CASE REPORT

An uncommon case of retinitis pigmentosa patients based on clinical and genetic study

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SUMMARY

Inherited retinal dystrophy (IRD) is a group of phenotypes caused by mutations in visual pathways-related genes, mostly occurring at photoreceptors. This heterogeneous group includes retinitis pigmentosa (RP) recognised by bone spicule at the peripheral retina and the other is Stargardt with macular pisiform flecks. In this study, a 20-year-old male patient with RP symptoms was accompanied by a yellowish pisiform flex in the macula. However, his brother, mother and aunty have typical Stargardt disease. This study involved four persons, two males (cases 1 and 2), their mother (case 3) and aunt (case 4). Initially, cases 1 and 2 came to the clinic, case 1 was diagnosed as RP and macular dystrophy, and case 2 was diagnosed as Stargardt disease. On the follow-up, cases 1 and 2 as well as their father, mother and other family members underwent comprehensive eye examination, including fundus, Snellen, OCT, OCT-A and HFA, and found an uncommon macular abnormality besides typical RP appearance in case 1. The father is healthy while the mother and one of his aunties were diagnosed as Stargardt. A genetics analysis was conducted in case 1, finding various mutations associated with IRD mutation at the cone protein-encoded gene that concentrated at the central and rod protein-encoded gene concentrated at the peripheral retina. Whether the combination of multiple or the same mutations is responsible for this RP phenotype needs further analysis and validation. Cases 2 and 3 genetic analysis showed similar mutation results but with a healthy peripheral retina and only represented Stargardt. Case 1 is considered as RP with macular dystrophy, while cases 2, 3 and 4 are confirmed as Stargardt.

INTRODUCTION

Inherited retinal dystrophy (IRD) is a group of diseases with heterogeneous manifestations and genetic backgrounds. So far, 281 genes have been associated with IRD (https://web.sph.uth.edu/RetNet/home.htm). The damaged retinal cells compromise the patient's visual field partially or completely. At least 20 IRD sub-types were identified, including retinitis pigmentosa (RP), Stargardt, rod-cone dystrophy (RCD), Leber Congenital Amaurosis (LCA), etc.[1]

The clinical features of IRD can be varied among individuals. The key features of each type of IRD are unique. RP is recognised as a condition characterised by arteriolar attenuation, retinal pigmentary changes (hypopigmentation/hyperpigmentation of bone-spicule and pigment clumping) and waxy disc pallor caused mostly by mutation of *USH2A* or *RHO*. Patients with Stargardt disease present pigment mottling, frank macular atrophy, a bull's eye maculopathy and fundus flecks, which are mostly caused by *ABCA4* mutation.[2]

These genetically heterogeneous retinal dystrophies present significant challenges in predicting the causative mutation since mutations can be expected in any of 8 to 61 genes. The high resolving power of whole exome sequencing (WES) solves almost all IRD cases.[3] The remaining unsolved cases are suggested to undergo further genetic analysis, such as whole genome sequencing (WGS) or multiplex ligation-dependent probe amplification (MLPA). The *ABCA4* mutation is well established causing the Stargardt phenotype[4] and limited RP.[5] *ABCA4* mutation is found in around 1 to 250,000 RP cases and causes an RP subtype 19 (RP19; MIM: 601718).6

CASE PRESENTATION

This study involved a family consisting of four persons, two male sons (cases 1 and 2), their mother (case 3) and aunt (case 4). Initially, a 20-year-old male (case 1) visited the eye clinic at the Sardjto General Hospital for evaluation, with a referring diagnosis of hereditary eye disease based on findings seen during a fundus examination. Case 1 suffered from tunnel vision and blurry night vision began 13 years ago. Case 2, a 27-year-old male, the brother of case 1 had blurry vision which was not corrected with an eyeglass and inconveniences under bright light starting from 14 years ago. Case 2 also underwent a fundus examination after finding that case 1 was RP. In further examination of those patients and their family members found that case 1 was confirmed as RP and case 2, his mother (case 3) and aunt (case 4) were confirmed as Stargardt. These patients have a family history of inherited retinal dystrophy shown in their pedigree, which implies that the traits are carried from the maternal lineage. At the first eye evaluation, case 1 visual acuity was best

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Table I: The summary of the clinical assessment of IRD patients' family in this research

Parameters	Case 1, son	Case 2, son	Case 3, mother	Case 4, aunt	father	Aunt
Diagnosis	RP-macular dystrophy	Stargardt	Stargardt	Stargardt	Healthy	Healthy
Sex	Male	Male	Female	Female	Male	Female
Symptoms	Blurry vision, inconvenience at bright light	Blurry vision, inconvenience at bright light	Blurry vision	Blurry vision	Healthy	Healthy
Quality of life	Disturbed	Amenable	Amenable	Amenable	normal	normal
ETDRS	1/60 1/60	6/60 6/60	1/60 1/60	1/60 1/60	normal	6/6 6/6
Macula	Pisiform fleck	Pisiform fleck	Pisiform fleck	Pisiform fleck	Healthy	Healthy
Peripheral	Bone spicule	Clear	Clear	Clear	Clear	Clear
Possible causative mutations	SLC7A14, RHO, ABCA4	ABCA4	ABCA4	-	-	

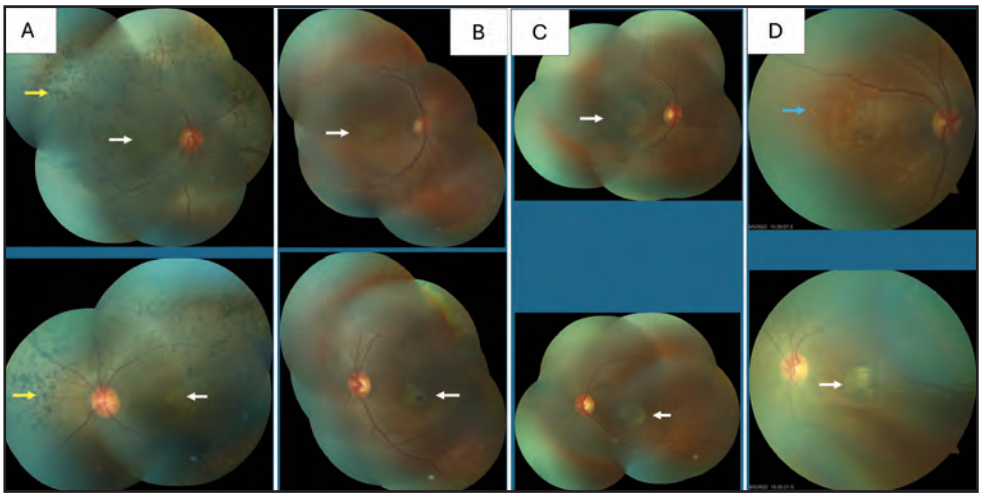

Fig. 1: The fundus image of the studied family. (A) Fundus with pisiform flecks across the macula (white arrow), in both eyes, the patient had a bone spicule in the periphery retina (yellow arrow). (B to C) Stargardt patient, brother (case 2), mother (case 3) has pisiform flecks in both eyes, and (D) aunty (case 4) has lipofuscin accumulation in the macula right eye (blue arrow) and pisiform fleck in the left eye (white arrow) with healthy peripheral retina.

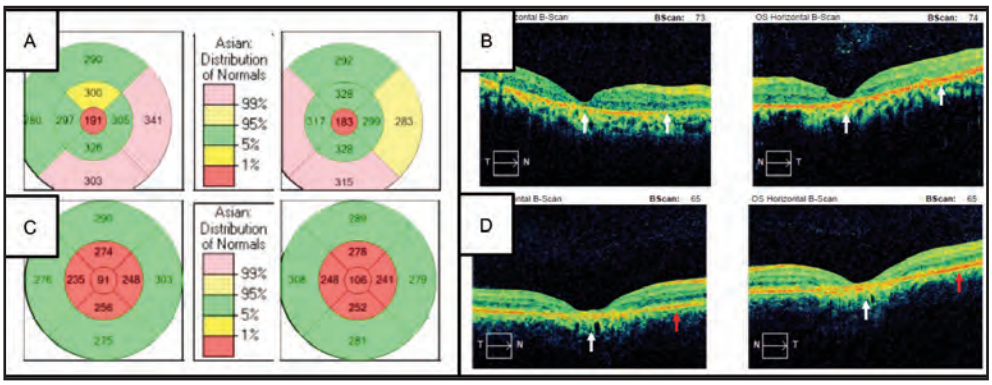

Fig. 2: The OCT image shows the abnormality of the macula as well as the periphery retina of the retinitis pigmentosa-macular dystrophy in case 1. (A) The central red circle indicates a central macular thickness (CMT) which is thiner than normal and (B) white arrow indicates a disturbed photoreceptor layer across the retina. The OCT image shows the abnormality of the macula of Stargardt in case 2. (C) The central and surrounding red circles indicated a central macular thickness (CMT) which thinner than normal and (D) The white arrow indicated disturbed while the red arrow indicated normal photoreceptor layer.

corrected to Snellen 1/60 in both eyes. Anterior segment examination was unremarkable. Fundus and OCT tests initially suggest that case 1 was RP with macular dystrophy. At the follow-up, all subjects underwent comprehensive ophthalmic tests at Sardjito General Hospital. During examination, blood samples and baseline data were also collected. The ophthalmic test was initiated with dilation using topical tropicamide (1%) and phenylephrine hydrochloride (2.5%). The imaging studies were conducted using wide-angle colour fundus image (serial no.: 1103524, VISUCAM NM/FA, Zeiss), optical coherence tomography (Serial No.: 5000-21320, Cirrus 5000, Zeiss) and Humphrey field analyser 3 (HFA) (Serial No.: 860-18955, HFA 3, Zeiss). DNA was isolated from blood samples using QIAamp DNA Mini Kit (Qiagen; Cat.No.: 51306). The library was prepared according to the Illumina protocol. The library was sequenced using Nextseq 550.

Case 1 had a macular abnormality. Dilated fundus examination revealed a pattern of yellow pisiform flecks across the macula, sparing the juxta-papillary region in both eyes. The foveal region exhibited a hyperpigmented appearance and bone spicule in the peripheral region (Figure 1A). Case 2 also showed pisiform fleck sparing the juxta-papillary region in both eyes. The foveal region exhibited a hyperpigmented appearance while the periphery is healthy (Figure 1B).

Case 3 was 53-years-old. She was adapted to her condition and invited to the hospital after her two sons (case 1 and 2) were diagnosed with IRD. She was diagnosed as Stargardt. Her visual acuity was best corrected to Snellen 1/60 right (RE) and 0.5/60 left eye (LE) (Table I). Dilated fundus examination revealed a pattern of yellow pisiform flecks across the macula, sparing the juxta-papillary region in both eyes (Figure 1C). The foveal region exhibited a hyperpigmented appearance. Case 4 was 47-years-old and adapted to her condition; she was invited to the hospital after her two nieces (case 1 and 2) who were diagnosed with IRD. She was diagnosed as Stargardt. Her visual acuity was best corrected to Snellen 1/60 in both eyes (Table I). Dilated fundus examination revealed a pattern of pisiform and yellow flecks across the macula's right eye with a pisiform fleck in the left eye (Figure 1D).

Another aunt of cases 1 and 2 was healthy. No signs of abnormalities were found during her fundus examination. After finishing the eye examination of all family members, they get an education regarding the conditions. The affected member was educated to avoid direct sunlight and received a vitamin. Further follow-up will be conducted to reassess their condition the disease progression.

The second assessment uses The OCT scan for the right (RE) and left eye (LE) of cases 1 and 2. The analysis shows a low CMT score in both eyes (RE: 191 and LE: 183), signifying a decrease in foveal thickness. Inferior and Nasal of RE and LE underwent thickening (Figure 2A). The OCT in case 2 shows an even wider decrease in foveal thickness (CMT RE: 91 and LE: 106) and parafovea (274 and 256) perifovea (235 248) (Figure 2C).

Irregular photoreceptor form was seen in the case 1 (Figure2B). For case 2, irregular in the central while the periphery is normal. Specifically, it shows an irregular photoreceptor form, and the inner foveal pitch is gone at the macula (Figure 2D).

Interestingly, the visual field analysis showed a significant visual field defect, especially in the inferior-temporal region in both the right and left eyes of case 1. The inferior-temporal region undergoes constriction (Figure 3A and B). In case 2, the perimetry analysis unfortunately showed a high rate of fixation losses. However, it showed a defect in almost all peripheral fields, but mostly in the inferior and nasal regions.

The genetic analysis using whole exome sequencing was performed to elucidate the causative mutation of the RP case. After sorting, around 200 variants associated with IRD were found. Then, further filtering was conducted to exclude variants not associated with the RP and Stargardt.

Notably, ABCA4, USH2A, FLVCR1, SLC7A14, RP and RHO were reported to cause IRD cases in the population. RHO was associated with autosomal dominant RP. The found mutation was heterozygote but not in the regulatory region. The FLVCR1 (Feline Leukaemia Virus Subgroup C Receptor 1) gene encodes a protein that exports heme and regulates intracellular heme concentration. This gene was reported with syndromic RP.

The SLC7A14 is responsible for cationic amino acid transporter. Mutations in SLC7A14 were reported to cause autosomal recessive RP in Chinese patients.[7] Knockdown of slc7a14 in zebrafish results in peripheral photoreceptor defects, which are most likely from the rod cell.[8] A Slc7a14 knockout in mice led to a thinning retinal layer and compromised electroretinography (ERG).[7] CRISPR–Cas9–mediated knock in p.Gly330Arg mice not only shows a thinning retinal layer but also auditory impairment.[9] The genetic analysis of case 2 and 3 was then filter out other mutations besides *ABCA4*. The exact causative mutations should be interpreted carefully in the future.

DISCUSSION

After conducting clinical and genetics assessments of three IRD cases, the results showed interesting findings. Fundus examination of case 1 showed three main features of RP including bone spicule, attenuate vascular and pallor optic disc with a yellowish fleck in the macula. OCT scan showed a decrease in foveal thickness in both eyes. Visual field tests also found defects, especially in the inferotemporal aspect. His brother (case 2), mother (case 3) and aunt (case 4) had Stargardt's main feature and pisiform flex. Genetic tests of case 1 showed multiple mutations at both RP (SLC7A14, RHO) and Stargardt (ABCA4) pathological gene. Cases 2 and 3 had many mutations in Stargardt i.e. ABCA4 and EVOL4. The mode of inheritance of IRD needs to be studied further. The mother and aunty do not have an RP feature. The RP in case 1 was most possibly in recessive form. The case 1 most likely resulted from genetic interplay between SLC7A14 and ABCA4, with missense mutations at ABCA4 found together with heterozygote SLC7A14.

The family history plays a part in this case. First, the vision impairment of the mother and aunt was not treated and neglected then they were unaware of sibling vulnerability of having the same diagnosis. This results in a more severe condition in the case 1 and 2. A serious care such as avoiding direct sunlight and vitamin A supplementation might postpone the onset of IRD condition. This care also halts the progression of IRD.

Special attention was given to case 1 because several IRD cases might exhibit similar late-stage phenotypes, with features such as severe retinal cell death, extensive atrophy of the retina, and irreversible visual loss. However, RP and Stargardt2 had two differentiated diagnoses and pathways. Peripheral visual loss and inconvenience under bright light are most likely due to rod cell death in the rear retina since the rod was more abundant in the rear and the function of a dime light acceptor. Noticed RP cases with a shared genetic background with Stargardt should be interpreted cautiously. This case is most likely under recessive inheritance due to non-RP parents. The previous study also reports the different IRD subtypes expressed in the same offspring.[10]

CONCLUSION

In this study, an uncommon case of retinitis pigmentosa (RP) with macular lesions is described which is most likely in a recessive fashion. This family is affected by two subtypes of Inherited retinal dystrophy, RP and Stargardt.

ETHICS STATEMENT

The study was approved by the ethical committee of the Faculty of Medicine, Public Health, and Nurses, Universitas Gadjah Mada (No.: KE/FK/1315/EC/2021). The retinitis pigmentosa patient and family were examined at Sardjito General Hospital, Yogyakarta.

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