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Deep-intronic and frameshift *DSG1* variants associated with atypical severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome in a Chinese family

Background: Severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome comprise a rare genodermatosis associated with biallelic (homozygous or compound heterozygous) mutations in the *DSG1* (desmoglein-1) gene, or heterozygous mutations in the *DSP* (desmoplakin) gene. To date, while many patients with SAM syndrome have been described, the number of cases with SAM syndrome with deep-intronic variants, together its genetic aetiology, remain limited. **Objectives:** We report the case of a five-year-old Chinese boy with atypical SAM syndrome. **Materials & Methods:** Relevant blood specimens from the family were collected. DNA isolation, RNA isolation and cDNA synthesis, and next-generation sequencing using a multi-gene panel were applied to verify the pathogenic gene. To test the functional consequences and pathogenic mechanism of the deep-intronic mutation *in vitro*, a mini gene strategy was constructed. **Results:** A heterozygous *DSG1* deletion (c.2437_2450delACCTATCCCTCGGG: p.Tyr814Trpfs*6) and a deep-intronic (c.1688-30A > T) variant were identified. The identified intronic variant was shown to create an alternative splice site, leading to nonsense-mediated mRNA decay of the aberrant transcript. **Conclusion:** This is the first study to demonstrate a causal role for a deep-intronic *DSG1* mutation in a patient with SAM syndrome. Our findings underline the need to analyse the intronic regions of *DSG1* in patients with SAM syndrome. Improved diagnosis and a better understanding of prognosis will lead to clearer a picture of the concept of atypical SAM syndrome.

Key words: deep-intronic mutation, *DSG1* variants, genodermatosis, minigenes

Article accepted on 14/08/2020

Severe dermatitis, multiple allergies and metabolic wasting (SAM) syndrome (OMIM #615508) is a rare genetic disorder caused by mutations in desmoglein 1 (*DSG1*) or desmoplakin (*DSP*). Since its identification in three affected children from two consanguineous families, a total of 14 individuals with SAM syndrome have been reported. In 14 individuals, cases were attributed to recessive homozygous or compound heterozygous loss-of-function mutations in *DSG1* [1-7], while five cases were caused by dominant heterozygous mutations in *DSP* [8-11]. These reports show that SAM syndrome manifests with a broad spectrum of skin phenotypes and multi-system complications. Main cutaneous manifestations of SAM syndrome include erythroderma, palmoplantar keratoderma, and ichthyosis. Other cutaneous manifestations include pruritus, hypotrichosis, woolly hair, and nail abnormalities. In addition, dental abnormalities, cardiac abnormalities, recurrent infections, failure to thrive,

gastrointestinal problems, developmental delay, brain abnormalities, immunoglobulin E elevation, eosinophilia, immunoglobulin E sensitization to allergens, food sensitization or sensitization to other allergens, ophthalmic abnormalities and a hoarse voice can also be observed in SAM syndrome [1-11].

Desmosomes consists of DSGs and desmocollins, two transmembrane glycoproteins (cadherins) that serve as an anchor for adjacent epithelial cells to link to one another. The keratin-based skeleton is integrated by the cytoskeleton domains of these cadherins to stable cell-cell contact sites. This results in enhanced function of tensile tissue, such as that found in the epidermis and heart [12, 13]. *DSG1* is essential for the adhesion function in the epidermis and can be observed in the upper suprabasal and granular layer, where terminal differentiation takes place. *DSG1* not only maintains the integrity of epidermal tissue but also supports the differentiation of keratinocytes and monitors the morphology of the suprabasal layer [14].

In this study, we report on a novel, deep-intronic heterozygous mutation, c.1688-30A > T (predicted protein change: p.Leu563*), and a c.2437_2450delACCTATCCCTCGGG

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variant (p.Tyr814Trpfs*6) found in intron 11 and exon 15 of *DSG1*, respectively. The patient with SAM syndrome in whom the variants were identified displayed congenital erythroderma with flares of generalized dermatitis, ichthyosis, palmoplantar keratoderma, hypotrichosis and nail dystrophy. We also describe the molecular basis of *DSG1* dysfunction resulting from the novel deep-intronic mutation and suggest a possible pathogenic model related to this patient.

Materials and methods

Patient and sample collection

A five-year-old Chinese boy with a severe cutaneous phenotype was examined under the procedure approved by the institutional review board of the Xinhua Hospital, Shanghai Jiaotong University School of Medicine. Written consent was obtained from all family members.

Immunohistochemistry of skin biopsy specimens

A pathological analysis of paraffin-embedded skin samples was performed based on haematoxylin-eosin (HE) and immunohistochemistry (IHC) staining, as previously described [15]. Desmoglein 1 monoclonal antibody (27B2, Thermo Fisher Scientific, Waltham, MA, USA) was used as the primary antibody.

Molecular genetics analysis

DNA isolation, RNA isolation and cDNA synthesis

Briefly, DNA and RNA were isolated from the peripheral blood of the patient. RNA samples were reverse transcribed and a cDNA library generated. Sequencing of the cDNA library was achieved using an ABI PRISM 3730XL automated sequencer (Applied Biosystems, Foster City, CA). Identified variants can be found under Genbank Accession Number NM_001942.4.

Next-generation sequencing using a multi-gene panel, data analysis and interpretation

Using next-generation sequencing technology applied to a 596-gene panel associated with monogenic hereditary diseases, genomic DNA was analysed and interpreted as previously described [16].

Sanger sequencing

Direct Sanger sequencing was applied to verify the identified variants using an ABI PRISM 3730XL automated sequencer (Applied Biosystems).

Minigene construct, transfection and PCR

The wild-type gene and c.1688-30A>T variant were amplified using PrimeSTAR GXL DNA polymerase (TaKaRa Bio-engineering Co. Ltd.) and the concentration of the DNA products were measured using NanoDrop2000 (Thermo Scientific, Waltham, USA). Fragments of interest were cloned into a pEGFP-C3 plasmid via XhoI/EcoRI

using Hieff Clone Plus One Step Cloning Kit (Yeasen Biotech Co. Ltd.) and the vectors were transformed into *Escherichia coli* DH5 α cells. Sequences were confirmed by DNA sequencing. The plasmids were extracted by Plasmid Maxi Preparation Kit or Plasmid Maxiprep Kit (Beyotime, Shanghai, China), and subsequently transfected into HEK-293T cells with EndoFectin-MAX (GeneCopoeia, Rockville, MD, USA). The wild-type and mutant plasmids are referred to as pEGFP-C3-WT and pEGFP-C3-MUT2, respectively.

Results

Clinical description

The patient was the first child of non-consanguineous healthy parents, and was delivered with normal weight and length at 40 weeks. Delivery was by means of Caesarean section because of reduced amniotic fluid. Three days post-delivery, severe dermatitis manifested as generalized skin flushing covered by fine scales. The cutaneous phenotype consisted of erythroderma with skin scaling and peeling, severe palmoplantar keratoderma (PPK), ichthyosis, and hypotrichosis (*figure 1A-E*). Skin lesions, characterized by generalized dry scaly patches of skin and partial mild exudation, were detected and associated with intense and intractable itching. PPK had an early-onset and was aggravated with age. Symptoms were worst in the extensor side of lower limbs and plantar regions, presenting with yellowish hyperkeratotic plaques with little palmar involvement. PPK also strongly impeded walking and refined motor function. Severe dermatitis was diagnosed using the SCORAD (SCORing Atopic Dermatitis) index (72.5) and total (90.5) scores [17]. In addition, the boy showed growth retardation and developmental delay with a height of 80 cm (-3 SD) and weight of 15 kg (-2 SD). However, his teeth were normal, and no observable cardiac defects or digestive system disorders were present. Clinical investigations did not detect any infection, or food or other allergies. No skin lesions were identified in his parents upon clinical examination. In addition, there was no personal or family history of atopic dermatitis or other atopic disorders. Laboratory findings showed that the patient had elevated eosinophils (relative percentage: 16.30%, normal value: 0.5%–5%; absolute quantity: $2.76 \times 10^9/L$, normal value: $0-0.7 \times 10^9/L$) and reduced 25-hydroxy-vitamin D (11.76 nmol/L, normal value: >50 nmol/L). Immunoglobulin E (IgE) levels were normal as measured by Immucap. Tandem mass analysis of dried blood samples revealed decreased concentrations of multiple amino acids. Gas chromatography analysis showed slightly increased 3-hydroxybutyric acid. Eruptions and skin pruritus were not relieved following a four-week treatment period with oral acitretin, gabapentin, and topical pimecrolimus.

Histopathological and immunohistochemical findings

Histopathological examination of the skin specimen showed psoriasiform hyperplasia and hyperkeratosis with large subcorneal neutrophil accumulations (*figure 1H*).

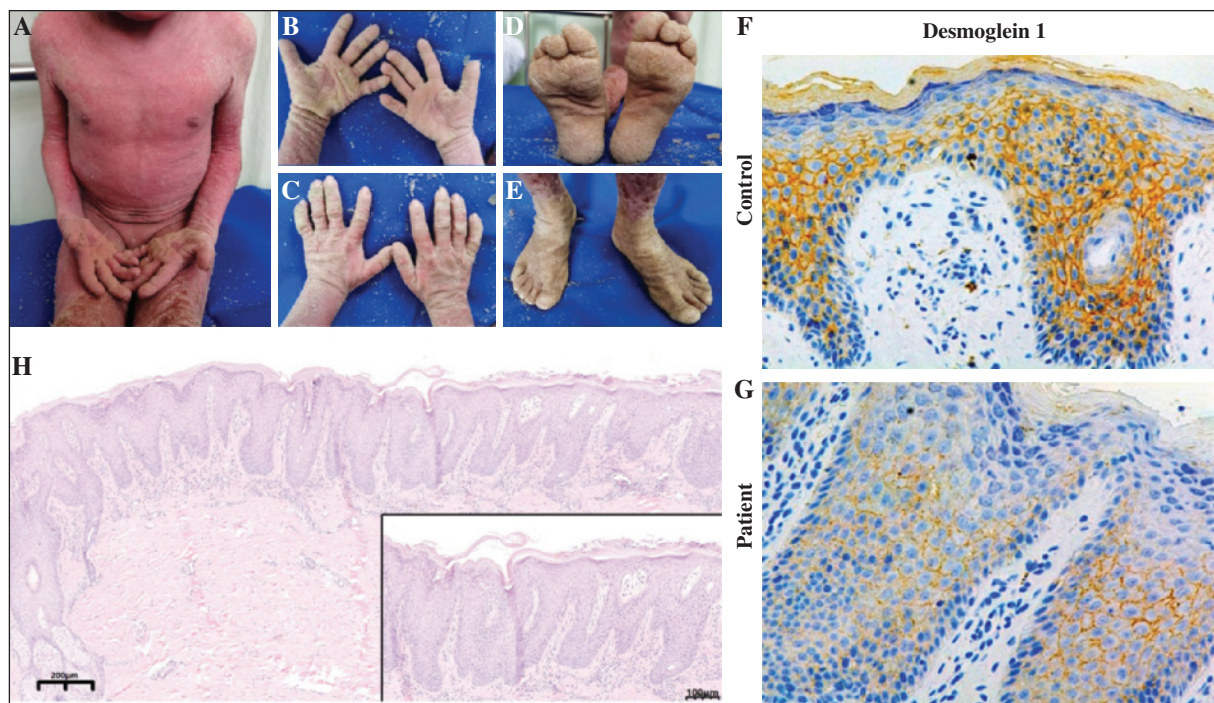


Figure 1. Clinical manifestation and dermatopathology. **A-E)** Images of the proband show generalized congenital erythroderma with fine scales, palmoplantar keratoderma with nail dystrophy and ichthyosis. **F-G)** Immunohistochemistry shows a marked decrease in DSG1 in the proband specimens compared to normal controls. **H)** Haematoxylin-eosin staining of a skin specimen shows psoriasiform hyperplasia and hyperkeratosis with large subcorneal neutrophil accumulations.

Immunohistochemistry was used to compare the expression of *DSG1* in the epidermis between the proband and healthy controls. The results showed a remarkable discrepancy as the expression of *DSG1* was significantly reduced in the patient's skin biopsy specimens (figure 1F) compared to the control samples (figure 1G).

Molecular genetics

By employing a multi-gene panel strategy, a paternally inherited deletion mutation, c.2437_2450delACCTATCCCTCGGG, was identified in exon 15 of *DSG1*, and a maternally derived deep-intronic mutation in intron 11, c.1688-30A > T, was identified in the proband (figure 2A). The latter variant was strongly linked to pathogenicity. No mutation was detected in *DSP*. The c.2437_2450delACCTATCCCTCGGG variant is expected to result in a frame-shift event and premature termination of protein translation (p.Tyr814Trpfs*6), however, the genetic changes resulting from c.1688-30A > T remain unclear.

To confirm the effect of the deep-intronic mutation, RNA was extracted from blood obtained from the family. While sequencing of the cDNA further confirmed the presence of the deletion mutation c.2437_2450delACCTATCCCTCGGG in exon 15 of *DSG1* in both the proband and his father (figure 2B, C), c.1688-30A > T could not be detected in either the proband or his mother (figure 2B, D).

In vitro minigene results

In vitro hybrid minigenes were constructed to simulate aberrant splicing and investigate the pathogenic mechanism related to the c.1688-30A > T mutation. The wild-type *DSG1* product (corresponding to 430 bp, 134 bp, and 34 bp fragments, expected from exons 11, 12, and 13, respectively) consisted of a single band of 598 bp. However, the transcriptional products derived from the mutant variant revealed a shorter transcript of 464 bp (figure 3E) with a complete loss of exon 12 (figure 3A). Direct sequencing of the transcripts from the mutant variant demonstrated that exon 12 skipping caused exon 13 to directly follow exon 11, generating a 'TGA' stop codon and resulting in premature termination of protein translation (figure 3C, D).

Discussion

In the current report, we present a novel case in addition to those published in 2016 and 2019 from the same research group [1,8]. Based on a summary of data from all patients reported with SAM syndrome, it was found that: (1) all patients had conspicuous skin features typically accompanied with congenital erythroderma and PPK; (2) not all patients presented with food sensitization or sensitization to other allergens (44.4%, 8/18 patients), elevated IgE (77.8%, 14/18 patients) or severe metabolic wasting (50%, 9/18 patients); and (3) only a small number of patients showed multi-system syndromes (supplementary tables 1-3).

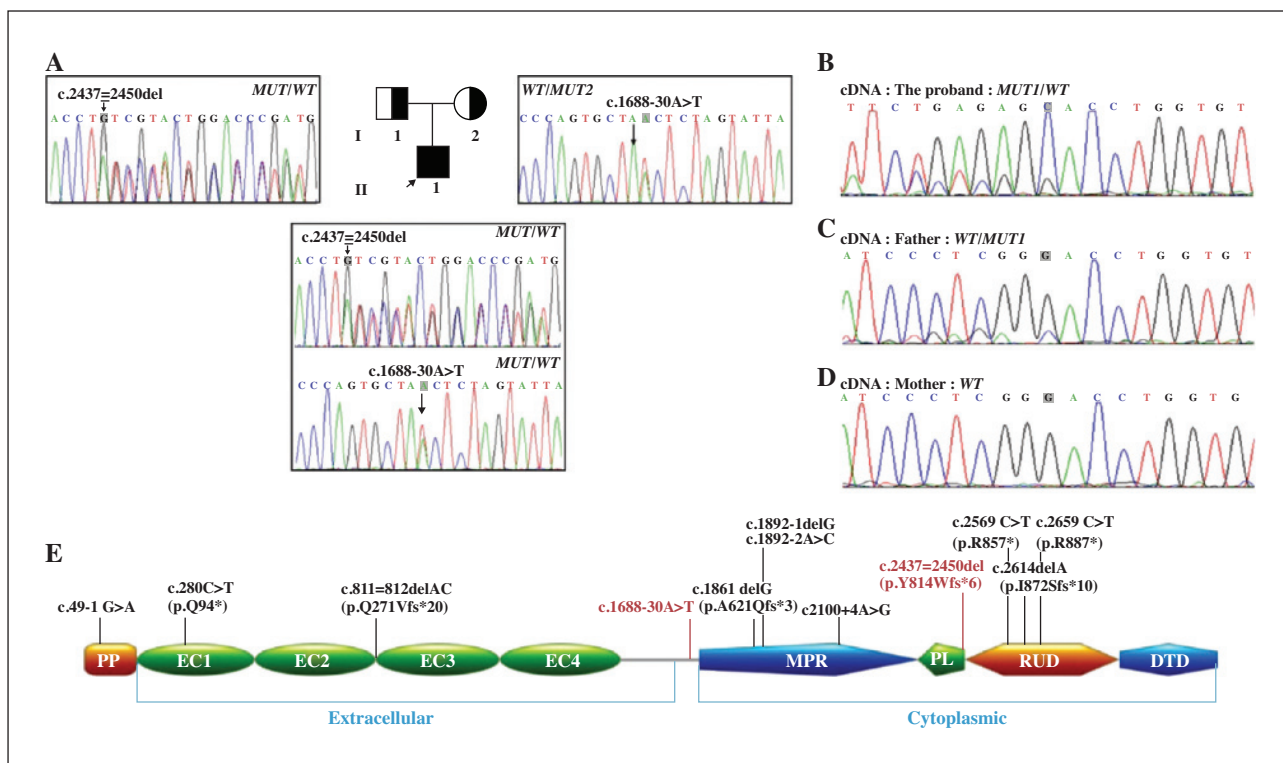


Figure 2. Mutation and immunohistochemical analysis of the SAM family. **A)** The pedigree illustrates the inheritance pattern of the SAM nuclear family. Squares represent males and circles represent females. Half-filled shapes indicate heterozygous parents while black shapes indicate affected patients. As reflected in the pedigree, DNA sequencing of the proband identified the paternally derived c.2437_2450delACCTATCCCTCGGG (I:1) and maternally derived c.1688-30A > T variants (I:2). **B-D)** cDNA sequencing of the family. **E)** SAM syndrome-causing mutations known to occur in *DSG1* are depicted along a schematic representation of *DSG1*. Mutations identified in this report are depicted in red, and previously described mutations are presented in black. PP: pro-peptides; EC: extracellular domains; MPR: membrane proximal region; PL: proline-rich linker; RUD: repeated unit domain; DTD: desmoglein-specific terminal domain.

According to the tentative diagnostic criteria [3], and based on the accurate genetic diagnosis, SAM syndrome should include symptoms of severe dermatitis (predominantly ichthyosis, and palmoplantar keratoderma striata), multiple allergies for which total IgE or specific IgE elevation should be considered, and metabolic wasting. The latter includes failure to thrive, developmental delay, malabsorption, and hypoalbuminemia. The absence of any of these three manifestations would indicate an atypical type. According to these criteria, 10 patients with SAM syndrome and *DSG1* mutations and one with *DSP* mutations were confirmed as atypical cases. Four SAM syndrome patients with *DSG1* mutations and three with *DSP* mutations were considered to be typical forms of SAM syndrome. Comparatively, while the patient included in this report presented with a severe cutaneous phenotype with intractable itching and severe metabolic wasting, no food sensitization or elevated IgE were observed. The case reported here therefore illustrates the clinical manifestation of an atypical form of SAM syndrome.

We propose the following modifications to two concepts related to SAM syndrome. Firstly, in previous studies, congenital ichthyosis was used to describe the dry, thickened and scaly skin of patients [3]. However, true ichthyosis is typically an autosomal recessive congenital condition [18]. In order to avoid confusion, we suggest the use of the word “ichthyosiform dry skin” to describe the lesion. Secondly,

concerning allergology, the diagnosis of a food allergy is not that easy or straightforward. Double-blinded, placebo-controlled food challenges (DBPCFCs) are considered the gold standard to accurately diagnose a food allergy [19]. It is not always possible to provide conclusive answers based on modern molecular diagnoses or component-resolved diagnoses. Food allergies cannot be diagnosed merely on medical history or the presence of specific IgE antibodies [20]. We therefore suggest the use of food sensitization, instead of food allergy, as part of the diagnostic criteria. The main cutaneous feature of SAM syndrome results from a keratosis abnormality. Ras/Erk mitogen-activated protein kinase (MAPK) signalling has been explicitly discussed in epidermal homeostasis, constituting a critical component of morphological and biochemical transformations of nascent keratinocytes [21]. Dislodging the distinctive C-terminal domain (PL, RUD and TD) of *DSG1* eliminates the integration of *DSG1* and Erbin. Erbin is a known ERK signal transduction and promote differentiation of the epidermis [22]. Both mutations detected in this study resulted in distinctive dislodging of the C-terminal domain, possibly affecting epidermal differentiation and leading to abnormal keratinization.

Pre-mRNA splicing is mediated by *cis*-elements which involves a branch point sequence (BPS), the polypyrimidine tract, the 5' and 3' splice sites, and enhancers/silencers

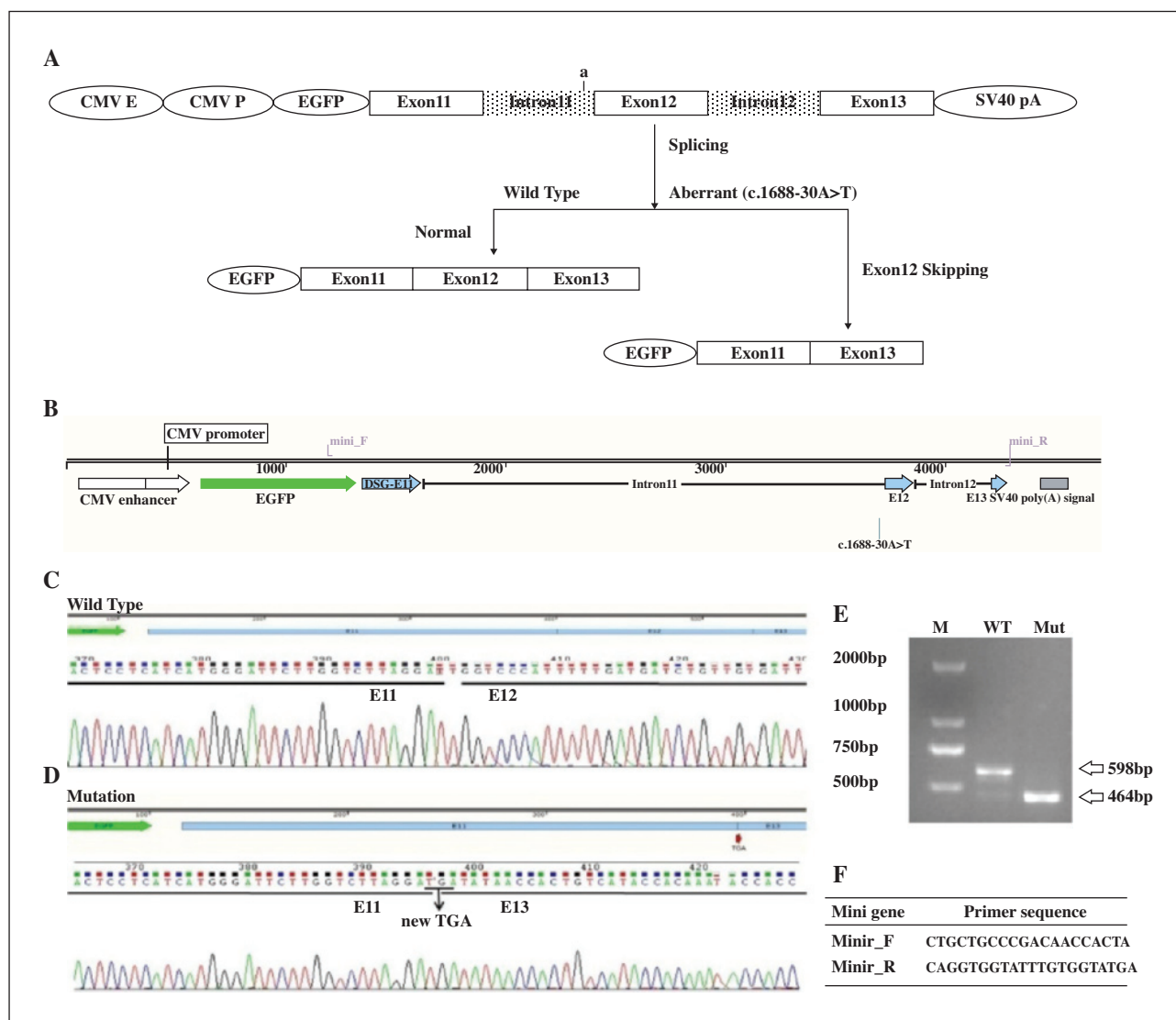


Figure 3. *In vitro* minigene assay for the c.1688-30A > T mutation. **A**) Schematic representation of splicing. **B**) A 2,930-bp genomic fragment, spanning exons 11 through to 13 and introns 11–12, was amplified and cloned into the pEGFP-C3 vector via XhoI/EcoRI cut sites. Exons are represented by thick blue arrows and introns by black lines. The short purple lines denote the positions of primers used for PCR. The blue arrow points to c. 1688-30A > T. **C-D**) Sequencing results of PCR fragments reveals that the mutation elicited a ‘TGA’ stop codon and skipped the whole of exon 12. **E**) Analysis of PCR products amplified from HEK-293T cells transfected with pEGFP-C3-WT and pEGFP-C3-MUT. The transcription of the wild-type minigene shows a band with a normal spliced size of 598 bp. On the contrary, the mutant vector shows a smaller band of 464 bp. M: marker; WT: wild-type; Mut: mutant. **F**) CR forward and reverse primer sequences.

of exonic/intronic splicing. *Homo sapiens* carry a conserved BPS of ‘yUnAy’ with a highly conserved ‘A’, located 21–34 nucleotides upstream of the 3’ terminal of an intron [23]. In this study, c.1688-30A > T is located in the BPS of intron 11, where the highly conserved ‘A’ was replaced by ‘T’ (c.1688-30A > T). The change in the BPS may disrupt early steps associated with spliceosome assembly, impede pre-mRNA splicing procedures and, as observed in this study, cause the skipping of an entire exon (exon 12 in this instance). Reassembly of exons 11 and 13 generated a ‘TGA’ stop codon that resulted in premature termination of protein translation. However, c.1688-30A > T mRNA was not detected *in vivo*. Here, nonsense-mediated mRNA decay may possibly explain the absence of the mutant mRNA,

since these mechanisms are believed to degrade eukaryotic mRNAs that encode truncated proteins with no function or unexpected functions [24].

The association between human diseases and mutations located outside coding regions appears to be growing rapidly. In this study, we report on a Chinese boy with an atypical case of SAM syndrome and confirmed the presence of compound heterozygous variants. Our findings underline the need to analyse the intronic regions of *DSG1* in patients with SAM syndrome, especially those in whom no coding region mutation is found. The deep-intronic (c.1688-30A > T) variant in *DSG1* should be included in the diagnostic testing and carrier screening for SAM syndrome. We also present the concept of atypical SAM

syndrome which is important as a guide for diagnosis and prognosis.

Acknowledgements and disclosures. Acknowledgements: we thank the patient and his family members for their cooperation and participation in this study. This study was supported by the Nature Science Foundation of China (81874239), Shanghai Health System Excellent Academic Leader Training Project (2018BR22), and Pujiang Talents Program (18PJ1407300). Conflicts of interest: none.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1684/ejd.2021.4012. Table S1: Reported patients with atypical SAM syndrome phenotypes associated with *DSG1* mutations. Table S2: Reported patients with typical SAM syndrome phenotypes associated with *DSG1* mutations. Table S3: Reported patients with SAM syndrome phenotypes associated with *DSP* mutations.

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Supplementary material

Supplementary table 1. Reported patients with atypical SAM syndrome phenotypes associated with *DSG1* mutations.

Patient no./(Homo/Hete)		1/(present case (Hete))		2/(Homo)	3/(Homo)	4/(Homo)	5/(Homo)	6/(Homo)	7/(Hete)		8/(Homo)	9/(Homo)	10/(Homo)
Variant		c.2437_2450del (p.Y814Wfs*6)	c.1688-30A>T	c.1892-1delG	c.280C>T (p.Q94*)	c.2659 C>T (p.R887*)	c.2614delA (p.I872Sfs*10)	c.2614delA (p.I872Sfs*10)	c.811_812delAC (p.Q271Vfs*20)	c.2100+4A>G	c.2569 C>T (p.R887*)	c.2569 C>T (p.R887*)	c.1892-2A>C
Exon/Intron number		15 (Exon)	11 (Intron)	14 (Exon)	4 (Exon)	15 (Exon)	15 (Exon)	15 (Exon)	7 (Exon)	14 (Intron)	15 (Exon)	15 (Exon)	13 (Intron)
Age (years)		5		13	2.5	17	27	11	2		*	*	19
Sex		Male		Female	Male	Female	Male	Male	Female		Female	Female	Female
Cutaneous phenotypes	Congenital erythroderma	+		+	+	+	+	+	+	+	+	+	+
	PPK	+		+	+	+	+	+	+	+	+	+	+
	Ichthyosis	+		-	+	+	+	+	-		+	-	-
	Pruritus	+		-	-	-	-	-	-		+	+	-
	Woolly hair	-		+	-	+	-	-	+		-	-	-
	Hypotrichosis	+		-	+	-	-	-	-		-	-	-
	Nail/teeth abnormality	+/-		+/-	-/-	-/-	+/-	-/-	-/-		-/-	-/-	-/-
Immunity-associated disorders	Food or other sensitizations	-		-	-	+	+	+	-		-	+	-
	IgE elevation/IgE titres (IU/ml)	-/ <4.63		-	-	+/-131000	+/-954	+/-1634	+/-109.4		+/-395	+/-1952	+/-531
	Recurrent infections	-		+	-	-	-	-	-		-	-	-
Severe metabolic wasting	Failure to thrive	+		-	-	-	-	-	-		-	-	-
	Developmental delay	+		-	-	-	-	-	-		-	-	-
	Malabsorption	+		-	-	-	-	-	-		-	-	-
	Hypoalbuminemia	+		-	-	-	-	-	-		-	-	-
Alimentary system associated disorders	Eosinophilic esophagitis	-		-	-	-	-	-	-		-	-	-
	Severe oesophageal reflux	-		-	-	-	-	-	-		-	-	-
Others	Hypernatremia	-		-	-	-	-	-	-		-	-	-
	Microcephaly	-		-	-	-	-	-	-		-	-	-
	Cardiac defects	-		-	-	-	-	-	-		-	-	-

+: positive; -: negative; Homo: homozygosity; Hete: heterozygosity; PPK: palmoplantar keratoderma; IgE: immunoglobulin E

Supplementary table 2. Reported patients with typical SAM syndrome phenotypes associated with *DSG1* mutations.

Patient no./ (Homo/Hete)		1/(Homo)	2/(Homo)	3/ (Homo)	4/(Homo)
Variant		c.49-1G>A	c.49-1G>A	c.1861delG (p.A621Qfs*3)	c.1892-1delG
Exon/Intron number		1 (Intron)	1 (Intron)	13 (Exon)	14 (Exon)
Age (years)		7	3	0.75	7
Sex		Female	Female	Female	Male
Cutaneous phenotypes	Congenital Erythroderma	+	+	+	+
	PPK	+	+	+	+
	Ichthyosis	–	–	+	+
	Pruritus	–	–	–	–
	Woolly hair	–	–	–	+
	Hypotrichosis	+	+	–	–
	Nail/teeth abnormality	–/–	–/–	–/–	–/–
Immunity-associated disorders	Food or other sensitizations	+	+	+	–
	IgE elevation/IgE titres (IU/mL)	+/10900	+/266	+/1438	+/>25000
	Recurrent infections	+	+	+	+
Severe metabolic wasting	Failure to thrive	+	+	+	+
	Developmental delay	+	+	+	+
	Malabsorption	+	+	+	–
	Hypoalbuminemia	+	+	+	–
Alimentary system associated disorders	Eosinophilic esophagitis	+	–	–	–
	Severe oesophageal reflux	–	+	–	–
Others	Hypnatremia	+	+	+	–
	Microcephaly	+	+	–	–
	Cardiac defects	–	+	–	–

+: positive; –: negative; Homo: homozygosity; Hete: heterozygosity; PPK: palmoplantar keratoderma; IgE: immunoglobulin E

Supplementary table 3. Reported patients with SAM syndrome phenotypes associated with *DSP* mutations.

Patient no./ (Homo/Hete)		1/(He te)	2/(He te)	3/(He te)	4/(He te)
Variant		c.175 7A>C (p.H5 86P)	c.174 8T>C (p.L5 83P)	c.182 8T>C (p.S6 10P)	c.175 6C>T (p.H5 86Y)
Exon/Intron number		14 (Exon)	14 (Exon)	14 (Exon)	14 (Exon)
Age (years)		6	10	2	2
Sex		Male	Fema le	Fema le	Male
Cutaneous phenotypes	Congenital erythroderma	+	+	+	+
	PPK	+	+	+	+
	Pustulosis	+	+	+	+
	Ichthyosis	+	+	+	+
	Pruritus	+	–	+	+
	Hypotrichosis/Woolly hair	+/+	+/+	+/-	+/-
	Nail/teeth abnormality	+/+	-/+	+/+	+/-
Immunity-associated disorders	Food or other sensitizations	+	–	–	–
	IgE elevation/IgE titres (IU/ml)	+/611 000	–	+/895	+/958
	Recurrent infections	+	+	–	+
Severe metabolic wasting	Failure to thrive	+	+	+	+
	Developmental delay	+	+	+	+
Alimentary system associated disorders	Eosinophilic esophagitis	–	–	–	–
	Severe oesophageal reflux	–	–	–	–
Others	Hypernatremia	–	–	–	–
	Microcephaly	+	–	–	–
	Cardiac defects	–	+	–	–
	Brain abnormalities	–	–	–	+
	Ophthalmic abnormalities	+	–	–	–

+: positive; –: negative; *Homo*: homozygosity; *Hete*: heterozygosity; *PPK*: palmoplantar keratoderma; *IgE*: immunoglobulin E.