7) and 31 (n = 7) constitute 53.8% of EB-OU mutations. The mutations and their respective case description with citations are outlined in Table I.

nail dystrophy was noted. A previous voiding cystourethrogram and renal ultrasound were normal. Notably, workup involving any instrumentation of the urinary tract was avoided due to the risk of epithelial damage and thus exacerbation of urological manifestations of the disease. Due to a high clinical suspicion that the urinary symptoms were related to EB and the lack of previous testing, a targeted next-generation sequencing 27-gene EB panel was performed.* While awaiting results, the patient was hospitalized with severe dysuria and hematuria. Renal ultrasound revealed new bilateral hydroureteronephrosis, irregular thickening of the bladder wall, and decreased renal function due to obstructive nephropathy.

Next-generation sequencing revealed that the patient was heterozygous for the 3793+1G>A pathogenic variant and the R252L variant of uncertain significance in the *ITGB4* gene. The patient's mother was a carrier of the R252L variant, but lacked the 3793+1G>A variant. The patient's father was unavailable for testing. Given that that 3793+1G>A frameshift mutation has been noted in previous cases of EB-PA with varying presentations, it was hypothesized that the combination of this mutation and the novel p.R252L variant led to the atypical phenotype (mild cutaneous but severe urological involvement) in our patient. The patient was subsequently diagnosed with epidermis bullosa-pyloric atresia with obstructive uropathy (EB-PA-OU).

*Using genomic DNA from the proband, the exonic regions and flanking splice junctions of the genome were captured using the SureSelect Human All Exon V4 (50 Mb). Massively parallel (NextGen) sequencing was done on an Illumina system with 100bp or greater paired-end reads. Reads were aligned to human genome build GRCh37/UCSC hg19, and genes of interest were analyzed for sequence variants using a custom-developed analysis tool. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page.

The clinical course is significant for recurrent bilateral hydronephrosis, microscopic hematuria, nocturnal enuresis, dysuria, and chronic kidney disease stage 2 attributed to hydronephrosis from underlying EB. A follow-up skin examination was largely unremarkable, without blisters or erosions.

DISCUSSION

ITGB4 encodes integrin $\beta 4$, a subunit of the hemidesmosomal $\alpha 6\beta 4$ integrin, which is critical for cellular signaling and anchoring basal keratinocytes to the underlying basement membrane in the epidermis. Because hemidesmosomal $\alpha 6\beta 4$ integrin is also expressed in gastrointestinal and urinary tract epithelium, certain mutations may also cause disease in these systems, albeit by varying levels of severity. Of the subtypes of EB, junctional EB (JEB) is most commonly accompanied by urological complications, with urethral meatus stenosis noted in 11.6% of patients with JEB compared with 8.0% of patients with dystrophic EB.[2] Urinary retention, hydronephrosis, and bladder hypertrophy are known to occur in JEB patients at rates of 9.3%, 7.0%, and 4.6%, respectively.[2]

The Human Gene Mutation Database cites a total of 114 mutations in *ITGB4*. In previous studies, poor prognosis has been linked to deleterious *ITGB4* mutations that were either PTC (premature termination codon)/PTC or PTC/missense, particularly when the missense mutations occurred in highly conserved or protein-binding domains.[3] Cases with nonlethal outcomes had mutations limited to the caudal part of *ITGB4* (introns 14-36), resulting in normal or slightly reduced integrin expression rather than complete absence.[3]

In our patient with compound heterozygous mutations in *ITGB4*, the splice site mutation on intron 30, 3793+1G>A, is a previously confirmed pathogenic variant, which encompasses a G-to-A transition. This mutation causes destruction of the splice donor site on intron 30, creating a cryptic splice site which then results in a downstream PTC. The PTC may cause mRNA degradation prior to translation, or, if the mRNA is translated, will result in an abnormal (but partially functional) product due to truncation of the protein. The case of a PTC with partially translated mRNA resulting in a partially functional product may account for the associated milder phenotypes that have been previously described in several cases with mild cutaneous disease similar to our patient. Included in these cases is a patient who was compound heterozygous for the 3793+1G>A variant and a novel nonsense mutation W1478X in exon 36, which caused an initial presentation with scant blisters on the limbs, dystrophic nails, and PA.[4] Similarly to our patient, skin blistering resolved shortly after birth, with urinary manifestations (bladder wall hemorrhage and blistering, bilateral ureteric reflux, and unstable detrusor contractions) appearing later.[4] Since W1478X is likely a null allele, the mild phenotype might be due to some read-through of 3793+1G>A, resulting in some functional protein in this case. Another patient with the same 3793+1G>A variant on one allele and a novel missense mutation (D453G) on the other presented at 6 months of age with nonscarring traumatic cutaneous blistering, nail dystrophy, corneal erosions, dysuria, and PA. Cutaneous symptoms resolved, but at 20 months, he developed macrohematuria, left vesicoureteral reflux, dilatation of the posterior urethra and urethral bulb, bilateral ureteral stenosis, hydronephrosis, ureterectasia, and a thickened bladder wall.[5] In another mild case, a 3793+1G>A mutation on one allele and a missense mutation (R60C) on the other caused mild palmoplantar blistering and duplicated renal collecting sytems.[6] Lastly, a case of homozygous 3793+1G>A mutations led to a lethal phenotype of EB-PA with a complete absence of expression of integrin $\beta 4$, suggesting that 3793+1G>A has a variable expression in different patients given its other associations with milder phenotypes.[7] It appears compound heterozygous mutations containing 3793+1G>A have a predilection for urogenital sequelae accompanied by mild cutaneous manifestations.

The novel missense mutation on the other *ITGB4* allele of our patient has not been previously reported. The mutation, p.R252L of exon 8, constitutes a nonconservative amino acid substitution likely affecting secondary protein structure. Although the R252L variant has not been previously described, another missense mutation affecting the same amino acid residue (R252C) was found in a patient with mild, nonlethal EB-PA-OU.[8] In this case, compound heterozygous missense mutations (R252C and R1281W of exons 8 and 31, respectively) caused mild cutaneous blistering with severe obstructive uropathy similar to our patient, but with additional respiratory complications.[8] The R252C mutation was also described in a lethal case of EB-PA in which combined R252C missense/658delC PTC mutations caused blistering, bronchopulmonary dysplasia, and PA.[9] In this case, a skin biopsy revealed markedly reduced hemidesmosomes along the dermal-epidermal junction.[9] The patient was born premature and died at 147 days of age due to complications with the PA repair, prior to the appearance of any urinary abnormalities (which, in other cases, normally begin to appear closer to the age of 2). This comparison of R252C mutations not

Table I. Reported 3793+1G>A (underlined) & *R252* (italics) mutations in the literature and reported *ITGB4* gene mutations associated with varying presentations of the EB-PA-OU spectrum

Case (Reference)	Mutation	Consequence	Clinical presentation
This Case	3793+1G>A/*R252L* Intron 30/Exon 8	PTC/Missense	**Mild nonlethal EB-PA-OU. Minimal blistering, pyloric atresia, and obstructive uropathy progressed to Stage 2 CKD.**
Mellerio et al.[4]	3793+1G>A/W1478X Intron 30/Exon 36	PTC/PTC	**Mild nonlethal EB-PA-OU. Scanty cutaneous blistering at birth with pyloric atresia. Hematuria and dysuria at age 3 found to be due to bladder wall hemorrhage and blistering, bilateral ureteric reflux, and unstable detrusor contraction.**
Mellerio et al.[4]	C38R/4776delG Exon 3/Exon 36	Missense/PTC	**Mild nonlethal EB-OU. Mild skin fragility as well as hydronephrosis**
Lee et al.[5]	3793+1G>A/D453G Intron 30/Exon 11	PTC/Missense	**Nonlethal EB-OU. Nonscarring blistering, nail dystrophy, corneal erosions, and dysuria. Hematuria and left vesicoureteral reflux, bladder spasm, urethral dilatation, ureteral stenosis, hydronephrosis, hydroureterosis, ureterestasia, and thickened bladder wall at 20 months.**
Varki et al.[6]	R60C/3793+1G>A Exon 4/Intron 30	Missense/PTC	**Mild nonLethal EB-PA-OU. At 8 years of age presented with mild blistering on hands and feet only, dystrophic nails, duplicated renal collecting system, and cavities.**
Varki et al.[6]	2250+1G-A/3793+1G>A Intron 19/Intron 30	Splice/PTC	Nonlethal EB-PA. No phenotypic description provided
Pulkinnen et al.[7]	3793+1G>A/3793+1G>A Intron 30/Intron 30	PTC/PTC	Lethal EB-PA at 1 month. Extensive skin defects, respiratory distress, cardiovascular problems
Wallerstein et al.[8]	*R252C*/R1281W Exon 8/Exon 31	Missense/Missense	**Nonlethal EB-PA-OU. Pyloric atresia at birth and subsequent skin blistering in the following days. Obstructive uropathy, urethral epithelial sloughing, and additional respiratory complications.**
Dang et al.[9]	658delC/*R252C* Exon 7/Exon 8	PTC/Missense	Lethal EB-PA. Blisters and skin fragility appearing 2 days after birth at 30 weeks gestation, PA, stage 3 bronchopulmonary dysplasia, and death at 147 days old due to complications of previously repaired PA
Dang et al.[9]	G273D/3903dupC Exon 8/Exon 31	Missense/PTC	**Lethal EB-PA-OU. Widespread cutaneous fragility, pelvicalyceal dilatation, tortuous ureters, complicated PA. Death day 2.**
Pulkkinen et al.[10]	R1281W/R1281W Exon 31/Exon 31	Missense/Missense	**Nonlethal EB-PA-OU. Pyloric atresia and subsequent skin blistering at birth. Severe nephrotic syndrome at the age of 3 months.**
Schumann et al.[11]	P200L/P305L Exon 7/Exon 8	Missense/Missense	**Nonlethal EB-OU. Mild blistering, duodenal atresia, dysuria, and vesicoureteral occlusion.**
Schumann et al.[11]	P305L/S306L Exon 8/Exon 8	Missense/Missense	**Nonlethal EB-OU. Mild blistering along with chronic cystitis with bladder wall changes, urothelium erosions, dysuria, and hematuria.**
Schumann et al.[11]	R1281P/T1434LfsX69 Exon 31/Exon 33	Missense/Frameshift	**Nonlethal EB-OU. Mild blistering on the hands and feet at 2 months old and then hematuria, dysuria, hydronephrosis, and bladder wall ulceration at 3 years old.**
Nakano et al.[12]	D131Y/G273D Exon 5/Exon 8	Missense/Missense	**Lethal EB-PA-OU. Aplasia cutis congenita, multicystic dysplasia of the left kidney, hydronephrosis of the right kidney, and involvement of the bladder, esophagus, trachea, and small intestine.**

Continued

Table I. Cont'd

Case (Reference)	Mutation	Consequence	Clinical presentation
Nakano et al.[12]	L336P/R1225H Exon 9/Exon 30	Missense/Missense	**Nonlethal EB-PA-OU. Blistering, urologic obstruction, laryngeal obstruction, ocular involvement, and scarring on legs.**
Chavanas et al.[13]	3977-19T-A/3793+1G>A Intron 31/Intron 30	PTC/PTC	**Nonlethal EB-OU. Widespread blistering at birth that decreased in severity with age as well as urethrovesicular occlusion.**
Inoue et al.[14]	G931D/G931D Exon 25/Exon 25	Missense/Missense	**Nonlethal EB-OU. Chronic skin fragility and nail dystrophy. Recurrent urethral stenosis and hair loss.**
Salvestri et al.[15]	3940-3942del/3940-3942del Exon 31/Exon 31	In-frame Deletion/In-frame Deletion	**Nonlethal EB-OU. Skin fragility, protein losing enteropathy, anasarca, as well as bladder wall thickening, bullae and trabeculation**

Bolded text, OU cases.
Select genotypic and phenotypic data found from a literature search utilizing Pubmed, Google Scholar, and Science Direct. For our case, known or expected pathogenic mutations were confirmed using a traditional Sanger sequencing method. 17 patients with 34 *ITGB4* mutations resulting in obstructive uropathy were identified in the review. An additional 3 patients who did not exhibit OU but had similar mutations as our patient are also provided in this table.
EB, Epidermolysis bullosa; *PA*, pyloric atresia; *OU*, obstructive uropathy; *PTC*, premature termination codon.

only supports that PTC mutations cause a greater impact on integrin structure and expression than missense mutations, but also that R252 site mutations may predict urological involvement, though with limited data this is a difficult conclusion to make with utmost certainty.

A review of 16 reported EB-OU cases with collectively 32 different mutations reveals several trends (Table I, Fig 1).[4-15] 81.3% (n = 26) of EB-OU mutations are exonic in nature with 76.9% of the mutations involving exons 7-9 (n = 9) and 30-36 (n = 11). Certain exons have a higher predictive value for EB-OU than others, as exons 8 (n = 7) and 31 (n = 7) constitute 53.8% of EB-OU mutations alone. Interestingly, all heterozygous intronic mutations were nonlethal and our own case's nonlethal prognosis corroborates Mylonas et al.'s finding that caudal *ITGB4* mutations result in more favorable prognoses.[3]

New mutations in the *ITGB4* gene are continuing to be reported. Although urologic involvement is less common than cutaneous, the urothelium may suffer the greatest insult from genetic aberrations, as seen in our patient. Our case, like all other heterozygous 3793+1G>A mutations cited, resulted in nonlethal EB. Although we know the heterozygous 3793+1G>A mutation is associated with the unique phenotype of mild cutaneous and severe urological disease, 3793+1G>A in the homozygous form is lethal. Therefore, it is compound heterozygosity with the novel missense R252L mutation that is responsible for our patient's phenotype. This further strengthens the theory that missense mutations result in milder EB-PA-OU phenotypes.

Further large-scale research and gene-mapping is needed to confirm these observations and to improve prognostic capability for EB patients. Although there is a correlation between types of mutations and severity of disease in different organ systems, there is not enough evidence to suggest a correlation between cutaneous and urological severity. As more genotype-phenotype correlations are reported, this theory will become clearer. However, a high clinical suspicion for multisystemic EB involvement should be maintained in patients with a history of skin blistering and subsequent gastrointestinal or genitourinary abnormalities, as cases caused by milder, missense variants may present conspicuously with minimal cutaneous involvement but progrsessively severe nephropathy.

Conflicts of interest

Dr Lee has been an investigator in the past in the subject area of epidermolysis bullosa for Castle Creek, Scioderm, and Amaryt. She received research funding for all of these. Ryan is a Genetic Counselor employed by GeneDx, Inc. Author Ellis, Dr Eason, author Snyder, Dr Siegel, Dr Pai, and Dr Pfendner have no conflicts of interest to declare.

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