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H. 知的所有権の出願・登録状況

1．特許取得 7件

・「磁性ナノ微粒子を用いたバイオターゲティング」（16年国際特許申請中）

・「光干渉断層計による厚みのリング状表示、および眼底写真の重ね合わせ」（16年特許申請中）

・「遺伝子導入方法及び薬剤導入方法」
（現在公開中：公開番号・特開2001-37476）

・「水晶体細胞の作成方法、およびこの方法によって得られる水晶体細胞」
（特許出願：出願番号 特願2003-96002）
（PCT出願：国際出願番号 PCT/JP2004/003848）

・「Low Vision Evaluator」 (Patent No US6802608B1)

・「希少糖の生理活性作用の利用方法および希少糖を配合した組成物」
2003年5月22日特許出願
（整理番号：ＰＣＴ-03-Ｒ Ｓ01）

・その他1件

2．実用新案登録 1件

・硝子体手術用コンタクトレンズ補助リング（14年5月）

3．その他 なし

Ⅲ. 分担研究報告

1. 東北大学における網膜色素変性の実態調査～中間報告～

渡辺亜希[1)]、和田裕子[1)]、板橋俊孝[1)]、佐藤　肇[1)]、川村后幸[1)]、遠藤麻衣[1)]
多田麻子[1)]、玉井　信[1)]、大森　芳[2)]、辻　一郎[2)]
([1)]東北大、[2)]東北大公衆衛生学)

研究要旨　我々は、網膜色素変性の予後決定に関する因子を調査するため2004年4月から12月まで東北大学眼科外来を受診した網膜色素変性患者143名に臨床調査を行った。調査項目は、夜盲・視野狭窄・視力低下を自覚した時期、遺伝形式、白内障の有無、眼底所見、網膜電位図、ゴールドマン動的量的視野検査、薬物治療の有無を調査、心理状況を調べるためにＰＯＭＳ（Profile Of Mood States）を施行した。結果、初発症状は夜盲の自覚が先行し、常染色体優性遺伝患者が比較的早期に自覚をしていた。これは、遺伝形式から病気に対する知識の加味も考えられた。POMS（Profile Of Mood States）により患者のストレス・心理状況を調査してみると、網膜色素変性の患者では健常人に比べ鬱症状が強く活気が少ないことが判明した。また50歳代女性および60歳代男女では比較的情動の変化が少ないことがわかり、病名告知からの時期との関与も示唆された。

A．研究目的

網膜色素変性は表現型の多様性がみられる疾患である。その進展速度も遺伝形式、同一家系内でもさまざまである。我々は、病気の進行を促進する因子を解明していく一環として、予後決定因子に関する疫学研究および実態調査を施行した。今回はその中間報告を行う。

B．研究方法

2004年4月から11月までに東北大学眼科を受診し臨床調査に同意が得られた患者143人(男性68名、女性75名)、年齢13～77歳（平均44歳)。

上記対象に対し　夜盲・視野狭窄・視力低下を自覚した時期、遺伝形式、白内障の有無、眼底所見、網膜電位図、ゴールドマン動的量的視野検査、薬物治療の有無を調査、患者の気分・心理状況を調べるためにＰＯＭＳ（Profile Of Mood States）を施行した。[1)]

C．研究結果

遺伝形式別では、常染色体優性遺伝が42名（29.3%)、常染色体劣性遺伝19名（13.3%)、X染色体劣性遺伝2名（1%)、弧発例69名（48.2%）であった。夜盲を自覚している人は126名と全体の9割近くであり、この自覚時期の平均年齢は常染色体優性遺伝で18.7歳と最年少、常染色体劣性遺伝で20.6歳、弧発例で26.5歳であった。初診時に白内障を有したものは108眼（38%）であったが2004年には161眼（56%）であった。眼底所見では、骨小体様色素沈着を有する患者は初診時59名（41%）が2004年には123名（86%)、黄斑

浮腫は初診時8名（5.6%）が2004年には20名（14%）、視神経萎縮は初診時1眼（0.6%）が2004年では8眼（5.6%）であった。網膜電位図（ＥＲＧ）で初診時non recordableだったのは44名（30%）、ゴールドマン視野検査で正常範囲を示したのはＶ－4－ｅで32名（11.6%）、Ｉ－4－ｅで2名(0.7%)、Ｉ－2－ｅでは0名であった。[2)]

投薬状況は、ヘレニエン投与者44名(30%)、ビタミンAおよびビタミンAを含むサプリメント服用者は28名（19.6%）であった。

＜ＰＯＭＳ結果＞

全年齢層で、健常人よりDepressionの値が高く、19歳以下の女性を除いてVigorが低値であった。男性では特に20歳代のDepressionの値が高く、５０歳代のVigorの低下が顕著であった。女性では20歳代のAnger－Hostility、30歳代のConfusionが高値であり、50歳代女性および60歳以上男女では比較的健常人と並行する穏やかな波形となった。(図１、図2)

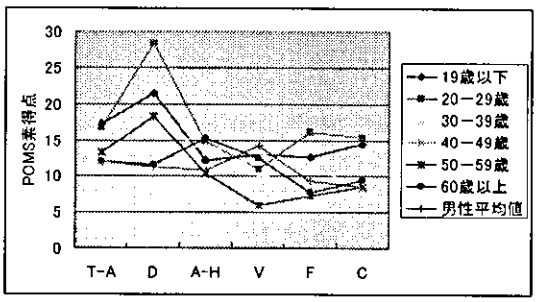

図１．男性年代別POMS結果

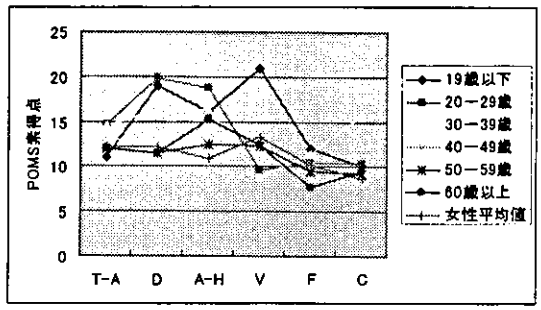

図２．女性年代別POMS結果

D．考察

実態調査の結果、初発症状は夜盲の自覚が先行し、さらに常染色体優性遺伝患者では比較的早期に夜盲を自覚していることが判明した。これは同じ病気の近親者がいることで、網膜色素変性に対する知識が関与していることが示唆された。視力の低下自体は個々様々であり、遺伝形式ごとの有意差を見出すことはできなかった。

薬物療法に関しては、当科では経過観察のみを施行している例が半数以上であった。POMSの結果、網膜色素変性の患者全般に鬱傾向が強いと言えるが、治療法のない進行性の病気であることから、病名告知から日が浅い若年患者や鬱世代である壮年男性に顕著に現れたと言える。50歳代女性および60歳代男女の波形は比較的穏やかであったが、病名告知から年月を経ることで、残された視機能に対して受容の境地が勝っているとも考えられた。

F．健康危険情報

なし

G．研究発表

1．論文発表

なし

２．学会発表

なし

Ｈ．知的財産権の出願・登録状況

１．特許取得

なし

２．実用新案登録

なし

３．その他

なし

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2. 遺伝性網膜変性疾患の遺伝子解析と遺伝子異常をもつ症例の臨床像の検討　～東北大学におけるまとめ～

和田裕子、板橋俊隆、佐藤　肇、川村后幸、多田麻子、遠藤麻衣、玉井　信
（東北大）

研究要旨　1990 年に、ロドプシン遺伝子の点突然変異が常染色体優性網膜色素変性を起こす事が報告されて以来、遺伝性網膜変性疾患に関して、遺伝子異常と臨床像の報告が国内外から多数行われている。我々は 1999 年より、東北大学眼科で登録されている遺伝性網膜変性疾患患者に対して遺伝子解析を施行し、遺伝子変異と臨床像について臨床眼科”眼と遺伝病“で報告してきた。今回は 6 年間の遺伝子解析の結果と臨床像について報告する。遺伝性網膜変性疾患 541 家系を対象とし、各疾患ごとに原因遺伝子をスクリーニングした。常染色体劣性網膜色素変性、Leber 先天盲、Usher 症候群に関しては原因遺伝子変異を確認できたのは 10%以下であった。一方、若年性網膜分離症、眼底白点症、クリスタリン網膜症、小口病、ノリエ病に関してはほぼ 100%に変異を確認し、遺伝子診断が有用である事が判明した。さらにクリスタリン網膜症、小口病、眼底白点症に関しては日本人高頻度変異が存在するために、遺伝子解析へのアプローチが容易であると考えた。網膜色素変性へ遺伝的異質性に富み、日本人の主要な原因遺伝子解明のため、連鎖解析を用いたアプローチが必要になると考えられた。

A．研究目的

網膜色素変性をはじめとする遺伝性網膜変性疾患に対して遺伝子解析が国内外で施行され遺伝子変異と臨床像が蓄積されている。我々は、1999 年 9 月より現在に至るまで、臨床眼科　“眼と遺伝病“で遺伝子解析結果と、臨床像の報告を行ってきた。今回の研究目的は、我々が施行した遺伝子解析のまとめを示し、遺伝性網膜変性疾患における遺伝子解析の意義と遺伝子変異が引き起こす臨床像について詳細に検討する事である。

B．研究方法

東北大学眼科を受診し、遺伝子解析に同意が得られた遺伝性網膜変性疾患 541 家系を対象とした。（図１）

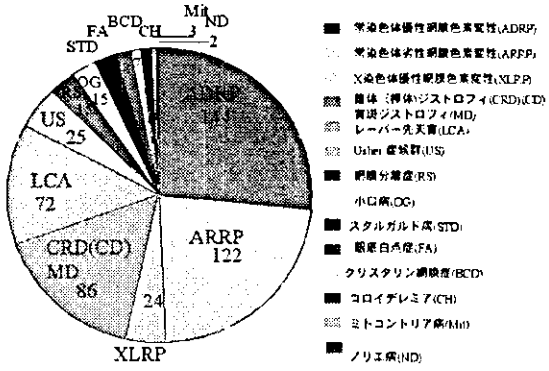

図１　遺伝子解析をおこなった症例数

症例の末梢血より DNA を抽出し、Rhodopsin,, Peripherin/RDS, FSCN2, RP1, HPRP3, PRPF31, NRL, PRPC8, CA4, IMPDH1, RPGR, RP2, CRX, RPE65, GUCY2D, AIPL1, RDH12, CYP4V2, ABCA4, RS1, ND, Rep-1, Arrestin, RDH5, MERTK, の全ての

エクソンについてプライマーをデザインし、PCR-Direct sequence 法を用いてスクリーニングを施行した。

図２　対象とした遺伝子

C．研究結果

網膜分離症、小口病、眼底白点症、クリスタリン網膜症に関しては、ほぼ 100%に原因遺伝子変異を確認した、一方、網膜色素変性、錐体桿体ジストロフィ、Usher 症候群、Leber 先天盲の症例では原因遺伝子変異を確認できない症例が大多数であった。また FSCN2, RDH5, HPRP3, IMPDH1, CYP4V2, Arrestin 遺伝子は日本人患者の変異のホットスポットが存在した。[1)-6)]

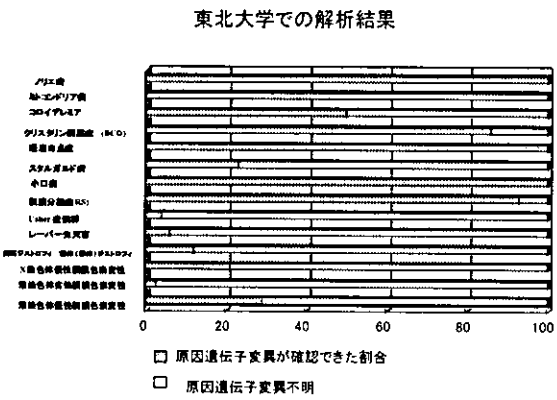

図３　遺伝子解析の結果

D．考察

網膜色素変性は、遺伝的異質性に富み、遺伝子変異から臨床像を、臨床像から遺伝子変異を端的に解明する事は不可能である。さらに海外の報告と遺伝子変異の種類、頻度は日本人患者では大きな違いが認められる。

眼底白点症、小口病、クリスタリン網膜症、網膜分離症では遺伝子解析を行う事で診断が確定できる。今後は、未知の原因遺伝子を解明するために日本人患者における連鎖解析が必要になると思われる。

E．結論

網膜色素変性の症例では遺伝子変異の解明は低頻度であった。今後は日本の多施設で各疾患を分類して効率的にスクリーニングを行う事が必要になると思われる。

F．健康危険情報

なし

G．研究発表

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Ｈ．知的財産権の出願・登録状況

１．特許取得

なし

２．実用新案登録

なし

３．その他

なし

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century to treat urinary tract infections and is currently recommended for the treatment and prophylaxis of cystitis.[1,2] Uva ursi contains arbutin, which is cleaved and conjugated to form hydroquinones, which are known inhibitors of tyrosine kinase and thus melanin synthesis. These agents are marketed in topical form as whitening creams, and uva ursi is known to cause depigmentation.[3] Tyrosine kinase is a ubiquitous enzyme whose structure and function is preserved in the retinal pigment epithelium and choroidal melanocytes. Since melanin is present in these ocular layers, ingestion of uva ursi may alter melanin metabolism with secondary visual effects.

This patient demonstrated a constellation of findings including a bull's-eye maculopathy, paracentral scotomas with a decrease in sensitivity, reduction in electroretinography (ERG) amplitude, and retinal thinning on OCT. Paracentral scotomas are an early manifestation of toxicity in patients with chloroquine and hydroxychloroquine toxicity.[4] In the early stages of these disorders the ERG can be normal, although patients can develop a decrease in ERG amplitude later accompanied by an increase in latency.[4] Use of a multifocal ERG can detect abnormalities at an early stage.[5] Since toxicity develops in the retinal pigment epithelium and outer retina, the OCT changes we observed are likely attributable to thinning of the outer retina although we cannot discern this directly because of limitation of instrument resolution.

While several cases of acute quinone toxicity have been documented, there have not been any case reports of ocular toxicity from chronic quinone use. Other drugs such as chloroquine and hydroxychloroquine, which differ in chemical structure from quinones, are known to cause bull's-eye maculopathy. The maculopathy described in this patient was suspected to result from ingestion of the herbal supplement uva ursi on the basis of its ability to inhibit melanin synthesis. It is important for ophthalmologists to obtain a complete history when evaluating patients with retinal dysfunction, including eliciting a history of herbal supplement use.

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Novel 2336-2337delCT Mutation in RP1 Gene in a Japanese Family With Autosomal Dominant Retinitis Pigmentosa

Miyuki Kawamura, MD, Yuko Wada, MD, Yoshihiro Noda, MD, Toshitaka Itabashi, MD, Soh-Ichiro Ogawa, MD, Hajime Sato, MD, Kenji Tanaka, MD, Tasturo Ishibashi, MD, and Makoto Tamai, MD

PURPOSE: To determine the frequency and kinds of mutations in the RP1 gene, and to characterize the clinical features of a Japanese family with autosomal dominant retinitis pigmentosa (ADRP) with a novel 2336 to 2337delCT mutation in the RP1 gene.

DESIGN: Case reports and results of DNA analysis.

METHODS: Mutational screening by direct sequencing was performed on 96 unrelated patients with ADRP. The clinical features were determined by complete ophthalmologic examinations.

RESULTS: A novel 2336 to 2337delCT mutation in the RP1 gene was identified in two patients from a Japanese family with ADRP. In addition, three families with ADRP carried a previously reported nonpathogenic Arg1933X mutation. The ophthalmic findings with a 2336 to 2337delCT mutation were similar to those of typical retinitis pigmentosa with rapid progression after age 40 years.

CONCLUSIONS: The most common Arg677X mutation in the white population was not found in the Japanese population; instead a novel mutation was found. (Am J Ophthalmol 2004;137:1137–1139. © 2004 by Elsevier Inc. All rights reserved.)

IN 1999, TWO GROUPS REPORTED ON A FOURTH GENE, THE RP1 gene, that was associated with autosomal dominant retinitis pigmentosa (ADRP), and that it was located on chromosome 8q12 with four exons.[1,2] This gene probably plays a role in the regulation of the retinal oxygen levels, and might be involved in photoreceptor metabolism and development.

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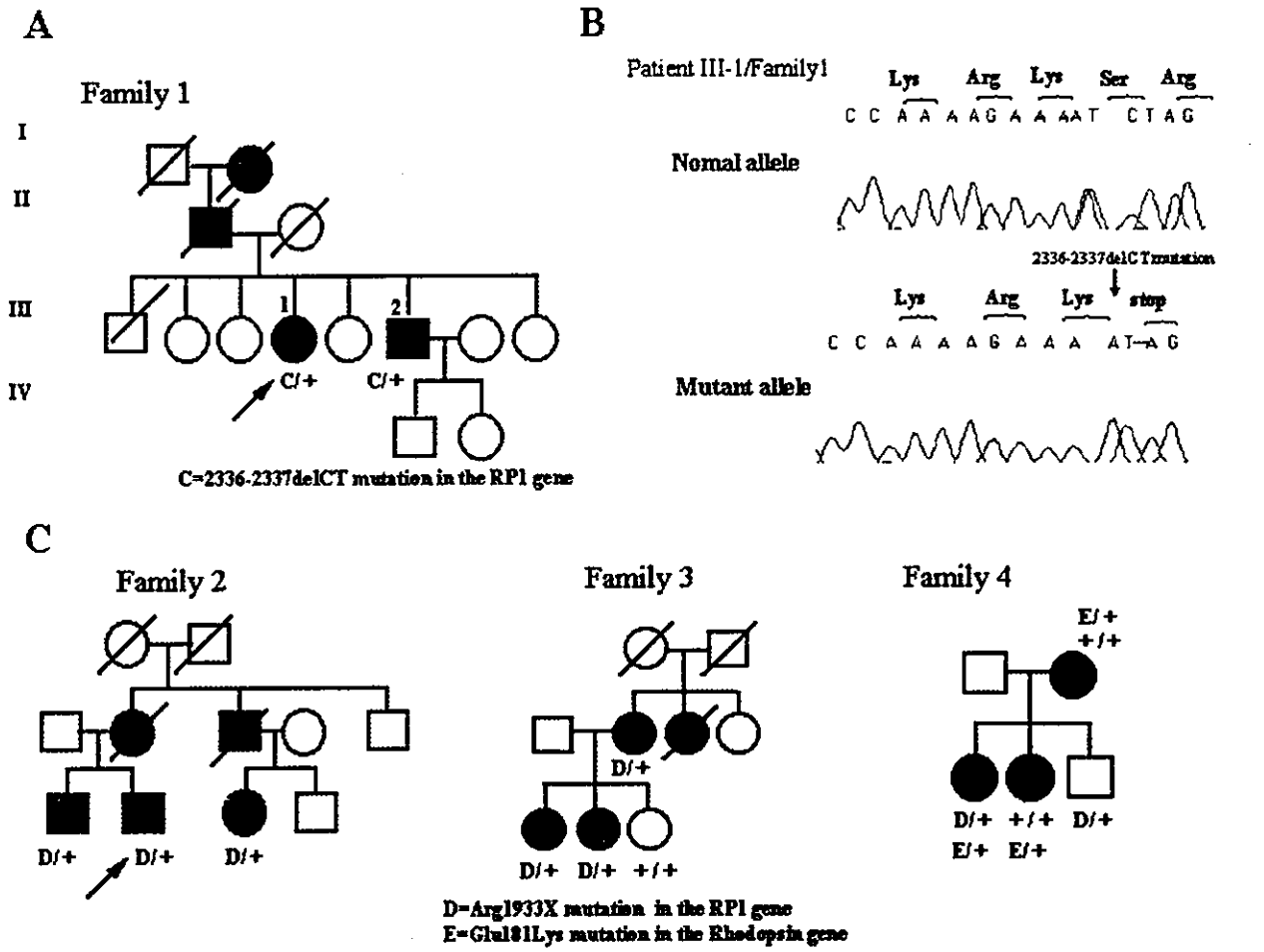

FIGURE 1. (A) Pedigree of a Japanese family with autosomal dominant retinitis pigmentosa associated with the 2336 to 2337delCT mutation in the RP1 gene. The affected (solid symbols) and unaffected (open symbols) members are shown. Arrow = proband; circles = female members; slash = deceased; squares = male members; X = individuals examined in this study; + = normal allele. (B) Results of nucleotide sequencing analysis. Family 1 has a 2336 to 2337delCT mutation. The upper sequence is the normal allele, and the lower sequence is the mutant allele from patient III-1. (C) Pedigrees of three Japanese families with Arg1933X mutation.

All of the reported mutations in the RP1 gene led to a premature termination, and most were located between codon 500 and 1053, especially within 663 to 777.[1-5] Among the pathogenic mutations, the most common mutation, Arg677X, was present in approximately 3% to 4% patients with ADRP in the United States, and this mutation is the third most common ADRP mutation.

To determine if mutations in the RP1 gene were present in the Japanese population, 96 genomic DNA samples from 96 unrelated patients with ADRP were screened. Informed consent was obtained from all patients after an explanation of the purpose of this study and the procedures to be performed.

Twenty-one sets of primer pairs were employed to cover the entire coding region in the RP1 gene for direct sequencing with an ABI sequencer (model 3100) (Applied Biosystems, Foster City, California, USA). The results of mutational screenings demonstrated that Japanese patients did not have the common Arg677X mutation; instead, two members from one family with ADRP were found to have a 2336 to 2337delCT mutation, and three unrelated families with ADRP had an Arg1933X mutation in the RP1 gene (Figure 1).

For the heterozygous 2336 to 2337delCT mutation, the amplified DNA fragments were subcloned using pGEM to confirm this mutation. Ten subcloned polymerase chain reaction products were then sequenced. The novel 2336 to 2337delCT mutation resulted in a frame shift and premature termination at the next codon.

The Arg1933X mutation was reported to be nonpathogenic in Chinese patients despite its stop codon, because it was detected in one normal control, a patient with Stargardt disease, and his two relatives.[5] In our cases, this mutation segregated with the phenotype from two unre-

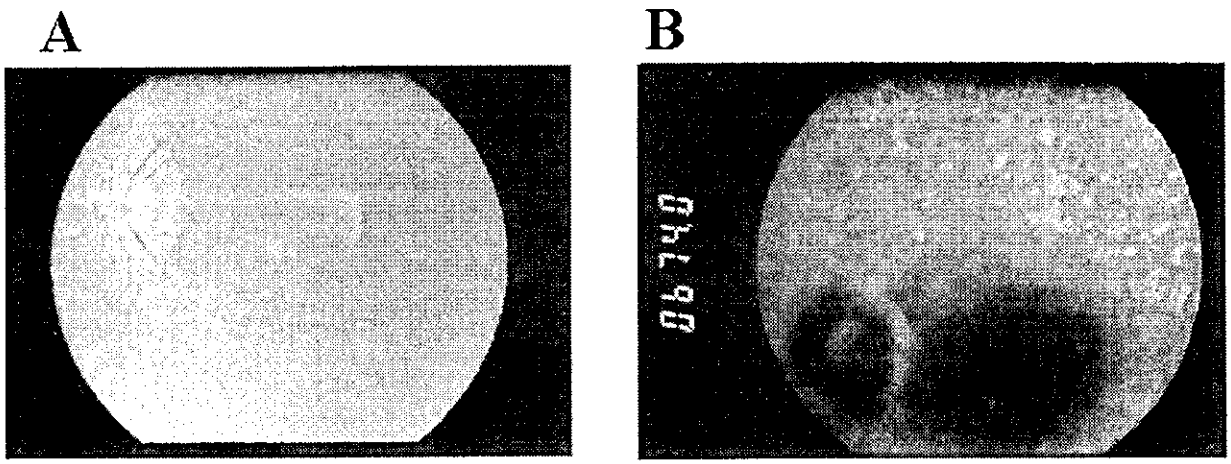

FIGURE 2. Fundus photographs of patient III-2 of family 1. The mottled appearance of the retinal pigment epithelium, attenuated retinal vessels, and edematous macula can be seen bilaterally. (A) Fluorescein angiogram of patient III-2 of family 1 shows granular hyperfluorescence from the posterior pole to the midperipheral area.

lated families with ADRP, and one family was also found to have a rhodopsin mutation (Figure 1). The Arg1933X mutation has not been detected in the white population, and these findings suggest that this mutation has a common sequence variant in Japanese and Chinese people.

The clinical features of the two affected members (68 and 64 years old) with the 2336 to 2337delCT mutation were typical of patients with retinitis pigmentosa. Both patients had night blindness that was first noted at approximately age 40 years. Their visual acuity ranged from 0.02 to 0.3, and both noted visual disturbances after age 40 years with severe clinical features after age 50. These findings suggested that Japanese patients with the RP1 mutations have a rapid and progressive decline of visual functions after age 40.

Fundus examination disclosed bilateral pigmentary retinal degeneration, attenuation of the retinal arteries, and edematous macula (Figure 2).

The Pro23His and Pro347Leu mutations in the rhodopsin gene, the Arg677X mutation in the RP1 gene, and the Asp226Asn mutation in the IMPDH1 gene are representative mutations for ADRP, but the Arg677X in the RP1 gene and Asp226Asn mutation in the IMPDH1 gene have not been reported, and mutations in the rhodopsin gene are very rare in Japanese patients with ADRP. These findings support our hypothesis that the kinds and frequency of mutations depend on the ethnic population.

In conclusion, mutations in the RP1 gene are relatively rare in Japanese patients with ADRP; this mutation has been found in 1.6% of unrelated patients with ADRP.

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Optical Coherence Tomography Findings in Patients With Late Solar Retinopathy

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PURPOSE: To investigate the morphologic appearance on optical coherence tomography (OCT) of the macula in

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TABLE 1. Baseline Examination and Study Examination Findings in Patients With Solar Retinopathy

	Patient 1	Patient 2	Patient 3	Patient 4
Baseline examination				
Age (yr), gender, study eye	42, F, RE	16, F, RE	15, F, RE	28, F, LE
Date of presenting examination	November 2000	March 2003	December 2002	June 2002
Past medical history	Sun gazing since childhood	Gazing at the sun and at fluorescent lights	Sun gazing	Sun gazing
BCVA, study eye	20/60	20/20	20/20	20/25-2
Study examination				
Period from baseline	32 mo	4 mo	7 mo	12 mo
BCVA, study eye	20/60	20/20	20/20	20/20
Amsler grid	Central metamorphopsia	Central metamorphopsia	Central metamorphopsia	Unremarkable
Macular findings	Small reddish spot	Mild RPE mottling	Small yellowish dot	Mild RPE mottling
Fluorescein angiography	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Optical coherence tomography				
Photoreceptor outer segments	Absence of signal	Absence of signal	Absence of signal	Fragmentation/fusion
Inner high reflective layer	Interruption	Interruption	Small breaks	Fragmentation/fusion
Photoreceptor inner segments	Absence of signal	Presence of signal	Presence of signal	Presence of signal

BCVA = best-corrected visual acuity; F = female; LE = left eye; RE = right eye; RPE = retinal pigment epithelium.

patients with late solar retinopathy and its association with visual acuity.

DESIGN: **Observational case series.**

METHODS: **All patients with solar retinopathy evaluated between 1998 and 2003 at one institution were invited to participate in an ophthalmic evaluation.**

RESULTS: **In all four affected eyes of four patients, OCT demonstrated abnormal reflectivity at the outer foveal retina, such as fragmentation or interruption of the inner high reflective layer corresponding to the junction between the photoreceptor inner and outer segments. Involvement of the entire photoreceptor reflective layer at the fovea was observed in the patient with decreased visual acuity (20/60).**

CONCLUSIONS: **Optical coherence tomography demonstrated abnormalities in the outer foveal retina. The OCT findings suggest that decreased visual acuity may be associated with full-thickness involvement of the photoreceptors. (Am J Ophthalmol 2004;137:1139–1142.**

SOLAR RETINOPATHY IS CHARACTERIZED BY VISUAL DISTORTION and a small yellow or red foveal lesion in patients with a history of sun gazing.[1–4] Fluorescein angiography may demonstrate leakage in the foveal region in the acute phase (hours to days) of the disease.[1,2] In the latter phases of the disease, angiography may demonstrate transmission of fluorescence from the affected areas (window defects), but is often unremarkable.[2] Optically clear spaces within the fovea,[2] transient increase in foveal reflectivity,[3] and reduced reflectivity from the retinal pigment epithelium[4] have been described in solar retinopathy using first generation optical coherence tomography (OCT).[2–4] The current study investigates the morphologic appearance by third generation OCT of the macula in patients with late solar retinopathy and its association with visual acuity.

Institutional review board approval for the study was obtained. All patients evaluated at the University of São Paulo Department of Ophthalmology with a diagnosis of solar retinopathy between 1998 and 2003 were invited to participate in a comprehensive examination including best-corrected Early Treatment Diabetic Retinopathy Study visual acuity, Amsler grid testing, fundus photography, fluorescein angiography, and OCT. Four of the six patients identified participated in the study (one patient could not be contacted, and the other was unable to travel to the clinic).

The study included four patients with visual symptoms and examination findings consistent with solar retinopathy in one eye (examination of the fellow eye was unremarkable) (Table 1). Optical coherence tomography abnormalities were similar and limited to the outer aspect of the foveal retina in all patients, but subtle morphologic peculiarities allowed differentiation of three patterns of abnormalities: 1) optically clear spaces within the entire photoreceptor reflective band (patient 1); 2) optically clear spaces at the level of the reflective band corresponding to photoreceptor outer segments with maintenance of reflective signal from photoreceptor inner segments (patients 2 and 3); and 3) fragmentation of the photoreceptor reflective layer with loss of the double high-reflective-layer pattern (patient 4). Involvement of the entire photorecep-

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SHORT COMMUNICATION

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Clinical features of a Japanese family with autosomal dominant retinitis pigmentosa associated with a Thr494Met mutation in the *HPRP3* gene

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Abstract *Purpose:* To determine the clinical features of a Japanese family with autosomal dominant retinitis pigmentosa (ADRP) associated with a Thr494Met mutation in the *HPRP3* gene. *Methods:* Mutational screening by direct sequencing was performed on 96 unrelated patients with ADRP. The clinical features were determined by visual acuity, slit-lamp biomicroscopy, electroretinography, fluorescein angiography, and kinetic visual field testing. *Results:* A Thr494Met mutation in the *HPRP3* gene was found in one family and it cosegregated with ADRP in the three affected members. The ophthalmic findings were those of typical retinitis pigmentosa with rapid progression after 40-years-of-age. One patient also had retinoblastoma as a child. *Conclusion:* We conclude that the Thr494Met mutation in the *HPRP3* gene causes ADRP in Japanese patients. This mutation was found in 1% of patients with ADRP in Japan.

Introduction

Retinitis pigmentosa (RP) is an inherited retinal disease with considerable genetic heterogeneity; to date, 10 candidate genes have been identified for autosomal dominant retinitis pigmentosa (ADRP) [7]. In 2001, two groups reported that genes related to splicing factors, e.g., *PRPF31* and *PRPF8* genes, were candidate genes for RP11 and RP13, respectively [5, 8]. Several kinds of disease-causing mutations in the *PRPF31* and *PRPF8* genes have been identified in patients with ADRP.

The *HPRP3* gene is the third candidate gene for ADRP that is related to the pre-mRNA splicing factor after the *PRPF31* and *PRPC8* genes [8]. The *HPRP3* gene is located within the RP18 interval, which supported the suggestion that the *HPRP3* gene was a candidate gene for RP18 mapped to chromosome 1q21.1.

In 2002, two kinds of pathogenic mutations in the *HPRP3* gene, designated Thr494Met and Pro493Ser, were identified in patients with ADRP and simplex RP [1]. At present, the phenotypes associated with mutations in the *HPRP3* gene in patients with ADRP have not been reported.

We have characterized the clinical features of Japanese patients associated with the Thr494 Met mutation in the *HPRP3* gene.

Methods

Subjects and mutation analysis

We screened genomic DNA samples from 96 unrelated patients with ADRP for mutations of the *HPRP3* gene. Genomic DNA was isolated from leukocytes prepared from each patient's blood (10–15 ml). Informed consent was obtained from all patients before their entry into this study.

The sequence from exon 1 to exon 16 of the *HPRP3* gene was amplified by polymerase chain reaction. For screenings, 16 sets of oligonucleotide primer pairs were used from the genomic sequence of the *HPRP3* gene. The PCR products were sequenced directly in the forward and reverse directions on an ABI sequencer (model 3100; Applied Biosystems, Foster City, CA). We further screened our patients for mutations in the *peripherin/RDS*, *rhodopsin*, *RP1*, *NRL*, *FSCN2*, *PRPF31*, *PRPF8*, and *IMPDH1* genes. The members of one pedigree, who were identified as having a Thr494Met mutation, became the focus of this study (Fig. 1).

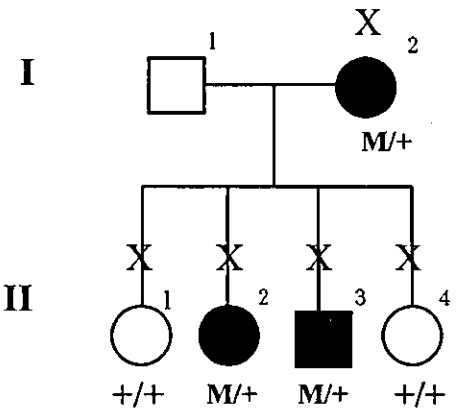

Fig. 1 Pedigree of a Japanese family with ADRP associated with the Thr494Met mutation in the *HPRP3* gene showing affected (*solid symbols*) and unaffected (*open symbols*) members. *Squares* male members, *circles* female members, *X* individuals examined in this study, *slash* deceased, *arrow* proband, *M* mutant allele, + normal allele

Clinical examination

We examined three affected and two non-affected members (Fig. 1) of our family. The ophthalmic examination included best-corrected visual acuity, slit-lamp biomicroscopy, kinetic visual field examination, fundus examination, fluorescein angiography (FA), and electroretinography (ERG). Ophthalmoscopic findings were recorded by color fundus photography. Kinetic visual field examination was performed on a Goldmann perimeter with the V-4-e, IV-4-e, I-4-e, I-3-e, and I-2-e targets.

The ERGs were recorded under conditions that conformed to the standards of the International Society of Clinical Electrophysiology of Vision [3]. The photopic ERGs were elicited by a single flash or a 30 Hz flickering red light under light-adapted conditions. Rod-isolated responses were elicited by a dim blue flash under dark-adapted conditions (30 min in the dark). A bright white flash (20 J) in the dark-adapted state was used to elicit the mixed rod–cone response.

Results

DNA analysis

The three affected members with ADRP (Fig. 1) were found to have a Thr494Met mutation in the *HPRP3* gene. The abnormal nucleotide sequence was a transversion of cytosine to thymine in the second nucleotide at codon 494 (Fig. 2). This alteration caused an amino acid substitution of a threonine residue, resulting in a methionine at codon 494. The non-affected members did not have this mutation, and this mutation cosegregated with the phenotype of this Japanese family. No mutation was detected in the *peripherin/RDS*, *rhodopsin*, *RP1*, *NRL*, *FSCN2*, *PRPF31*, *PRPF8*, and *IMPDH1* genes.

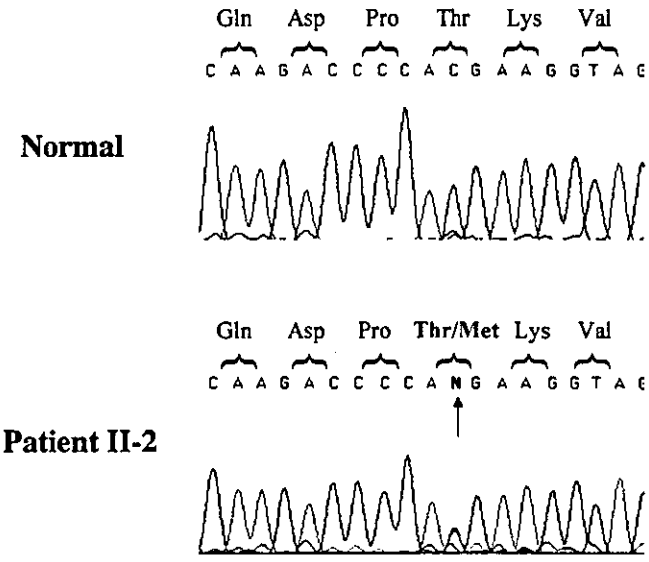

Fig. 2 Results of nucleotide sequencing analysis. The abnormal nucleotide sequence of patient II-2 was a heterozygous transversion of cytosine to thymine in the second nucleotide at codon 494

Case reports

Case 1

Patient I-2 was a 48-year-old woman, the proband, who noted a decrease in visual acuity, night blindness, and constricted visual field when she was 15 years old. She was diagnosed as having retinitis pigmentosa by a local ophthalmologist at that time. She reported that her visual acuity and visual field became worse after age 40 years. In 2003, her visual acuity was hand motion in both eyes. Slit-lamp biomicroscopy disclosed normal-appearing cornea, anterior chamber, iris, and lens in both eyes.

Fundus examination showed severe attenuation of the retinal vessels, bone spicule pigmentation, mottled appearance of the retinal pigment epithelium, and chorioretinal atrophy, bilaterally (Fig. 3a). FA disclosed hypofluorescence corresponding to the chorioretinal atrophy, and hyperfluorescence in the posterior pole (Fig. 4a).

The scotopic, photopic, bright flash, and 30-Hz flicker ERGs were non-recordable. Kinetic visual field testing showed severe constriction of the visual fields with the V-4-e, IV-4-e, and III-4-e targets (Fig. 5a).

Case 2

Patient II-2 was the 21-year-old daughter of patient I-2. When she was 2 years old, she was diagnosed with retinoblastoma in the left eye, and the eye was enucleated. The ophthalmologic examination disclosed that she also had retinitis pigmentosa.

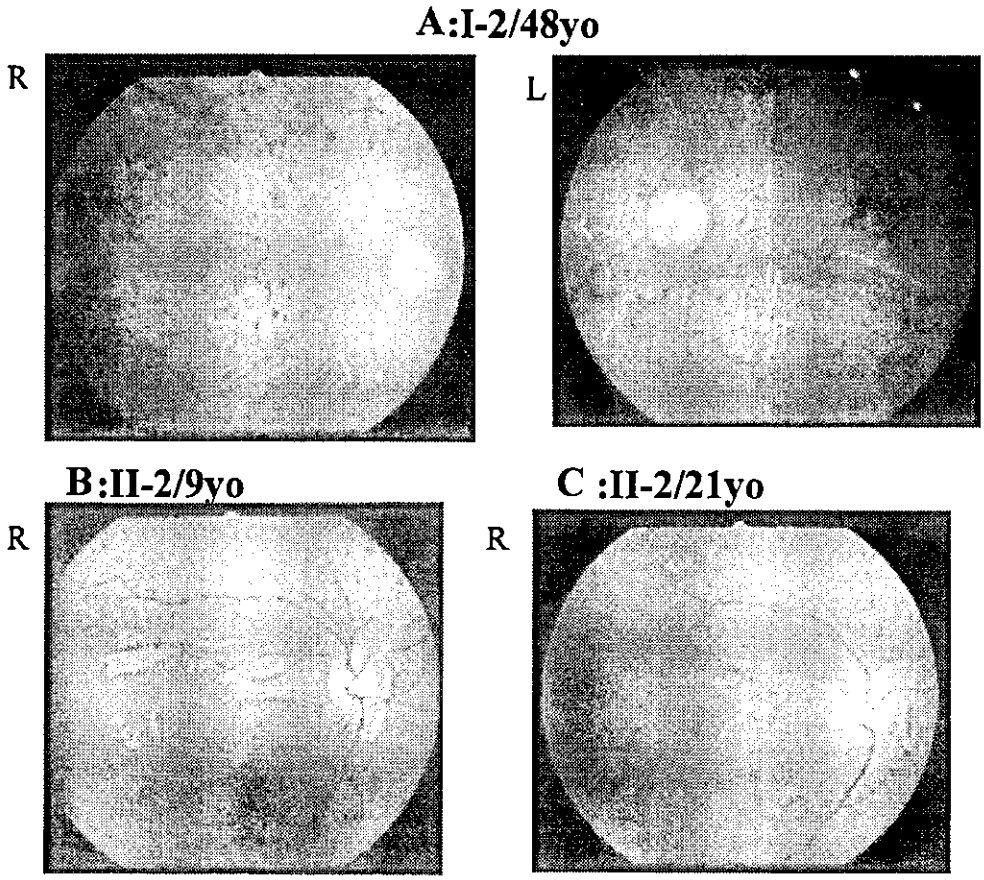

Fig. 3 **A** Fundus photographs of patient I-2. Severe attenuation of retinal vessels, atrophy of the retinal pigment epithelium, and chorioretinal atrophy can be seen. **B** Fundus photograph of patient II-2 at age 9 years showing a mottled appearance of the retinal pigment epithelium and mild attenuation of the retinal vessels. **C** Fundus photograph of patient II-1 at age 21 years. No remarkable change can be seen

Her visual acuity at age 21 years was 0.9 in the right eye. Slit-lamp biomicroscopic examination showed normal-appearing cornea, anterior chamber, iris, lens, and vitreous. Fundus examination showed mild attenuation of the retinal vessels and a mottled appearance of the retinal pigment epithelium. A comparison of fundus photographs at ages 9 years and 21 years indicated that no remarkable progression occurred during this 12-year period (Fig. 3b,c). FA disclosed granular hyperfluorescence from the posterior pole to the peripheral area (Fig. 4b).

Her scotopic, photopic, bright flash, and 30-Hz flicker ERGs were non-recordable. Kinetic visual field testing disclosed a constricted visual field and a paracentral scotoma with the I-4-e, I-3-e, and I-2-e targets (Fig. 5b).

Case 3

Patient II-3 was the 19-year-old son of patient I-2. He noticed that he had poor night vision when he was 7 years old. As his mother and his elder sister (patient II-2) were diagnosed as having retinitis pigmentosa in our clinic, he also visited our clinic at 7 years of age. His best-corrected visual acuity was 0.4 with −5.75 to 3.5×160° OD and 0.4 with −5.25 to 3.0×180° OS. Slit-lamp biomicroscopic findings of the cornea, anterior chamber, iris, and lens were normal in both eyes. Fundus examination disclosed a mottled appearance of the retinal pigment epithelium, mild attenuation of retinal vessels, and bone spicule pigmentation, bilaterally.

Discussion

The *HPRP3* gene encodes the U4/U6 snRNP-associated splicing factor, Hprp3p, has 16 exons, and is located on chromosome 1q21.1. Hprp3p is a 77-kDa protein that interacts with Hprp4p and plays an important role in RNA splicing [2,10]. At present, two mutations, designated Thr494Met and Pro493Ser, have been reported in the *HPRP3* gene. These two codons are highly conserved and are located at the C-terminal region of the protein. They have been considered to play a role in the binding of Hprp3p with Hprp4p [4]. The Thr494Met mutation is considered to be a common mutation in the *HPRP3* gene because it has also been found in English, Danish, and Spanish families [1].

Chakarova et al. reported two different haplotypes of the Thr494Met mutation in the *HPRP3* gene that coseg-

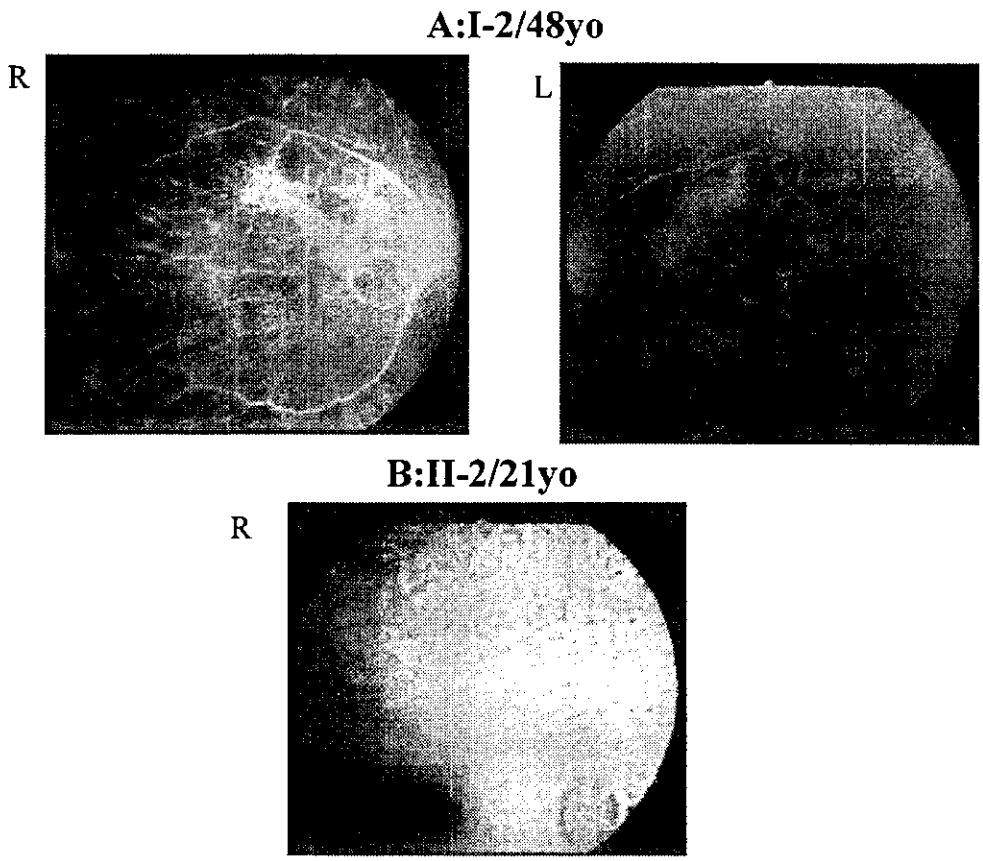

Fig. 4 A Fluorescein angiograms of patient I-2. Hypofluorescence corresponding to the chorioretinal atrophy can be seen. B Fluorescein angiogram of patient II-2 shows the granular hyperfluorescence in the posterior pole

regated with ADRP in English and Danish families [1], as it did in our Japanese family.

Previous studies on the phenotype–genotype correlation of inherited retinal degeneration in the Japanese population demonstrated that Japanese patients had unique mutations. Thus, the 1147delA mutation in the arrestin gene and the 208delG mutation in the FSCN2 gene have been found only in Japanese patients [6, 11]. On the other hand, the Pro23His and Pro347Leu mutations in the *rhodopsin* gene and the Arg677X mutation in the *RP1* gene are the most common mutations for ADRP in the United States, and yet Japanese patients with ADRP rarely have these mutations. These findings suggested that the type and frequency of mutations depend on the ethnic population.

To date, there is only one clinical report on patients with the Thr494Met mutation, and that in Spanish patients. The findings were late onset and a less severe phenotype than the ADRP patients with mutations in the *PRPF8* and *PRPF31* genes [4].

In general, our patients noted visual disturbances and were diagnosed with RP from the first decade of life, but the severe clinical features did not develop until after 40 years of age.

In conclusion, we have identified a Thr494 Met mutation in 1% of patients with ADRP, which suggests that this mutation might be relatively rare in Japanese patients with ADRP. Additional families with ADRP are being screened for this mutation to ascertain the phenotype–genotype correlation and to identify the haplotype of the *HPRP3* gene in the Japanese population.

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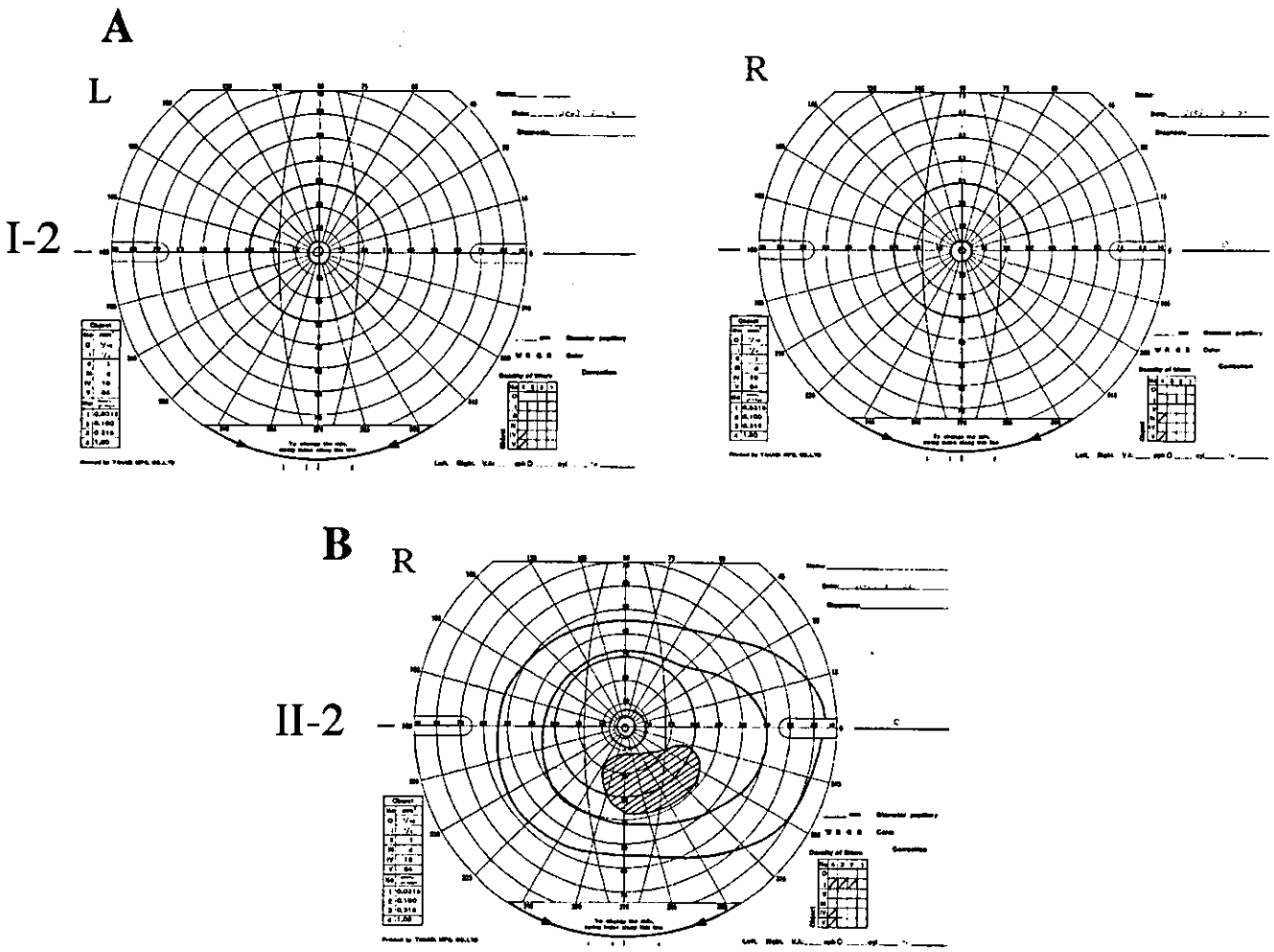

Fig. 5 Results of Goldmann visual field testing of patient I-2 (**A**) and patient II-2 (**B**). Patient I-2 shows constricted visual field with V-4-e, IV-4-e, and III-4-e targets. Patient II-2 shows constricted visual field and paracentral scotoma with I-4-e, I-3-e, and I-2-e targets. *L* Left eye, *R* right eye

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