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RESEARCH ARTICLE

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Two novel *SASH1* mutations in Chinese families with dyschromatosis universalis hereditaria

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Abstract

Background: Dyschromatosis universalis hereditaria (DUH) is a rare genodermatosis characterized by hyper- and hypo-pigmented macules on the face, trunk, and extremities. The condition causes severe cosmetic problem which can lead to significant psychological distress to the patients and bear a negative impact on society. DUH is a condition with genetic heterogeneity. The *SASH1* gene was recently identified as pathogenic genes in DUH patients.

Methods: Two families clinically diagnosed with dyschromatosis universalis hereditaria were enrolled. Whole-exome sequencing combined with Sanger sequencing and bioinformatics analysis was performed in the probands. MutationTaster, CADD, SIFT, PolyPhen-2, and LRT software, and The American College of Medical Genetics and Genomics Standards and Guidelines were employed to assess the pathogenicity of detected missense mutations. One hundred healthy unrelated Chinese individuals were used as controls. All participants signed an informed consent form.

Results: Genetic screening revealed a heterozygous *SASH1* c.1547G>A (p.Ser516Asn) mutation for patients in family 1, and *SASH1* c.1547G>T (p.Ser516Ile) for family 2. Both such *de novo* mutations are located in a highly conserved SLY domain in *SASH1*, have not been previously reported in any publication, and were not detected in any control databases.

Conclusions: The novel heterozygous mutations, *SASH1* c.1547G>A and c.1547G>T, are likely responsible for the DUH phenotype in these two families. Our study expands the mutation spectrum of DUH. Whole-exome sequencing showed its efficiency in the diagnostic of hereditary skin disorders.

KEYWORDS
dyschromatosis universalis hereditaria, missense mutation, *SASH1*, SLY domain, whole-exome sequencing

Jia-Wei Liu and Xiaerbati Habulieti contributed equally to this work.

1 | INTRODUCTION

Dyschromatosis universalis hereditaria (DUH, MIM# 127500) is a rare pigmented genodermatosis whose clinical features include asymptomatic hyper- and hypopigmented macules that emerge in infancy or early childhood, develop after puberty, and persist throughout life without a significant change in color or distribution. The macules occur in a diffused distribution over the trunk, limbs, and sometimes the face.[1] Skin appendages involvements have been reported, such as dystrophic nails with hyperpigmentation and pterygium formation[2] and the diffuse thinning of scalp hair.[3] Aside from skin changes, DUH may be associated with systemic abnormalities, including small stature, ocular albinism, and high-tone deafness.[2,4,5] DUH is a condition with genetic heterogeneity, with both autosomal dominant and autosomal recessive inheritance patterns reported. *ABCB6* and *SASH1* were recently identified as pathogenic genes in several DUH families with an autosomal dominant pattern and several patients with sporadic disease.

In this study, we report two cases of DUH from two Chinese families. Whole-exome sequencing combined with Sanger sequencing revealed that two novel *SASH1* mutations may be responsible for their condition. Whole-exome sequencing showed its efficiency in the molecular diagnosis of hereditary skin disorders. We also discussed the possible mechanism of *SASH1* heterozygous mutations that result in DUH phenotype, which shed light on the pathogenesis of the condition.

1.1 | MATERIALS AND METHODS

1.2 | Patient recruitment, blood sampling, and DNA extraction

Careful physical examinations and hospital medical record reviews were conducted for each affected member to confirm the clinical diagnosis. Digital photographs were taken, and peripheral blood samples were collected from the two patients and their family members. Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Blood MiNi Kit (Qiagen, Hilden, Germany) following the standard instructions of the manufacturer. All individuals who participated in this study gave informed consent, and the study was approved by the ethics committee of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College.

1.3 | Whole-exome sequencing

An Agilent liquid capture system (Agilent SureSelect Human All Exon V6; Santa Clara, CA, USA) was employed to enrich exome sequences from 0.4 μg genomic DNA using the manufacturer's protocol. Firstly, qualified genomic DNA was randomly fragmented to an average size of 180–280 bp by a Covaris S220 ultrasonicator (Woburn, MA, USA). This was followed by A-tailing and ligation at the 3'ends with paired-end adaptors (Illumina, San Diego, CA, USA). DNA fragments were then end repaired and phosphorylated. In a PCR reaction, DNA fragments with adapter molecules attached at both ends were selectively enriched. After PCR reaction, libraries were hybridized with a biotin-labeled probe to the liquid phase, and gene exons captured with magnetic beads and streptomycin. Products were quantified using an Agilent high sensitivity DNA assay on an Agilent Bioanalyzer 2100 system, and purified using an AMPure XP system (Beckman Coulter, Beverly, MA, USA). Finally, a DNA library was sequenced on an Novaseq (Illumina, San Diego, CA, USA) for paired-end 150-bp reads. After quality control, effective sequencing data were mapped to the reference genome (GRCh37/hg19) by Burrows–Wheeler Aligner software to obtain the original mapping result in BAM format. Reading segments aligned to exon regions were collected for mutation identification and subsequent analysis.

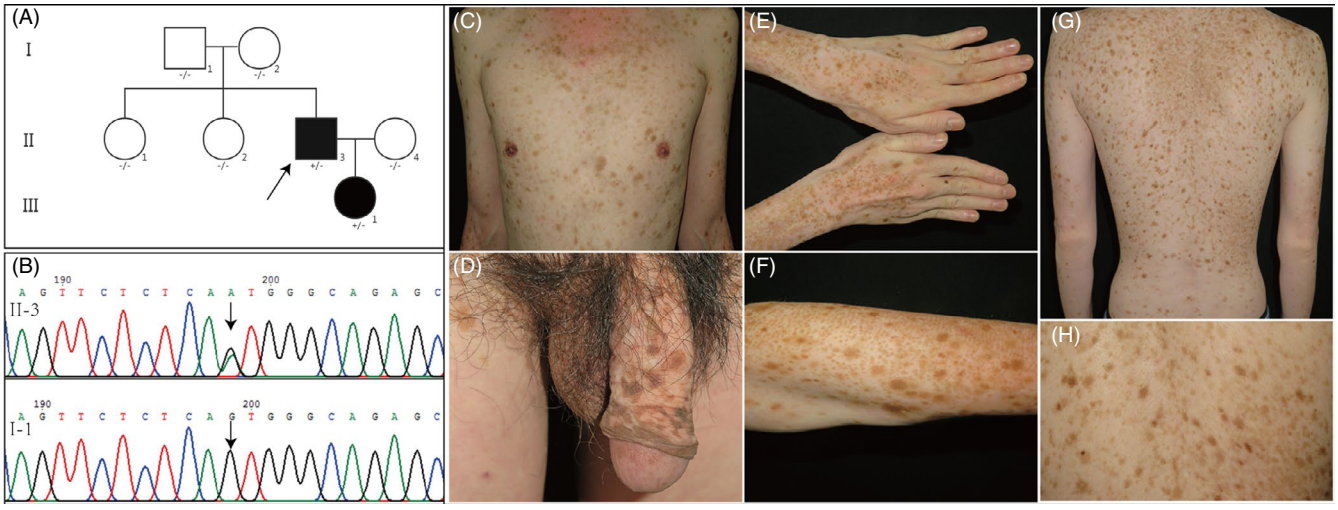

FIGURE 1 A, Pedigree of family 1; B, Electropherograms showing the sequence of the heterozygous *SASH1* c.1547G>A (p. Ser516Asn) mutation for the patient in family 1 (upper panel) and healthy individuals (lower panel); C-H, Clinical pictures of the proband in family 1 with *SASH1* mutation. Hyper- and hypopigmented macules spreading over his trunk, limbs, and external genitalia

1.4 | Sanger sequencing for mutation verification

A screening process for whole-exome sequencing (WES) mutation loci and the gene sequence were obtained from the University of California Santa Cruz Genome Browser database (http://genome.ucsc.edu). Prime3 Input (version 0.4.0) (http://bioinfo.ut.ee/primer3-0.4.0/) online software was used to design primers. Primers used for amplification of the *SASH1* gene were 5′-GGCCAAGTCGCTGAGAATTA-3′ (forward) and 5′-TCACCTTGAAATTGCCTGAA-3′ (reverse) in both families. Sanger sequencing was used for verifying the mutations in patients and their families, as well as for the normal controls.

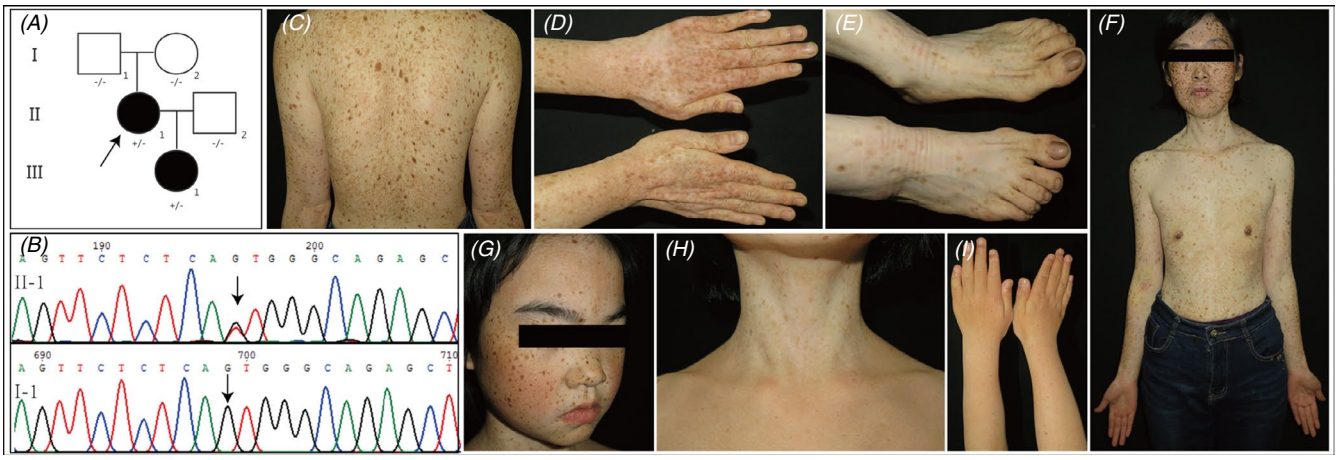

FIGURE 2 A, Pedigree of family 2; B, Electropherograms showing the sequence of the heterozygous *SASH1* c.1547G>T (p.S5er16Ile) mutation for the patient in family 2 (upper panel) and healthy individuals (lower panel); C-I, Clinical pictures of the proband in family 2 (C-F) and her daughter (III-1) (G-I) with *SASH1* mutation. Hyper- and hypopigmented macules were observed all over the body and were more prominent on the face, upper body, and extensor aspects of upper limbs

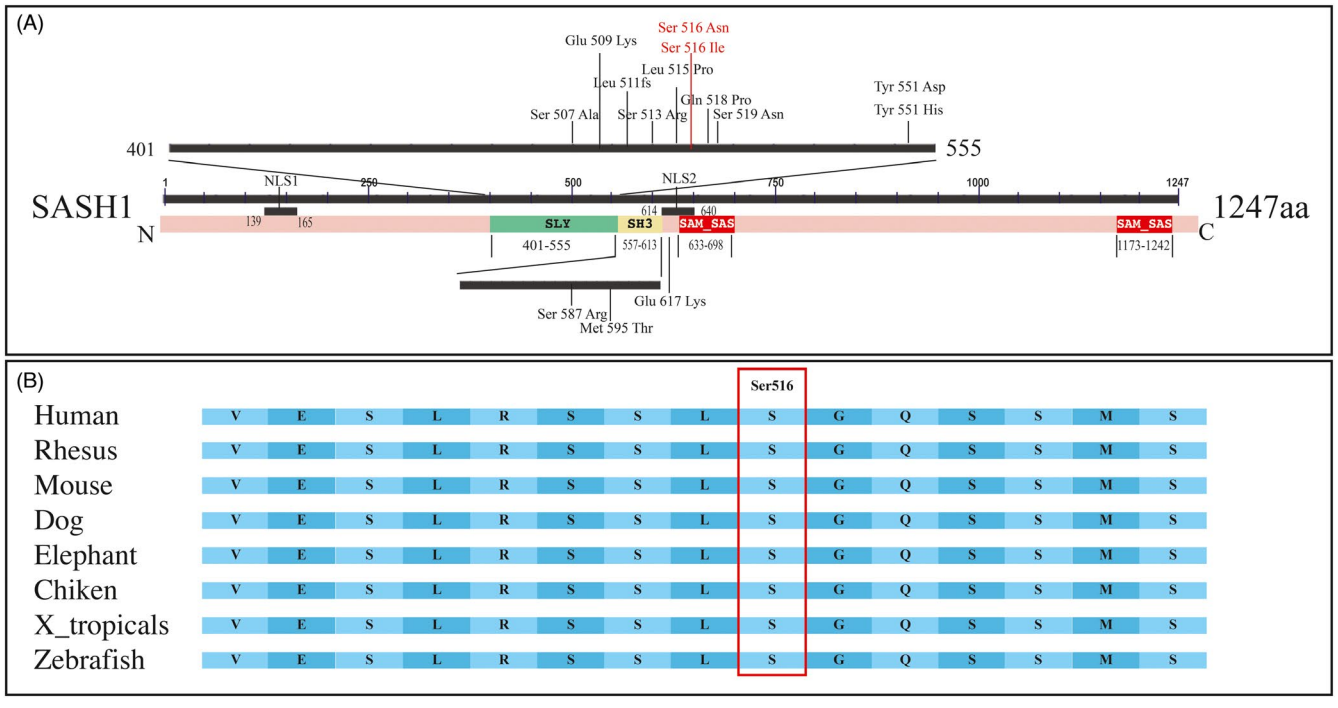

FIGURE 3 A, Schematic view of SASH1 deletion constructs and localization of rare nonsynonymous variations reported so far (red ones indicate the variations identified in this study). SASH1 contains two predicted nuclear localization signals (NLS1 and NLS2), one conserved SLY domain, Src-homology 3 domain (SH3) and the two sterile alpha-motifs (SAM1 and SAM2). Amino acid residues of the SASH1 coding sequence are indicated. B, SASH1 sequence comparison among multiple species showed Ser516 was located in highly conservative regions

1.5 | Bioinformatics analysis

The effect of the mutations was assessed using The American College of Medical Genetics and Genomics (ACMG) Standards and Guidelines and combined with the automatic tools, MutationTaster, SIFT, CADD, Polyphen-2, and LRT, to predict the possible effect of amino acid substitutions.

1.6 | RESULTS

1.7 | Clinical features

Proband 1 (II-3, Figure 1A) in family 1 was a 26-year-old Chinese male who presented with asymptomatic hypo- and hyperpigmented macules spread over almost the entire body (Figure 1C–H). The lesions first appeared on his abdomen when he was 1-year-old and then extended gradually to his back, limbs, face, and neck over the years. He was otherwise healthy with no associated abdominal or cardiac symptoms. He did not have a history of photosensitivity. He also denied a history of drug intake. The family history was negative for skin cancer and consanguinity. His mental and physical development was normal. His daughter, who was 1-year-old, possessed similar skin lesions, which indicated a possible autosomal dominant inheritance pattern.

Physical examination showed his skin was generally covered with hyper- and hypopigmented macules and patches of various shapes and sizes. The patient had normal vision, visual field, and ocular structure on examination. The palms and soles, mucous membranes, teeth, nails, and hair appeared normal. The patient had fair skin with Fitzpatrick skin type I. No telangiectasia, xerosis, or atrophy was observed.

Proband 2 (II-1, Figure 2A) was a young Chinese female who presented with dyschromatosis and who was born to a nonconsanguineous family. The lesion initially appeared on her dorsal hands when she was an infant and has progressively extended to her face, neck, trunk, and limbs over the years. The lesion was more prominent on the face, upper body, and extensor aspects of the upper

TABLE 1 *SASH1* variants

Patient	Ethnicity	Chromosomal position (GRCh37)	Transcript alteration (NM_015278)	Protein alteration (NP_056093 3)	SASH1 domain affected	Variant type
1	Chinese	Chr6:148852752	c.1519T>G	p. Ser507Ala (Wang J et al.2017)	SLY domain	Missense
2	American	Chr6:148852758	c.1525G>A	p. Glu509Lys (Zhou D et al.2013)	SLY domain	Missense
3	Chinese	Chr6:148852760-148852763	c.1527_1530dup	p. Leu511fs (Zhang J et al.2013)	SLY domain	Frameshift
4	Chinese	Chr6:148852770	c.1537A>C	p. Ser513Arg (Zhang J et al.2016)	SLY domain	Missense
5	Chinese	Chr6:148852777	c.1544T>C	p. Leu515Pro (Zhou D et al.2013)	SLY domain	Missense
6	Chinese	Chr6:148852780	c.1547G>A	p. Ser516Asn This study	SLY domain	Missense
7	Chinese	Chr6:148852780	c.1547G>T	p. Ser516Ile This study	SLY domain	Missense
8	Chinese	Chr6:148852786	c.1553A>C	p. Gln518Pro (Nan Wu et al. 2020)	SLY domain	Missense
9	Hispanic	Chr6:148852789	c.1556G>A	p. Ser519Asn (Shellman et al. 2015)	SLY domain	Missense
10	Chinese	Chr6:148854019	c.1651T>C	p. Tyr551His (Zhong WL et al. 2019)	SLY domain	Missense
11	Chinese	Chr6:148854019	c.1651T>G	p. Tyr551Asp (Zhou D et al.2013)	SLY domain	Missense
12	Chinese	Chr6:148854933	c.1761C>G	p. Ser587Arg (Cui HZ et al. 2020)	SH3 domain	Missense
13	Chinese	Chr6:148854956	c.1784T>C	p. Met595 Thr (Zhong WL et al. 2019)	SH3 domain	Missense
14	Moroccan	Chr6:148855021	c.1849G>A	p. Glu617Lys (Courcet JB et al. 2015)	-	Missense

Abbreviations: AD, Autosomal dominant; AR, Autosomal recessive.

limbs, while it presented with a more scattered distribution on the trunk, and flexor aspects of the limbs. She did not report any pain or pruritus. Similar lesions also appeared in her daughter since she was 1-year-old (Figure 2G–I). Physical examination revealed mingled hyper- and hypopigmented macules of irregular size on her face, limbs, and trunk (Figure 2C–F). No abnormality was detected on systemic examination. There was also no hair, nail, teeth, palm, sole, or mucous membrane involvement.

1.8 | Identification of pathogenicity mutations

Of all identified variants, those with frequency of more than 1% in control databases or not functionally affecting the protein were considered as benign and filtered out. After the filtration, we focused on Online Mendelian Inheritance in Man (OMIM) disease genes: a heterozygous mutation on *SASH1* (c.1547G>A) in patient 1 (Figure 1B) and a heterozygous mutation on *SASH1* (c.1547G>T) in patient 2 (Figure 2B) remained as candidate disease-causing mutations. No possible pathogenic mutations in other known pigmentation disorder-causing genes were detected. These two mutations have not been reported in any previous publication and were not detected in any control databases. Results from Sanger sequencing validation were consistent with next generation sequencing results and were absent from the normal controls. In addition, the mutation was co-segregated in each family, with the parents of patients 1 and 2 negative for the presence of these two mutations, indicating they occurred *de novo* for these two patients. The choice of transcript for variant reporting was based on transcript and protein lengths, and expression in blood cells according to Blueprint data.[6] Five *SASH1* transcripts exist, with NM_015278 the longest transcript in blood cells (209206 bp, corresponding to a protein with the expected 1247 aa length).

The two novel mutations in the *SASH1* gene were predicted to be pathogenic by prediction programs (MutationTaster, 1.000 disease-causing; PolyPhen2, 1.0 probably_damaging; LRT, 0.000 deleterious; SIFT, 0.003 damaging for *SASH1* c.1547G>A and 0.008 damaging for

CADD score	Inheritance	SIFT	PolyPhen−2	LRT	Diagnosis
25.9 Damaging	AD	0.002 Damaging	0.994 Probably damaging	0.000 Deleterious	Multiple Lentigines
35 Damaging	AD	0.002 Damaging	0.998 Probably damaging	0.000 Deleterious	Dyschromatosis
-	AD	-	-	-	Dyschromatosis
32 Damaging	AD	0.001 Damaging	0.999 Probably damaging	0.000 Deleterious	Lentiginous
29.9 Damaging	AD	0.001 Damaging	1.0 Probably damaging	0.000 Deleterious	Dyschromatosis
33 Damaging	AD	0.003 Damaging	0.998 Probably damaging	0.000 Deleterious	DUH
34 Damaging	AD	0.008 Damaging	0.999 Probably damaging	0.000 Deleterious	DUH
29.4 Damaging	AD	0.001 Damaging	1.0 Probably damaging	0.000 Deleterious	DUH
32 Damaging	AD	0.002 Damaging	0.998 Probably damaging	0.000 Deleterious	Lentiginous
27.6 Damaging	AD	0.002 Damaging	0.999 Probably damaging	0.000 Deleterious	DUH
29.6 Damaging	AD	0.001 Damaging	0.999 Probably damaging	0.000 Deleterious	Dyschromatosis
28.8 Damaging	AD	0.002 Damaging	0.998 Probably damaging	0.000 Deleterious	Lentiginous
22.3 Damaging	AD	0.185 Tolerable	0.568 Possibly damaging	0.000 Deleterious	DUH
24.6 Damaging	AR	0.007 Damaging	0.797 Possibly damaging	0.000 Deleterious	Cancer, Alopecia, Pigment Dyscrasia, Onychodystrophy, and Keratoderma

SASH1 c.1547G>T; CADD, 33 damaging for *SASH1* c.1547G>A and 34 damaging for *SASH1* c.1547G>T). According to ACMG Standards and Guidelines,[7] the two variations were both classified as PM6, PM2, PP1, PP2, and PP3. In conclusion, the variants should be considered as likely pathogenic variants (Likely Pathogenic, 2 Moderate [PM1–PM6] and ≥2 Supporting [PP1–PP5]).

1.9 | DISCUSSION

In this study, patients from the two families all showed a phenotype with a generalized dyschromatosis skin lesion. A clinical diagnosis of DUH was made. Through WES, we confirmed the diagnosis by identifying two novel, likely pathogenic, mutations in the *SASH1* gene.

SASH1 is a gene containing 20 exons that maps to chromosome 6q24.3 and encodes sterile alpha motif- and SH3 domain-containing protein 1 (Figure 3A).[8-16] SASH1 is expressed in many human tissues including cultured human epidermal keratinocytes, dermal fibroblasts, and melanocytes in the skin.[16] *SASH1* is also a candidate tumor suppressor gene,[17] which regulates TLR4 signaling through its formation of a molecular complex with TRAF6.[18] The *SASH1* gene has recently been found to be a pathogenic gene for DUH and generalized lentiginous in an autosomal dominant inherence pattern. The two variations identified in this study were both located in the highly conserved SLY domain, and the amino acid Ser516 in SASH1 is highly conserved among species (Figure 3B). Functional consequence predictions with PolyPhen2, SIFT, and MutationTaster were all damaging, according to ACMG Standards and Guidelines: both variations should be considered as likely pathogenic variants. To date, only 14 mutations have been reported in *SASH1* gene (Table 1). Eleven of the heterozygous variations are located in the SLY domain[9-12,14,15,] and two in the SH3 domain[14,15] have followed an autosomal dominant inheritance pattern and led to pigmented dermatosis. Only one homozygous mutation in *SASH1* in a family showed an autosomal recessive inheritance pattern and this was characterized by dyschromatosis, alopecia, palmoplantar keratoderma, ungueal dystrophy, teeth abnormalities, and a predisposition to spinocellular carcinomas.[16] These findings confirm the phenotypic heterogeneity of *SASH1*-associated pigmented dermatosis.

The mechanism of melanogenesis, which refers to melanin synthesis, transport, distribution, and metabolism, is very complex with many determinants involved in the process. Upregulation of p53 can promote the expression of many cytokines in keratinocytes, with or without ultraviolet irradiation. Pro-opiomelanocortin (POMC), a multi-component precursor for α-melanotropic (MSH), adrenocorticotropic hormone, and β endorphin, is a cytokine promoted by p53 that can combine with receptors on the surface of melanocytes to promote the proliferation of melanocytes and melanogenesis, and eventually leads to pigmentation.[19] Melanocortin receptor 1 (MC1R) is a G-protein–coupled receptor that can induce concentrations of intracellular cyclic adenosine monophosphate (cAMP) by combining with a ligand, such as α-MSH and α subunits of heterotrimeric G proteins (Gαs). Cyclic AMP, an important second messenger of melanogenesis, can be upregulated by the binding of MC1R and Gαs and is involved in both melanin synthesis and the movement of melanosomes. The increase in cAMP results in the activation of mitogen-activated protein kinase (MAP kinase or MAPK), and extracellular-regulated protein kinases (ERK1, also known as MAPK3),[20] indicating the MAPK pathway is involved in melanogenesis. Taken together, these components can form a p53–POMC–MC1R cascade to regulate melanogenesis.

The exact pathogenesis of the heterozygous mutation in *SASH1* that results in a DUH clinical phenotype is as yet unclear. However, many studies have given us some insight into the possible mechanism. First, recent studies have shown that SASH1 is a member of the p53–POMC–MC1R cascade.[21] SASH1 responds to MC1R stimulation by POMC through their medium interacting protein, Gαs. *SASH1* mutations intensify activation of the p53–POMC–MC1R–SASH1 cascade. This can result in upregulating premelanosomes, mature melanosome-resident proteins (tyrosinase-related protein 1 and melanocyte protein), and melanosome transporters to enhance the biosynthesis and transport of melanin in melanocytes.[21] Second, SASH1 combines with MAP2K2 (a member of the MAP kinase family that can activate ERK1) through its SH3–SAM domain to form SASH1/MAP2K2 that regulates phosphorylation levels of ERK1. *SASH1* mutations can enhance their binding to further activate the MAPK/ERK pathway to induce hyperpigmentation.[22] Lastly, *SASH1* mutations boost melanocyte migration from basal to suprabasal layers. E-cadherin is required for melanocyte adhesion to the basal layer. *SASH1* mutations in the SLY domain decrease E-cadherin expression and induce concomitant increased melanocyte migration, indicating the SLY domain has a crucial role in the movement of melanosomes.[10] All these may contribute to the phenotype of DUH patients with *SASH1* mutations.

The main feature of DUH is a generalized distribution of mottled hypopigmented and hyperpigmented macules. The clinical features of DUH show some overlap with variants of hereditary or non-hereditary skin disorders, which can be limited to skin or with systemic involvement. These include dyschromatosis symmetrica hereditaria, dyskeratosis congenita, epidermolysis bullosa simplex with mottled pigmentation, reticulate acropigmentation of Kitamura, acropigmentation of Dohi, Dowling-Degos disease, xeroderma pigmentosum, Carney syndrome, LEOPARD syndrome, and amyloidosis. Although DUH caused by a hereditary *SASH1* mutation usually manifests as pigmented abnormalities found only in the skin and rarely affects other systems, a differential diagnosis should still be made to distinguish from the condition with potential malignant transformation and systemic defects. Careful clinicpathological examination, together with gene detection, can lead to an accurate diagnosis and further enables the physician to provide etiotropic suggestions. Such uncomely conditions impose a severe psychological burden on affected families. Therefore, completing a genetic diagnosis for patients is necessary for appropriate clinical and genetic counseling. Although Sanger sequencing remained the gold standard for variants detection in patients, whole-exome sequencing provides a more time-saving, yet cost-effective method of

gene screening, especially when faced up with clinical integrity with an uncertain diagnosis.

In conclusion, this study investigated two pedigrees with DUH by whole-exome sequencing and confirmed the clinical diagnosis by the identification of two novel likely pathogenic heterozygous mutations in the SLY domain of *SASH1*. This further strengthens the evidence that *SASH1* is one of the genes responsible for pigmented dermatosis and that its SLY domain is prone to being a mutational hotspot region. This study expanded the spectrum of *SASH1* mutations in DUH patients, and the mechanism of *SASH1* heterozygous mutations leading to DUH phenotype was discussed, which contributes to our understanding of the pathomechanism of this condition. Also the efficiency of whole-exome sequencing in this study further validates its essential role in diagnosing genodermatosis, demonstrating WES can therefore be implemented as a reliable diagnostic test in clinical work.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

All data are available from the corresponding author by request.

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