Identification of *POLA1* gene deep intronic mutation confirms diagnosis of X-linked reticulate pigmentary disorder in a Chinese patient

Dear Editor,

X-linked reticulate pigmentary disorder (XLPDR, OMIM#301220) is a rare disease characterised by diffuse reticulate dyschromatosis, distinctive facial features, ectodermal dysplasia, and immune dysregulation. In 2016, *POLA1* was identified as the causative gene for XLPDR.[1] *POLA1* encodes the catalytic subunit of DNA polymerase-alpha, crucial for DNA replication.

A 24-year-old Chinese male presented with characteristic facial features and recurrent respiratory infections since six months of age, which improved after the age of 10. Hypopigmented macules appeared when he was six years old, and by 13 years of age, he began to experience vision loss, strabismus, and photophobia, which progressed to corneal scarring. He also had hypohidrosis affecting his head, face, and neck since birth with normal growth and mental development.

In 2015, the patient was clinically diagnosed with XLPDR after a dermatological examination that revealed generalised dark hyperpigmentation with reticular patterns and hypopigmented macules on the face and extremities [Figures 1a and 1b]. Routine laboratory tests, including blood, biochemical, and immunological tests, were normal. Ocular examination revealed corneal scarring, corneal edema on fundus photography and thickened corneal epithelial layers with scarring on optical coherence tomography [Figures 2a, 2b, and 2c]. Skin histopathology indicated hyperkeratosis

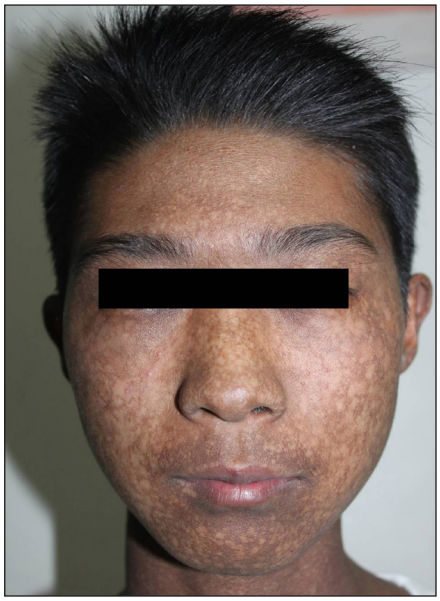

Figure 1a: Upswept hair, flared eyebrows, reticulate hyperpigmentation, and hypopigmentation on the face.

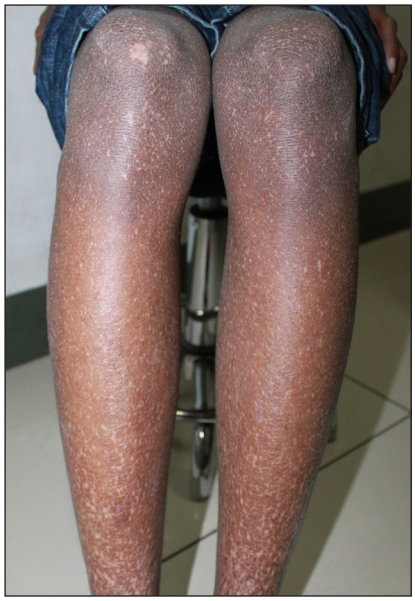

Figure 1b: Reticulate hyperpigmentation and hypopigmented patches on lower limbs.

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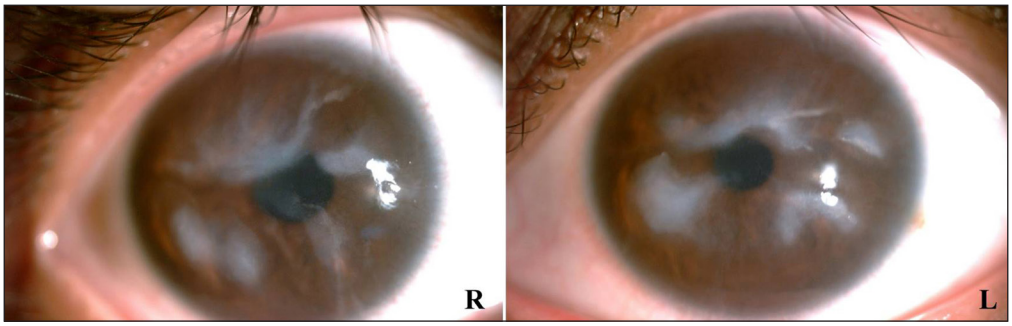

Figure 2a: Anterior segment examination at slit-lamp exhibiting corneal opacities.

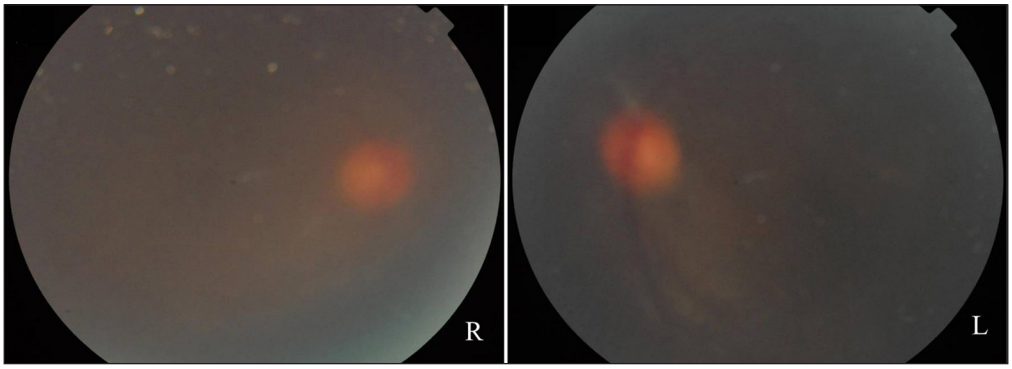

Figure 2b: Fundus photography showing a blurred fundus with a faintly visible normal optic disc.

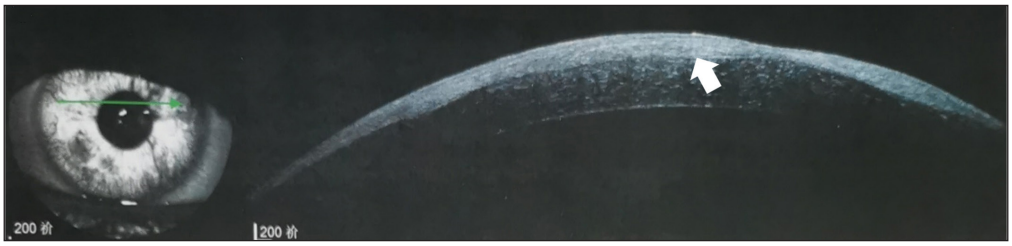

Figure 2c: Optical coherence tomography revealing oedematous thickening of the cornea (arrow), involving the corneal epithelial layer and scarring.

of the epidermis and increased melanin granules in the keratinocytes of both the basal and spinous cell layers [Figure 3a]. Upon follow-up in 2023, there were no significant changes in skin or ocular symptoms, hypohidrosis, or immune profile.

Whole-exome sequencing (WES) in 2015 did not identify any pathogenic variants. However, a follow-up reanalysis in 2023 using Sanger sequencing identified a previously reported deep intronic variant, c.1393-354A>G, in *POLA1*, which was inherited from the patient's phenotypically normal mother [Figure 3b]. Notably, this deep intronic variant is classified as a pathogenic variant according to American College of Medical Genetics and Genomics (ACMG) guidelines, and it is the only known variant causing XLPDR.

XLPDR is an exceptionally rare inherited pigmentary condition. Initially, Petro Starokadomskyy *et al.* identified the *POLA1* gene variant c.1393-354A>G as the genetic cause of XLPDR in affected patients[1]. To date, approximately 30 cases have been reported but only seven of them had detailed clinical and genetic information documented. The clinical manifestations of these seven patients are summarised in Table 1. Functional experiments demonstrated that this deep intronic variant introduces a new exon, leading to a decrease in the expression level of the POLA1 protein, diminishing cytosolic RNA: DNA hybrids and further activating the transcription of the type I interferon gene. The type I interferon receptor amplifies signals through the JAK/STAT signalling pathway, triggering lymphocytes to produce excessive cytokines and inflammatory factors, resulting in a series of clinical symptoms.[2]

Pigmentary abnormalities in XLPDR typically appear between the ages of four months and five years.[3] However, our patient did not exhibit skin lesions in infancy. Hypopigmented spots emerged at six years old, progressing to reticular hyperpigmentation, which is an atypical presentation not previously reported. Throughout the two visits, the patient's generalised skin reticular pigment abnormality showed no significant worsening over an eight-year period. Currently,

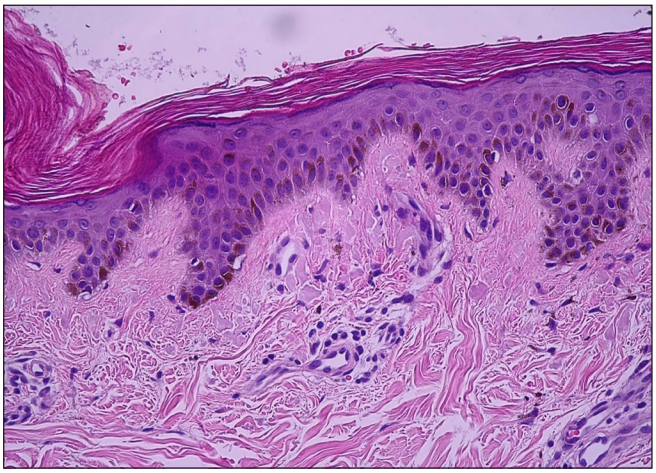

Figure 3a: Mild epidermolytic hyperkeratosis, basal layer hyperpigmentation, and scattered melanophages in the dermal papillae (Haematoxylin and eosin, 200x).

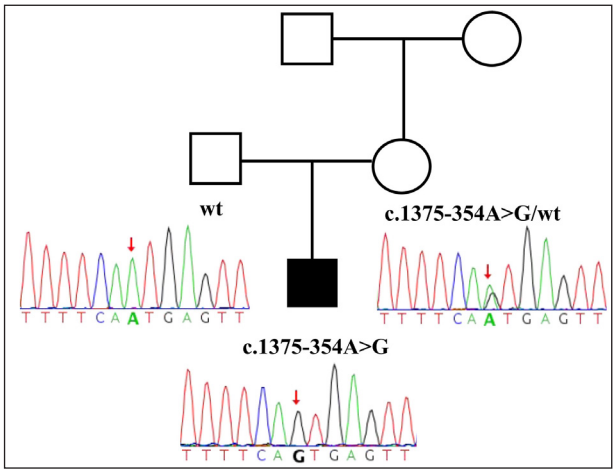

Figure 3b: Sanger sequencing results of the patient and his parents. The red arrow indicates the position of the mutation in the sequence. (R: right; L: left. A: Adenosine, C: Cytosine, T: Thymine, G: Guanine).

Table 1: Clinical manifestations of all XLPDR patients with *POLA1* mutation and detailed phenotype information

Patient	1	2	3	4	5	6	7
Reference	Starokadomskyy P *et al*.	Xu Z *et al*.	Légeret C *et al*.	Zhao YK *et al*.	Li XY *et al*.	Li MWY *et al*.	Present case
Origin	American	Chinese	Caucasian	Chinese	Chinese	Vietnamese	Chinese
Year of publication	2017	2019	2021	2022	2023	2024	N/A
Pubmed ID	28407217	30714101	32989594	35645674	37851432	38165470	N/A
Variant	c.1393-354A>G	c.1393-354A>G	c.1393-354A>G	c.1393-354A>G	c.1393-354A>G	c.1393-354A>G	c.1393-354A>G
Method of identification	N/A	N/A	Targeted sequencing	Exome sequencing	Genome sequencing	Genome sequencing	Sanger sequencing
Sex	Male	Male	Male	Male	Male	Male	Male
Age	13y	26y	12y	4y	7y	9y	24y
Corneal scar	N/A	N/A	N/A	N/A	N/A	Yes	Yes
Photophobia	Yes (7y)	N/A	N/A	Yes (3m)	Yes	Yes	Yes
Skin Hyperpigmentation	Yes (1y)	Yes (early childhood)	Yes	Yes (6m)	Yes (1y)	Yes (8m)	Yes
Hypohidrosis	Yes	Yes (early childhood)	N/A	Yes	Yes	Yes	Yes
Upswept frontal hairline	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Flared eyebrows	Yes	Yes	Yes	Yes	N/A	Yes	Yes
Respiratory System Infections	Yes (1y)	Yes (childhood)	Yes (18m)	No	Yes	Yes	Yes
Gastrointestinal Issues	Yes (1y)	N/A	Yes (18m)	N/A	Yes	Yes	No
Urogenital involvement	Yes (1y)	N/A	No	N/A	N/A	No	No

N/A: Not available; y: year; m: month; XLPDR: X-linked reticulate pigmentary disorder.
The onset time of the symptoms is indicated in parentheses.

all patients with XLPDR have skin pigmentation. The *POLA1* gene is mainly involved in DNA replication and is significantly expressed in skin melanocytes. However, there are few studies on how the *POLA1* gene affects the function of melanocytes and the mechanism of melanin production, and further research is needed to clarify this.

Initially, whole-exome sequencing in 2015 failed to identify a causative variant for our patient, relying solely on clinical assessment for diagnosis. Subsequent Sanger sequencing, performed several years later, confirmed the presence of the *POLA1* variant c.1393-354A>G, which had been previously reported in other XLPDR patients. Direct detection of this variant proves to be a more effective and cost-efficient approach for individuals with XLPDR. Our experience underscores the importance of reanalysis for cases with initially negative results in genetic diagnosis. Currently, over half of patients with rare genetic disease remain undiagnosed after WES testing, partly due to the limited scope of the targeted regions in exome sequencing.[4] Similarly, the pathogenic deep intronic variant in *POLA1* was identified

only through whole-genome sequencing (WGS) years after clinical diagnosis.[1] Furthermore, with the increasing number of individuals undergoing genetic testing, approximately 300 or more new gene–disease associations are discovered annually.[5] Therefore, reanalysis of patients with negative genetic diagnoses every two to five years has a high potential for uncovering new genetic etiologies.[6,7]

Currently, there is no effective treatment for XLPDR. However, our patient exhibited well-controlled lesions with no evident progression and were observed with strict sun protection. Prompt consultation with a doctor is essential for any eye symptoms to prevent irreversible corneal scarring due to recurrent keratitis. Basic supportive treatment is essential for managing multisystemic involvements, and it is noted that multisystemic infections may naturally improve with age.

In conclusion, our study underscores the importance of reanalysis and the role of deep intronic variants in rare genetic disorders like XLPDR, contributing valuable insights to the growing understanding of how such genetic variations can be overlooked in initial diagnostic attempts.

Ethical approval: The research/study was approved by the Institutional Review Board No.: TREC2023-KYS088; dated 2023.02.24 at the Ethics Committee of Beijing Tongren Hospital, Capital Medical University.

Declaration of patient consent: The authors certify that they have obtained all appropriate patient consent.

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