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Identification of mutation c.632G>A (p.G211D) in the *ATP2A2* gene and genotype–phenotype correlation in a large Chinese family with Darier's disease

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Conflicts of interest: None.

Abstract

Darier's disease (DD, MIM 124200) is an autosomal dominant inherited skin disease. Mutations in the *ATP2A2* gene, which encoded the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase isoform 2 (SERCA2), are responsible for this skin disorder. Here we report the clinical, genetic, and molecular characterization of a large Chinese family with DD. We identified mutation c.632G>A (p.G211D) in the *ATP2A2* gene in this family. Genotype–phenotype correlation in available family members provided helpful genetic counseling information for mutation carriers.

Introduction

Darier's disease (DD, MIM 124200) is an autosomal dominant inherited skin disease. Warty papules and plaques clinically characterize this disease in seborrheic areas, such as the central trunk, flexures, scalp and forehead, palmoplantar pits, and distinctive nail abnormalities.[1,2] Previous studies have demonstrated that mutations in the *ATP2A2* gene, which encoded the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase isoform 2 (SERCA2), was responsible for this skin disorder.[2]

There are at least 150 *ATP2A2* mutations documented in patients with DD at present.[2–11] However, almost all identified mutations are family specific and are not observed in multiple families.[12] Additionally the relationship between genotype and DD phenotype has not been sufficiently established.

In this study, we report the clinical, genetic, and molecular characterization of a large Chinese family with DD. Histological examination and mutational analysis of the *ATP2A2* gene were performed. The transmission of the identified mutation c.632G>A was also traced in the pedigree to reconstruct the genotype–phenotype relationship.

Patients and methods

A three-generation Chinese family (Fig. 1a) with DD was identified at the clinical center of the First People's Hospital of

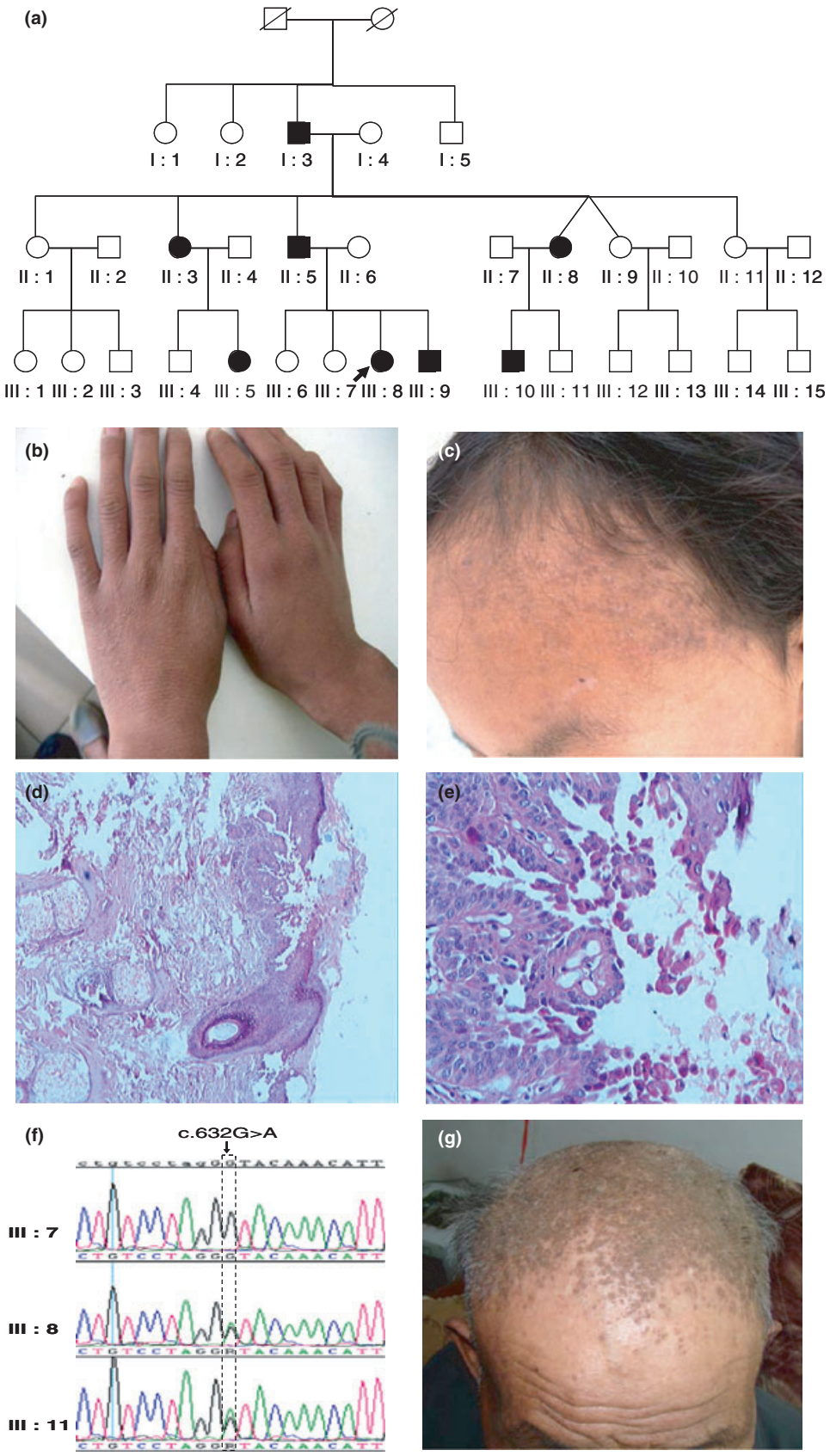
(a)
I : 1
I : 2
I : 3
I : 4
I : 5
II : 1
II : 2
II : 3
II : 4
II : 5
II : 6
II : 7
II : 8
II : 9
II : 10
II : 11
II : 12
III : 1 III : 2 III : 3
III : 4
III : 5
III : 6 III : 7 III : 8 III : 9
III : 10 III : 11 III : 12 III : 13 III : 14 III : 15
(b)
(c)
(d)
(e)
(f)
c.632G>A
III : 7
III : 8
III : 11
(g)

Figure 1 Clinical and genetic characterization of a Chinese family with Darier's disease. (a) Pedigree information showing the penetrance of the disease. The affected individuals are marked by filled symbols. The arrow indicates the proband whose entire exons and exon–intron boundaries in the *ATP2A2* gene were sequenced. (b–e) Clinical skin lesions and skin biopsy of the proband III:8. The proband's hands densely covered with multiple hyperkeratotic papules (b), and her scalp covered with brown hyperkeratotic papules and greasy crusts (c). Skin biopsy of proband III:8 shows suprabasal clefting and dyskeratosis with round dyskeratotic keratinocytes under a light microscope (d,e). Slides stained with hematoxylin and eosin; original magnification ×40 (d) original magnification ×100 (e). (f) Sequencing chromatogram of the proband showing the heterozygous mutation c.632G>A in the *ATP2A2* gene. A healthy individual without the mutation (III:7), the proband (III:8), and subject III:11 who harbors this mutation without expressing DD. The sequences were aligned together with the *ATP2A2* gene reference sequence (GenBank accession number: NG_007097). (g) Skin lesions in the scalp of the proband's grandfather (I:3)

Table 1 Summary of clinical information of a Chinese family with Darier's disease

				Phenotype[b]	
Sample	**Gender**	**Age**	**c.632G>A[a]**	**Onset age**	**Skin lesion**
I:3	Male	74	Yes	20	+++
I:4	Female	66	No	–	–
II:1	Female	45	No	–	–
II:3	Female	41	Yes	5	++++
II:5	Male	39	Yes	14	++++
II:8	Female	34	Yes	17	+++
II:9	Female	34	No	–	–
II:11	Female	32	No	–	–
III:5	Female	17	Yes	15	++
III:6	Female	16	No	–	–
III:7	Female	14	No	–	–
III:8	Female	12	Yes	9	+++
III:9	Male	10	Yes	8	+
III:10	Male	9	Yes	9	+
III:11	Male	5	Yes	?[c]	–
III:12	Male	13	No	–	–
III:13	Male	7	No	–	–
III:14	Male	13	No	–	–
III:15	Male	7	No	–	–

[a]Mutation c.632G>A was presented heterozygously in all family members with the mutation.
[b]The skin lesions in this family are classified as mild (+), moderate (++), severe (+++), and most severe (++++). Patients with mild disease expression (+) developed keratotic papules, which were scattered in one to two places. Patients with both keratotic papules and plaque or had 3–5 skin lesions are classified as moderate (++). Patients with keratotic papules and plaque with greasy crusts or with more than five skin lesions are regarded as severe (+++). Patients with more than five skin lesions in the trunk region, or those with keratotic papules and plaque with greasy crusts or offensive odors are considered as the most severe (++++).
[c]This boy harbors mutation c.632G>A (p.G211D) without expressing Darier's disease at the time of sample collection. He may develop the disease later in life but the exact onset age is unknown.

Qujing City. We were able to collect blood samples from eight patients and 11 normal individuals in this family (Table 1). The proband (III:8) was a 12-year-old girl. She began suffering from painless, brown warty papules on the post aurem, head, and scalp when she was nine years old. These lesions exacerbated with age. At present, her scalp, neck, and limbs are covered with brown hyperkeratotic papules and greasy crusts are confluent in larger areas. The shape and size of the lesions are irregular but symmetrically distributed (Fig. 1b,c) and agree with the typical features of DD.[1]

A biopsy specimen was taken from the proband III:8. A histological examination revealed typical features of hyperkeratosis, with parakeratosis, suprabasal clefts, acantholysis of the epidermis and dyskeratotic cells, and corps rounds and grains (Fig. 1d,e). Genomic DNAs were extracted from whole blood samples of all available family members by using a standard phenol/chloroform method. The entire 21 exons of the *ATP2A2* gene, including the exon–intron boundaries with a coverage of 4–217 bp, were amplified in the proband using previously published primers and amplification conditions[3] with some modification (see Table S1). PCR products were analyzed on 1.5% agarose gels, purified on spin columns (Tiangen Biotech Co., Beijing, China), and were directly sequenced using BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on a 3730 sequencer (Applied Biosystems).

Results

Similar multiple keratotic papules in seborrheic areas, the typical clinical phenotype of DD, were present in eight members of this family, albeit at different levels of severity (Table 1). We sequenced the entire 21 exons and exon–intron boundaries of the *ATP2A2* gene in proband III:8 and only identified a heterozygous nucleotide change of G to A at the 632nd position in the *ATP2A2* cDNA encoding sequence (Fig. 1f). This alteration caused an amino acid substitution from glycine (G) to aspartic acid (D) at position 211 (p.G211D). This mutation was previously described in European patients with DD[8] but was not found in reported Chinese patients with DD. None of the other *ATP2A2* gene mutations (among the available mutation list that is up to 150)[2–11] were found in our patient with DD except for c.632G>A (p.G211D). The presence of this mutation was further screened in

the other 18 members of this family. All the remaining seven patients harbored this mutation in a heterozygous status, whereas all normal individuals (except for one 5-year-old boy who also harbored a heterozygous mutation; III:11) were without this mutation (Table 1; Fig. 1f).

Discussion

In this study, we performed a clinical, genetic, and molecular characterization of a large Chinese family with DD. Eight members of this family showed similar multiple keratotic papules in seborrheic areas as the typical clinical phenotype. Sequencing of the entire exons and the exon–intron boundaries of the *ATP2A2* gene identified a heterozygous point mutation, c.632G>A (p.G211D) in the eighth exon in the proband. Although this mutation has been reported by Ruiz-Perez *et al.*[8] in a European family, it was not reported in Chinese patients. The mutation c.632G>A (p.G211D) is located in the β-strand region of the SERCA2, which is highly conserved among vertebrate species.[2] The functional importance of p.G211D in SERCA2 was previously explored by Miyauchi *et al.*;[13] the SERCA2 expression level decreased 50%, and the ATPase activity was completely abolished when this mutant was transfected into *Cos-1* cells compared with wild type. This implicated that mutation p.G211D probably disrupted the process of Ca^{2+} homeostasis and exhausted more ATP resulting in reduced energy charge in the cell.[13] Evidently, the dysfunction of the SERCA2 caused by p.G211D may be the cause of DD in this Chinese family.

In order to discern the transmission of mutation p.G211D and the potential correlation of genotype-phenotype in this family, we screened the presence of this mutation in available family members with or without DD (Table 1). Our results show that all patients harbor this mutation heterozygously, whereas all normal individuals (except for a five-year-old boy; III:11) are without this mutation (Fig. 1f and Table 1). Intriguingly, one of the twin samples (II:8) has the mutation, whereas the other one (II:9) does not, suggesting that they are dizygotic twins. The five-year-old boy (III:11) possesses the heterozygous mutation but did not express any DD phenotype at the time of the study. The onset age of DD is usually in adolescence; the clinical expression is age-related[14] and demonstrates a hormone-induced pattern.[15] Therefore, it is not surprising that there is no expression of p.G211D in proband III:11 at the present time. Note that the grandfather of the boy (I:3) also had a relatively late onset of DD at 20 years of age. Although the DD is a chronic syndrome typically causing lifelong deterioration and currently lacks effective prevention methods, detection of the mutation in this "normal" boy may offer insight into protection and prevention treatments. Reducing the frequency of exposure to sunlight and ultraviolet as well as decreasing sweating or overheating potentially play roles in postponing disease expression.

In summary, we identified a mutation c.632G>A (p.G221D) in the *ATP2A2* gene that was previously reported in European patient in a three-generation Chinese family with DD. Tracing the mutation in available members of the family may provide helpful genetic counseling information for them, especially for the future protection of the mutation-carrying 5-year-old boy.

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Supporting information

Additional supporting information may be found in the online version of this article:

Table S1 Primer sequences for amplification and sequencing the entire 21 exons of the *ATP2A2* gene and the exon/intron boundaries.

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Supplementary Table 1. Primer sequences for amplification and sequencing the entire 21 exons of the *ATP2A2* gene and the exon/intron boundaries

Primer name	Exon	Sequences (5′→3′)	PCR length (bp)	Position of primer in the intron[a]	Annealing temperature (°C)
1F	1	CGAGGCGGAGGCGAGGAG	271	–60	55
1R		GGAGCCGAAGCCCACGCG		+93	
2–3F	2, 3	ACCTCCCTCTTGACACATTG	464	–45	55
2–3R		GACAACTCCTAACCACACTG		+227	
4F	4	CGTGCCATTTCTCTTCTAG	222	–20	55
4R		CTCAACACATCAGGAAAAACAG		+98	
5F	5	AGTGTCAGGCAGGTCTTTAC	368	–45	55
5R		AGGAAGGGAGGTGCTAAAAC		+184	
6F	6	AGCCTCATTCTCTTCCTTCC	455	–224	55
6R		ATGGAGCGAGACTAAAGCAC		+150	
7F	7, 8	CTTGGTGTGGGTCGCAGAG	1722	–50	52
8R		GAACAAAGAACCACGACACG		+45	
8F		GTTGTATGGCTGGTTGCTTG			
9F	9	GGTTGTTTGCCTTTGTCCTAA	426	–98	50
9R		ATAACAAACACAAATCCCTCTT		+239	
10F	10, 11	GGCGACCATACCCTGCTC	1125	–42	55
11R		CTGTAAGTTTGAGGAGATAAGG		+121	
10–11n		AAAGGCACAGAAAGTAGAA			
12–13F	12, 13	ATTGCCACCCAGTAGTATCC	568	–39	55
12–13R		GAACTGTTTGACCTTTTGCTTG		+88	
14F	14	CTAGAACTTGCCACTTTTATTTA	436	–43	50
14R		GAGGCTACTATGTGCTTGTG		+57	
15F	15, 16	TTTCCTCCTGCTTCCCATTC	1424	–91	55
16R		CATCTCTGTCTTTTGCTACCC		+127	
16F		TCATTTATTTTTCTGGAGGAGG			
15–16n		TGCTGTCAGGAAAATACTG			
17F	17, 18	TGATCTTCGTCCTTGTGGGG	704	–94	55
18R		TTTTGGGAAGGGAAGAACTGT		+114	
19–20F	19, 20	TCCCCACCTCTCCTTGCTC	609	–24	55
19–20R		CCTCCATCACCAGCCAGTAT		+115	
21F	21	GTTCCTTTTCATCTGTCGCTG	1040	–105	55
21Rn		CTAGTCACATGGCCACCCT		+136	

All the primers were taken from Sakuntabhai *et al.*,[3] unless otherwise stated. Primers used for PCR amplification were named by the exon number followed by "F" or "R" to designate the forward/upstream or reverse/downstream, respectively. These primers newly designed in this study was further marked by adding "n" after the primer name. We used the amplification primers for sequencing. For some larger fragments, we also included some inner primers (8F, 10-11n, 15–16n, and 16F) for sequencing.

[a]"–" denotes the nucleotide position of the 5′-end of the forward primer, which was scored relative to the first nucleotide of the exon; "+" denotes the nucleotide position of the 5′-end of the downstream primer, which was scored relative to the last nucleotide of the exon. For these fragments covered two exons, "–" was scored relative to the first nucleotide of the first exon, "+" was scored relative to the last nucleotide of the second exon.