視力低下を訴える66歳女性で，検眼鏡的所見，蛍光眼底造影所見に異常がみられない。全視野ERGでは杆体系，錐体系ともに正常反応で，黄斑部局所ERGの反応が消失しておりオカルト黄斑ジストロフィと診断される。*RP1L1*遺伝子には原因と考えられる変異が認められなかった。OCTにおける異常所見は中心小窩でみられるISeラインの部分欠損のみであり，*RP1L1*遺伝子変異をもつ症例のOCT所見とは明らかに異なっている。電気生理学的に診断されるオカルト黄斑ジストロフィには，病因を異にする複数の疾患が関与している可能性が考えられる。

まとめ

オカルト黄斑ジストロフィは，眼底所見および全視野ERGが正常であるため，多くの患者が原因不明の視神経疾患，弱視，非器質性（心因性）視力障害などと診断されてきた。黄斑部の機能低下は多局所ERGや黄斑部局所ERGによって容易に検出できるが，一般眼科においてそれらを施行することは困難と思われる。ただし，フーリエドメインOCTを観察することで黄斑部の異常を容易に検出できるため，早期から正確な診断に近づける可能性は格段に高くなったと思われる。

文献

1) Miyake Y, Ichikawa K, Shiose Y et al：Hereditary macular dystrophy without visible fundus abnormality. Am J Ophthalmol **108**：292-299, 1989
2) Miyake Y, Horiguchi M, Tomita N et al：Occult macular dystrophy. Am J Ophthalmol **122**：644-653, 1996
3) Akahori M, Tsunoda K, Miyake Y et al：Dominant Mutations in *RP1L1* Are Responsible for Occult Macular Dystrophy. Am J Hum Genet **87**：424-429, 2010
4) Tsunoda K, Usui T, Hatase T et al：Clinical characteristics of occult macular dystrophy in family with mutation of RP1l1 gene. Retina **32**：1135-47, 2012
5) Kabuto T, Takahashi H, Goto-Fukuura Y et al：A new mutation in the RP1L1 gene in a patient with occult macular dystrophy associated with a depolarizing pattern of focal macular electroretinograms. Molecular vision **18**：1031-1039, 2012
6) Ahn SJ, Cho SI, Ahn J et al：Clinical and genetic characteristics of Korean occult macular dystrophy patients. Invest Ophthalmol Vis Sci **54**：4856-4863, 2013
7) Davidson AE, Sergouniotis PI, Mackay DS et al：RP1L1 variants are associated with a spectrum of inherited retinal diseases including retinitis pigmentosa and occult macular dystrophy. *Hum Mutat* **34**：506-514, 2013
8) Fujinami K, Tsunoda K, Hanazono G et al：Fundus Autofluorescence in Autosomal Dominant Occult Macular Dystrophy. Arch. Ophthalmol **129**：597-602, 2011

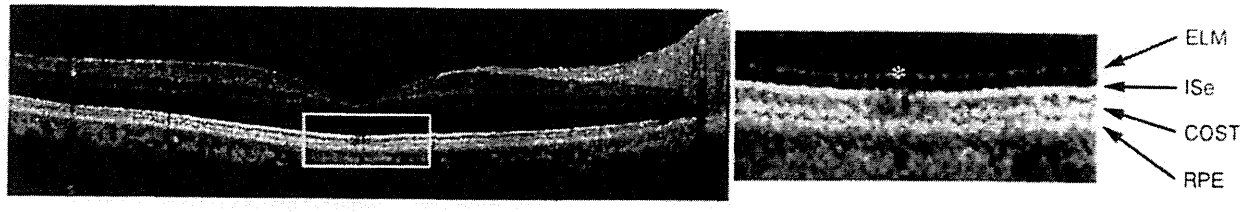

図 3 *RP1L1* 変異のない，孤発例のオカルト黄斑ジストロフィ（66 歳，女性）
症状経過および電気生理学的検査所見からオカルト黄斑ジストロフィと診断されるが，OCT における異常所見は中心小窩における ISe ラインの部分欠損のみであり，優性遺伝の典型例とは異なっている。*RP1L1* 変異のない症例の OCT 所見には，このほかにもいくつかのパターンがみられる。

わち，オカルト黄斑ジストロフィには *RP1L1* 遺伝子を原因とするタイプの他に，異なる原因による疾患群が含まれていると考えられる。

Practice

オカルト黄斑ジストロフィの検査所見

①自覚的検査

矯正視力は，通常は運転免許の取得に問題の生じる 0.7 未満に低下してから受診する場合が多い。他の黄斑ジストロフィと異なり網膜色素上皮の萎縮をきたすことがないため，最終視力が 0.1 を下回るケースはない。80 歳の時点でも 1.0 の視力を維持している患者もおり，進行には大きな個体差がある[4]。

視野検査では中心比較暗点が検出されるが，Goldmann 動的視野計でも進行例を除いて異常を検出できないことも多い。軽症例では，Humphrey 自動視野計「中心 30-2」でも中心比較暗点が明瞭に検出できず，「中心 10-2」でようやく暗点が検出される例も多い。黄斑部以外の周辺視野は進行例でも正常に保たれている。

②他覚的検査

検眼鏡的所見（図 1a），フルオレセイン蛍光眼底造影，インドシアニングリーン蛍光眼底造影ともに正常である。眼底自発蛍光は正常であることが多いが（図 1b），近視の強い症例や長期間罹患している症例では中心小窩に軽度の過蛍光を認めることがある[5]。ただし，他の黄斑ジストロフィ，錐体・杆体ジストロフィ，Stargardt 病などに特徴的な強い過蛍光や低蛍光などの異常所見はみられない。

全視野 ERG では，杆体系，錐体系反応ともに正常に記録されるが，黄斑部局所 ERG あるいは多局所 ERG で黄斑部の反応が減弱しており，これがオカルト黄斑ジストロフィの確定診断となる。

遺伝子検査で診断が確定したオカルト黄斑ジストロフィでは，OCT で特徴的な黄斑部の網膜外層構造異常が観察される（図 1c）[4]。すなわち，黄斑部に限局した錐体視細胞外節先端部（cone outer segment tip：以下，COST）ラインの消失，視細胞内節 ellispoid ライン（ISe ライン；別称 IS/OS 接合部ライン）の不明瞭化などである。また，OCT の所見は発症から長期間経過するにつれて次第に変化していく（図 2）[4]。すなわち，発症から 10〜20 年ほどまでは中心窩の COST ラインの消失，および ISe ラインの境界不明瞭化（厚く，膨潤したように見える）がみられるものの，中心窩網膜厚はほぼ正常である。さらに長期間経過すると，中心窩で ISe ラインの分断がみられるようになり，外顆粒層は徐々に菲薄化していく。一方，黄斑部以外の視細胞構造は長期間経過しても正常である（図 2）。ただし COST ラインについては，高度近視，光量不足，固視不良など測定条件が悪い場合，正常者でも明瞭に観察されないことがあるので注意が必要である。

一方，家族歴のない孤発例のオカルト黄斑ジストロフィには，上記と全く異なる OCT 所見を示す症例も多い。図 3 は両眼にゆっくりと進行する

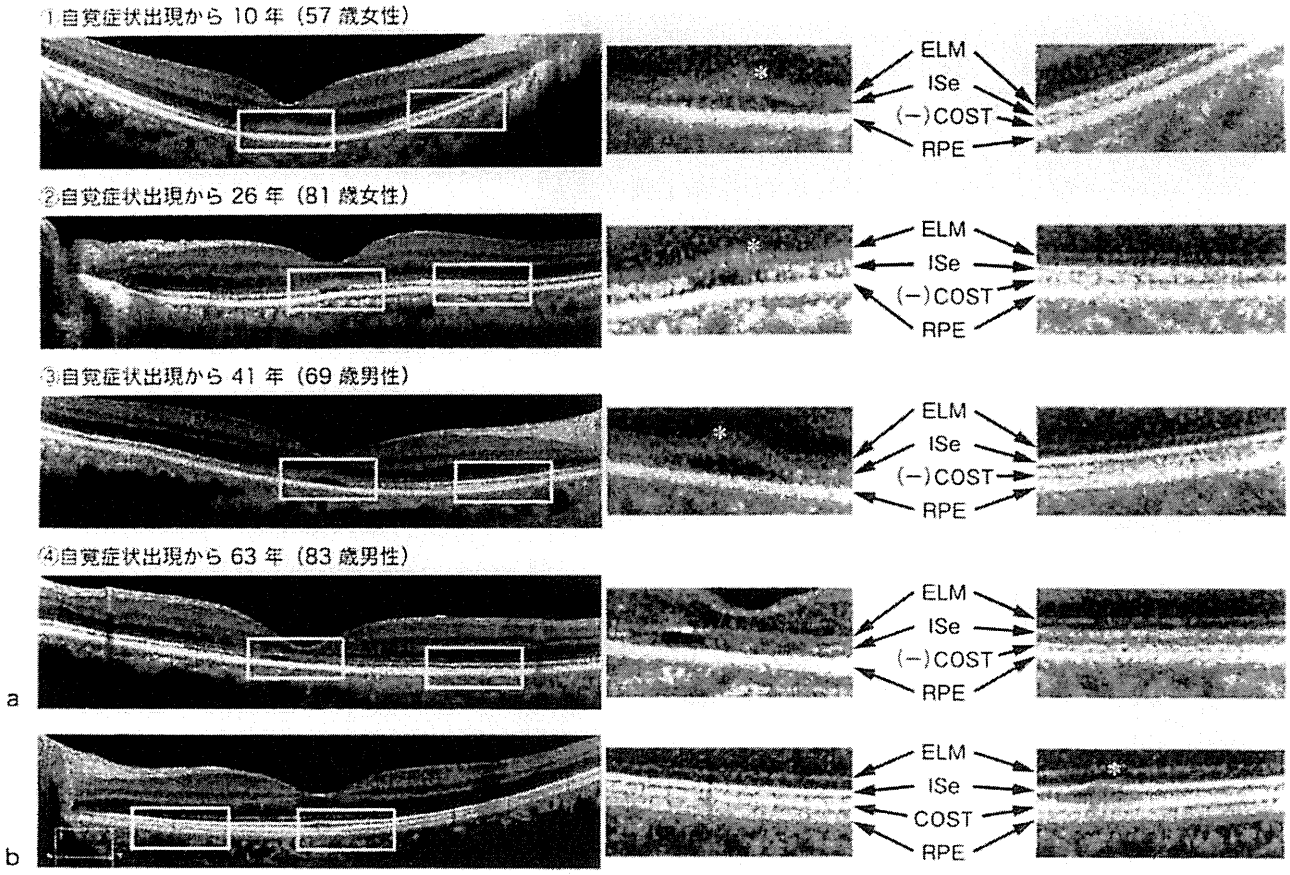

図 2　*RP1L1* 変異を有するオカルト黄斑ジストロフィ患者（*RP1L1*, p.Arg45Trp, heterozygous）（a）における，推定罹患期間（視力低下を自覚し始めてからの期間）による OCT 所見の違い

発症から 20 年ほどまでは，中心窩の COST ラインの消失および ISe ラインの境界不明瞭化がみられるものの，中心窩網膜厚はほぼ正常である。さらに長期間経過すると，中心窩で ISe ラインの分断がみられるようになり，外顆粒層が菲薄化していく。一方，黄斑部以外の視細胞構造は長期間経過しても正常である。中心窩と黄斑部周囲の画像を右に拡大で示した。b：健常者（22 歳，女性）の所見。

ていく。両眼がほぼ同時に進行する例が多いが，自覚症状の出現や視力低下の進行が左右眼で数年以上異なるケースもある。根本的な治療法はないが，ほとんどの患者は拡大鏡などを用いることにより日常の読み書きをこなしている。周辺視野は末期でも正常に保たれるため，歩行時に大きな困難はきたさない。

三宅病の原因は？

2010 年に東京医療センターを中心とした研究グループにより，優性遺伝タイプのオカルト黄斑ジストロフィの原因遺伝子として 8 番短腕に位置する *RP1L1* が同定された[3]。その後，国内外においても同様の遺伝子異常の報告がなされている[5〜7]。ヒトにおける *RP1L1* の機能はいまだ完全に解明されていない。これまでの研究では，霊長類では錐体および杆体視細胞の特に内節に発現しており，視細胞内節・外節の構造維持，細胞内輸送に大きな役割を果たしていると考えられている。当院におけるこれまでの調査では，優性遺伝家系のオカルト黄斑ジストロフィにおいてはほぼ全例で *RP1L1* 遺伝子の変異が確認できる。ただし，孤発例の症例のなかには *RP1L1* 遺伝子に異常がみられない症例や，後述するように OCT 所見が典型的なタイプと異なる症例も含まれる。すな

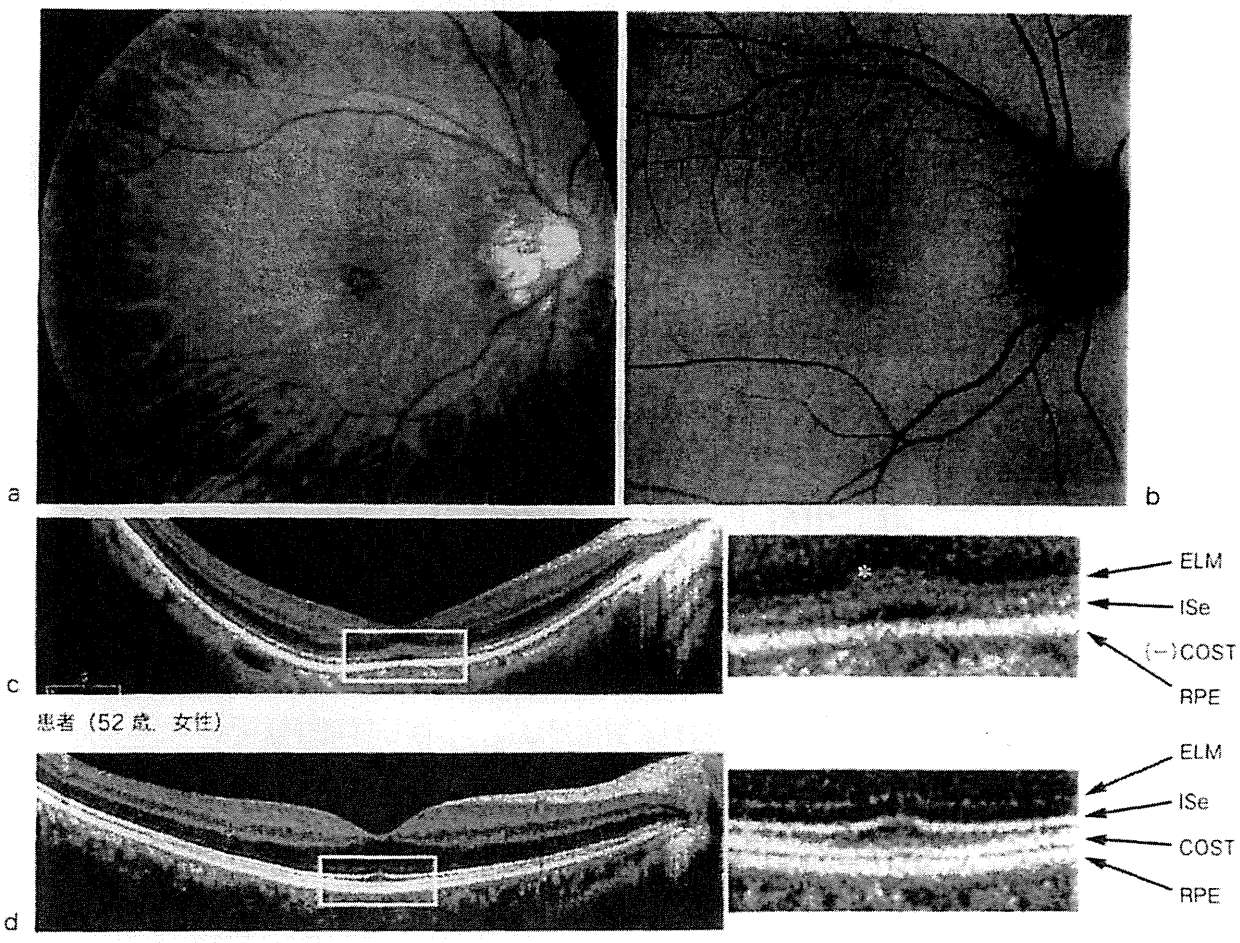

図 1　オカルト黄斑ジストロフィの所見
オカルト黄斑ジストロフィ患者（52歳，女性。*RP1L1* p.Arg45Trp heterozygous）の眼底写真（a），眼底自発蛍光（b）およびOCT所見（c）。矯正視力は左右とも0.15。dは健常者（45歳，男性）のOCT所見。
眼底写真および眼底自発蛍光では異常を認めない。OCTでは，黄斑部でCOSTラインが消失し，さらに中心窩のISeラインが不明瞭となっている（＊）。網膜色素上皮は正常である。なお，この患者は−9.0D程度の高度近視のため，COSTラインは後極部全体で不明瞭となっている。

オカルト黄斑ジストロフィの症状および経過

オカルト黄斑ジストロフィとは，1989年に名古屋大学の三宅養三[1]によって「眼底所見に異常の見られない家族性黄斑症」として初めて紹介された疾患である。その後，正常な眼底所見によって網膜の異常が隠されていることから，オカルト（occult＝目に見えない）黄斑ジストロフィと命名された[2]。さらに発見からほぼ20年を経過した2010年には，本疾患の原因遺伝子として8番染色体短腕に*RP1L1*（retinitis pigmentosa 1 like-1）が特定された[3]。現在では発見者の名前から「三宅病（Miyake's disease）」と呼ばれることもある。

黄斑部，特に中心窩の視細胞機能が局所的に低下するため，視力低下および中心比較暗点が主な症状である。視力障害は徐々に進行し，最終的に0.1〜0.2程度まで低下することがある[4]。羞明を訴える患者も多い。進行は非常にゆっくりであるため，自覚症状の出るかなり以前から黄斑部の機能低下は始まっていると考えられる。自覚症状を訴える時期は10歳ごろ〜60歳以上までと非常に幅があり，両眼の視力がきわめてゆっくりと低下し

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何が見える？何がわかる？OCT

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Series 2 応用編

第16回

オカルト黄斑ジストロフィ（三宅病）のOCT所見

角田和繁　東京医療センター

Point

- オカルト黄斑ジストロフィは検眼鏡的所見に異常がなく，しばしば視神経疾患などと間違われる。
- 詳細なERG検査によって確定診断が可能である。
- OCTを用いると，黄斑部視細胞層構造の異常がほぼ全例で確認できる。

Basic Lecture

眼底検査で異常が見つからない網膜疾患

視力不良を訴える患者において前眼部検査や眼底検査で異常が見つからないとき，通常は視神経疾患，頭蓋内疾患，弱視，あるいは非器質性（心因性）視力障害などを疑い，さらに詳細な検査を行うことが多い。しかし，検眼鏡的所見に異常がみられない網膜疾患は決して稀ではなく，初診時に網膜疾患を除外してしまうと最終診断を誤る可能性が非常に高くなる。具体的には，一部の錐体ジストロフィ，初期の網膜色素変性，先天性停止性夜盲症，急性帯状潜在性網膜外層症（acute zonal ocault outer retinopathy：AZOOR），初期の癌関連網膜症，悪性黒色腫関連網膜症，回復期の網膜血管閉塞症，一部の薬剤性網膜障害（ジゴキシン，タモキシフェン，クロロキン）など，多岐にわたる。これらの疾患の多くは杆体系あるいは錐体系ERG（electroretinogram）において何らかの異常をきたすことが多いが，なかでも全視野ERGに全く異常がみられず診断が困難であるのがオカルト黄斑ジストロフィである。

OCT Findings in Occult Macular Dystrophy, Miyake's Disease

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5. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (*ABCR*) is mutated in recessive Stargardt macular dystrophy. Nat Genet 1997;15: 236–46.
6. Cremers FP, van de Pol DJ, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene *ABCR*. Hum Mol Genet 1998;7:355–62.
7. Rozet JM, Gerber S, Souied E, et al. Spectrum of *ABCR* gene mutations in autosomal recessive macular dystrophies. Eur J Hum Genet 1998;6:291–5.
8. Fishman GA, Stone EM, Grover S, et al. Variation of clinical expression in patients with Stargardt dystrophy and sequence variations in the *ABCR* gene. Arch Ophthalmol 1999;117: 504–10.
9. Lewis RA, Shroyer NF, Singh N, et al. Genotype/phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, *ABCR*, in Stargardt disease. Am J Hum Genet 1999;64:422–34.
10. Briggs CE, Rucinski D, Rosenfeld PJ, et al. Mutations in *ABCR (ABCA4)* in patients with Stargardt macular degeneration or cone-rod degeneration. Invest Ophthalmol Vis Sci 2001;42:2229–36.
11. Martinez-Mir A, Paloma E, Allikmets R, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene *ABCR* [letter]. Nat Genet 1998;18:11–2.
12. Burke TR, Fishman GA, Zernant J, et al. Retinal phenotypes in patients homozygous for the G1961E mutation in the *ABCA4* gene. Invest Ophthalmol Vis Sci 2012;53:4458–67.
13. Burke TR, Tsang SH. Allelic and phenotypic heterogeneity in *ABCA4* mutations. Ophthalmic Genet 2011;32:165–74.
14. Schindler EI, Nylen EL, Ko AC, et al. Deducing the pathogenic contribution of recessive *ABCA4* alleles in an outbred population. Hum Mol Genet 2010;19:3693–701.
15. Zernant J, Schubert C, Im KM, et al. Analysis of the *ABCA4* gene by next-generation sequencing. Invest Ophthalmol Vis Sci 2011;52:8479–87.
16. Fishman GA, Stone EM, Eliason DA, et al. *ABCA4* gene sequence variations in patients with autosomal recessive cone-rod dystrophy. Arch Ophthalmol 2003;121:851–5.
17. Maugeri A, Klevering BJ, Rohrschneider K, et al. Mutations in the *ABCA4 (ABCR)* gene are the major cause of autosomal recessive cone-rod dystrophy. Am J Hum Genet 2000;67: 960–6.
18. Rivera A, White K, Stohr H, et al. A comprehensive survey of sequence variation in the *ABCA4 (ABCR)* gene in Stargardt disease and age-related macular degeneration. Am J Hum Genet 2000;67:800–13.
19. Simonelli F, Testa F, Zernant J, et al. Genotype-phenotype correlation in Italian families with Stargardt disease. Ophthalmic Res 2005;37:159–67.
20. Cella W, Greenstein VC, Zernant-Rajang J, et al. G1961E mutant allele in the Stargardt disease gene *ABCA4* causes bull's eye maculopathy. Exp Eye Res 2009;89:16–24.
21. Aguirre-Lamban J, Gonzalez-Aguilera JJ, Riveiro-Alvarez R, et al. Further associations between mutations and polymorphisms in the *ABCA4* gene: clinical implication of allelic variants and their role as protector/risk factors. Invest Ophthalmol Vis Sci 2011;52:6206–12.
22. Wiszniewski W, Zaremba CM, Yatsenko AN, et al. *ABCA4* mutations causing mislocalization are found frequently in patients with severe retinal dystrophies. Hum Mol Genet 2005;14:2769–78.
23. Maugeri A, van Driel MA, van de Pol DJ, et al. The 2588G–>C mutation in the *ABCR* gene is a mild frequent founder mutation in the Western European population and allows the classification of ABCR mutations in patients with Stargardt disease. Am J Hum Genet 1999;64:1024–35.
24. Westeneng-van Haaften SC, Boon CJ, Cremers FP, et al. Clinical and genetic characteristics of late-onset Stargardt's disease. Ophthalmology 2012;119:1199–210.
25. Rosenberg T, Klie F, Garred P, Schwartz M. N965S is a common ABCA4 variant in Stargardt-related retinopathies in the Danish population. Mol Vis [serial online] 2007;13: 1962–9. Available at, http://www.molvis.org/molvis/v13/a221/. Accessed April 7, 2013.
26. Shroyer NF, Lewis RA, Lupski JR. Complex inheritance of *ABCR* mutations in Stargardt disease: linkage disequilibrium, complex alleles, and pseudodominance. Hum Genet 2000;106: 244–8.
27. Fukui T, Yamamoto S, Nakano K, et al. *ABCA4* gene mutations in Japanese patients with Stargardt disease and retinitis pigmentosa. Invest Ophthalmol Vis Sci 2002;43:2819–24.
28. Simonelli F, Testa F, Zernant J, et al. Association of a homozygous nonsense mutation in the *ABCA4 (ABCR)* gene with cone-rod dystrophy phenotype in an Italian family. Ophthalmic Res 2004;36:82–8.
29. McBain VA, Townend J, Lois N. Progression of retinal pigment epithelial atrophy in Stargardt disease. Am J Ophthalmol 2012;154:146–54.
30. Tsunoda K, Watanabe K, Akiyama K, et al. Highly reflective foveal region in optical coherence tomography in eyes with vitreomacular traction or epiretinal membrane. Ophthalmology 2012;119:581–7.
31. Sergouniotis PI, Holder GE, Robson AG, et al. High-resolution optical coherence tomography imaging in *KCNV2* retinopathy. Br J Ophthalmol 2012;96:213–7.
32. Marmor MF, Fulton AB, Holder GE, , et alInternational Society for Clinical Electrophysiology of Vision. ISCEV standard for full-field clinical electroretinography (2008 update). Doc Ophthalmol 2009;118:69–77.
33. Bach M, Brigell MG, Hawlina M, et al. ISCEV standard for clinical pattern electroretinography (PERG): 2012 update. Doc Ophthalmol 2013;126:1–7.
34. Lois N, Holder GE, Bunce C, et al. Phenotypic subtypes of Stargardt macular dystrophy-fundus flavimaculatus. Arch Ophthalmol 2001;119:359–69.
35. Jaakson K, Zernant J, Kulm M, et al. Genotyping microarray (gene chip) for the *ABCR (ABCA4)* gene. Hum Mutat 2003;22: 395–403.
36. O'Sullivan J, Mullaney BG, Bhaskar SS, et al. A paradigm shift in the delivery of services for diagnosis of inherited retinal disease. J Med Genet 2012;49:322–6.
37. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 2003;31: 3812–4.
38. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations [letter]. Nat Methods 2012;7:248–9.
39. Biswas EE, Biswas SB. The C-terminal nucleotide binding domain of the human retinal ABCR protein is an adenosine triphosphatase. Biochemistry 2000;39:15879–86.
40. Molday RS, Zhong M, Quazi F. The role of the photoreceptor ABC transporter ABCA4 in lipid transport and Stargardt macular degeneration. Biochim Biophys Acta 2009;1791: 573–83.

(1.08 and 2.0), type 3 fundus appearance, type 3 AF pattern, and ERG group 3.

Patient 4, homozygous for p.Glu905fsX916, showed a severe phenotype: age of onset (5 years), visual acuity loss (1.0 for both eyes), type 2 fundus appearance, type 2 AF pattern, and ERG group 3. Patient 13, homozygous for p.Arg1300X, had a very severe disease, with early onset (8 years), visual acuity loss (1.3 and 1.0), type 3 fundus appearance, type 2 AF pattern, and ERG group 3. A severe phenotype also was observed in patient 14, homozygous for c.4253+4C>T, with early onset (8 years), visual acuity loss (1.0 both eyes), type 2 fundus appearance, type 2 AF pattern, and ERG group 3. Four patients (patients 15, 16, 17, and 18), homozygous for p.Gln2220X, from 2 families demonstrated a severe phenotype: early onset (<10 years) in 3 patients, visual acuity (1.0–3.0), type 1 fundus appearance with vessel attenuation or type 3 fundus appearance, and ERG group 3.

Discussion

This study assessed the detailed phenotypic features of a cohort of patients with homozygous *ABCA4* alleles, allowing a detailed clinical assessment of the effect of each individual allele on retinal structure and function. The effects of 8 variants are reported in the homozygous state for the first time in this study (Table 4, available at http://aaojournal.org).

Patients with homozygous missense variants had variable phenotypes, ranging from very severe and early onset to relatively mild and later onset. Three patients, homozygous for p.Gly1961Glu, had localized dysfunction confined to the macula with no evidence of generalized retinal dysfunction, consistent with previous reports.[12,20] Burke et al[12] reported that patients homozygous for p.Gly1961Glu usually have milder disease, with severe phenotypes linked to the presence of additional *ABCA4* variants. The 2 previously reported variants (p.Asn96Lys and p.His1838Asp) in complex with p.Gly1961Glu that were associated with a very severe phenotype were not detected in our patients.[12] We observed a particularly late-onset mild disorder, with numerous flecks and foveal sparing, associated with p.Leu2027Phe, suggesting primary disease of the parafoveal RPE with preservation of foveal structure, a "foveal sparing" phenotype. This supports previous reports of p.Leu2027Phe in heterozygous patients.[14,23] Of note, p.Gly1961Glu and p.Leu2027Phe are both situated in the nucleotide-binding domain 2 subunit, shown to be the site of adenosine triphosphatase activity (Fig 5, available at http://aaojournal.org).[39,40]

Subjects homozygous for p.Arg212Cys had phenotypes of moderate severity, and these findings are generally in keeping with previous reports.[19] Patient 5, homozygous for p.Val931Met and p.Arg1705Gln in complex, and subjects 6 and 7, homozygous for p.Cys1488Arg, had "typical" Stargardt's disease fundus and AF findings with macular atrophy surrounded by flecks with electrophysiologic evidence of generalized rod and cone system dysfunction.

A very severe phenotype was observed in 2 patients with homozygous missense variants in this study. Patient 3, homozygous for p.Leu541Pro and p.Ala1038Val, had a very early onset associated with severe disease. These findings are similar to genetically identical patients reported by Wiszniewski et al.[22] A very severe phenotype also was observed in patient 8 (p.Arg1640Trp), in keeping with the severe findings of genetically identical subjects, homozygous for p.Arg1640Trp, in a previous report by Briggs et al.[10]

In contrast, patients with homozygous null variants consistently showed a very severe phenotype: 6 of 7 had early-onset disease (<10 years), and all 7 had electrophysiologic evidence of generalized rod and cone system dysfunction. These findings support previous familial reports[6,10,27] and highlight the fact that generalized functional loss (ERG) precedes fundus or AF abnormalities, as observed in 5 pediatric patients.

Haplotype analysis in 2 probands homozygous for p.G1961E and 2 homozygous for p.Gln2220X suggested a founder effect for these mutations in these consanguineous families from South Asia, although some of the screened variants were not rare. Concordance in phenotypic features between patients with an identical disease-causing variant was observed in individuals homozygous for p.Arg212Cys, p.Cys1488Arg, p.Gly1961Glu, and p.Gln2220X, suggesting that few modifiers exist.

In conclusion, 2 patients were homozygous for 2 variants previously reported as associated with disease (patient 3, p.Leu541Pro and p.Ala1038Val; patient 5, p.Val931Met and p.Arg1705Gln; Table 4, available at http://aaojournal.org). However, p.Ala1038Val and p.Val931Met were present in a relatively high number of chromosomes on the Exome Variant Server database and were predicted to be tolerant by Sorting Intolerant from Tolerance analysis. It remains to be seen whether these variants are merely neutral polymorphisms in cis with disease-causing ones or these families have 2 independently damaging missense variants. These data help to provide insight into the retinopathy caused by specific *ABCA4* alleles in this clinically variable disorder and may guide clinicians in the selection of patients for therapeutic clinical trials for *ABCA4*-related retinal disease in the future.

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References

1. Stargardt K. Über familiäre, progressive Degeneration in der Maculagegend des Auges. Albrecht Von Graefes Arch Ophthalmol 1909;71:534–50.
2. Webster AR, Heon E, Lotery AJ, et al. An analysis of allelic variation in the *ABCA4* gene. Invest Ophthalmol Vis Sci 2001;42:1179–89.
3. Michaelides M, Hunt DM, Moore AT. The genetics of inherited macular dystrophies. J Med Genet 2003;40:641–50.
4. Fujinami K, Akahori M, Fukui M, et al. Stargardt disease with preserved central vision: identification of a putative novel mutation in ATP-binding cassette transporter gene [letter] [report online]. Acta Ophthalmol 2011;89:e297–8.

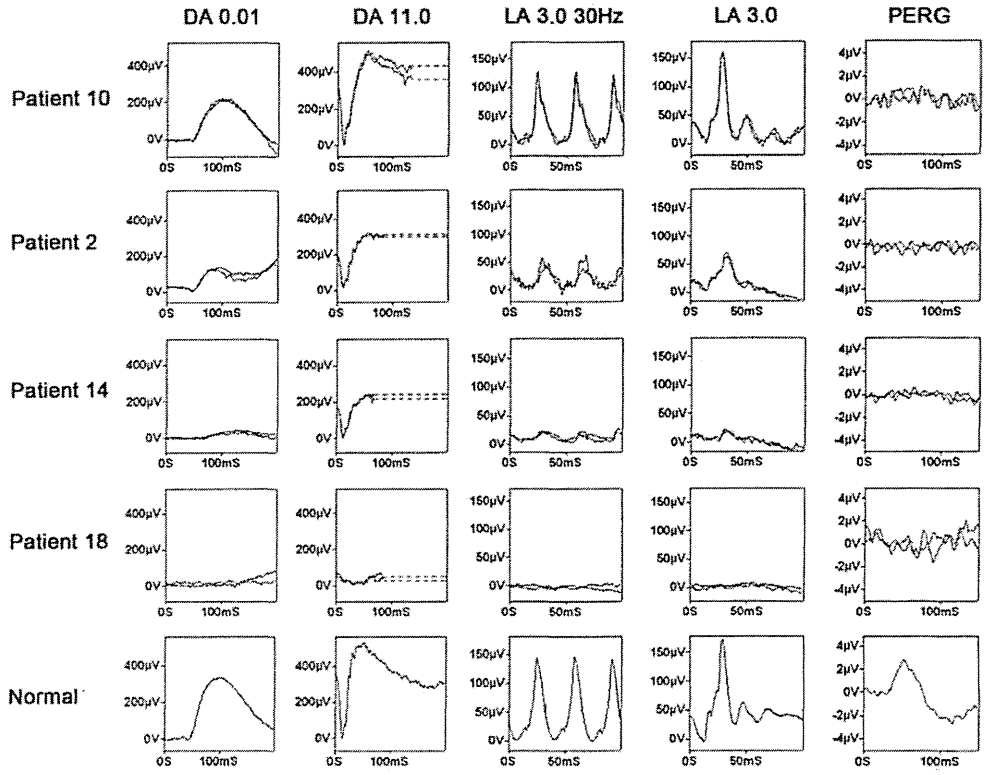

Figure 4. Full-field electroretinograms (ERGs) and pattern ERGs (PERGs) from 4 representative cases of Stargardt's disease harboring homozygous *ABCA4* alleles (patients 10, 2, 14, and 18). Patient 10 demonstrates undetectable PERG and normal full-field ERGs, consistent with ERG group 1. Patient 2 has undetectable PERG and abnormal responses in light-adapted (LA) single flash ERG (LA 3.0) and LA 30 flicker ERG (LA 3.0 30 Hz), whereas responses in dark-adapted (DA) dim flash ERG (DA 0.01) and DA bright flash ERG (DA 11.0) are slightly abnormal. Patient 14 has undetectable PERG, subnormal DA ERGs (DA 0.01 and DA 11.0), and mildly delayed and markedly subnormal LA ERGs (LA 3.0 and LA 3.0 30 Hz). Patient 18 demonstrates undetectable responses in PERG and full-field ERGs. The electrophysiologic findings of patients 2, 14, and 18 are classified into group 3. Normal traces are shown in the *bottom* row for comparison.

To define the chromosomal haplotype for the alleles detected in this study, 9 *ABCA4* polymorphisms were examined in probands from 10 families (Table 5, available at http://aaojournal.org). In 8 of 10 probands, all variants were homozygous, suggesting autozygosity and defining the haplotype for these polymorphisms (Table 5, available at http://aaojournal.org). Two probands harbored heterozygous changes: patient 5 from family 4 with no known consanguinity and patient 12 from family 9 with no consanguinity. Three heterozygous changes were found in patient 12, suggesting that the p.Leu2027Phe had occurred recurrently on 2 distinct chromosomal backgrounds. In patient 5, 1 of 9 polymorphisms was heterozygous, c.1356+5delG, 29.7 kb from the disease-causing variant (p.Val931Met). Further genotyping might determine whether the mutation occurred recurrently or c.1356+5delG occurred on an ancient common haplotype.

Genotype–Phenotype Association

The phenotype severity associated with each variant and concordance between patients with identical disease-causing variants was investigated (Tables 2–4, available at http://aaojournal.org). Two missense alleles were associated with a mild phenotype, and 3 missense alleles were linked to a moderate phenotype (Table 4, available at http://aaojournal.org). A severe phenotype was associated with 2 missense alleles and 4 null alleles (Table 4, available at http://aaojournal.org).

Three patients from 2 families (patients 9, 10, and 11), homozygous for p.Gly1961Glu, showed similar mild findings: adult onset (17–30 years), type 1 fundus appearance, type 1 or 2 AF pattern, and ERG group 1 but variable visual acuity and OCT findings. A very mild phenotype was observed in patient 12, homozygous for p.Leu2027Phe, with late onset (44 years), well-preserved visual acuity (0.18 and 0.0 for each eye), type 2 fundus appearance, type 2 AF pattern with spared AF signal at the fovea, and normal full-field ERGs (group 1).

Two siblings (patients 1 and 2), homozygous for p.Arg212Cys, showed a similar moderate findings: age of onset (11 years), logMAR visual acuity (1.0), type 2 fundus appearance, type 2 AF pattern, and atrophic change at the fovea on OCT, but ERG grouping was discordant (group 1 and 3). Patient 5, homozygous for both p.Val931Met and p.Arg1705Gln, had moderate findings: age of onset (10 years), visual acuity (1.0 and 1.08 for each eye), type 2 fundus appearance, type 2 AF pattern, and ERG group 3. Two siblings (patients 6 and 7), homozygous for p.Cys1488Arg, also had a similar moderate findings: age of onset (10 years), type 2 fundus appearance, type 2 AF pattern, and ERG group 3 but variable visual acuity.

Patient 3, homozygous for both p.Leu541Pro and p.Ala1038Val, showed a very severe phenotype: age of onset (3 years), visual acuity (1.2 for both eyes), type 2 fundus appearance, type 2 AF pattern, and ERG group 3. Patient 8, homozygous for p.Arg1640Trp, had severe disease but of adult onset: visual acuity

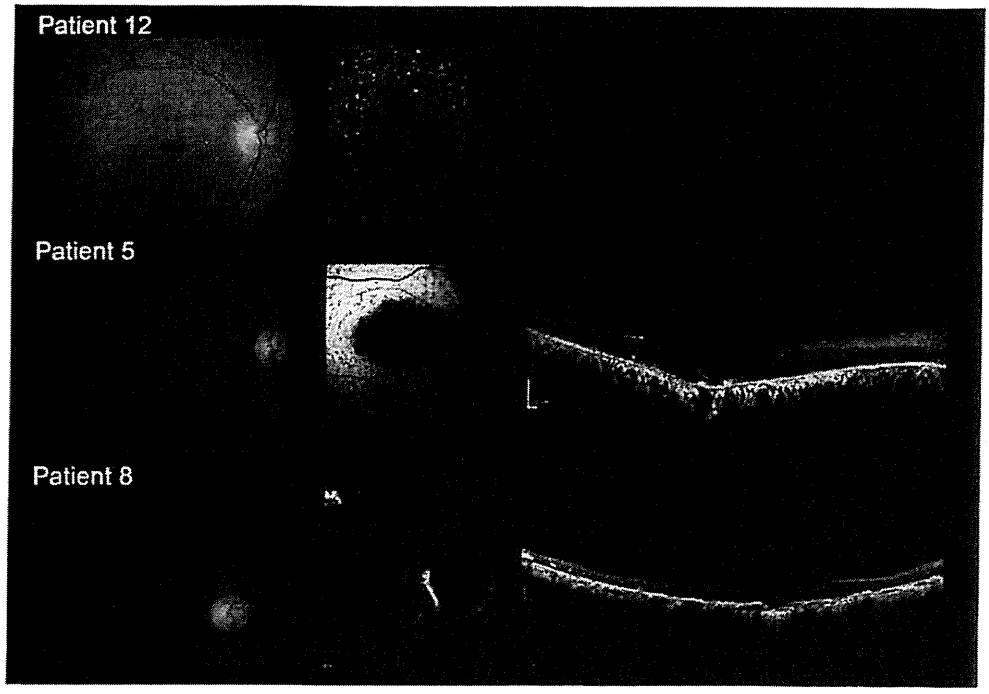

Figure 2. Color fundus photographs (left), autofluorescence (AF) images (middle), and optical coherence tomography (OCT) images (right) of 3 representative cases with Stargardt's disease harboring homozygous missense alleles, showing a mild phenotype in patient 12, a moderated phenotype in patient 5, and a severe phenotype in patient 8.

14 patients (Table 3). One patient (7%) had a type 1 AF pattern, 12 patients (86%) had a type 2 pattern, and 1 patient (7%) had a type 3 pattern. Optical coherence tomography images were available in 8 patients (Table 3). Central foveal thickness ranged from 31 to 128 μm. Six patients (75%; patients 1, 2, 3, 5, 11, and 13) showed foveal atrophy in both the neurosensory retina and the RPE, with disruption observed mainly in outer retinal layers at the macula; 2 patients (25%; patients 8 and 9) had a disrupted outer retinal layer with preserved inner retinal layers at the macula. Electrophysiologic assessment was available in 18 patients (Table 3). Five patients (28%) had a group 1 ERG, and 13 patients (72%) had a group 3 ERG.

Molecular Genetics

Mutational screening was performed with the APEX technology in 12 probands (patients 1, 3, 5, 6, 8, 9, 11, 12, 13, 14, 15, and 18), and the high-throughput NGS was applied to 1 proband (patient 3). Ten homozygous *ABCA4* variants that likely cause disease were detected in 18 patients. Detailed results including in silico analysis to predict pathogenicity are shown in Table 4 (available at http://aaojournal.org).

Eleven patients (61%) had homozygous missense variants, and 7 (39%) were homozygous for presumed null variants (Table 3). Of 13 variants identified, 9 were missense variants and 4 were presumed null variants. The 4 null variants include 1 frame shift (p.Glu905fsX916), 2 stops (p.Arg1300X and p.Gln2220X), and 1 intronic variant (c.4253+4C>T), which is likely to disrupt the splice-donor site of intron 28. Coexistence of 2 homozygous missense variants was observed in 2 patients (patients 3 and 5). One putative novel variant (p.Glu905fsX916) was identified by NGS, and 7 have not been described in the homozygous state before: p.Val931Met, p.Cys1488Arg, p.Arg1705Gln, p.Leu2027-Phe, p.Arg1300X, c.4253+4C>T, and p.Gln2220X (Table 4, available at http://aaojournal.org). A schematic of the *ABCA4* protein structure showing the position of the missense variants detected in this study is presented in Figure 5 (available at http://aaojournal.org).

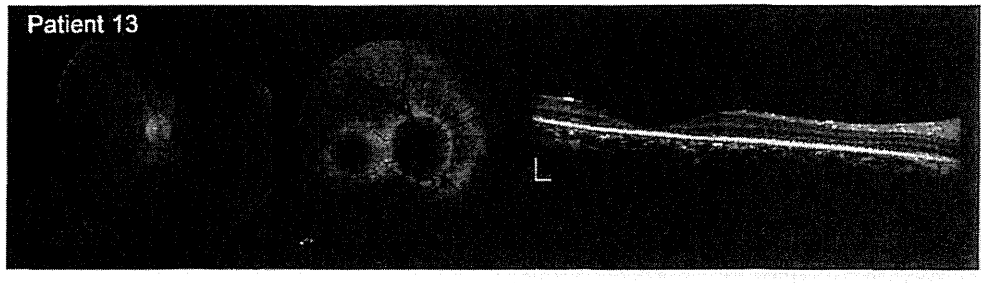

Figure 3. Color fundus photographs (left), autofluorescence (AF) images (middle), and optical coherence tomography (OCT) images (right) of 1 representative case with a severe phenotype of Stargardt's disease harboring homozygous null alleles (patient 13).

Sorting Intolerant from Tolerance (available at: http://sift.jcvi.org/; accessed November 1, 2012)[37] and PolyPhen2 (available at: http://genetics.bwh.harvard.edu/pph/index.html; accessed November 1, 2012).[38] A frequency for each allele was estimated with reference to the Exome Variant Server (National Heart, Lung, and Blood Institute Exome Sequencing Project, Seattle, WA; available at: http://snp.gs.washington.edu/EVS/; accessed November 1, 2012).

Previously reported variants that likely do not cause disease (polymorphisms) also were screened by the APEX technology in probands of each family to define the chromosomal haplotype for the alleles detected in this study.[2,12,21]

Results

Clinical Findings

Eighteen patients from 13 families were included in the study. The clinical, genetic, and electrophysiologic findings are summarized in Table 3. There were 12 male and 6 female patients. Fourteen patients from 9 families are originally from South Asia, 2 patients from 2 families are from Middle East Asia, and 2 patients from 2 families are from Europe. There were 3 sibling pairs (patients 1 and 2, 6 and 7, and 9 and 10) and a further family with 2 affected siblings and an affected cousin (patient 16, 17, and 18 respectively; Fig 1, available at http://aaojournal.org). Consanguinity was reported in 11 families: 7 first cousin marriages, 1 uncle-niece marriage, 1 second cousin once removed marriage, and 2 unknown related marriages (Table 3). One patient had delayed developmental milestones as an infant (patient 3).

All affected patients except for 1 (patient 12) had central visual loss, with the age of onset ranging from 3 to 30 years (Table 3). One individual with few symptoms was referred because of fundus abnormality at the age of 40 years (patient 12). Seven patients had early onset (<10 years) (patients 3, 4, 13, 14, 15, 16, and 17). The age at examination ranged from 7 to 48 years, and the duration of disease ranged from 0 to 39 years. The logMAR visual acuities ranged from 0.0 to 3.0, and 3 patients (17%; patients 10, 11, and 12) had a logMAR visual acuity better than 1.0 in at least 1 eye. One patient (patient 12) had preserved logMAR visual acuity, with 0.18 in the right eye and 0.0 in the left eye.

Color fundus photographs, AF images, and OCT images of representative cases with each variant are shown in Figures 2 and 3: 3 representative cases with missense variants in Figure 2 and 1 case with presumed null variants in Figure 3. Electrophysiologic traces of 4 representative cases are shown in Figure 4.

Of 18 patients, 5 (28%) had a type 1 fundus appearance, 9 (50%) had a type 2 appearance, and 4 (22%) had a type 3 appearance (Table 3). Autofluorescence images were available in

Table 3. Summary of Clinical and Molecular Status of 18 Patients with *ABCA4* Homozygous Variants

Pt No.	FM No.	Sex	Ethnicity	Consanguinity	Age at Onset (yrs)	Age (yrs)	Duration of Disease (yrs)	LogMAR VA		Fundus Appearance	AF Pattern	ERG Group	OCT Central Foveal Thickness (μm)		Mutation Status
								RE	*LE*				*RE*	*LE*	
1	1	M	S Asian	Yes (1st cousin)	11	33	22	1.0	1.0	2	2	1	64	69	c.634 C>T, p.Arg212Cys
2	1	F	S Asian	Yes (1st cousin)	11	36	25	1.0	1.0	2	2	3	31	41	c.634 C>T, p.Arg212Cys
3*	2	M	European	No	3	8	5	1.2	1.2	2	2	3†	41	36	c.1622 T>C, p.Leu541Pro / c.3113 C>T, p.Ala1038Val
4	3	F	S Asian	Yes (2nd cousin once removed)	5	8	3	1.0	1.0	2	2	3	NA	NA	c.2713delG, p.Glu905fsX916
5	4	M	S Asian	Yes (unknown)	10	25	15	1.0	1.08	2	2	3	64	60	c.2791 G>A, p.Val931Met / c.5114 G>A, p.Arg1705Gln
6	5	M	S Asian	Yes (1st cousin)	10	30	20	1.0	1.0	2	2	3	NA	NA	c.4462 T>C, p.Cys1488Arg
7	5	F	S Asian	Yes (1st cousin)	10	22	12	2.0	1.0	2	2	3	NA	NA	c.4462 T>C, p.Cys1488Arg
8	6	M	ME Asian	Yes (1st cousin)	30	36	6	1.08	2.0	3	3	3	103	95	c.4918 C>T, p.Arg1640Trp
9	7	F	S Asian	Yes (2nd cousin)	19	27	8	1.78	2.0	1	2	1	128	90	c.5882 G>A, p.Gly1961Glu
10	7	M	S Asian	Yes (2nd cousin)	30	34	4	0.48	0.48	1	2	1	NA	NA	c.5882 G>A, p.Gly1961Glu
11	8	F	S Asian	Yes (1st cousin)	17	26	9	0.78	0.78	1	1	1	54	47	c.5882 G>A, p.Gly1961Glu
12	9	F	European	No	44	44	0	0.18	0.0	2	2	1	NA	NA	c.6079 C>T, p.Leu2027Phe
13	10	M	ME Asian	Yes (1st cousin)	5	11	6	1.3	1.0	3	2	3	62	68	c.3898 C>T, p.Arg1300X
14	11	M	S Asian	Yes (unknown)	8	11	3	1.0	1.0	2	2	3	NA	NA	c.4253+4 C>T
15	12	M	S Asian	Yes (1st cousin)	9	48	39	3.0	3.0	3	NA	3	NA	NA	c.6658 C>T, p.Gln2220X
16	13	M	S Asian	Yes (uncle and niece)	4	7	3	1.08	1.08	1	NA	3	NA	NA	c.6658 C>T, p.Gln2220X
17	13	M	S Asian	Yes (uncle and niece)	6	8	2	1.08	1.0	1	NA	3	NA	NA	c.6658 C>T, p.Gln2220X
18	13	M	S Asian	Yes (1st cousin)	17	25	8	1.78	1.78	3	NA	3	NA	NA	c.6658 C>T, p.Gln2220X

AF = autofluorescence; ERG = electroretinography; F = female; FM No. = family number; LE = left eye; LogMAR VA = logarithm of the minimum angle of resolution; M = male; ME Asian = middle east Asian; NA = not applicable; OCT = optical coherence tomography; Pt = patient; RE = right eye; S Asian = south Asian.

The age of onset was defined as either the age at which visual loss was first noted by the patient, or as the age at the latest examination for patients who were asymptomatic. The duration of the disease was calculated as the difference between age at onset and age at the latest examination. The central foveal thickness was defined as the distance between inner retinal surface and inner border of retinal pigment epithelium.

*Patient 3 had delayed developmental milestones as an infant.

†The single flash cone ERG and scotopic bright flash ERG were electronegative in patient 3.

alleles by means of clinical features, retinal imaging, and electrophysiologic examination.

Methods

Patients

Patients with homozygous *ABCA4* alleles were identified from a cohort of 276 patients with a clinical diagnosis of *ABCA4*-related retinal disease presenting to the retinal genetics clinics at Moorfields Eye Hospital. After informed consent was obtained, blood samples were taken from all individuals for DNA extraction and mutation screening of *ABCA4*. The protocol of the study adhered to the provisions of the Declaration of Helsinki and was approved by the Ethics Committee of Moorfields Eye Hospital.

Clinical Assessment

A detailed medical history was obtained, and a comprehensive ophthalmological examination was performed. The age of onset was defined as the age at which visual loss was first noted by the patient or the age at the latest examination for patients who were asymptomatic. The duration of the disease was calculated as the difference between age at onset and age at the latest examination. Clinical assessment included best-corrected Snellen visual acuity (converted to equivalent logarithm of the minimum angle of resolution [logMAR]), ophthalmoscopy, fundus photography, autofluorescence (AF) imaging, spectral-domain optical coherence tomography (SD-OCT), and electrophysiologic assessment.

Color fundus photography was performed with the TRC-50IA Retinal Fundus Camera (Topcon, Inc., Tokyo, Japan), and patients were divided into 1 of 3 types, in keeping with a previous classification of ophthalmoscopic findings[8]: (1) patients with an atrophic-appearing foveal lesion(s) with or without perifoveal yellowish-white deposits; (2) subjects with an atrophic-appearing foveal/macular lesion and numerous yellowish-white deposits throughout the posterior pole, extending anteriorly to the vascular arcades and nasally to the optic disc; and (3) individuals with extensive atrophic-appearing changes of the retinal pigment epithelium (RPE) throughout the posterior pole, extending beyond the vascular arcades (Table 1, available at http://aaojournal.org).

Autofluorescence imaging was performed using Spectralis with viewing module version 5.1.2.0 (Heidelberg Engineering, Heidelberg, Germany; excitation wavelength, 488 nm; barrier filter, 500 nm; field of view, 30×30° and 55×55°) after pupillary dilation. Patients were classified into 1 of 3 patterns, which was partially modified according to a previous report based on the AF findings[29]: (1) patients with a localized low AF signal at the fovea surrounded by a homogeneous background with or without perifoveal foci of high or low signal; (2) subjects with a localized low AF signal at the macula surrounded by a heterogeneous background and widespread foci of a high or low AF signal extending anterior to the vascular arcades; and (3) individuals with multiple areas of low AF signal at the posterior pole with a heterogeneous background with or without foci of a high or low AF signal (Table 1, available at http://aaojournal.org).

Spectral-domain OCT imaging was obtained with Spectralis with viewing module version 5.1.2.0. Central foveal thickness was defined as the distance between the inner retinal surface and the inner border of the RPE.[30,31] HEYEX software interface (version 1.6.2.0; Heidelberg Engineering) was used for obtaining a thickness measurement.[31]

Electrophysiologic assessment included full-field electroretinography (ERG) and pattern electroretinography (PERG) incorporating the standards of the International Society for Clinical Electrophysiology of Vision.[32,33] Electroretinography examination included (1) dark-adapted (DA) dim flash 0.01 $cd \cdot s \cdot m^{-2}$ (DA 0.01), (2) dark-adapted bright flash 11.0 $cd \cdot s \cdot m^{-2}$ (DA 11.0), (3) light-adapted (LA) 3.0 $cd \cdot s \cdot m^{-2}$ 30 Hz flicker ERG (LA 30 Hz), and (4) light-adapted 3.0 $cd \cdot s \cdot m^{-2}$ at 2 Hz (LA 3.0). Pattern ERG P50 was used to measure macular function; in this study, PERG reduction was considered severe if the P50 component was 0.6 μV or less (lower limit of normal, 2.0 μV). The electrophysiologic phenotype was based on a previous classification[34]: (1) patients with PERG P50 abnormality with normal ERGs, (2) subjects with PERG P50 abnormality and additional generalized cone ERG abnormality (assessed with LA 30 Hz and LA 3.0), and (3) individuals with PERG P50 abnormality and additional generalized cone and rod ERG abnormality (the latter assessed using DA 0.01 and DA 11.0) (Table 1, available at http://aaojournal.org).

The classification of severity for the phenotype of *ABCA4*-related retinal disease was performed on the basis of the clinical findings: age of onset, logMAR visual acuity, fundus appearance, AF pattern, and electrophysiologic grouping (Table 2).

Mutation Screening

Mutation analysis was performed with the solid-phase arrayed primer extension (APEX) technology (ABCR400 chip, Asper Ophthalmics, Tartu, Estonia) in 12 probands[35] and with high-throughput next-generation DNA sequencing (NGS) in 1 proband.[36] Direct Sanger sequencing was performed in siblings, parents, and other relatives of the probands when available to confirm segregation of alleles.

Null variants were defined as those expected to affect pre-mRNA splicing of the transcript or introduce a premature truncating codon. The term *variants* for purposes of this study includes those sequence changes previously shown to be enriched in patients with Stargardt's disease from prior studies. Missense variants were analyzed using 2 software prediction programs:

Table 2. Classification of Severity for the Phenotype of *ABCA4*-Related Retinal Disease

	Onset of Disease (yrs)	LogMAR VA in the Better Eye	Fundus Appearance	AF Type	ERG Group
Mild phenotype	Later onset (>15)	<0.78	1	1	1
Moderate phenotype	Patients who did not meet at least 2 criteria of either mild phenotype or severe phenotype were classified into the moderate phenotype subgroup.				
Severe phenotype	Early onset (<10)	>1.0	3	3	3

AF = autofluorescence; ERG = electroretinography; LogMAR VA = log minimum angle of resolution visual acuity.
For the purpose of this study, patients who met at least 2 criteria of mild phenotype were classified into the mild phenotype subgroup and those who had at least 2 features of severe phenotype were classified into the severe phenotype subgroup.

The Clinical Effect of Homozygous *ABCA4* Alleles in 18 Patients

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Purpose: To describe the phenotypic presentation of a cohort of individuals with homozygous disease-associated *ABCA4* variants.

Design: Retrospective case series.

Participants: Eighteen affected individuals from 13 families ascertained from a total cohort of 214 families with *ABCA4*-related retinal disease presenting to a single center.

Methods: A detailed history was obtained, and color fundus photography, autofluorescence (AF) imaging, optical coherence tomography (OCT), and electrophysiologic assessment were performed. Phenotypes based on ophthalmoscopy, AF, and electrophysiology were assigned using previously reported characteristics. *ABCA4* mutation detection was performed using the ABCR400 microarray (Asper Biotech, Tartu, Estonia) and high-throughput DNA sequencing, with direct sequencing used to assess segregation.

Main Outcome Measures: Detailed clinical, electrophysiologic, and molecular genetic findings.

Results: Eleven disease-associated homozygous *ABCA4* alleles were identified, including 1 frame shift, 2 stops, 1 intronic variant causing splice-site alteration, 2 complex missense variants, and 5 missense variants: p.Glu905fsX916, p.Arg1300X, p.Gln2220X, c.4253+4 C>T, p.Leu541Pro and p.Ala1038Val (homozygosity for complex allele), p.Val931Met and p.Arg1705Gln (complex allele), p.Arg212Cys, p.Cys1488Arg, p.Arg1640Trp, p.Gly1961Glu, and p.Leu2027Phe. Eight of these 11 homozygous alleles have not been reported previously. Six of 7 patients with homozygous null alleles had early-onset (<10 years) disease, with all 7 having a severe phenotype. Two patients with homozygous missense variants (p.Leu541Pro and p.Ala1038Val [complex], and p.Arg1640Trp) presented with a severe phenotype. Three patients with homozygous p.Gly1961Glu had adult-onset disease and a mild phenotype. One patient with homozygous p.Leu2027Phe showed a spared fovea and preserved visual acuity.

Conclusions: The phenotypes represented in patients identified as homozygous for presumed disease-associated *ABCA4* variants gives insight into the effect of individual alleles. Null alleles have severe functional effects, and certain missense variants are similar to nulls, suggesting complete abrogation of protein function. The common alleles identified, p.Gly1961Glu and p. Leu2027Phe, both have a mild structural and functional effect on the adult retina; the latter is associated with relatively retained photoreceptor architecture and function at the fovea.

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Stargardt's disease is the most common form of inherited macular dystrophy and is caused by recessive mutations in the gene *ABCA4* (Online Mendelian Inheritance in Man 601691; cytogenetic location, 1p 22.1; genomic coordinates [GRCh37], 1:94,458,392–94,586,704).[1–3] More than 600 variants have been reported to date.[4–22] Marked genetic heterogeneity confounds attempts to establish genotype–phenotype correlations. The lack of a readily exploitable functional assay of adenosine triphosphatase activity also limits the ability to investigate the functional consequences of individual *ABCA4* variants.[13]

Nevertheless, many reports have attempted to investigate the relationship between genotype and phenotype.[7,9,12,14,16,19,20,23,24] Determining the severity of specific alleles is confounded by the high prevalence of individuals who have compound heterozygous variants in *ABCA4*.[2,13] In only a few studies is it possible to evaluate the effects of specific variants.[12,14,20,22,23,25] Individuals who have homozygous variants in *ABCA4* offer a valuable opportunity to investigate the phenotype associated with specific single variants. There are a number of reports of families or small case series describing the phenotypic features of homozygous patients,[6,10,11,22,25–28] and 1 report features homozygous patients with 1 specific common allele (p.Gly1961Glu).[12] However, studies of large cohorts are still lacking because of the rarity of such homozygous cases.

We examined a cohort of homozygous patients to gain insights into the retinopathy caused by specific *ABCA4*

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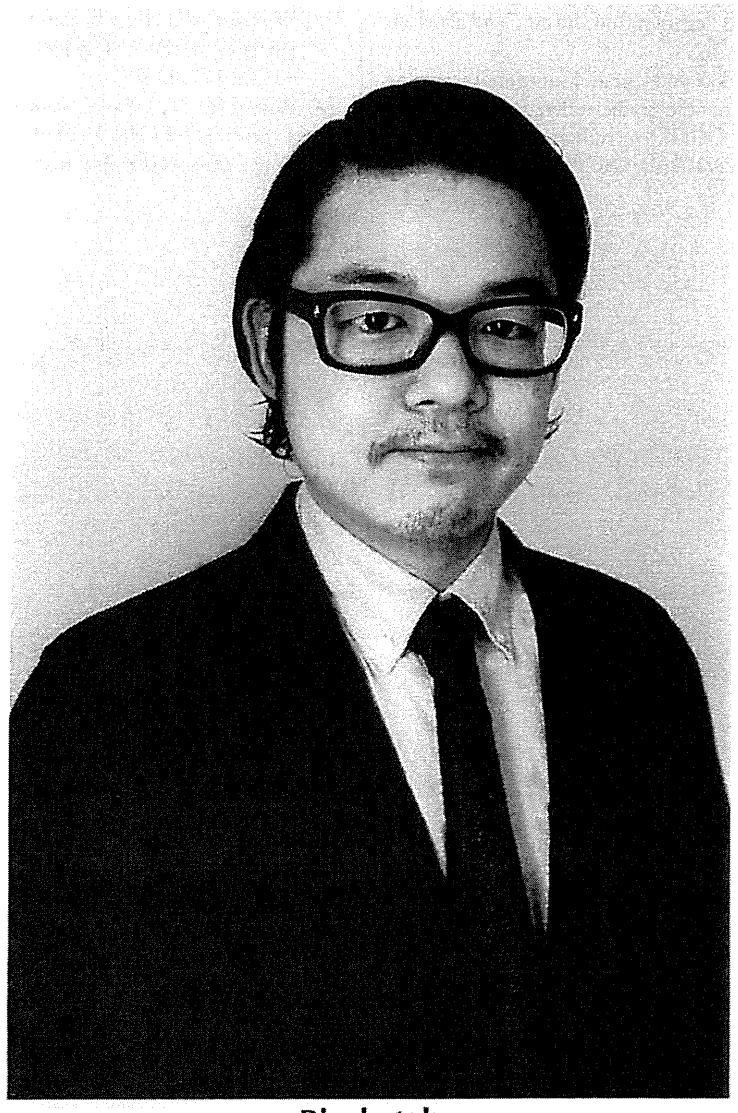

Biosketch

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degeneration or cone-rod degeneration. *Invest Ophthalmol Vis Sci* 2001;42(10):2229–2236.
52. Fumagalli A, Ferrari M, Soriani N, et al. Mutational scanning of the ABCR gene with double-gradient denaturing-gradient gel electrophoresis (DG-DGGE) in Italian Stargardt disease patients. *Hum Genet* 2001;109(3):326–338.
53. Webster AR, Heon E, Lotery AJ, et al. An analysis of allelic variation in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2001; 42(6):1179–1189.
54. Rosenberg T, Klie F, Garred P, Schwartz M. N965S is a common ABCA4 variant in Stargardt-related retinopathies in the Danish population. *Mol Vis* 2007;13:1962–1969.

19. Burke TR, Rhee DW, Smith RT, et al. Quantification of peripapillary sparing and macular involvement in Stargardt Disease (STGD1). *Invest Ophthalmol Vis Sci* 2011;52(11): 8006–8015.
20. Klevering BJ, Blankenagel A, Maugeri A, Cremers FP, Hoyng CB, Rohrschneider K. Phenotypic spectrum of autosomal recessive cone-rod dystrophies caused by mutations in the ABCA4 (ABCR) gene. *Invest Ophthalmol Vis Sci* 2002;43(6):1980–1985.
21. Michaelides M, Chen LL, Brantley MA Jr, et al. ABCA4 mutations and discordant ABCA4 alleles in patients and siblings with bull's-eye maculopathy. *Br J Ophthalmol* 2007; 91(12):1650–1655.
22. Burke TR, Tsang SH, Zernant J, Smith RT, Allikmets R. Familial discordance in Stargardt disease. *Mol Vis* 2012;18: 227–233.
23. Burke TR, Fishman GA, Zernant J, et al. Retinal phenotypes in patients homozygous for the G1961E mutation in the ABCA4 gene. *Invest Ophthalmol Vis Sci* 2012;53(8): 4458–4467.
24. Cella W, Greenstein VC, Zernant-Rajang J, et al. G1961E mutant allele in the Stargardt disease gene ABCA4 causes bull's eye maculopathy. *Exp Eye Res* 2009;89(1):16–24.
25. Allikmets R. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 1997;17(1):122.
26. Cremers FP, van de Pol DJ, van Driel M, et al. Autosomal recessive retinitis pigmentosa and cone-rod dystrophy caused by splice site mutations in the Stargardt's disease gene ABCR. *Hum Mol Genet* 1998;7(3):355–362.
27. Martinez-Mir A, Paloma E, Allikmets R, et al. Retinitis pigmentosa caused by a homozygous mutation in the Stargardt disease gene ABCR. *Nat Genet* 1998;18(1):11–12.
28. Fishman GA, Stone EM, Eliason DA, Taylor CM, Lindeman M, Derlacki DJ. ABCA4 gene sequence variations in patients with autosomal recessive cone-rod dystrophy. *Arch Ophthalmol* 2003;121(6):851–855.
29. Klevering BJ, Yzer S, Rohrschneider K, et al. Microarray-based mutation analysis of the ABCA4 (ABCR) gene in autosomal recessive cone-rod dystrophy and retinitis pigmentosa. *Eur J Hum Genet* 2004;12(12):1024–1032.
30. Riveiro-Alvarez R, Aguirre-Lamban J, Lopez-Martinez MA, et al. Frequency of ABCA4 mutations in 278 Spanish controls: an insight into the prevalence of autosomal recessive Stargardt disease. *Br J Ophthalmol* 2009;93(10): 1359–1364.
31. Schindler EI, Nylen EL, Ko AC, et al. Deducing the pathogenic contribution of recessive ABCA4 alleles in an outbred population. *Hum Mol Genet* 2010;19(19):3693–3701.
32. Aguirre-Lamban J, Gonzalez-Aguilera JJ, Riveiro-Alvarez R, et al. Further associations between mutations and polymorphisms in the ABCA4 gene: clinical implication of allelic variants and their role as protector/risk factors. *Invest Ophthalmol Vis Sci* 2011;52(9):6206–6212.
33. Zernant J, Schubert C, Im KM, et al. Analysis of the ABCA4 gene by next-generation sequencing. *Invest Ophthalmol Vis Sci* 2011;52(11):8479–8487.
34. Robson AG, Egan CA, Luong VA, Bird AC, Holder GE, Fitzke FW. Comparison of fundus autofluorescence with photopic and scotopic fine-matrix mapping in patients with retinitis pigmentosa and normal visual acuity. *Invest Ophthalmol Vis Sci* 2004;45(11):4119–4125.
35. Sergouniotis PI, Davidson AE, Lenassi E, Devery SR, Moore AT, Webster AR. Retinal structure, function, and molecular pathologic features in gyrate atrophy. *Ophthalmology* 2012;119(3):596–605.
36. Sergouniotis PI, Holder GE, Robson AG, Michaelides M, Webster AR, Moore AT. High-resolution optical coherence tomography imaging in KCNV2 retinopathy. *Br J Ophthalmol* 2012;96(2):213–217.
37. Tsunoda K, Watanabe K, Akiyama K, Usui T, Noda T. Highly reflective foveal region in optical coherence tomography in eyes with vitreomacular traction or epiretinal membrane. *Ophthalmology* 2012;119(3):581–587.
38. Zweifel SA, Engelbert M, Laud K, Margolis R, Spaide RF, Freund KB. Outer retinal tubulation: a novel optical coherence tomography finding. *Arch Ophthalmol* 2009;127(12): 1596–1602.
39. Bach M, Brigell MG, Hawlina M, et al. ISCEV standard for clinical pattern electroretinography (PERG): 2012 update. *Doc Ophthalmol* 2013;126(1):1–7.
40. Marmor MF, Fulton AB, Holder GE, Miyake Y, Brigell M, Bach M. ISCEV standard for full-field clinical electroretinography (2008 update). *Doc Ophthalmol* 2009;118(1): 69–77.
41. Hood DC, Bach M, Brigell M, et al. ISCEV standard for clinical multifocal electroretinography (mfERG) (2011 edition). *Doc Ophthalmol* 2012;124(1):1–13.
42. Bellmann C, Neveu MM, Scholl HP, et al. Localized retinal electrophysiological and fundus autofluorescence imaging abnormalities in maternal inherited diabetes and deafness. *Invest Ophthalmol Vis Sci* 2004;45(7):2355–2360.
43. Jaakson K, Zernant J, Kulm M, et al. Genotyping microarray (gene chip) for the ABCR (ABCA4) gene. *Hum Mutat* 2003; 22(5):395–403.
44. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003;31(13):3812–3814.
45. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Methods* 2010;7(4):248–249.
46. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 1997;277(5333):1805–1807.
47. Rozet JM, Gerber S, Souied E, et al. Spectrum of ABCR gene mutations in autosomal recessive macular dystrophies. *Eur J Hum Genet* 1998;6(3):291–295.
48. Lewis RA, Shroyer NF, Singh N, et al. Genotype/phenotype analysis of a photoreceptor-specific ATP-binding cassette transporter gene, ABCR, in Stargardt disease. *Am J Hum Genet* 1999;64(2):422–434.
49. Rivera A, White K, Stohr H, et al. A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration. *Am J Hum Genet* 2000;67(4):800–813.
50. Birch DG, Peters AY, Locke KL, Spencer R, Megarity CF, Travis GH. Visual function in patients with cone-rod dystrophy (CRD) associated with mutations in the ABCA4 (ABCR) gene. *Exp Eye Res* 2001;73(6):877–886.
51. Briggs CE, Rucinski D, Rosenfeld PJ, et al. Mutations in ABCR (ABCA4) in patients with Stargardt macular

in the foveal-sparing phenotype compared to the typical phenotype; there was also clear concordance in sibships with the foveal-sparing phenotype, although a possible influence of genetic/environmental modifiers cannot be excluded.

The present study identifies distinct clinical features and molecular findings in patients with Stargardt disease and foveal sparing. It has highlighted that this phenotype is associated with a later onset of disease, better visual acuity (≥0.48 logMAR), often normal full-field ERGs, and evidence of outer retinal tubulation. These data assist genetic counseling and may help in the appropriate selection of patients for therapeutic clinical trials for ABCA4 retinopathy.

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REFERENCES

1. Stargardt K. Uber familiare progressive degeneration in der makulagegend des auges. *Albrecht von Grafes Arch Klin Ophthalmol* 1909;71:534–550.
2. Allikmets R, Singh N, Sun H, et al. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat Genet* 1997; 15(3):236–246.
3. Michaelides M, Hunt DM, Moore AT. The genetics of inherited macular dystrophies. *J Med Genet* 2003;40(9): 641–650.
4. Burke TR, Tsang SH. Allelic and phenotypic heterogeneity in ABCA4 mutations. *Ophthalmic Genet* 2011;32(3):162–174.
5. Rotenstreich Y, Fishman GA, Anderson RJ. Visual acuity loss and clinical observations in a large series of patients with Stargardt disease. *Ophthalmology* 2003;110(6):1151–1158.
6. Fujinami K, Akahori M, Fukui M, Tsunoda K, Iwata T, Miyake Y. Stargardt disease with preserved central vision: identification of a putative novel mutation in ATP-binding cassette transporter gene. *Acta Ophthalmol* 2011;89(3):297–298.
7. Armstrong JD, Meyer D, Xu S, Elfervig JL. Long-term follow-up of Stargardt's disease and fundus flavimaculatus. *Ophthalmology* 1998;105(3):448–458.
8. Fishman GA, Stone EM, Grover S, Derlacki DJ, Haines HL, Hockey RR. Variation of clinical expression in patients with Stargardt dystrophy and sequence variations in the ABCR gene. *Arch Ophthalmol* 1999;117(4):504–510.
9. Lois N, Holder GE, Bunce C, Fitzke FW, Bird AC. Phenotypic subtypes of Stargardt macular dystrophy-fundus flavimaculatus. *Arch Ophthalmol* 2001;119(3):359–369.
10. Yatsenko AN, Shroyer NF, Lewis RA, Lupski JR. Late-onset Stargardt disease is associated with missense mutations that map outside known functional regions of ABCR (ABCA4). *Hum Genet* 2001;108(4):346–355.
11. McBain VA, Townend J, Lois N. Progression of retinal pigment epithelial atrophy in stargardt disease. *Am J Ophthalmol* 2012;154(1):146–154.
12. Westeneng-van Haaften SC, Boon CJ, Cremers FP, Hoefsloot LH, den Hollander AI, Hoyng CB. Clinical and genetic characteristics of late-onset Stargardt's disease. *Ophthalmology* 2012;119(6):1199–1210.
13. Testa F, Rossi S, Sodi A, et al. Correlation between photoreceptor layer integrity and visual function in patients with Stargardt disease: implications for gene therapy. *Invest Ophthalmol Vis Sci* 2012;53(8):4409–4415.
14. Lenassi E, Jarc-Vidmar M, Glavac D, Hawlina M. Pattern electroretinography of larger stimulus field size and spectral-domain optical coherence tomography in patients with Stargardt disease. *Br J Ophthalmol* 2009;93(12):1600–1605.
15. Gomes NL, Greenstein VC, Carlson JN, et al. A comparison of fundus autofluorescence and retinal structure in patients with Stargardt disease. *Invest Ophthalmol Vis Sci* 2009;50(8): 3953–3959.
16. Lazow MA, Hood DC, Ramachandran R, et al. Transition zones between healthy and diseased retina in choroideremia (CHM) and Stargardt disease (STGD) as compared to retinitis pigmentosa (RP). *Invest Ophthalmol Vis Sci* 2011; 52(13):9581–9590.
17. Genead MA, Fishman GA, Anastasakis A. Spectral-domain OCT peripapillary retinal nerve fibre layer thickness measurements in patients with Stargardt disease. *Br J Ophthalmol* 2011;95(5):689–693.
18. Chen Y, Ratnam K, Sundquist SM, et al. Cone photoreceptor abnormalities correlate with vision loss in patients with Stargardt disease. *Invest Ophthalmol Vis Sci* 2011;52(6):3281–3292.

TABLE 4. Comparison of the Most Prevalent *ABCA4* Variants' Frequency Between the Cohort With the Foveal-Sparing Stargardt Disease and the Group With the Typical Stargardt Disease (Without Evidence of Foveal Sparing)

	Number of Alleles and Those Frequencies	
	Foveal-Sparing Stargardt Disease (n = 31, Total 30 Variants in 62 Alleles)	Typical Stargardt Disease (n = 140, Total 72 Variants in 280 Alleles)
c.2588G>C, p.Gly863Ala	4 (6.45%)	19 (6.79%)
c.4139C>T, p.Pro1380Leu	2 (3.23%)	14 (5.00%)
c.6079C>T, p.Leu2027Phe	4 (6.45%)	10 (3.57%)
c.6089G>A, p.Arg2030Gln	4 (6.45%)	3 (1.07%)
c.5461-10T>C	3 (4.84%)	23 (8.21%)
c.5882G>A, p.Gly1961Glu	1 (1.61%)	17 (6.07%)

p. Pro1380Leu, c.5461-10T>C, and p.Gly1961Glu, occurring in 19, 14, 23, and 17 patients, respectively. Sequence variant frequencies were compared between the 2 groups of patients and there was a suggestion of a higher frequency of the variant p.Arg2030Gln in the cohort with the foveal-sparing phenotype compared to the group with typical Stargardt disease (Table 4).

DISCUSSION

THIS REPORT DESCRIBES THE DETAILED CLINICAL AND molecular genetic characteristics of 40 patients with Stargardt disease and foveal sparing on AF imaging; at least 1 disease-causing *ABCA4* allele was identified in 78% of subjects.

The median age of onset was 43.5 years, with a median visual acuity of 0.18, and 13 patients (32%) were asymptomatic, in keeping with previous reports.[5,6,12,22] Thirty-eight of 40 patients (98%) had logMAR visual acuity better than or equal to 0.48, with the remaining 2 subjects also having a visual acuity better than or equal to 0.48 at the initial consultation. Unsurprisingly, the normal AF signal at the fovea is strongly associated with relative visual acuity preservation.

Four previously reported fundus patterns were identified, with 22 patients (55%) showing patchy parafoveal atrophy surrounded by numerous yellow-white flecks in our cohort.[5,12] The median age of onset and median visual acuity associated with each pattern did not differ significantly. These findings suggest that late presentation of disease and preserved visual acuity are characteristic features of the foveal-sparing phenotype, whereas it may be associated with a variable fundus appearance.

Morphologic preservation of foveal structure was present in SDOCT in most cases. The 6 patients with foveal thinning (less than 100 μm central foveal thickness) also had more severe visual acuity loss (0.48) and a longer duration of disease (12.8 years). Evidence of outer retinal tubulation was identified in 15 of 33 patients (45%). Outer retinal tubulation is believed to occur in response to RPE loss/dysfunction, since these rosettes may represent degenerating photoreceptor cells becoming arranged in a circular or ovoid fashion to promote survival.[35,38] The high incidence of outer retinal tubulation in the foveal-sparing phenotype cohort is in keeping with predominant RPE cell failure in the foveal-sparing phenotype of Stargardt disease.[35,38]

In consideration of the electrophysiological findings, the majority (67%) of patients were in ERG group 1 (localized macular dysfunction with normal ERG). It is, however, noteworthy that 8 of 33 subjects (24%) were in ERG group 3 (macular dysfunction and additional generalized cone and rod dysfunction), indicating that foveal sparing can occur even in the context of generalized retinal dysfunction.

Perimetry data were not collected on this cohort. Although some data are available on foveal-sparing patients,[6,12] more detailed analysis on a larger group of patients early in the disorder would be informative. This might determine the specific region of the macula where dysfunction starts and, if scotopic and photopic thresholds are measured separately, the relative vulnerability of rod and cone systems.

Thirty likely disease-causing variants were identified in 31 patients, including 29 previously reported disease-causing variants and the 1 novel putative disease-causing splice site variant, c.1760+1G>T.[8,20,46–54] Interestingly, there was a suggestion of a higher frequency of the substitution p.Arg2030Gln in the foveal-sparing cohort (incidence ratio: 6.5% for the foveal-sparing phenotype and 1.1% for typical Stargardt), with a possible lower incidence of p.Gly1961Glu in the foveal-sparing cohort (incidence ratio: 1.6% for the foveal-sparing phenotype and 6.1% for typical Stargardt). Although these molecular differences did not meet statistical significance, larger cohorts of patients will help to establish their importance. Typical Stargardt disease is characterized by early foveal cone failure, in direct contrast to the foveal-sparing phenotype, suggesting that there may be more than 1 mechanism influencing cell death, which could either relate to the specific sequence variant or other genetic/environmental modifiers. In support of a direct genotype-phenotype association, a different preponderance of variants was identified

TABLE 3. Allele Frequencies of 72 *ABCA4* Variants Identified in a Comparison Group[a] With the Typical Stargardt Disease (140 Patients Without Evidence of Foveal Sparing on Autofluorescence Imaging)

Exon	Nucleotide Substitution and Amino Acid Change	Number of Alleles	Allele Frequency
2	c.71G>A, p.Arg24His	1	0.36%
2	c.161G>A, p.Cys54Tyr	3	1.07%
3	c.223T>G, p.Cys75Gly	1	0.36%
5	c.455G>A, p.Arg152Gln	1	0.36%
5	c.454C>T, p.Arg152*	1	0.36%
5	c.466 A>G, p.Ile156Val	2	0.71%
6	c.634C>T, p. Arg212Cys	3	1.07%
6	c.656G>C, p.Arg219Thr	1	0.36%
6	c.666_678delAAAGACGGTGCGC, p.Lys223_Arg226delfs	2	0.71%
6	c.768G>T, Splicing site	4	1.42%
8	c.1037A>C, p.Lys346Thr	1	0.36%
10	c.1222C>T, p.Arg408*	3	1.07%
12	c.1622T>C, p.Leu541Pro	2	0.71%
12	c.1648 G>T, p.Gly550*	1	0.36%
13	c.1804C>T, p.Arg602Trp	1	0.36%
13	c.1817G>A, p.Gly606Asp	1	0.36%
13	c.1922G>C, p.Cys641Ser	1	0.36%
Int 13	c.1937+1G>A, Splicing site	2	0.71%
14	c.1957C>T, p.Arg653Cys	2	0.71%
17	c.2588G>C, p.Gly863Ala	19	6.79%
18	c.2701A>G, p.Thr901Ala	1	0.36%
19	c.2791G>A, p.Val931Met	2	0.71%
19	c.2894A>G, p.Asn965Ser	1	0.36%
20	c.2966T>C, p.Vla989Ala	3	1.07%
20	c.2971G>C, p.Gly991Arg	2	0.71%
21	c.3056C>T, p.Thr1019Met	1	0.36%
21	c.3113C>T, p.Ala1038Val	3	1.07%
21	c.3064G>A, p.Glu1022Lys	2	0.71%
22	c.3211_3212insGT, p.Ser1071Cysfs	6	2.14%
22	c.3259G>A, p.Glu1087Lys	4	1.43%
22	c.3292C>T, p.Arg1098Cys	1	0.36%
22	c.3322C>T, p.Arg1108Cys	5	1.79%
22	c.3323G>A, p.Arg1108His	1	0.36%
23	c.3364G>A, p.Glu1122Lys	1	0.36%
23	c.3386G>A, p.Arg1129His	1	0.36%
24	c.3602T>G, p.Leu1201Arg	3	1.07%
27	c.3898C>T, p.Arg1300*	2	0.71%
28	c.4139C>T, p.Pro1380Leu	14	5.00%
28	c.4222T>C, p.Trp1408Arg	1	0.36%
28	c.4234C>T, p.Gly1412*	1	0.36%
28	c.4253+5G>T, Splice site	1	0.36%
28	c.4253+4C>T, Splice site	1	0.36%
29	c.4283C>T, p.Thr1428Met	1	0.36%
29	c.4319T>C, p.Phe1440Ser	1	0.36%
29	c.4462T>C, p.Cys1488Arg	1	0.36%
30	c.4469G>A, p.Cys1490Tyr	5	1.79%
30	c.4537_4538insC, p.Gly1513Profs	1	0.36%
31	c.4577C>T, p.Thr1526Met	2	0.71%
33	c.4715C>T, p.Thr1572Met	1	0.36%

Continued on next page

TABLE 3. Allele Frequencies of 72 ABCA4 Variants Identified in a Comparison Groupa With the Typical Stargardt Disease (140 Patients Without Evidence of Foveal Sparing on Autofluorescence Imaging) (*Continued*)

Exon	Nucleotide Substitution and Amino Acid Change	Number of Alleles	Allele Frequency
Int 33	c.4773+48C>T	1	0.36%
34	c.4793C>A, p.Ala1598Asp	1	0.36%
35	c.c.4918C>T, p.Arg1640Trp	1	0.36%
Int 35	c.5018+2T>C, Splice site	2	0.71%
36	c.5114G>A, p.Arg1705Gln	2	0.71%
37	c.5222_5233delTGGTGGTGGGC, p.Lys1741Hisfs	1	0.36%
37	c.5281_5289delCTT CCT GCC, p.Pro1761_Leu1763del	2	0.71%
Int 38	c.5461-10T>C	23	8.21%
Int 39	c.5585-1G>A, Splice site	1	0.36%
Int 40	c.5714+5G>A, Splice site	5	1.79%
42	c.5882G>A, p.Gly1961Glu	17	6.07%
43	c.5908C>T, p.Leu1970Phe	2	0.71%
43	c.5917delG, p.Val1973*	1	0.36%
44	c.6079C>T, p.Leu2027Phe	10	3.57%
44	c.6089G>A, p.Arg2030Gln	3	1.07%
44	c.6118C>T, p.Arg2040*	1	0.36%
45	c.6148G>C, p.Val2050Leu	3	1.43%
46	c.6286G>A, p.Glu2096Lys	1	0.36%
46	c.6320G>A, p.Arg2107His	4	1.43%
47	c.6445C>T, p.Arg2149*	1	0.36%
47	c.6449G>A, p.Cys2150Tyr	3	1.07%
48	c.6658C>T, p.Gln2220*	3	1.07%
48	c.6709_6710insG, p.Thr2237Serfs	1	0.36%

Int = Intron.

[a]The comparison group consisted of all patients without evidence of foveal sparing on autofluorescence imaging and also harbored at least 1 *ABCA4* disease-causing variant following screening with the arrayed primer extension microarray. One hundred and forty subjects from a total cohort of 438 individuals fulfilled these criteria.

and 1 uncertain effect (c.5461-10T>C); and 23 non-null variants (Table 2). Twenty-nine previously reported disease-causing variants and 1 novel putative disease-causing variant were identified (c.1760+1G>T). The most common variants identified were p.Gly863Ala, c.5461-10T>C, p.Leu2027Phe, and p.Arg2030Gln, occurring, respectively, in 4, 3, 3, and 4 patients with the foveal-sparing phenotype of Stargardt disease. One patient was identified to be homozygous for the p.Leu2027Phe variant; none had 2 or more null variants.

Molecular genetic data for the 140 individuals with typical Stargardt disease (without foveal sparing / with foveal atrophy) are summarized in Table 3. Seventy-two likely disease-causing variants were identified: 23 null variants, including 7 predicted to affect splicing; and 49 non-null variants. The 4 most prevalent variants were p.Gly863Ala,

TABLE 2. Investigation of the Pathogenicity of *ABCA4* Variants Identified in 40 Patients With the Foveal-Sparing Phenotype of Stargardt Disease

Exon	Nucleotide Substitution and Amino Acid Change	Number of Alleles	Het/Homo	Previous Report	SIFT* Pred.	SIFT* Index (0-1)	Polyphen 2* Pred.	Polyphen 2* Hum Var Score (0-1)	HSF Matrix* Site Affected	HSF Matrix* Wt CV	HSF Matrix* Mt CV	HSF Matrix* CV % Variation	Allelic Frequency Observed by EVS*	Reference
2	c.71G>A, p.Arg24His	1	Het	Lewis[45]	Tol.	NA	PRD	0.98				No change	ND	
6	c.768G>T, Splice site	1	Het	Klevering[70]	Tol.	0.56	NA		Donor	70.4	58	Site broken (−17.51)	ND	
6	c.658C>T, p.Arg220Cys	1	Het	Webster[53]	Tol.	NA	Benign	0.39				No change	ND	
11	c.1411G>A, p.Glu471Lys	1	Het	Allikmets[46]	Tol	NA	Benign	0.01	Acceptor	71.7	43	Site broken (−40.4)	11/13006	db SNP (rs1800548)
12	c.1622T>C, p.Leu541Pro	1	Het	Fishman[8]	Intol.	0.00	PRD	0.961				No change	2/13006	db SNP (rs61751392)
Int 12	c.1760+1G>T, Splice site	1	Het	This study	NA		NA		Donor	84.6	58	WT site broken (−31.72)	ND	
13	c.1805G>A, p.Arg602Gln	2	Het	Webster[53]	Tol.	NA	PRD	0.513		48.9	78	New site (+59.14)	2/13006	db SNP (rs61749410)
14	c.1957C>T, p.Arg653Cys	2	Het	Rivera[49]	Tol.	0.10	PRD	0.999				No change	ND	
17	c. 2588G>C, p.Gly863Ala	4	Het	Allikmets[46]	Intol.	0.01	PRD	0.996				No change	68/13006	db SNP (rs76157638)
21	c.3113C>T, p.Ala1038Val	1	Het	Webster[53]	Tol.	NA	Benign	0.014	Donor	43.5	70	New site (+61.72)	22/13006	db SNP (rs61751374)
24	c.3602T>G, p.Leu1201Arg	2	Het	Lewis[45]	Tol.	NA	Benign	0.052	Donor	61.3	74	New site (+20.08)	416/13006	db SNP (rs61750126)
27	c.3898C>T, p.Arg1300*	1	Het	Rivera[49]	NA		NA						ND	
28	c.4139C>T, p.Pro1380Leu	2	Het	Lewis[45]	Intol.	0.01	Benign	0.377				No change	2/13006	db SNP (rs61750130)
28	c.4222 T>C, p.Trp1408Arg	2	Het	Lewis[45]	Tol.	NA	PRD	0.845				No change	ND	dbSNP (rs61750135)
29	c.4319T>C, p.Phe1440Ser	1	Het	Lewis[45]	Tol.	NA	PRD	0.744				No change	ND	dbSNP (rs61750141)
30	c.4469G>A, p.Cys1490Tyr	1	Het	Webster[53]	Intol.	0.03	PRD	0.994				No change	ND	dbSNP (rs61751402)
31	c.4577C>T, p.Thr1526Met	1	Het	Lewis[45]	Intol.	0.00	PRD	0.91				No change	ND	db SNP (rs61750152)
31	c.4594G>T, p.Asp1532Asn	3	Het	Lewis[45]	Tol.	NA	PRD	0.853				No change	ND	
33	c.4685T>C, p.Ile1562Thr	1	Het	Allikmets[46]	Tol.	NA	Benign	0.034				No change	18/13006	db SNP (rs1762111)
35	c.4956T>G, p.Tyr1652*	1	Het	Fumagalli[52]	NA		NA		Acceptor	43	72	New site (+67.36)	ND	
35	c.4918C>T, p.Arg1640Trp	2	Het	Rozet[47]	Intol.	0.00	PRD	1				No change	ND	dbSNP (rs61751404)
35	c.4926C>G, p.Ser1642Arg	1	Het	Birch[50]	Tol.	0.68	Benign	0.116				No change	ND	db SNP (rs61753017)
Int 35	c.5018+2T>C, Splice site	1	Het	Fumagalli[52]	NA		NA		Donor	81.2	54	WT site broken (−33.07)	ND	
Int 38	c.5461-10T>C	3	Het	Briggs[50]	NA		NA					No change	3/13006	db SNP (rs1800728)
40	c.5693G>A, p.Arg1898His	2	Het	Allikmets[46]	NA		Benign	0.00				No change	25/13006	db SNP (rs1800552)
42	c.5882G>A, p.Gly1961Glu	1	Het	Allikmets[46]	Tol.	0.18	PRD	1				No change	41/13006	db SNP (rs1800553)
44	c.6079C>T, p.Leu2027Phe	4	Homo	Lewis[45]	Intol.	0.02	PRD	0.999				No change	4/13006	db SNP (rs61751408)
44	c.6089G>A, p.Arg2030Gln	4	Het	Lewis[45]	Tol.	NA	PRD	0.995				No change	8/13006	db SNP (rs61750641)
44	c.6118C>T, p.Arg2040*	1	Het	Rosenberg[54]	NA		NA						ND	
46	c.6320G>A, p.Arg2107His	1	Het	Fishman[8]	Intol.	0.00	PRD	0.996				No change	91/13006	db SNP (rs62642564)

EVS = Exome Variant Server; HSF = Human Splicing Finder program; Hum var score = Human var score; Int = intron; Intol = intolerant; Mt CV = mutant consensus value; NA = not applicable; ND = not detected; PRD = probably damaging; Pred. = prediction; SIFT = Sorting Intolerant from Tolerance program; Tol. = tolerant; Wt CV = wild-type consensus value.

*SIFT (version 4.0.4) results are reported to be tolerant if tolerance index >0.05 or intolerant if tolerance index <0.05. PolyPhen-2 (version 2.1) appraises mutations qualitatively as Benign, Possibly Damaging, or Probably Damaging based on the model's false-positive rate. The cDNA is numbered according to Ensemble transcript ID ENST00000370225, in which þ1 is the A of the translation start codon. Human Splicing Finder (HSF, version 2.4.1) reports the results from the HSF matrix: the higher the consensus value, the stronger the predicted splice site. The values for the wild-type and mutant sequences are shown; the larger the difference between these values, the greater the chance that the variant can affect splicing. Minor allele frequency for each allele was estimated with reference to the EVS (NHLBI Exome Sequencing Project, Seattle, Washington, USA).