A de novo missense mutation in the NC1 domain of type VII collagen leads to dystrophic epidermolysis bullosa

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Dystrophic epidermolysis bullosa (DEB) is a genetic mechanobullous skin disorder that manifests at birth or in early infancy. The hallmarks of DEB are blister formation, skin fragility, and nail dystrophy following minor trauma. The disorder results from mutations in the type VII collagen gene (*COL7A1)* encoding the type VII collagen protein (C7). C7 is a major component of anchoring fibrils (AFs) [1], which is critical for attachment of the epidermis to the dermis. Dysfunction or loss-of-function of C7 leads to DEB. For instance, the complete loss of C7 causes the Hallopeau-Siemens type of DEB – the most severe phenotype. The inheritance pattern of mutated *COL7A1* is either autosomal dominant (DDEB, OMIM 131750) or autosomal recessive (RDEB, OMIM 226600). However, de novo spontaneous mutations of *COL7A1* are rarely reported in the population. Herein, we describe a DEB patient with a mild phenotype caused by a de novo missense mutation in the amino-terminal non-collagenous (NC)1 domain of C7.

The proband was a 4-year-old female single child born to non-consanguineous parents without any family history of blistering disease. The patient visited the clinic of the Institute of Dermatology, Chinese Academy of Medical Sciences and Peking Union Medical College, Nanjing, China in September 2017. The chief complaint was blisters in response to skin trauma and dystrophic toenails since birth. Her parents were free of symptoms of DEB, but the child suffered from blistering limited to trauma-exposed sites and toenail dystrophy. At the time of the medical examination, the skin sites were healed and covered with atrophic scars. No other malformation of the patient was detected.

In order to determine the pathogenesis, we collected the peripheral blood from the patient, and extracted DNA from lymphocytes for sequencing. We sequenced all 118 exons, exon-intron borders, and the promoter region of *COL7A1* using PCR amplicons according to a published paper [2]. Sequencing results and mutational analysis revealed a heterozygous mutation of C to T in the *COL7A1* gene, located at position 2183 of exon 17 (Figure 1). No other known mutations in the *COL7A1* gene were found. Further analyses revealed that the mutation resulted in the S728F variation in protein C7. The mutation was not detected in the patient's parents, indicating the mutation was indeed de novo. More importantly, we sequenced 100 random healthy volunteers and failed to find this mutation. By searching a genome database, gnomAD (http://gnomad.broadinstitute.org/) [3], we were able to confirm that this mutation had not been reported.

By taking the advantages of existing databases, we sought the biological significance of the mutation. The 2183C>T mutation has been predicted to be detrimental by PolyPhen-2 (score = 0.05) (http://genetics.bwh.harvard.edu/pph2/) [4] and CADD (Phred-score = 23.8) (http://cadd.gs.washington.edu/snv). Furthermore, we used SWISS-MODEL website [5] and Maestro software [6], respectively, to predict the 3D model of C7 (SMTL id: 1fnf.1) and observe the structure related to amino acid residue 728. We found that the hydrogen bond between the side chain of Ser728 and Ser718 in the wild type collagen was broken in the mutated protein, probably causing protein instability (Figure 1).

In addition to advice on standard skin care for the patient, we also suggested that the parents take genetic consultation during the patient's child bearing ages. The patient has been followed up since discharge (September 2017), during which the symptoms have not exacerbated.

DEB is a group of genetic conditions whose hallmarks are skin fragility and nail deformity. The pathogenesis of DEB is mutations in *COL7A1* leading to dysfunctional C7

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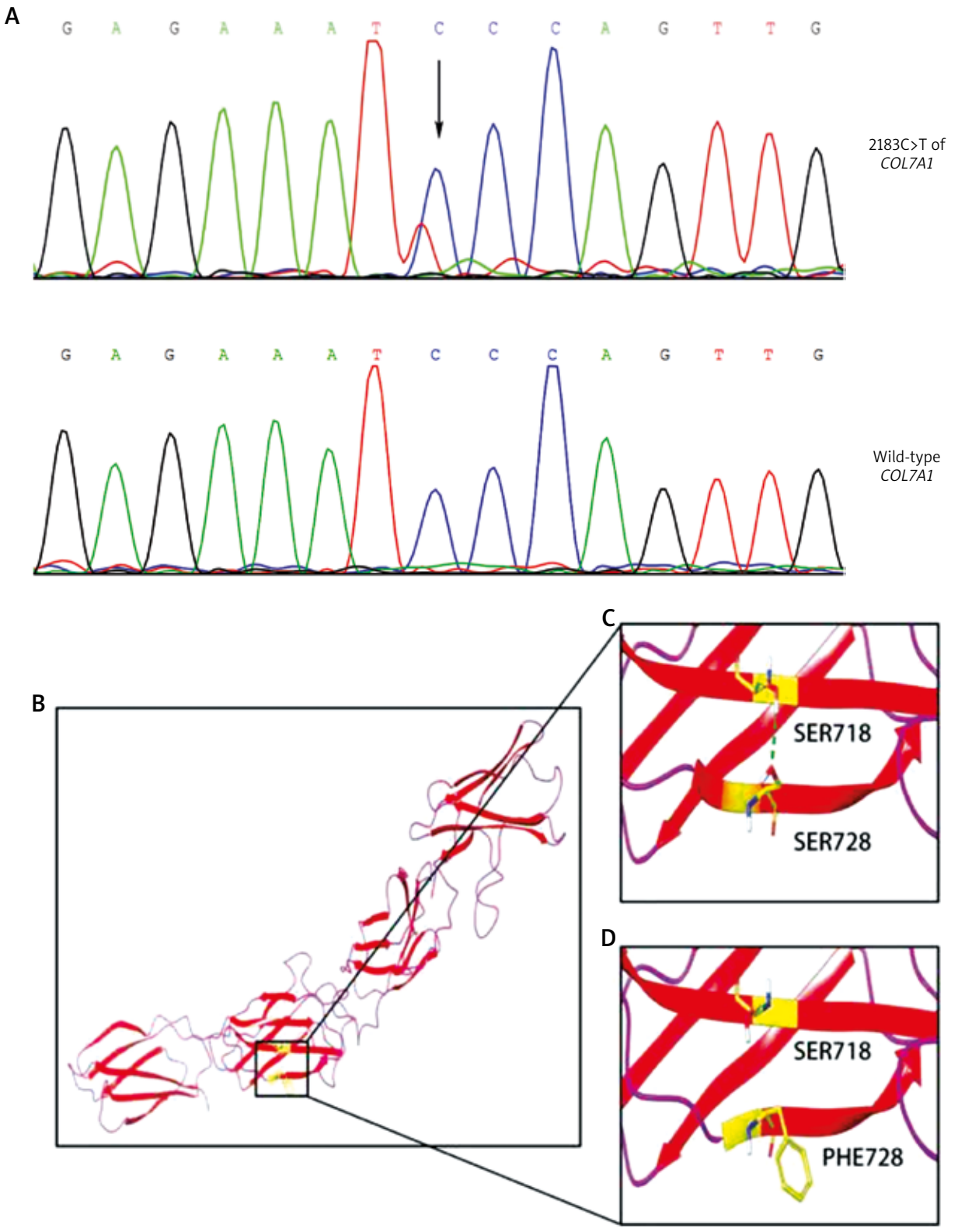

Figure 1. Sequencing results of the mutation and predicted variation in C7. **A** – Heterozygous mutation of 2183C>T of COL7A1 (the top panel). The sequence of the same region in a health volunteer (the lower panel). **B** – Overall view of the predicted structure of the NC1 domain of wild-type C7, with the β-sheets in red and the loops in pink. **C** – Local view showing the hydrogen bond (green dotted line) between Ser718 and Ser728 of wild-type C7. **D** – Partial view showing the mutant domain with Phe728 and the loss of the hydrogen bond

Table 1. Phenotypes and genotypes of DEB patients with missense mutation in NC1 domain of the *COL7A1* gene [9]

Patient	Diagnosis	Allele1	Consequence	Exon	Allele2/Allele3	Consequence	Exon
1	RDEB	p.R28G	MS	1	p.G2366A	MS	92
2	RDEB	p.D44N	MS	2	p.E2857X	PTC	116
3	RDEB	p.S47L	MS	2	p.R2492X	PTC	98
4	RDEB	p.S48P	MS	2	c.3625del11	PTC	27
5	RDEB	p.R51G	MS	2	p.R2492X	PTC	98
6	RDEB	p.A80P	MS	2	p.Q1211X	PTC	27
7	RDEB	p.G150R	MS	4	c.682 + 1G>A	PTC	IVS5
8	RDEB	p.G174R	MS	4	p.G174R	MS	4
9	DDEB	P.V760M	MS	17	/c	/	/
10	RDEB	p.G798R	AS	18	c.2621ins5(GCTTC)	PTC	20
					p.Q1286X	PTC	31
11	RDEB	p.R886P	MS	20	c.497insA	PTC	4
12	RDEB	p.R910P	MS	21	p.K142R	AS	3
13	RDEB	p.G923R	MS	21	c.7371insA	PTC	96
14	RDEB	p.R990Q	MS	22	p.R2008H	MS	73
15	RDEB	p.Y1250S	MS	28	p.P2028S	MS	73

RDEB – recessive DEB, DDEB – dominant DEB, MS – missense mutation, AS – alternative splicing, PTC – premature termination codon. / absent.

or a completely loss of C7. C7 acts as a key member of AF, residing underneath the lamina densa in the papillary dermis. C7 connects to laminin-332 and type IV collagen in the epidermal basement membrane through C7's NC1 domain [7], and interacts with type I collagen in the dermis [8]. C7 is a homotrimer that is comprised of three identical α unit chains. Each α chain is composed of a 145-kDa central collagenous triple-helical domain (TH) with the characteristic repeating Gly-X-Y amino acid sequence. A 145-kDa amino terminal, named NC1, and a 34-kDa carboxyl-terminal NC2 flanks the TH domain.

Although it has been reported that approximately 600 mutations in the *COL7A1* gene cause DEB, there are fifteen missense mutations found within the NC1 domain (Table 1) [9]. There is only one missense mutation reported within the NC1 domain causing DDEB. This mutation is widely considered to be associated with glycine substitutions in the TH domain of C7. As the second identified missense mutation to cause DDEB within the NC1 domain, S728F mutation is also the first in the fourth-last fibronectin type III-like repeat, which results in a mild disease phenotype.

We were unable to determine the biological effects of the S728F mutation, which require multidisciplinary collaborations. Given a predicted loss of the hydrogen bond between Ser728 and Ser718, it is reasonable to speculate that the mutation causes impaired stability of C7. Also, the probability cannot be excluded that the S728F mutation might interfere in the structure of the TH domain or the interaction between the NC1 domain and extracellular matrix components.

In conclusion, the de novo missense mutation S728F observed in the NC1 domain of C7 from this patient may result in a relatively mild phenotype. However, the prevalence and genotype-phenotype correlation of this mutation in the NC1 domain should be explored further.

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Conflict of interest

The authors declare no conflict of interest.

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