

Mosaicism for *ATP2A2* Mutation and Mutant Allelic Fractions Detected by Droplet Digital PCR in Simple Segmental Darier Disease

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Darier disease (DD; Online Mendelian Inheritance in Man #124200) is an autosomal dominant disorder that presents in teenagers or adults, with multiple papules or plaques in seborrhoeic areas. Histology shows suprabasal acantholysis in the epidermis with overlying dyskeratotic cells. DD was caused by *ATP2A2* mutations, which encodes the sacro/endoplasmic reticulum Ca²⁺ ATPase isoform 2 (SERCA2) (1). This protein serves to actively pump Ca²⁺ out of the cytoplasm and plays an important role in regulation of intracellular Ca²⁺ stores and subsequent epidermal cell-cell adhesion/differentiation.

The term mosaicism refers to the biological phenomenon in an individual with 2 or more genetically distinct cell populations derived from a single zygote. There are 2 patterns of mosaicism in autosomal dominant skin conditions, referred to as simple segmental mosaicism and superimposed mosaicism, previously termed type 1 and type 2 segmental mosaicism, respectively (2). Simple segmental mosaicism is caused by an autosomal dominant postzygotic mutation during embryogenesis in 1 allele of a gene that is otherwise normal in the affected individual, leading to mosaic skin involvements that correspond to the distribution of cells with the mutation. Superimposed mosaicism occurs in patients of generalized autosomal dominant genodermatosis with an additional postzygotic mutation that leads to loss of the normal allele, resulting in superimposed segmental manifestations. It may develop prior to the diffuse non-segmental disease. To date, the molecular basis of simple segmental DD has been reported in 6 cases (3–8).

Superimposed mosaicism in DD is extremely rare, with only one published case in the literature (9).

We describe here the clinical and molecular characteristics in an additional Japanese woman with simple segmental DD, and the nature of underlying postzygotic mutation, which underscores its importance in clinical genetics.

CASE REPORT

A 57-year-old Japanese woman presented with a longer than 30-year history of keratotic papules and plaques that were limited to her left trunk and upper extremity (Fig. 1A). The patient was treated with topical steroid therapy without apparent benefits. Steroid therapy was discontinued, based on the patient's personal reasons, and the lesions progressively increased in number following Blaschko's lines. She had no history of neuropsychiatric abnormalities. No other family member, including her daughters, who were in their 30s, was affected. On initial presentation at the age of 57 years, a biopsy specimen was taken from her left anterior chest. Histology showed suprabasal acantholysis with dyskeratotic cells with eosinophilic cytoplasm (corps ronds) and focal hyperkeratosis with pyknotic retained nuclei (grains), confirming the diagnosis of segmental DD (Fig. 1B). Treatment with a combination of systemic retinoid (0.4 mg/kg/day) and topical maxacalcitol was started, and the skin lesions improved significantly. Over the course of 2 years, the patient has been maintained on a regimen of 0.2 mg/kg retinoid once a week to control the disease.

Materials and methods

After obtaining ethics approval and informed consent, genetic analysis was performed using genomic DNA from blood, the affected and unaffected skin of the patient in accordance with the

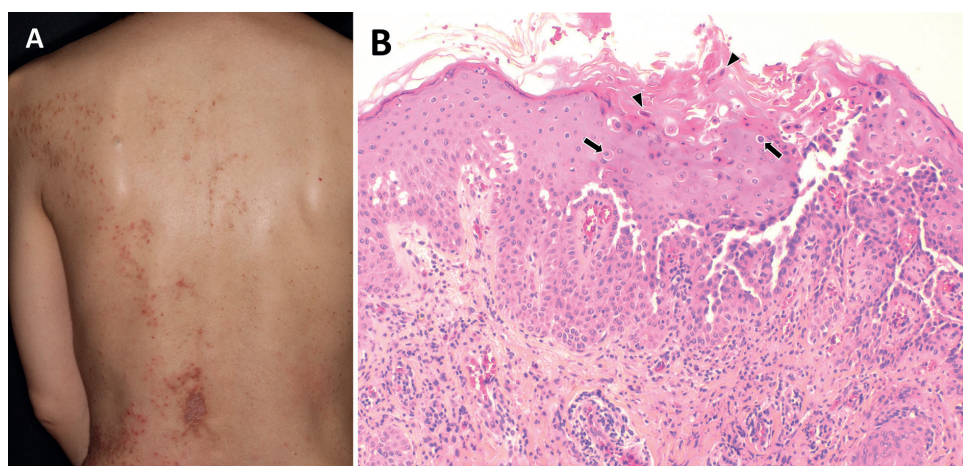


Fig. 1. Clinico-pathological assessment of the current patient with simple segmental Darier disease. (A) Keratotic papules and plaques on the left side of her back following Blaschko's lines. (B) Light microscopy reveals irregular acanthosis of the epidermis, suprabasal acantholytic dyskeratosis with corps ronds (arrows), and focal hyperkeratosis with grains (arrowheads; haematoxylin-eosin; original magnification $\times 200$).

principles of the Declaration of Helsinki. The blood sample was taken from the right median cubital vein of the unaffected side of the body. The affected skin of the left anterior chest was prepared originally for routine light microscopy and the unaffected skin was taken from the right anterior chest. Both skin tissues contained epidermis and dermis. The patient's daughters were not available for any genetic investigations. For mutation analysis, all coding exons and situated in flanking introns of *ATP2A2* were amplified by PCR using exon-specific primer pairs. Sanger sequencing was performed on an ABI 3130 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). Droplet digital PCR (ddPCR) was performed on the Bio-Rad QX200 system (Bio-Rad Life Science, Hercules, CA, USA) using the ddPCR Supermix for Probes (No dUTP). Primers and probes were custom designed by Bio-Rad as ddPCR assay dHsaMDS457132439 based on the variant identified in Sanger sequencing. Two negative controls were included with each experiment. Data were analysed with QuantaSoft™ Analysis Pro (Bio-Rad).

RESULTS

Sanger sequencing revealed a heterozygous substitution in exon 1 of *ATP2A2*, c.68G>A, p.Gly23Glu (rs28929478) from the affected skin, whereas signals of the mutated allele were not visible in blood and the unaffected skin, suggesting that discrepancy of these results demonstrates the presence of postzygotic mutation in this patient (Fig. S1). The mutation has been previously reported in a case with generalized autosomal dominant DD (1).

The ddPCR was used to quantify the mutant allelic fractions (MAFs) in each sample of the patient. The MAFs in these tissues were 22.3% (affected skin), 0.22% (unaffected skin), and 0.52% (blood), respectively (Table I).

DISCUSSION

A few molecular approaches, including pyrosequencing and next-generation sequencing, have been utilized for quantifying MAFs (of 37% and 12.7–39%, respectively) in affected skins with simple segmental DD (4, 6–8). We confirmed the presence of *ATP2A2* mutation via ddPCR in the affected skin from the current patient with MAF of 22.3%. This mosaicism level was relatively low compared with previous studies and sufficient to induce cutaneous DD lesions. It should be noted, however, that the MAF of the affected skin might be composed partially of mesenchymal cells originating from mesoderm (dermis and blood) relative to epidermis.

Table I. Summary of droplet digital PCR results in the tissues examined

	Number of positive droplets			MAFs (%)
	Total	Mutant	Wild-type	
Patient				
Affected skin	6,009	1,473	4,536	22.3
Unaffected skin	5,409	15	5,394	0.22
Blood	6,204	43	6,161	0.52
Control	4,621	0	4,621	0

MAFs: mutant allelic fractions.

Sanger sequencing has limited sensitivity, as only mosaicism with MAF >10% can be identified effectively (10). Pyrosequencing and next-generation sequencing can detect a low-level mosaicism (MAFs in the range of 5% and 1–2%, respectively) (11, 12). Although ddPCR requires the development of a custom assay, validation, and optimization for a single known mutation and lacks the ability to screen the entire genome for novel mutations, it improves more accurately and sensitively to detect MAFs through counting mutation positive and negative DNA fragments in thousands of droplets using a single amplicon. Using ddPCR, we verified the presence of low-level postzygotic mosaicism in genomic DNA from blood and the unaffected skin in the current patient, at MAFs of 0.52% and 0.22%, respectively.

Assessment of somatic mosaicism across ectodermal (epidermis or oral epithelium), mesodermal (dermis, blood or saliva), and endodermal (urothelium) origin DNA can be useful to determine at what stage of embryogenesis the variant arose. In this study, the mutation is present in tissues derived from ectoderm (epidermis) and mesoderm (dermis and blood), although urothelial DNA from this patient was not available for molecular analysis. The mosaicism observed here can be explained by a mutational event before differentiation of the embryonic epiblast in early embryogenesis. Investigation of gonadal mosaicism in females is not as straightforward as in males, due to the invasive procedure required when collecting oocytes, part of extraembryonic mesoderm, which also arise from differentiation of the embryonic epiblast. These observations are important for the purpose of genetic counselling, because the present case implies a risk of simultaneous gonadal mosaicism that may cause the full-blown phenotype in the next generation. Since DD usually presents in teenagers or adults, there remains debate as to whether offspring for DD predisposition should be offered genetic testing for the disease risk. Genetic testing is a way to learn of risk status, prevent exacerbation of disease, and reduce anxiety, whereas perceived disadvantages include negative emotions associated with the test results.

The authors have no conflicts of interest to declare.

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