

## Preimplantation Genetic Screening in Darier-White Disease: A Case Report

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### Abstract

Darier-White disease is a rare genodermatosis with many degrees of severity and clinical manifestations that extend beyond the limits of the skin. It is a genetic disorder with an autosomal dominant inheritance pattern that can be treated, but not cured. Our work aims to report unpublished aspects on genetics and clinical presentation of Darier-White disease in Brazil, presenting a new genetic variant found in a female patient that has not been described so far. We also demonstrated the efficacy of the preimplantation genetic diagnosis along with techniques in assisted reproduction to withhold the occurrence of the Darier-White disease in future generations.

**Keywords:** Darier disease, Darier-White, genodermatosis, new mutation, assisted reproduction, preimplantation genetic diagnosis.

### INTRODUCTION

Darier-White disease or Darier disease, also known as keratosis follicularis, is a rare genodermatosis with many degrees of severity and clinical manifestations that extend beyond the limits of the skin. It is a genetic disorder with an autosomal dominant inheritance pattern, and it can be treated, but not cured, in patients who have this medical condition [1]. The disease onset is usually before the third decade of life, with complete penetrance in adults and variable expression [2]. The prevalence of this disorder in the population is 1:55,000-100,000 [3]. Since this is a recurrent and long-term pathology, it could cause considerable social prejudice [4]. This work aims to present a case report exploring the applicability of the resources of preimplantation genetic diagnosis along with assisted reproduction techniques to prevent the transmission of Darier-White disease to one's descendants.

### DARIER-WHITE DISEASE

Darier-White disease is characterized by

hyperkeratotic papules in seborrheic regions, predominantly on the upper trunk and scalp, palmar pits; nail abnormalities and mucous membrane changes [5]. It shows up in many body sites due to the low adhesion of epidermal cells, but preferentially in these seborrheic areas, where the skin is greasy, having more sebaceous glands [3].

In 50% of patients with skin lesions, an oral involvement can be noticed with flat erythematous plaques, whether coalescent or not, that might become whitish. They affect the keratinized mucous membrane on the back of the tongue, palate and gums, similar to nicotine lesions [3]. Cases have been reported where, in addition to the skin lesions, the patients have fragile and brittle nails frequently associated with subungual hyperkeratosis and scalp lesions, in addition to several degrees of depression or mental disorders [4, 5]. In about 70% of the patients, the standard window of the disease symptoms' onset starts between the ages of 6 and 20 years with prevalence peaking during puberty (ages between 11 and 15 years old) [5, 6].

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Recent studies have shown that 96% of the patients have some lesion in the extremities; 13% have lesions in the oral mucous membrane, and 6% have hypertrophic lesions [7]. Quite often, the skin lesions get infected and have a fetid odor, causing significant discomfort to the patients [1, 2]. Typical disease lesions can be intensified by exposure to sunlight, artificial UVB radiation, heat exposure, sweating or friction. In periods of emotional stress, the lesions get worse, including any secondary manifestations such as brittle nails and pruritus[4].

### GENETICS OF DARIER-WHITE DISEASE

Darier-White disease occurs due to a change in the chromosome 12q23– 24.1, involved in keratin differentiation[8]. ATP2A2, which encodes the sarcoplasmic/endoplasmic reticulum Ca<sup>2+</sup> ATPase 2 (SERCA2) pump, has been characterized as the defective gene [9]. Mutations in the endoplasmic reticulum Ca<sup>2+</sup> ATPase ATP2A2 (product of SERCA2 protein) cause acantholysis and apoptosis, explaining the histological finding of acantholytic dyskeratosis in the disease. Since over 50% of the mutations led to a premature termination codon, haploinsufficiency is proposed as a common molecular mechanism for Darier-White disease. However, significant inter- and intrafamilial phenotypic variability of the disease was observed, suggesting that other factors could contribute to the clinical phenotype [2, 9]. Over 120 distinct ATP2A2 mutations have been identified, and, in general, there is no clear correlation between genotype and phenotype. Possible exceptions include specific ATP2A2 mutations associated with acrokeratosis verruciformis of Hopf (AVH) and acral hemorrhagic subtype of Darier-White disease [1, 10]. ATP2A2 mutations result in high cytosolic concentrations of Ca<sup>2+</sup> due to the inadequate provision of Ca<sup>2+</sup> reserves from the endoplasmic reticulum. Even though the isoforms SERCA2a and SERCA2b are found in the epidermis, SERCA2b is the primary product, and the dysfunction of this isoform itself can trigger Darier-White disease[2, 8].

SERCA2 is ubiquitously expressed, but the lack of a compensatory expression of SERCA3 in the keratinocytes could explain the specific vulnerability of these cells to SERCA2 deficiency. Several pathogenic mechanisms have been put forward as possible causes of acantholytic dyskeratosis in Darier-White disease. Defects in the intake of Ca<sup>2+</sup> in the endoplasmic

reticulum can produce acantholysis, jeopardizes the synthesis of junctional proteins (e.g. desmoplakin), as also their processing, folding and traffic. Unlike the reserves of Ca<sup>2+</sup> stored in the Golgi complex, the depletion of Ca<sup>2+</sup> in the keratinocytes of the endoplasmic reticulum is also linked to apoptosis. Apoptosis can be mediated by the accumulation of unfolded proteins inside the endoplasmic reticulum, that can generate two highly conservative stress responses: the “unfolded protein response” and, alternatively, the “endoplasmic reticulum overload response”. The Ca<sup>2+</sup> deregulation can result in the impairment of the cycle cell checkpoint control, and in a low threshold for skin trauma, triggering the keratinocyte apoptosis [1]. In Darier-White disease, it was found that ATP receptors thought to play a role in the apoptosis and the calcium signaling are abnormally located in the lesional epidermis, with a reduction of P2Y2 (a G protein-coupled receptor), but P2X7 (death-cell receptor) increases in the plasmatic membrane of acantholytic cells [8].

Darier-White disease is an excellent example of age-dependent penetrance because the first aspects of the disease usually appear only between the first and fourth decades of life. The age-dependent penetrance is challenging for genetic counselling and the establishment of an inheritance pattern of a given aspect, considering that some people can be too young to know whether they are affected or not. In particular, notably, the diagnosis of Darier-White disease in childhood and adolescence, before the onset of the symptoms, is only possible by genetic mapping, as that the patient has not yet developed the skin lesions that are typical of this disease [5, 11]. Therefore, the diagnosis is not possible using anatomopathology.

### CASE REPORT

We hereby state that all Bioethics guidelines have been followed in this case report, and the patient has signed the Informed Consent Form.

The patient is a 34-year-old female, white, married, a dental surgeon by profession; she was born in Rio de Janeiro, Brazil. She reports that the first onset of the condition was when she was nine years old, when xerosis was observed along with darkness of the neck and trunk, especially after exposure to sunlight. Later, a proliferation of scabs was seen in some seborrheic areas. At the same time, the onset of nail fragility was observed, with brittle nails. She denied systemic

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complaints and was subjected to many topical treatments with little improvement.

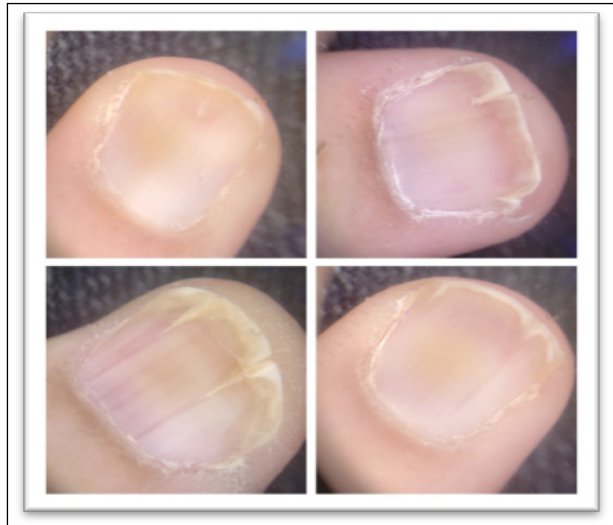
The dermatology exam showed keratotic papules, affecting mainly the neck and trunk (back, neck, and inframammary region) (Figure 1), and some of them were clustered, forming warty plaques. Scaly lesions in the scalp were observed (Figure 2), along with fragile nails with brittle aspect, longitudinal white streaks and V-shaped appearance, along the free edge of the nail (Figure 3). The patient mentioned that in periods of emotional stress, lesions appear in the oral and vaginal mucous membranes, disappearing under drug treatment (corticoid and retinoid therapy) and after the stress period.



**Figure 1.** *Inframammary region of the female patient with Darier-White disease.*



**Figure 2.** *Scaly lesion in the scalp of the female patient with Darier-White disease.*



**Figure 3.** *Longitudinal streaks with V-shaped notches in the female patient with Darier-White disease.*

A biopsy was conducted near the jaw angle. The histopathological exam showed hyperkeratotic epidermis, parakeratosis columns, granular aspect of the corneal layer, rounded bodies, predominantly suprabasilar acantholytic cleft, and the presence of perivascular lymphocytes in the superior reticular dermis.

The patient currently shows an 80% improvement of the lesions with the use of oral acitretin combined with topical corticotherapy, with rotation of the acitretin use periods, mainly to preserve liver function. Occasionally, in disease relapse periods, mainly in the summer, the patient develops bacterial infections that are managed with the use of oral antibiotic therapy and hygiene of the infected lesion sites.

In the disease history, she denied any comorbidities and identified similar conditions running in the family, including maternal grandfather, mother (Figure 4), aunt, and later, a cousin. The genogram of the family studies is shown in Figure 5. The first notice of the genetic disorder in the family history is Patient 0. This person has transmitted the pathology to two of his three daughters, propagating the disease through to the subsequent generations. The dominance previously described in the literature was identified, and the healthy children of a couple in which one of them has the disease did not transmit the pathology to the next generation. No specific cause for the onset of the disease was identified.



Figure 4. Lesions covering the ear cartilage entering the concha in the female patient's mother.

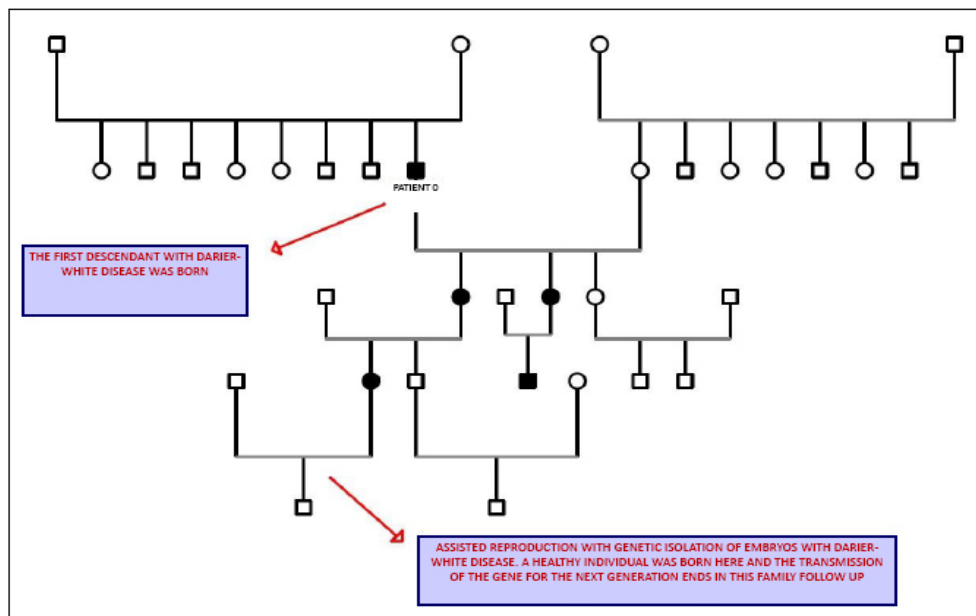


Figure 5. Genogram illustrating the onset and progression of the Darier-White within the same family through five generations. It shows the onset from Patient 0 until the disease withhold with isolation of the embryos through preimplantation genetic screening and subsequent assisted reproduction.

As the patient wanted to get pregnant without transmitting the disease to her descendants, her ATP2A2 gene was fully sequenced for Darier-White disease. The patient's peripheral blood was collected in EDTA tubes and sent to the Igenomix® laboratory to extract genomic DNA, prepare DNA libraries and conduct the NGS-based sequencing. Clinical interpretation based on comparative data analysis using HGMD® Professional, Online Mendelian Inheritance in Man® (OMIM®), Pubmed and other databases identified an undescribed splicing variant in heterozygosis,

identified as NM170665.3: c.2607+1\_2607+2insT and classified as a variant of unknown significance (VOUS). To find out whether this variant is also causing Darier-White disease together with the other variants as already described in medical literature, a segregation PCR study of the patient's family members, both affected and healthy, was subsequently conducted by the same laboratory using the peripheral blood samples of relatives of the patient, as follows: mother, maternal aunt, and maternal cousin, all presenting the same symptomatology for Darier-White disease. The

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patient's father, brother and another maternal cousin, none of whom show symptoms of the disease, were also genotyped. The screening technique analyzed people both healthy and ill, to avoid any potential doubt related to the discovery of this previously undescribed spliced VOUS. The study segregated all individuals affected by the disease, while in none of the asymptomatic individuals, including the patient's brother, was the VOUS found. Thus, this mutation has a putative role in the Darier-White disease.

The identification and confirmation of this VOUS allowed us to hamper the propagation of Darier-White disease to the offspring using the preimplantation genetic diagnosis (PGD) for monogenic diseases. Eleven ova were collected from the Darier-White disease patient, fertilized and divided into four cycles over three years. The period was reasonably long because the cost of all exams, drugs, and procedures was borne using the patient's resources; due to this, she paused the process a few times and kept the eggs frozen. The eleven ovules were fertilized with her husband's sperm; her husband does not have the disease.

The embryos were then submitted to a biopsy in the blastocyst stage and analyzed for this disease, following vitrification.

The genetic analysis of blastocysts' biopsies by Igenomix® using a PCR probe designed for the identified VOUS showed that eight of the eleven embryos fertilized harbored the mutation. The genome of the three remaining embryos did not have this VOUS; however, two failed the preimplantation genetic screening (PGS) because they were identified as aneuploids. Therefore, only one male embryo eligible for implementation remained for this couple.

For this patient, who was subjected to a process of assisted reproduction in order to block the transmission of the disease, we noticed that the disease dominance in the embryos' biopsies was of 72.7%, highly exceeding the expected rate of 50%.

On 12 July 2019, in São Paulo, Brazil, the patient gave birth to a healthy baby boy who does not have the disease.

### CONCLUSION

Darier-White disease is a serious genetic disease with an autosomal dominant inheritance pattern, complete penetrance and variable expressivity in adults, appearing in different degrees of severity,

which could cause considerable physical, mental, and social prejudice. The transmission of this monogenic disease to future generations can be hampered with PGD, PGS, and assisted reproduction. Combined with a genogram, these techniques enabled us to track a new genetic mutation causative of the Darier-White disease and select healthy embryos to make sure that the mutated cell lineage could be controlled in this family, and, thus, prevented in their subsequent offspring. One of the great advances of personalized medicine is the possibility of avoiding incurable diseases, which has successfully been achieved in this case.

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