我が国のX連鎖性網膜分離症の原因はXLRS1遺伝子異常による

Japanese juvenile retinoschisis is caused by mutations of the XLRS1 gene

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抄録

我々は日本人の若年網膜分離症患者のXLRS1遺伝子を検討した。互いに独立した10家系から1卵生双生児を含む13人の患者と、5人のキャリアーと、2人の正常者について、6つすべてのエクソンについてＰＣＲ法により増幅し、直接塩基配列を決めた。また、エクソン4、5、6について正常者の合計100アレルについても同様に塩基配列を決めた。13人の患者すべてにXLRS1遺伝子の変異（5種類のミスセンス変異と、1種類のナンセンス変異）を認め、5人のキャリアーでは変異をヘテロ接合体で認めた。家族歴のはっきりしている家系では変異は疾患と連鎖していた。Glu72Lys変異は、4家系に認められ、ホットスポットが疑われた。正常者には上記の変異を認めなかった。日本人の若年網膜分離症のほとんどは、XLRS1遺伝子異常によることが考えられ、遺伝子診断に有用と考えられた。

We investigated the XLRS1 gene in Japanese patients with juvenile retinoschisis(RS). All exons of the XLRS1 gene were amplified by the PCR and sequenced directly in 13 males, including a pair of monozygotic twins, five of thier carrier, mothers and normal individuals from 10 individual families with RS. The exons 4-6 were directly sequenced in a total of 100 alleles. Five kinds of missense mutations and a nonsense mutation were detected in all 13patients and carriers showed heterozygous mutations. All mutations in patients who have family histories were shown to segregate with the disease. A mutation, Glu72Lys was found in four families, suggesting a common mutation. The above mentioned mutations were not recognized in the normal individuals. Most of the Japanese retinoschisis is caused by the XLRS1 gene mutations suggesting that the XLRS1 gene analysis is useful for the DNA diagnosis for juvenile retinoschisis.

キーワード：若年網膜分離症、X連鎖性、ＸＬＲＳ遺伝子、ミスセンス変異、遺伝子診断
Key words: Juvenile retinoschisis, X-linked, XLRS1 gene, Missense mutation, DNA diagnosis

緒言

若年網膜分離症は、進行性網膜変性疾患であり、X連鎖性遺伝形式のまれな疾患である[1-2]。患者はほとんどが男性で、小児期に診断されるが、乳児期の報告もある[3]。その臨床像は多彩で、診断は、家族歴、眼底所見、網膜電図(electroretinogram, ERG)による。最近、X連鎖性先天網膜分離症において、XLRS1 遺伝子異常が高頻度に認めることが報告された[4]。今回我々は日本人の若年網膜分離症患者の白血球DNA よりXLRS1 遺伝子を検討したところ、すべての患者に異常を認めたので報告する。

対象と方法

互いに独立した日本人の若年網膜分離症の10家系から1卵生双生児を含む13人の患者と、5人のキャリアーと、2人の正常者について検討した。インフォームドコンセントの上で、末梢血の白血球よりＤＮＡ

を抽出した。XLRS1遺伝子の６つすべてのエクソンについて、Sauerらの報告したプライマーと条件４）によって、ＰＣＲ法により増幅した。ＰＣＲ産物をDye terminator法を用いてオートシークエンサーによって直接塩基配列を決めた。また、エクソン４、５、６について正常者の合計100アレルについても同様に塩基配列を決めた。

結果

13人の患者すべてに表に示すようなXLRS1遺伝子の変異（５種類のミスセンス変異Glu72Lys、Trp92Cys、Glu146Asp、Pro193Leu、Arg213Glnと、１種類のナンセンス変異Gln154stop）を認め、5人のキャリアーでは変異をヘテロ接合体で認めた。家族歴のはっきりしている３家系では変異は疾患と連鎖していた（図１、２）。Glu72Lys変異は、４家系に認められ、ホットスポットが疑われた。正常者には上記の変異を認めなかった。

考案

網膜分離症には、X連鎖性遺伝形式の先天性のものと、老人性や、牽引性の後天性にわけられる。X連鎖性の先天網膜分離症は、黄斑部に車軸状の特徴的な変化を示し、周辺部網膜分離は半数以上に認められるという[1,2]。最近、先天網膜分離症の原因遺伝子、XLRS1 遺伝子が解明された[4]。我が国では検討した１３家系すべてに６種類のXLRS1 遺伝子異常が認められた。欧米では１３カ国による共同研究がなされ、検討された２３４家系中、２１４家系（９１％）に種々のXLRS1 遺伝子異常が認められている[5]。このことから人種の差を越えて、ほとんどの網膜分離症の原因はXLRS1 遺伝子異常によることが示された。

X連鎖性網膜分離症は、乳児期の報告もある[3]。患児が幼少であると種々の検査は困難となる。詳細な眼底検査にも全身麻酔が必要となるし、ＥＲＧや、蛍光眼底撮影は難しいこともある。遺伝子診断は、採血のみで可能であり、もし異常を認めれば詳細な検査計画をたて、もし異常を認めなければ、そうした検査をする必要がないことがわかる。X連鎖性先天網膜分離症においては、XLRS1 遺伝子異常の頻度が極めて高いので遺伝子診断は極めて有用である。X連鎖性網膜分離症は、幼少時のスクリーニング検査や、臨症像が多彩で診断がなかなか困難な症例の場合などには、XLRS1遺伝子の検討は有用と考えられる。

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若年網膜分離症における*XLRS1*遺伝子変異

家系	エクソン	塩基置換	変異	効果
Sat, De, Na, Ta	4	GAG → AAG	Glu72Lys	Missense
Se	4	TAG → TGC	Trp92Cys	Missense
Ki	5	GAG → GAC	Glu146Asp	Missense
Mi	5	CAG → TAG	Gln154stop	Nonsense
Mo, Yo	6	CCC → CTC	Pro193Leu	Missense
Ka	6	CGG → CAG	Arg213Gln	Missense

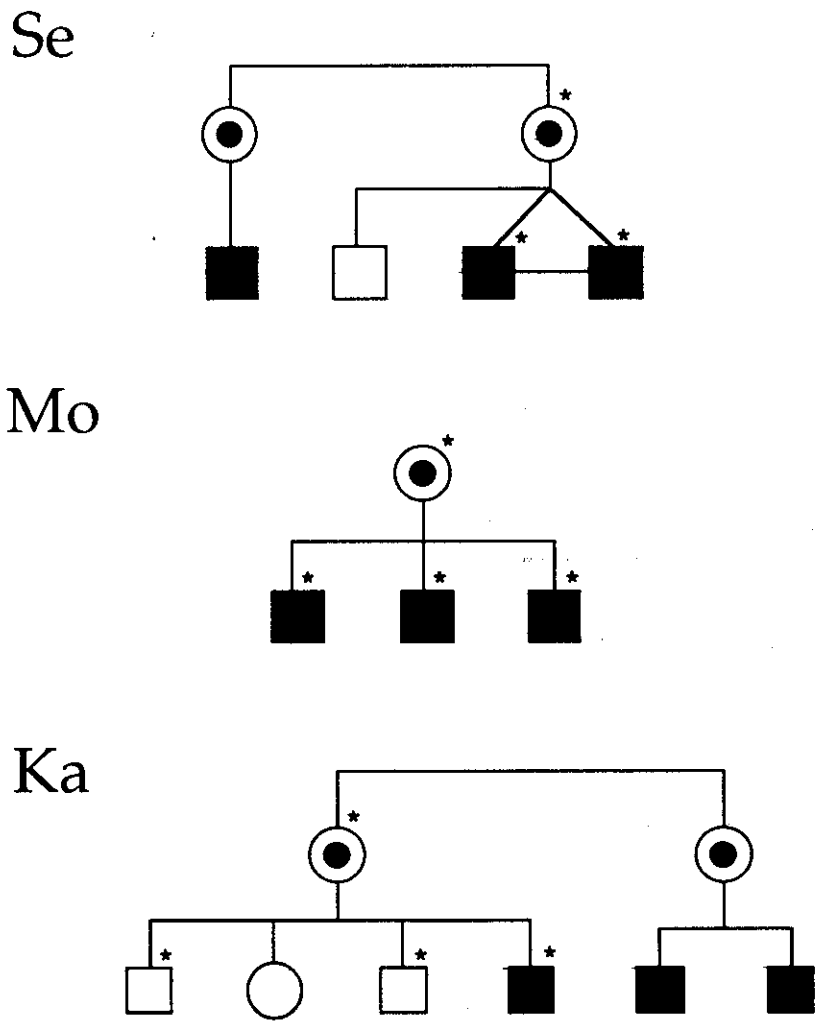

図1　家族歴のある若年網膜分離症の3家系。
■：患者、○□：正常者、白丸中の黒丸：キャリアー、
＊：検査済。Se家系、Mo家系、Ka家系ではそれぞれTrp92Cys、Pro193Leu、Arg213Gln変異を認めた。

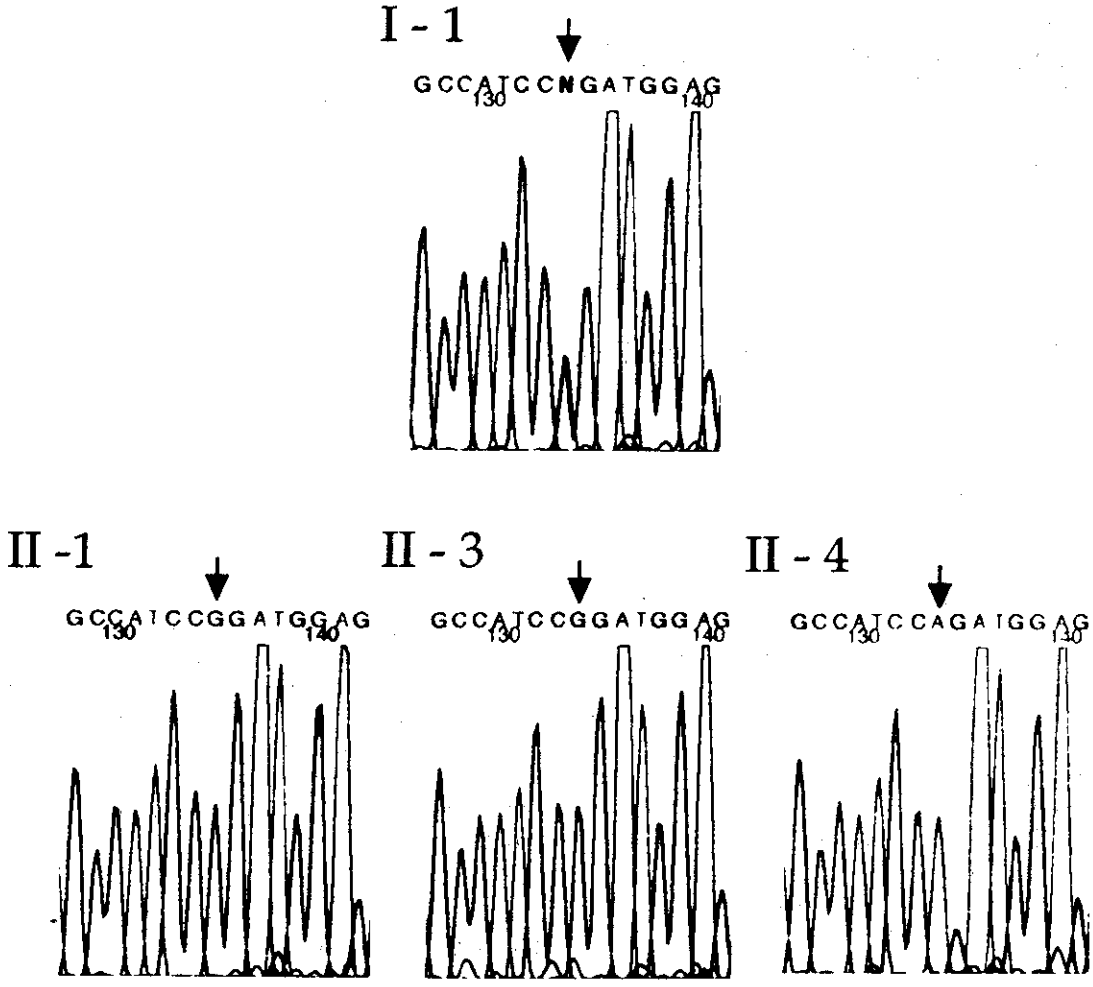

図2　オートシークエンサーによる塩基配列。

センスプライマーを用いて図1のKa家系のXLRS1遺伝子の塩基配列を決めた。矢印は変異の部分を示す。患者(II-4)、母親(I-1)ではそれぞれコドン213の、CGGからCAGの変異をヘミ接合体、ヘテロ接合体で認めた。正常者(II-1, II-3)には異常を認めなかった。

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Japanese juvenile retinoschisis is caused by mutations of the *XLRS1* gene

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ヒト網膜特異的アミンオキシダーゼ遺伝子：大腸菌および哺乳類培養細胞での発現システムの構築

Human retina-specific amine oxidase gene:
functional expression in E.coli and mammalian cell lines

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抄録

我々はサブトラクション法を改良した新しい方法を用いてヒト網膜に特異的に発現を認める新規遺伝子を単離した。今回RAOの機能解析を行う第1歩として，得られたRAO完全長cDNAを大腸菌および哺乳類培養細胞に発現させ，蛋白の分子量および酵素活性を測定した。IPTGにより誘導発現させたRAO蛋白はウエスタンブロット法で約70kDaの単一のバンドを示し，cDNAより予測される分子量とほぼ一致した。またヒト不死化細胞株HeLaにRAOを強制発現させ，細胞抽出液のアミンオキシダーゼ活性を測定し，ベクターのみを導入した細胞抽出液の測定値と比較した。この結果，RAO導入細胞は対象の約3.5倍の活性を示した。アミンオキシダーゼは胎生期の網膜でガンマアミノ酪酸の合成酵素であると考えられている。今後は胎生期での発現解析が，RAOの生体内での機能を知るうえで必要である。

We have employed a novel method using subtractive hybridization and isolated a novel gene whose expression was restricted to human retina. As a first step toward understanding the function of RAO in retina, we transfected expression vectors containing RAO full-length cDNA into E. coli and mammalian cell lines and analysed the molecular mass and enzymatic activity of the recombinant RAO protein. Western blot analysis of the IPTG-induced protrein showed single band at the molecular mass of 70kDa, which is almost identical to the predicted size from RAO cDNA. We induced RAO expression in human immortalized cell line, HeLa, and measured amine oxidase activity of cell fractures. The activity was 3.5-fold higher than that of control cells. Amine oxidase is considered to synthesize gamma-aminobutyric acid in embryonic retina. Expression anlysis of RAO in embryonic stage will be necessary to understand the function of RAO in vivo.

Keywords: Human retina-specific amine oxidase, functional expression , amine oxidase activity

目的

現時点で明らかとなった遺伝性網膜疾患の原因遺伝子は，その大半が網膜に優先的な発現を示し，網膜での固有の機能を担っている。我々はサブトラクション法により成人および胎児27臓器でヒト網膜にのみ発現する新規遺伝子を単離した1)。この遺伝子産物は大腸菌，酵母，植物から哺乳動物にいたる種で広く存在する銅依存性アミンオキシダーゼと塩基レベル，アミノ酸レベルできわめて高い類似性を示した。特に銅依存性アミンオキシダーゼの活性に必要なアミノ酸配列(Asn-Tyr-Asp-Tyr)および銅付着部位であるヒスチジン残基は正確に保存されていた。このためわれわれはこの遺伝子産物を網膜特異的アミンオキシダーゼ(retina-specific amine oxidase: RAO)と命名した。これまでにわれわれは完全長cDNA，遺伝子構造，染色体座位，およびmRNAの網膜内での発現部位を決定してきた1, 2)。今回われわれはRAOの機能解析の第1歩として，RAO cDNAの大腸菌および哺乳類培養細胞での発現システムを構築し，RAO蛋白の発現誘導および酵素活性を測定した。

方法

ヒト網膜cDNAライブラリーより単離されたRAO

完全長cDNAを大腸菌発現ベクターPSE420（Invitrogen）にサブクローニングした。この際，RAO蛋白のN末端にあるシグナルペプチドを除き，C末端には発現蛋白の標識および精製のために6つのヒスチヂン残基を結合させた。PSE420を導入した大腸菌を1mM IPTG下で培養し，RAO・6ヒスチヂン残基の融合蛋白を強制発現させ，融合蛋白の発現を抗ヒスチヂン抗体を用いたウエスタンブロット法で確認した。次にRAO完全長cDNAを哺乳類発現ベクターPCI-neoに組み込み，ヒト不死化細胞株HeLaおよびサル不死化細胞株Cos7にlipofection法で導入した。2日間の培養後，細胞を回収し超音波破砕後，抽出液を凍結保存した。10mMプトレッシンを基質とし，反応産物の過酸化水素をhorse radish peroxidaseおよびluminolを用いて検出する方法[3]で抽出液のアミンオキシダーゼ活性を測定した。

結果

1mM IPTG下の大腸菌の発現蛋白に約70kDaの陽性シグナルを認めた。これはRAO cDNAより予測される分子量とほぼ一致した。またアミンオキシダーゼ解析では，RAOを導入したHeLaおよびCos7の細胞のいずれにおいても対照群より高いアミンオキシダーゼ活性を認めた。特にRAO導入したHeLa細胞は対象の約3.5倍の活性を示した。

考案

我々は独自に開発した手法により，ヒト網膜のみで発現が認められる新しいアミンオキシダーゼ遺伝子を同定した。今回，われわれは大腸菌にRAO蛋白を発現させ分子量を確認した。さらに哺乳類培養細胞にRAO遺伝子を導入し，アミンオキシダーゼ活性を同定した。銅依存型アミンオキシダーゼは胎児網膜および副腎でGABAの合成酵素であることが報告されている4,5)。今後は胎生期でのRAOの発現の解析が生体内でのRAOの機能を決定するために重要であると考えられる。また現時点で17q21にマップされた遺伝性網膜・視神経疾患の家系は報告されていない（1999年1月現在）が，今後，RAOの機能を明らかにしながら各種病態との関連を追究していきたい。

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Human Retina-Specific Amine Oxidase: Genomic Structure of the Gene (AOC2), Alternatively Spliced Variant, and mRNA Expression in Retina (Short communication)

Yutaka Imamura, Setsuko Noda, Yukihiko Mashima, Jun Kudoh, Yoshihisa Oguchi, and Nobuyoshi Shimizu

Genomics. Volume 51, pp.293-298, 1998

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Human Retina-Specific Amine Oxidase (RAO): cDNA Cloning, Tissue Expression, and Chromosomal Mapping

Yutaka Imamura, Ryo Kubota, Yimin Wang, Shuichi Asakawa, Jun Kudoh, Yukihiko Mashima, Yoshihisa Oguchi, and Nobuyoshi Shimizu

Genomics. Volume 40, pp.277-283, 1997

癌関連網膜症患者血清が認識する新しい網膜変性症関連遺伝子のクローニング

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MOLECULAR CLONING OF NEW RETINAL AND NEURONAL AUTOANTIGENS RECOGNIZED BY SERUM OF A PATIENT WITH CANCER-ASSOCIATED RETINOPATHY

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厚生省特定疾患網膜脈絡膜・視神経萎縮症調査研究班
平成10年度　研究報告書

要約

癌関連網膜症（Cancer-Associated Retinopathy; CAR）は悪性腫瘍の浸潤や転移によらない遠隔効果によって惹起される傍腫瘍性症候群の一つである。CAR患者血清中には高い抗体価の網膜特異蛋白に対する自己抗体の存在が知られている。本研究の目的は一例のCAR患者血清が認識する複数の網膜特異抗原を同定することである。ヒトおよび新生児ラットの網膜由来のcDNAライブラリーを用いたスクリーニングを行い、3種類のクローンを単離した。そのうちの2種類のクローンはricoverinとtubby-like protein 1（TULP1）であった。RecoverinはCAR抗原としてよく知られている。TULP1は最近、常染色体劣性の網膜色素変性症の原因遺伝子の一つとして報告されている。もう一つのクローンはとpolypyrimidine-tract binding protein（ＰＴＢ）と相同性を有する未知の遺伝子（PTB-like protein; PTBLP）であった。PTBLPは532アミノ酸残基からなるタンパクでPTBとアミノ酸配列で73.5%の相同性を示し、PTBの機能ドメインである核移行性シグナルやRNA認識モチーフなどの部位はさらに高い相同性が保持されていた。これらの結果から患者血清はCARの確定診断のためだけでなく新しい網膜特異的遺伝子を検索するための有用なプローブになることを示唆している。

AbstractCancer-associated retinopathy (CAR) is one of the paraneoplastic syndromes caused by a remote effect of cancer without direct invasion or metastasis. The CAR syndrome is mediated high titer of autoantibodies specific to retinal proteins. The aim of this study was to identify multiple retinal autoantigens recognized by a serum sample of a patient with CAR. Expression screening of cDNA libraries from human adult retina and rat neonatal retina was carried out and three different types of cDNA were isolated. Two of the three different clones were identified as recoverin and tubby-like protein 1 (TULP1). Recoverin is well-known as a CAR antigen and TULP1 is recently reported as one of the canditate genes for autosomal recessive retinitis pigmentosa. The other clone was a new homologue of polypyrimidine-tract binding protein (PTB). This homologue, named PTB like protein (PTBLP), encodes a 532-residue protein, and has a 73.5% homology with PTB. Functional domains in the PTB, such as nuclear localization signal and RNA recognition motifs, were highly conserved. The results suggest that sera of CAR patients are useful as probes for molecular cloning of unknown retinal proteins as well as the diagnosis of the syndrome.

キーワード癌関連網膜症、網膜特異自己抗原、tubby 遺伝子ファミリー、TULP1遺伝子、RNA結合蛋白、Polypyrimidine-tract binding protein

目的

癌関連網膜症（Cancer -Associated Retinopathy: CAR）は悪性腫瘍の遠隔効果による網膜変性症候群である[1]。その発症機構は未だ不明であるが、CAR患者血清中には網膜神経細胞に対する自己抗体の上昇が報告され、しかも免疫抑制剤の投与によって眼症状の進行が弱まることから、本症が自己免疫機序によると考えられている[2)3)]。これまでの症例報告から、患者血清が複数の多様な抗原蛋白を認識することが示唆されている[4]。しかし、現在までのところCAR抗原としてrecoverin (23kDa)[5] とneuron specific enolase a (46kDa)[6] が同定されているのみである。今回、一人のCAR患者血清が認識する複数の網膜特異抗原蛋白を分子生物学的手法を用いて同時に単離・同定したので報告する。

実験方法

子宮内膜癌に伴うCARの1症例[7]より調整した患者血清を用いて以下の実験を行った。ラットの網膜、脳、肝臓および腎臓から抽出した蛋白をSDS-PAGEにかけ、患者血清を用いてWestern blot 分析を行った。1000倍希釈した患者血清を一次抗体とし、二次抗体は市販のHRP 標識した抗ヒトIgGウサギ抗体を用い、発色はDAB 法を行った。ヒトおよびラットの網膜由来のcDNAライブラリーから患者血清を用いて免疫化学的スクリーニング法により網膜特異抗原遺伝子の検索を行った。得られたクローンの全塩基配列はdye terminator cycle sequencing 法とオートシークエンサー（ABI-310）を用いて決定した。配列のホモロジー検索はFASTA および BLAST プログラムを用いた。患者血清の認識部位を検索するために、pET大腸菌発現ベクターにPCR法を用いて作製した種々の長さのcDNA断片を挿入して欠失変異した組み換え蛋白を大腸菌に産生させ、これらの蛋白を用いてWestern Blot分析を行い、抗体認識部位の検索を行った。

結果

Western blot 分析の結果を図1に示した。ラットの網膜抽出物では78、60 と23 kDa 、脳の抽出物では78と60 kDaのバンドが描出された。また、牛の網膜抽出物でも同様にWestern blot 法を行い、ラット網膜抽出物と同じ大きさの3本のバンドが検出された。患者血清を用いたcDNA クローニングの結果、ヒト網膜由来のcDNAライブラリーから7個のrecoverinおよび2個のtubby-like protein 1（TULP1)のクローンが分離された。新生児ラットの網膜由来のcDNAライブラリーから2個のrecoverinと16個の polypyrimidine-tract binding protein（PTB）と高い相同性を有する未知の遺伝子（PTB-like protein ; PTBLP）が単離された。PTBLP遺伝子とPTB遺伝子のアミノ酸配列を比較すると、全体で73.5%の高い相同性が保持されていた。PTB遺伝子の特徴的なドメイン構造（核移行性シグナルや4個のRNA認識モチーフ配列）はPTBLPにおいてもよく保存されていた。

TULP1およびPTBLPの欠失変異した組み換え蛋白を用いたWestern Blotの結果から、患者血清はTULP1ではtubby遺伝子と相同性の全くないアミノ末端部位、PTBLPではカルボキシ末端部位をそれぞれ認識することが明らかとなった。

結論

CAR 患者血清が認識する網膜・神経細胞特異抗原として新たにTULP1遺伝子とPTBLP1遺伝子を単離・同定することに成功した。TULP1遺伝子はtubby遺伝子ファミリーのひとつとしてヒト網膜から単離され[8]、常染色体劣性遺伝の網膜変性症の原因遺伝子の一つであることが報告されている[9)10)]。また、tubby遺伝子は肥満と視覚・聴覚異常を先天的に惹起するtubbyマウス（retina degeneration 5）の原因遺伝子としてpositional cloning によって単離された[11)12)]。これらの遺伝子産物の生理機能は不明であるが、感覚器官の神経細胞の生存維持に重要な役割を担っているものと思われ、自己抗体との反応によって生理機能が阻害され網膜損傷を惹起するのではないかと推察される。

PTB遺伝子はRNA結合核蛋白としてRNAスプライシング機構などの生理機能に関与している[13]。PTB遺伝子との高い相同性を考えるとPTBLP遺伝子も神経細胞において同様の機能に関与しているものと推測される。脳や神経系組織にみられる種々の自己免疫性神経症において患者抗体が認識する組織特異性抗原蛋白HuD、La、Ro等はRNA結合性核蛋白である。PTBLP遺伝子産物とCAR発症との関連を解析することはこれらの神経症疾患の解明に有用な情報を提供することが期待される。

今後は、これらの遺伝子産物を実験動物に免疫してCARの動物モデル系を確立し、CAR発症および網膜損傷機構を解析する予定である。

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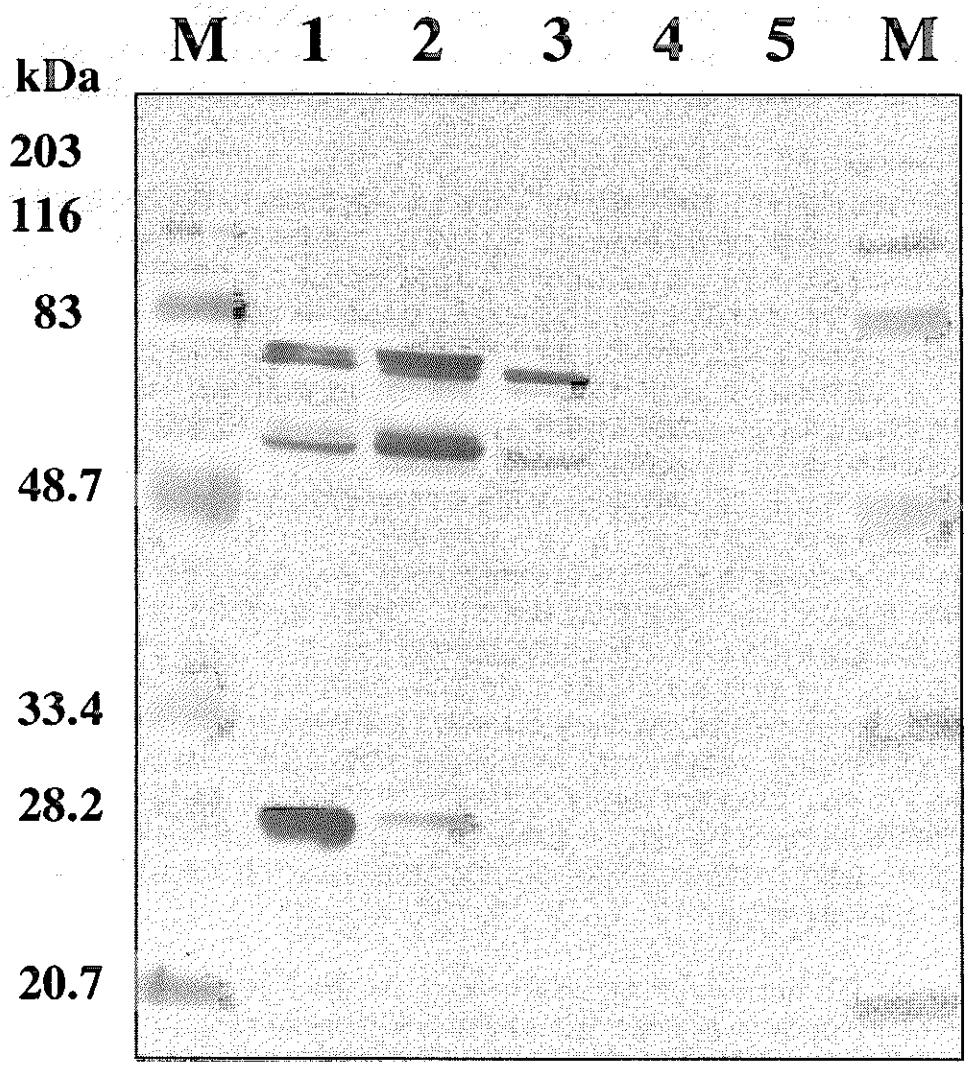

図1　Western blot 分析

ラットの種々の組織から抽出した蛋白試料を用い、一次抗体として1,000倍希釈した患者血清を4℃、16時間反応させた。レーン1：牛網膜、2：ラット網膜、3：ラット脳、4：ラット肝臓、5：ラット腎臓、M：Prestained broad range分子量マーカー（BioRad社製）。

白色光による網膜色素変性の光覚弁視力のグレード分類

GRADING DEVICE FOR LIGHT PERCEPTION WITH RETINITIS PIGMENTOSA

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目的：網膜色素変性の病気が進行し、数字で表わすことができない視力の場合、現在は自覚的症状をもとに、指数弁、手動弁、光覚弁の３段階で視力を評価している。しかし、このような患者の治療を試みる場合、または病状の進行を詳しく評価しようとすると、このような３段階ではあまりにも粗雑で変化を表しにくい。さらにこの疾患は電気生理学的にも反応をとることが出来ず、また強い光で刺激することは視細胞に対し障害を与えるおそれもあり困難であった。そのそれぞれの視力を細分化した評価はなされていない。今回、我々はそれらの視力をさらに簡便に細分化し、且つグレード分類を可能とする測定機器の開発を試みた。
対象と方法：網膜色素変性を中心とし、糖尿病網膜症、緑内障、網膜中心動脈閉塞症、等により、視力が手動弁以下に低下した症例を対象とした。検査装置は白色LEDによる刺激光を用い、眼前に来るようにゴーグルに固定した刺激装置を作成した。LED駆動電流を変化させ２桁の強度差の光で刺激し、発光時間は0.1秒、0.3秒、1秒の３段階で変化させ、２秒に１回（０．５ヘルツ）提示した。その結果９段階の刺激光を得ることが出来た。左右眼をランダムに各刺激光強度ごとに３回または５回行い、発光を認知した時、手に持ったスイッチを押し自動的に記録した。これらの刺激は正確を期し、又被験者の注意を集中させるためイヤホーンをとうしてクリック音を聞かせたあと０．７秒後に刺激光を提示した。また光を感じたときのみ反応しているかどうかを知る目的で、１０％のトライアルはクリック音のみで光を発光させず、そのときの反応の有無で信頼性を判断した。そのため合計刺激回数は60回又は100回（無発光刺激を含む）となった。
結果：直径３mmのLEDを使用し、強度を1、10，100cd／m2の３段階に設定した場合、主に手動弁におけるグレード分類が可能となった。この設定では指数弁及び光覚弁の詳細なグレード分類が不可能であった。強度を１０倍の 10,100,1000cd／m2にすると主に光覚弁のグレード分類をする事が出来る。
結論：この方法を検討し低視力を客観的に再現性のある方法で測定可能となれば重度視力障害の治療の効果判定に有用であることが示唆された。

Purpose: The goal of this study is to innovate a device for grading very low visual function expressed as light perception in patients such as retinitis pigmentosa (RP) and makes it possible to evaluate effects for various therapeutic modalities or their changes following the progress of the disease.Methods: The device was composed of a pair of goggles with a white light emission diode, 5mm in diameter, an earphone for each ear, a control cabinet which emits light and a clicking stimuli, a hand-held grip with a button and a recorder for calculating correct answer for stimuli. The light intensity and duration were changed in three levels, 10cd／m2(C), 100C, 1000C, and its duration, 0.1second(S), 0.3S, 1S independently. As a result, they were graded into nine different levels of intensities and delivered in a random sequence after a click sound of 0.7 sec prior to the stimulation. Patients were asked to push a button when they recognized these stimuli. Each eye was separately stimulated 5 times in each intensity and duration, 45 times per eye, therefore, a total of 90 times. Ten RP patients with light perception were examined and their visual ability was graded. The FP was examined before and after cataract extraction, stellate ganglion block and lipo-prostaglandin E1 was administered in two cases with RP and we studied the possibility of differentiating their functions and also reproducibility with this device.Results: We could differentiate changes of LP with this device and observe changes of very low grade vision with treatment.Conclusion: This device is valuable for evaluating low visual function such as LP of RP patients. It will be valuable for following their vision and also discriminating very small changes of visual function with medical and surgical treatment in the future.

GRADING DEVICE FOR LIGHT PERCEPTION WITH RETINITIS PIGMENTOSA

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Summary

Purpose: The goal of this study is to innovate a device for grading very low visual function expressed as light perception in patients such as retinitis pigmentosa (RP) and makes it possible to evaluate effects for various therapeutic modalities or their changes following the progress of the disease.
Methods: The device was composed of a pair of goggles with a white light emission diode, 5mm in diameter, an earphone for each ear, a control cabinet which emits light and a clicking stimuli, a hand-held grip with a button and a recorder for calculating correct answer for stimuli. The light intensity and duration were changed in three levels, 10cd/m2(C), 100C, 1000C, and its duration, 0.1second(S), 0.3S, 1S independently. As a result, they were graded into nine different levels of intensities and delivered in a random sequence after a click sound of 0.7 sec prior to the stimulation. Patients were asked to push a button when they recognized these sti muli. Each eye was separately stimulated 5 times in each intensity and duration, 45 times per eye, therefore, a total of 90 times. Ten RP patients with light perception were examined and their visual ability was graded. The FP was examined before and after cataract extraction, stellate ganglion block and lipo-prostaglandin E1 was administered in two cases with RP and we studied the possibility of differentiating their functions and also reproducibility with this device.
Results: We could differentiate changes of LP with this device and observe changes of very low grade vision with treatment.
Conclusion: This device is valuable for evaluating low visual function such as LP of RP patients. It will be valuable for following their vision and also discriminating very small changes of visual function with medical and surgical treatment in the future.

Introduction

Visual functions lower than expressing the ability to analyze an image (the minimum separable) is designated by the sensitivity of the retina in receiving the stimulus (the minimum visible) as light perception(LP) and non-light perception (NLP) when patients cannot discriminate between on and off settings of a test light1. At present, LP is difficult to grade. If a reliable measurement of the function with perimetry can be established, the level of sensation can be expressed by sensitivity of rod or cone system2. For patients who cannot detect visual stimuli, it has been impossible to accurately measure their visual impairment. In other words, we could only ask our patients whether the sensation of stimulated light was brighter or dimmer than the previous one. So, in daily practice, many patients with retinitis pigmentosa (RP) are diagnosed as "LP" by their physicians. Until recently we have had no problem with these methods even though patients wanted to know their residual functions more precisely. Naturally, it takes a long period for patients' visual function to decrease from LP to NLP. They are very eager to know how much and how rapidly their natural visual abilities are decreasing or increasing with their doctor's prescription or with their own health care.3 For eye doctors who have been trying to treat RP with new methods, it has been urgent to find a new evaluating method for measuring low vision ability, such as LP or NLP with RP. Because

it is very difficult or perhaps impossible to evaluate such effects with conventional electrophysiological methods or even other psychological ones. In this study, we tried to innovate a device for grading the LP in RP patients and evaluate the effects of treatment such as cataract extraction or with drugs.

Methods

From September 1997 to August 1998, we examined 10 patients whose visual function was LP due to RP in the Outpatient Clinic in the University Hospital. A Low Vision Evaluator, LoVE in short, was composed of goggles with a light emission diode(LED) and its generator, a set of earphones to hear a click before stimulation, a hand grip with a button and a printer for recording. The LED, 5mm in diameter, was set separately at the center of each goggle for both eyes. The goggles were affixed snugly to the skin with a sponge liner and was painted black to shield any inward scattering light. The distance between each goggle with LED could be adjusted depending on the patients' pupillary distance and to fit each orbital margin in order to block any scattered light from the other side of the stimulation. The stimulus light intensity was changed in three levels: 10cd/m2(C), 100C, 1000C, and their duration in 0.1second (S), 0.3S, 1S, respectively. Each eye was illuminated separately, so the patient was stimulated with 9 different kinds of stimulus intensity. Each stimulation was preceded 0.7 seconds by a click sent through the earphones to alert the patient of the forthcoming light stimulation. In one series of examinations, each eye was randomly stimulated 5 times with one intensity and duration, so 45 times per eye with a total of 90 times for both eyes. In another series of examinations, the click was delivered 10 times without light stimuli The purpose of these trials was to draw patients' attention to the examination and also to discriminate false positive responses. In total, 100 clicks with 90 light stimuli were delivered in one series. The number of incorrect responses (pushing the button when there was a click but no light stimulation) was printed out as X(score) /100. These scores were evaluated to assess the reliability of the data for each patient. In cases of over 5/100, we didn't tell the patients because we considered the data to be unreliable. Such cases, however, were very rare. So in once series of tests, 100 clicks with 90 light stimuli were delivered. Two RP patients, a 67-year-old man and a 57-year-old female with LP, had their cataracts extracted and the effect of surgery to their visual function was evaluated. They were administered lipoprostaglandin E1 for 10 days following surgery since this drug was reported to be effective to improve visual function in RP. 4 Also cervical stellate ganglion block and hyperbaric oxygen chamber were tried for the same purpose.5 Before this series of tests, the purpose of this study was fully explained to each patient and they gave their informed consent in writing.

Results

The sequential changes of the level of light perception of an RP patient are shown in Fig. 1. The right eye of a 67-year-old man operated on. Fig. 1a shows the result of the examination the day prior to the cataract extraction, 1b to 1e show the results from the 1st through 4th day after surgery. The level of light perception once decreased but gradually recovered to the pre-operative level at the 3rd day dramatically improved on the 4th day of surgery. With the conventional method using the light source of an indirect ophthalmoscope, his results showed the same LP before and after the operation.

With the administration of lipo-prostaglandin E1 which was started two weeks after the cataract extraction, his LP dramatically improved as shown in Figs. 2a to 2d. Fig. 2a and b are those of the right and left eye respectively before this treatment. After administration for 10 days, his LP improved, especially in the left eye. He could respond much better to the stimulation to100C and also some to 10C. The improved function was not kept so long and in the evening of the same day, his LP returned to the level before injection.

Three days after finishing the administration, he also got the stellate ganglion block following hyperbaric oxygen chamber. Fig. 3a and b are the right and left eye before treatment and 3c and d, those after, respectively. He showed no improvement in the left and decreased in the right as shown in Fig. 3d and 3c, so the trial was stopped.We repeated these trials with the other patient, a 57-year-old female with RP. Figure 4 shows the total number of correct answers in each trial before and after cataract extraction and administration of lipo-prostaglandin E1. Before surgery, her LP level 10 to 15 correct answers out of 45 trials in the left eye and none in the right. Cataract extraction was performed on her left eye on August 4th of this year as marked 1) in the figure. The level of LP improved the day following the surgery and remained so. On August 13th, she began the treatment of lipoprostaglandin E1 as shown 2) in the figure and continued for 10 days. The improvement was not so dramatic in the left eye but the LP in the right eye appeared in the second half of the treatment. The error score was 0-3/100 throughout these examinations.

Discussion

To our knowledge, residual visual functions of RP patients have been recorded with automated light- and dark-adapted perimetery or threshold response of cone sensitivity with Humphrey perimetry2. But in cases with very low vision such as light perception, it has been impossible to obtain reliable data with high reproducibility. With this device, it is possible to obtain a reproducible pattern to some extent of light perception of RP. For example, Fig. 1e, and 2a are the results of the same right eye, of the first case, without any treatment but two weeks interval. The number of correct answers was 37 out of 45 in Fig. 1e and 33 in Fig. 2a. In the former, he could answer at the lowest intensity of light, 10C more correctly than the latter. But to stimulations with 1000C and 100C, he could answer perfectly and his left eye maintained the level of LP at least to 100C, and partly to 10C. Fig.2c and 3a are also the same eye but after a 3-day interval.

The same consistent pattern of correctness can be seen.

This device has several advantages for recording low visual functions. They are:

1) There is no physical harm such as insertion of contact lens electrodes on the cornea or use of adhesive paste for decreasing resistance to the electrodes.

2) The goggles block any light from outside, so we can obtain a dark adapted condition after a certain period by wearing them. In other words, we don't need a special room to control the adaptation level.

3) Earphones can block most noise and the click draws the patient's attention to the test. Also, patients can concentrate during this examination even with a certain level of environmental noise, which occurs in most offices.

4) The trials with clicks without light stimulation made it possible to estimate the reliability of this examination in relation to the responses given for light stimulation. The level of error was relatively low throughout the test for each patient.

5) The stimulus intensity of 1000C was needed for grading the low visual function such as LP in RP patients. Since each stimulus was a flash of light, the adaptation level of the photoreceptors was not changed.

6) All stimuli and recordings are programmed in the control box and delivered automatically. It takes less than 7 minutes and anyone can perform this test, even RP patients by themselves.

In patients with RP, non- recordable electroretinograms have been emphasized as one of the characteristics in this clinical entity, even if they still have quite good vision. So it is difficult to differentiate their visual function, especially in such low vision with the amplitude of electrical responses. When we try to find or evaluate effects of some medical or surgical treatment for RP patients, it becomes a significant problem. The present results show this device can tell us the possibility of quantitative expression of the LP level, whether increased,

the same or decreased by the pattern of each record.The reason why the level of LP decreased after surgery in the first case is due to the postoperative inflammation and slight corneal edema. (Fig. 1) In the second case, we paid more attention to the duration of ultrasound application time for cataract extraction and tried to reduce the postinflammatory reaction with subconjunctival steroid administration. As a result, the day following the surgery, the LP improved much more than before. (Fig. 4). In reality, the second patient showed much less cell inflammation and flare in the anterior chamber and almost no corneal edema. The stellate ganglion block followed by the hyperbaric oxygen treatment has been reported to have some effect for improving visual function of RP patients.5 But this treatment is still controversial and finding no any positive effect, in fact, only a negative effect6, the trial was suspended. In both cases, the patients' visual function clearly improved comparing the before and after treatment with lipoprostaglandin E1 as shown in Figs. 2 and 4. Surprisingly, the patients did not feel that their light sensation had improved even when they were show the data which showed a and definite improvement. These results suggest the level of retinal perception (the minimum visible) and recognition with their visual cortex (the minimum cognizable) is not parallel. In the future, careful attention must be paid when evaluating the effects of new treatments, such discrepancies between real improvement of visual perception and the level of recognition.

Up to now, several medicines, such as helenine, have been used to improve visual field and dark adaptation, kallidinogenase to increase retinal blood flow, tocopherol acetate to improve peripheral blood circulation and inosine to activate cells in RP patients, at least in Japan. Idebenon (Co-Q10) and vitamin A are also administered to RP patients.7,8 With further study, this new device could prove to be a valuable instrument in evaluating the effect of currently prescribed medicine and/or vitamins.

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Fig.1

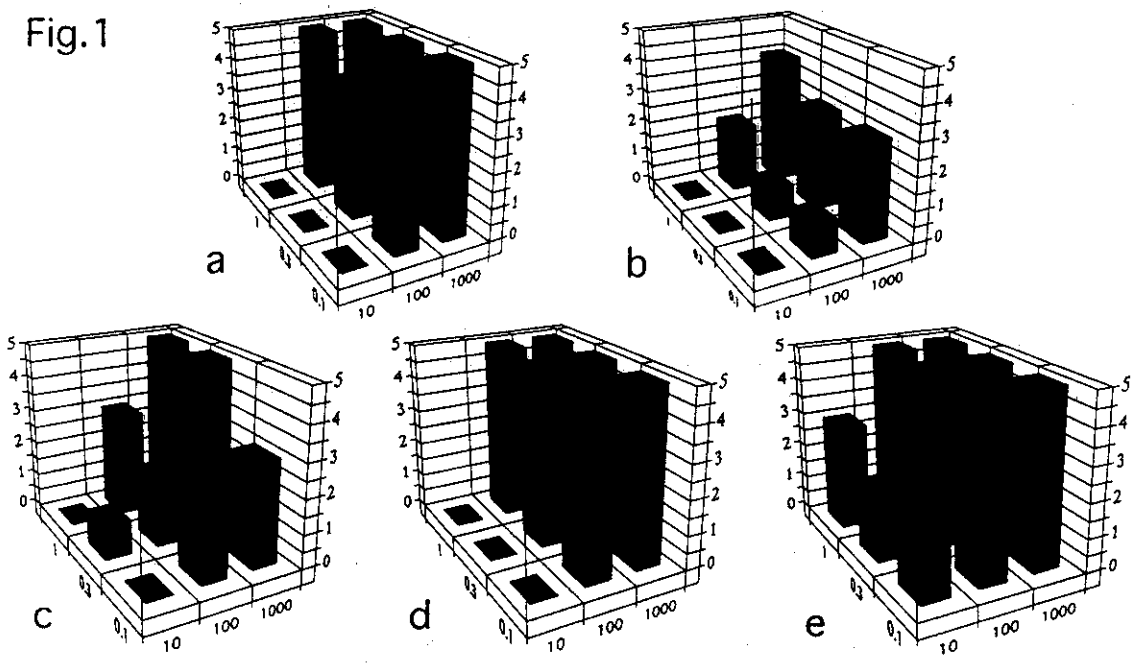

Fig. 1. The profile of light sensation with the LoVE device in the RP patient before (a) and after (b to e) cataract extraction. He is a 65-year-old male and the visual function before surgery was light perception. The visual field could not be detected and electroretinogram was non-recordable but he could correctly discriminate the light projection with conventional method which was performed with the bright light of an indirect ophthalmoscope. Before surgery, he could respond to the light stimulation with 1000C in any duration but partly failed with 100C and none with 10C. His error score was 1 in 100 trials which showed these results were very reliable. After surgery, his LP decreased once as shown in Figs. 1b and 1c (2 days following surgery) but recovered to the preoperative level at 3 (Fig. 1d) and better at 4 (Fig. 1e). His error score was 3/100, 0/100, 0/100 and 1/100 respectively. In this and the following figures, x-axis means the intensity of light , the y-axis, the duration of the stimulation and z-axis, the total number of correct answers, 45 in total trials to one eye.

Fig. 2

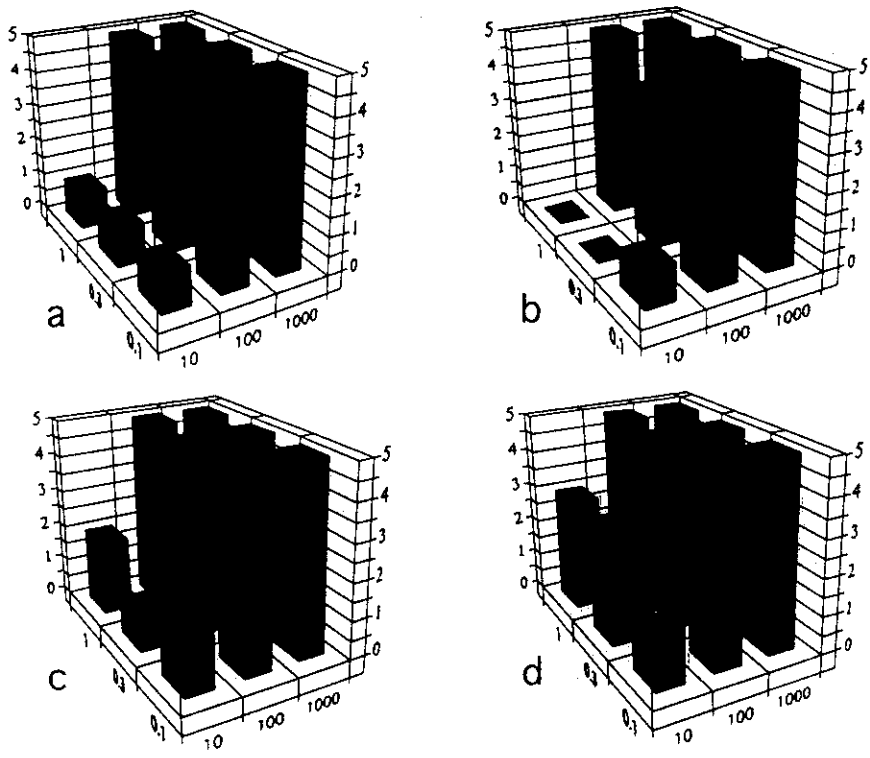

Fig. 2. The profile of light sensation with the LoVE device in the RP patient before (a and b) and after the treatment with lipoprostaglandin E1(c and d). The right eye shows Fig. 2a and 2c and the left, 2b and 2d. The effect of the drug was not clear in the right, but remarkable in the lefteye. The error score was 1/100, 0/100, 0/100, and 1/100, respectively.

Fig.3

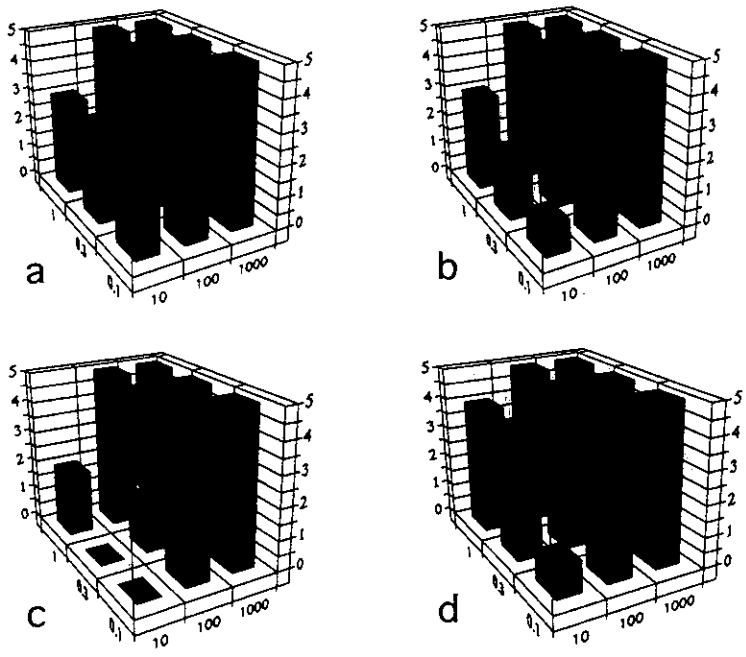

Fig. 3. The effect of cervical stellate ganglion block and hyperbaric oxygen chamber to the function of the RP patient. The level of LP was all correct with 1000C and 100C but difficult with 10C as shown in a (right) and b(left eye). After the block and oxygen treatment, the level of the LP was worse in the right (c) and no remarkable difference with 10C in the left (d), so the treatment was stopped.