Evaluation of reading speed ability in glaucoma patients with central visual field defects. 11th EGS, Nice. 2014.6.7-11

14. Maruyama Y, Ikeda Y, Mori K, Ueno M, Yoshikawa H, Kinoshita S. Comparison study of the intraocular-pressure reduction efficacy and safety between bimatoprost and latanoprost-timolol-fixed combination in Japanese open-angle glaucoma patients who switched from latanoprost. 11th EGS, Nice. 2014.6.7-11.

15. Morikawa K, Ikeda Y, Mori K, Ueno M, Yoshikawa H, Maruyama Y, Kato H, Yamamoto Y, Kinoshita S. Evaluation of Central Corneal Thickness Recovery Changes Associated with Discontinuance of Prostaglandin Eye-Drop Use Post Trabeculectomy. 11th EGS,

Nice. 2014.6.7-11.

16. Yamamoto Y, Ikeda Y, Mori K, Ueno M, Yoshikawa H, Maruyama Y, Yoshii K, Nakano M, Tashiro K, Kinoshita S. Analysis of Biochemical Markers in Pseudoexfoliation Glaucoma Patients. 11th EGS, Nice. 2014.6.7-11.

17. Cho Y, Mori K, Ikeda Y, Ueno M, Yamasaki T, Kinoshita S. 10-year retrospective trend analysis of the characteristics of elderly Japanese glaucoma patients requiring glaucoma surgery. 11th EGS, Nice. 2014.6.7-11.

18. Yamana M, Sugiura K, Mori K, Yoshikawa H, Maruyama Y, Ikeda Y, Koizumi N, Kinoshita S. Examination of Ganglion Cell Complex Thickness and Outer Retinal Layer Thickness in Glaucomatous Eyes. 11th EGS,

Nice. 2014.6.7-11

19. Ikeda Y, Mori K, Ueno M, Imai K, Yoshii K, Sato R, Sato F, Nakano M, Kinoshita S. Evaluation of IOP and Reduction Slopes Over a 16-Year Time Course in Japanese Glaucoma Patients. American Academy of Ophthalmology (AAO). Chicago. 2014.10.20

20. Nakano M, Ikeda Y, Tokuda Y, Adachi H, Ueno M, Imai K, Sato R, Omi N, Mori K, Kinoshita S, Tashiro K. Genome-wide association study of exfoliation syndrome/exfoliation glaucoma in a Japanese population. 64th Annual Meeting of the American Society of Human Genetics, San Diego Oct. 18-22, 2014.

21. 池田陽子、森　和彦 、上野盛夫 、吉川晴菜 、丸山悠子 、木下　茂. 線維柱帯切除術後のプロスタグラ

ンジン製剤点眼中止に伴う中心角膜厚変化の検討. 第37回日本眼科手術学会総会.　京都. 2014.1.17-19.

22. 森　和彦、池田陽子、上野盛夫、吉川晴菜、丸山悠子、木下　茂. 虹彩角膜内皮症候群に対するエクスプレスフィルトレーションデバイスを用いた濾過手術. 第37回日本眼科手術学会. 京都. 2014.1.17-19.

23. 三戸千賀子、池田陽子、森　和彦、山田裕美、津崎さつき、長谷川志乃、上野盛夫、中野正和、吉井健悟、木下　茂. 原発開放隅角緑内障と正常眼圧緑内障に関連する全身的要因の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

24. 張　佑子、森　和彦、池田陽子、上野盛夫、山崎俊秀、木下　茂. 当科における過去10年間の高齢者緑内障手術症例の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

25. 池田陽子、<u>森　和彦</u>、上野盛夫、今井浩二郎、吉井健悟、佐藤隆一、佐藤史子、中野正和、田代　啓、山崎俊秀、木下　茂. 広義原発開放隅角緑内障の過去16年間の経時的眼圧推移. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

26. 小田莉恵、<u>森　和彦</u>、吉井健悟、池田陽子、上野盛夫、吉川晴菜、丸山悠子、小泉範子、木下　茂. 日本人健常者における視神経乳頭サイズと乳頭回転角の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

27. 上野盛夫、池田陽子、<u>森　和彦</u>、中野正和、大見奈津江、佐藤隆一、佐藤史子、吉井健悟、田代　啓、木下　茂. CDKN2B-AS1ジェノタイプと原発開放隅角緑内障の量的形質との関連解析. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

28. 津崎さつき、池田陽子、<u>森　和彦</u>、上野盛夫、吉川晴菜、丸山悠子、今井浩二郎、木下　茂. 2種の視野検査計の信頼性スコアの比較検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

29. 高橋純子、今井浩二郎、<u>森　和彦</u>、加藤浩晃、池田陽子、上野盛夫、山村麻里子、木下　茂. 中心視野障害を有する緑内障患者の視野障害意識化による読字能改善効果の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

30. 日野智之、池田陽子、<u>森　和彦</u>、上野盛夫、吉川晴菜、丸山悠子、今井浩二郎、木下　茂. 正常者における垂直CD比の分布の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

31. 丸山悠子、池田陽子、<u>森　和彦</u>、上野盛夫、吉川晴菜、木下　茂. ラタノプロストからビマトプロストとラタノプロスト/チモロール配合剤への切替え効果. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

32. 荒木やよい、池田陽子、高橋純子、

森　和彦、上野盛夫、吉川晴菜、丸山悠子、今井浩二郎、木下　茂. 酒石酸ブリモニジンへの変更による眼圧下降効果の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

33. 森　千浩、池田陽子、森　和彦、中野恵美、津崎さつき、上野盛夫、今井浩二郎、吉川晴菜、丸山悠子、木下　茂. 点眼補助具使用の有無による1日当たり平均点眼使用料の差の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

34. 山本雄士、森　和彦、池田陽子、上野盛夫、吉川晴菜、今井浩二郎、木下　茂. トラベクレクトミー術後濾過胞形状に影響を及ぼす要因の検討. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

35. 今井浩二郎、森　和彦、稲富　勉、池田陽子、上野盛夫、吉川晴菜、丸山悠子、木下　茂. 線維柱帯切除後数年を経て発症した再発性デスメ膜剥離を前眼部OCTにて解析した一例. 第25回日本緑内障学会. 大阪. 2014.9.19-21.

36. 池田陽子、森　和彦、上野盛夫、今井浩二郎、佐藤隆一、佐藤史子、吉井健悟、中野正和、木下　茂, 早期結膜濾過胞機能不良に関連する因子の検討. 第38回手術学会. 京都. 2015.1.31.

H.　知的財産権の出願・登録状況

1.　特許取得
　　該当なし

2.　実用新案登録
　　該当なし

3.　その他
　　該当なし

厚生労働科学研究費補助金（障害者対策総合研究事業）

分担研究報告書

落屑緑内障に関連するコピー数バリアントの同定に向けた高精度データの取得

分担研究者　中野　正和

京都府立医科大学大学院　医学研究科　ゲノム医科学　准教授

研究要旨

　我々は、緑内障に関連する塩基配列の違い（バリアント）のゲノム情報と末梢血中のサイトカイン量や白血球のmRNA発現量の測定値とを組み合わせた緑内障統合的分子診断法の確立を目指している。昨年度は、コピー数の違いのバリアント（copy number variant, CNV）にも着目し、広義原発開放隅角緑内障（広義POAG）についてCNV情報に基づくゲノムワイド関連解析を実施した（分担・中野、田代）。本年度は、緑内障のもう1つの主要な病型である落屑緑内障に関連するCNVをゲノムワイド関連解析によって同定するための高精度なCNVデータの取得を実施した。

　まず、我々が独自に構築した大規模計算用の解析サーバー環境（分担・長崎）を用いて、アフィメトリクス社のDNAマイクロアレイにより取得した落屑緑内障群201例および対照群697例についての約200万プローブ／検体の蛍光強度の生データから、国際HapMapプロジェクトで公開されている270検体のコピー数データを基準として、各プローブのCNVデータ（0, 1, 2, 3, 4）を取得した。次に、取得した約18億データについては、速やかにゲノムワイド関連解析に移行するために専用データベースに格納した。

一方、症例群に特異的な循環血液中の白血球mRNAの遺伝子発現情報に基づく連続変数を加味するために、緑内障群2例、対照群2例の白血球由来のmRNAを用いた網羅的発現解析を実施した。しかし、各検体の白血球mRNAの遺伝子群の発現にばらつきが多く、これ以上症例数を上げても特異的な遺伝子発現ネットワークを同定することが困難であることが示唆されたため、緑内障統合的分子診断法の診断アルゴリズムの確立に向けてバリアントのジェノタイプ情報に基づく診断力の向上に注力することとした。

A　研究目的

　ゲノムワイド関連解析によって落屑緑内障に関連するCNVを同定するための高精度なCNVデータを取得する。また、白血球mRNAの遺伝子発現情報に基づく連続変数を加味するため症例群に特異的な遺伝子発現ネットワークを同定する。

B　研究方法

　落屑緑内障群201例および対照群697例について、アフィメトリクス社のDNAマイクロアレイ（Genome-Wide Human SNP Array 60）で取得した蛍光強度の生データ（約100万個のSNP/CNV兼用プローブ＋約100万個のCNV専用プローブ由来の総計約200万個のプローブデータ）をCNVデータ（0, 1, 2, 3, 4）に変換した。CNVデータの取得には、アフィメトリクス社が提供しているGenotyping Console 4.1.1（GTC）を用い、国際HapMapプロジェクトで公開されている270検体のコピー数データを基準とした。取得した大量のCNVデータについては、独自に構築した専用サーバー（分担・長崎）上にデータベース化した。

　網羅的遺伝子発現解析データは、緑内障群2例・対照群2例について微量な発現変動を捉えることに定評の

あるチップ（東レ・3D-Gene）を用いて取得した。

（倫理面への配慮）

本研究では患者由来の臨床情報とゲノム情報を取り扱うので、関連する倫理と法令を遵守しながら実行した。

C　研究結果

昨年度に加え、総計2,408検体（広義POAG群824例＋対照群686例、落屑緑内障群201例＋対照群697例）×約200万プローブ＝約48億個の高精度なCNVデータを取得した。取得したデータは、ゲノムワイド関連解析で実施する各種検定にも耐え得る専用データベースに格納した。

また、網羅的遺伝子発現解析については、緑内障群2例・対照群2例の白血球由来のmRNAを用いて取得したデータに基づきクラスター解析やアノテーション解析を実施したもの

の、各検体の白血球mRNAの遺伝子群の発現にばらつきが多く、症例群に特異的な遺伝子発現ネットワークを同定することは困難であった。

D　考察

大規模検体の全ゲノムにわたるプローブの蛍光強度データをコピー数データに変換する作業は、サーバーにかなりの負荷がかかることが予想されたが、大きな問題もなく全データを取得するに至った。今後、ゲノムワイド関連解析の結果も参照しながら、基準とするコピー数データの改善等、よりデータの品質を向上させる必要性が生じる可能性も考えられる。

一方、白血球mRNAの網羅的発現解析結果から、各検体の遺伝子群の発現データにばらつきが多く、これ以上症例数を上げても特異的な遺伝子発現ネットワークを同定することが困難であることが示唆された。

E　結論

ゲノムワイド関連解析によって落屑緑内障に関連するCNVを同定するための高精度なCNVデータを取得できた。また、緑内障統合的分子診断法の診断アルゴリズムの確立にはバリアントのジェノタイプ情報に基づく診断力の向上に注力することが得策であると判断した。

F 健康危険情報

該当なし

G 研究発表

1 論文発表

1. Aung T et al. (<u>Nakano M</u>, Mori K, Shigeru K, Tashiro K) A common variant mapping to CACNA1A is associated with susceptibility to exfoliation syndrome. Nat Genet. 2015 Feb 23. doi: 10.1038/ng.3226.

2. <u>Nakano M</u>, Ikeda Y, Tokuda Y, Fuwa M, Ueno M, Imai K, Sato R, Omi N, Adachi H, Kageyama M, Mori K, Kinoshita S, Tashiro K. Novel common variants and susceptible haplotype for exfoliation glaucoma specific to Asian population. *Sci. Rep.,* 4: 5340, 2014.

2 学会発表

1. <u>Nakano M.</u> Genetics study of Fuchs' endothelial corneal dystrophy: What we have learned from glaucoma genetics study. The 1st Advanced Biomedical Engineering Research Center Symposium, Kyotanabe (Feb. 21, 2015).

2. <u>Nakano M.</u> Genetics study of exfoliation syndrome/exfoliation glaucoma. Kyoto International Workshop in Visual Science 2015, Kyoto (Feb. 20, 2015).

3. <u>Nakano M</u>, Ikeda Y, Tokuda Y, Adachi H, Ueno M, Imai K, Sato R, Omi N, Mori K, Kinoshita S, Tashiro K. Genome-wide association

study of exfoliation syndrome/exfoliation glaucoma in a Japanese population. 64th Annual Meeting of the American Society of Human Genetics, San Diego (Oct. 18-22, 2014).

4. Ikeda Y, Mori K, Ueno M, Imai K, Yoshii K, Sato R, Sato F, Nakano M. Evaluation of intraocular-pressure and reduction slope over a 16-year time course in Japanese glaucoma patients. AAO 2014, Chicago (Oct. 18-21, 2014).

5. Sannohe C, Ikeda Y, Mori K, Yamada H, Tsuzaki S, Hasegawa S, Ueno M, Nakano M, Yoshii K, Kinoshita S. Comparison of the systemic risk factors, including body mass index, between primary open-angle glaucoma and normal tension glaucoma. 11th European Glaucoma Society Congress, Nice (June 7-11, 2014).

6. Ikeda Y, Mori K, Ueno M, Nakano M, Tokuda Y, Omi N, Sato R, Yoshii K, Tashiro K, Kinoshita S. Association Analysis between CDKN2B-AS1 Genotype and Quantitative Traits of Primary Open-Angle Glaucoma. Annual Meeting of the Association for Research in Vision and Ophthalmology, Florida (May 4-8, 2014).

7. Sannohe C, Ikeda Y, Mori K, Yamada H, Tsuzaki S, Hasegawa S, Ueno M, Nakano M, Yoshii K, Kinoshita S. Comparison of the systemic risk factors, including body mass index, between primary open-angle glaucoma and normal tension glaucoma. Annual Meeting of the Association for Research in Vision and Ophthalmology, Florida (May 4-8, 2014).

8. Li Z, Khor CC, Ikeda Y, Jia L, Chen LJ, Nakano M, George R, Do T, Abu-Amero

K, Tajudin LS, Kee C, Mineo O, Takanori M, Zhang M, Jonas JB, Chau TN, Fuse N, Wong TY, Liu JJ, Garway-Heath DF, Simmons CP, Vijaya L, Kinoshita S, Pang CP, Wang NL, Allingham RR, Hauser MA, Tashiro K, Aung T, Vithana EN. Exome-wide association study identifies a novel locus associated with primary open angle glaucoma. Annual Meeting of the Association for Research in Vision and Ophthalmology, Florida (May 4-8, 2014).

9. Nakano M. Exfoliation glaucoma-associated gene possibly related to autophagy. Special Interest Group: Genetics, Autophagy, and Glaucoma. World Ophthalmology Congress, Tokyo (Apr. 2-6, 2014, Panel Discussion).

10. 中川紘子, 中野正和, 奥村直毅, 池田陽子, 上野盛夫, 小

泉範子, 田代 啓, Baratz KH, Wieben ED, 木下茂. フックス角膜内皮ジストロフィの日本人患者におけるTCF4遺伝子のCTG反復配列. 角膜カンファランス2015, 高知 (2015年2月11日-13日).

11. 池田陽子、森和彦、上野盛夫、今井浩二郎、佐藤隆一、佐藤史子、吉井健悟、中野正和、木下茂. 早期結膜濾過胞機能不良に関連する因子の検討. 第38回日本眼科手術学会, 京都 (2015年1月30日-2月1日).

12. 池田陽子, 森和彦, 上野盛夫, 今井浩二郎, 中野正和, 徳田雄市, 吉井健悟, 佐藤隆一, 田代 啓, 木下茂. 1000Kマイクロアレイによる落屑緑内障のゲノムワイド関連解析. 第68回日本臨床眼科学会, 神戸 (2014年11月13日-16日).

13. 田代 啓, 中野正和, 池田陽子, 徳田雄市, 上野盛夫, 今

井浩二郎，佐藤隆一，大見奈津江，足立博子，森和彦，木下茂．落屑症候群／落屑緑内障のゲノムワイド関連解析．第87回日本生化学会大会，京都（2014年10月15日-18日）．

14. 大見奈津江，徳田雄市，池田陽子，<u>中野正和</u>，田代　啓．微量血液からの不死化B細胞株樹立における過剰抗凝固剤の影響の検討．第87回日本生化学会大会，京都（2014年10月15日-18日）．

15. 上野盛夫，池田陽子，森和彦，<u>中野正和</u>，大見奈津江，佐藤隆一，佐藤史子，吉井健悟，田代　啓，木下茂．CDKN2B-AS1ジェノタイプと原発開放隅角緑内障の量的形質との相関解析．第25回日本緑内障学会，大阪（2014年9月19日-21日）．

16. 池田陽子、森和彦、上野盛夫、今井浩二郎、吉井健悟、佐藤

隆一、佐藤史子、<u>中野正和</u>、田代　啓、山崎俊秀、木下茂．広義原発開放隅角緑内障の過去16年間の経時的眼圧推移．第25回日本緑内障学会，大阪（2014年9月19日-21日）．

17. 三戸千賀子、池田陽子、森和彦、山田裕美、津崎さつき、長谷川志乃、上野盛夫、<u>中野正和</u>、吉井健悟、木下茂．原発開放隅角緑内障と正常眼圧緑内障に関連する全身的要因の検討．第25回日本緑内障学会，大阪（2014年9月19日-21日）．

18. <u>中野正和</u>．大規模データに基づくゲノム医科学研究．宇宙航空研究開発機構（JAXA）バイオインフォマティクス勉強会，筑波（2014年9月17日）．

19. 足立博子，富永洋之，丸山悠子，米田一仁，丸山和一，<u>中野正和</u>，木下茂，田代　啓．出生前後のマウス網膜を用いた遺

伝子発現解析のための相対定

量PCR法の確立，第61回日本

生化学会近畿支部例会，京都

（2014年5月17日）

［Ⅲ］

研究成果の刊行に関する一覧表

1. Aung T et al.(Nakano M, Mori K, Kinoshita S, Tashiro K) A common variant mapping to CACNA1A is associated with susceptibility to exfoliation syndrome. Nat Genet. 2015 Feb 23. doi: 10.1038/ng.3226.

2. Nakano M, Ikeda Y, Tokuda Y, Fuwa M, Ueno M, Imai K, Sato R, Omi N, Adachi H, Kageyama M, Mori K, Kinoshita S, Tashiro K. Novel common variants and susceptible haplotype for exfoliation glaucoma specific to Asian population. Sci Rep . 2014 Jun 18;4:5340. doi: 10.1038/srep05340.

3. Koudouna E, Young RD, Ueno M, Kinoshita S, Quantock AJ, Knupp C. Three-dimensional architecture of collagen type VI in the human trabecular meshwork. Mol Vis. 2014 May 13;20:638-48. eCollection 2014.

4. Maruyama Y,Mori K, Ikeda Y, Ueno M, Kinoshita S. Effects of Long-Term Topical Prostaglandin Therapy on Central Corneal Thickness. J Ocul Pharmacol Ther. 2014 Apr 16.

5. Ueta M, Sawai H, Sotozono C, Hitomi Y, Kaniwa N, Kim MK, Seo KY, Yoon KC, Joo CK, Kannabiran C, Wakamatsu TH, Sangwan V, Rathi V, Basu S, Ozeki T, Mushiroda T, Sugiyama E, Maekawa K, Nakamura R, Aihara M, Matsunaga K, Sekine A, Pereira Gomes JÁ, Hamuro J, Saito Y, Kubo M, Kinoshita S, Tokunaga K. IKZF1, a new susceptibility gene for cold medicine-related Stevens-Johnson syndrome/toxic epidermal necrolysis with severe mucosal involvement. J Allergy Clin Immunol. 2015 Jan 27. pii: S0091-6749(14)03744-0. doi: 10.1016/j.jaci.2014.12.1916. [Epub ahead of print]

6. Ueta M, Kannabiran C, Wakamatsu TH, Kim MK, Yoon KC, Seo KY, Joo CK, Sangwan V, Rathi V, Basu S, Shamaila A, Lee HS, Yoon S, Sotozono C, Gomes JÁ, Tokunaga K, Kinoshita S. Trans-ethnic study confirmed independent associations of HLA-A*02:06 and HLA-B*44:03 with cold medicine-related Stevens-Johnson syndrome with severe ocular surface complications. Sci Rep. 2014 Aug 7;4:5981. doi: 10.1038/srep05981.

7. Ueta M, Mizushima K, Naito Y, Narumiya S, Shinomiya K, Kinoshita S. Suppression of polyI:C-inducible gene expression by EP3 in murine conjunctival epithelium. Immunol Lett. 2014 May-Jun;159(1-2):73-5. doi: 10.1016/j.imlet.2013.08.010. Epub 2013 Sep 12.

8. Ueta M, Kaniwa N, Sotozono C, Tokunaga K, Saito Y, Sawai H, Miyadera H, Sugiyama E, Maekawa K, Nakamura R, Nagato M, Aihara M, Matsunaga K, Takahashi Y, Furuya H, Muramatsu M, Ikezawa Z, Kinoshita S. Independent strong association of HLA-A*02:06 and HLA-B*44:03 with cold medicine-related Stevens-Johnson syndrome with severe mucosal involvement. Sci Rep. 2014 Apr

30;4:4862. doi: 10.1038/srep04862.

9. Yamada K, Ueta M, Sotozono C, Yokoi N, Inatomi T, Kinoshita S.Upregulation of Toll-like receptor 5 expression in the conjunctival epithelium of various human ocular surface diseases.BrOphthalmol.2014Aug;98(8):1116-9.doi: 10.1136/bjophthalmol-2013-304645. Epub 2014 May 12.PMID:24820048

10. 森　和彦.角膜疾患関連続発緑内障への対処法. あたらしい眼科　32(1): 83〜90, 2015

11. 吉川晴菜、池田陽子、外園千恵、森　和彦、上野盛夫、木下　茂.先天角膜混濁の超音波生体顕微鏡所見と臨床診断および眼圧の関係.日本眼科学会雑誌119(1):16-21,2015

12. 日野智之、森　和彦：緑内障と白内障同時手術派,IOL&RS Vol.28 No.4 :431-434,日本白内障屈折矯正手術学会雑誌編集部,Dec 2014

13. Tokuda Y, Tanaka M, Yagi T, Tashiro K. The defect of SFRP2 modulates an influx of extracellar calcium in B lymphocytes. *BMC Res. Notes,* 7: 780, 2014.

14. Nagasaki I and Ushitaki F. On G-bi-isovariant equivalence between G-representation spaces, 数理解析研究所講究録　1922: 60-64, 2014.

15. Yanagisawa, M., Aida, T., Takeda, Namekata, K., Harada, T., Shinagawa, R., Tanaka, K. Arundic acid attenuates retinal ganglion cell death by increasing glutamate/aspartate transporter (GLAST) expression neural cell death in a model of normal tension glaucoma. *Cell Death Dis* (in press).

16. Kimura, A., Guo, X., Noro, T., Harada, C., Tanaka, K., Namekata, K., Harada, T Valproic acid prevents retinal degeneration in a murine model of normal tension glaucoma. *Neurosci Lett* 588. 108-113, 2015.

17. Yamamoto T, Sawada A, Mayama C, Araie M, Ohkubo S, Sugiyama K, Kuwayama Y, on behalf of The Collaborative Bleb-Related Infection Incidence and Treatment Study Group. The 5-Year Incidence of Bleb-Related Infection and Its Risk Factors after Filtering Surgeries with Adjunctive Mitomycin C Collaborative Bleb-Related Infection Incidence and Treatment Study 2 Ophthalmology 2014; 121: 1001-1006

18. 池田陽子、中野正和. 緑内障に関連する遺伝子 緑内障診療クローズアップ（木内良明編）メジカルビュー社, 東京, 2014.

19. 張　祐子, 森　和彦：A 手術テクニックと手術用隅角鏡, 粘弾性物質. 第4章 原発閉塞隅角緑内障に対する治療, IV.隅角癒着解離術, 眼科臨床エキスパート All About閉塞隅角緑内障(澤口昭一、谷原秀信編)185-194, 医学書院, 東京, 2014

20. 丸山悠子, 森　和彦：角膜疾患に続発する緑内障, IV 病型別診断と治療／続発緑内障, 緑内障診療クローズアップ（木内良明編）242-247, メジカルビュー社, 東京, 2014

21. 多田香織, 森　和彦：続発緑内障の画像診断, 4 緑内障での使い方, 専門医のための眼科診療クオリファイ24 前眼部の画像診断(前田直之編)321-325, 中山書店, 東京, 2014

22. 成瀬繁太, 森　和彦：複数の点眼剤投与時の注意点, 特集点眼剤を実践活用するためのポイ

ント2, 薬局 65(5): 1809-1812, 2014

23. 木下　茂.【臨床医学の展望2014】眼科学Ophthalmology 日本医事新報（第4691号）,2014

［**IV**］

研究成果の刊行物・別刷

nature
genetics

A common variant mapping to *CACNA1A* is associated with susceptibility to exfoliation syndrome

Exfoliation syndrome (XFS) is the most common recognizable cause of open-angle glaucoma worldwide. To better understand the etiology of XFS, we conducted a genome-wide association study (GWAS) of 1,484 cases and 1,188 controls from Japan and followed up the most significant findings in a further 6,901 cases and 20,727 controls from 17 countries across 6 continents. We discovered a genome-wide significant association between a new locus (*CACNA1A* rs4926244) and increased susceptibility to XFS (odds ratio (OR) = 1.16, $P = 3.36 \times 10^{-11}$). Although we also confirmed overwhelming association at the *LOXL1* locus, the key SNP marker (*LOXL1* rs4886776) demonstrated allelic reversal depending on the ancestry group (Japanese: $OR_{A\ allele} = 9.87$, $P = 2.13 \times 10^{-217}$; non-Japanese: $OR_{A\ allele} = 0.49$, $P = 2.35 \times 10^{-31}$). Our findings represent the first genetic locus outside of *LOXL1* surpassing genome-wide significance for XFS and provide insight into the biology and pathogenesis of the disease.

XFS is a generalized disorder of the extracellular matrix that manifests most conspicuously in the eye. The exfoliation material consists of cross-linked, amyloid-like fibrillar material and glycoproteins. Apart from in ocular tissues, this material deposits around blood vessels, particularly in association with elastic connective tissue, and can be found in other organs[1]. The accumulation of exfoliation material deposits and pigment in the trabecular meshwork can damage this tissue and impede the drainage of aqueous humor from the eye, thus resulting in elevated intraocular pressure and glaucomatous optic neuropathy. Exfoliation glaucoma is the most serious known complication of XFS[2].

The first GWAS of XFS was reported in 2007 and successfully identified *LOXL1* as a major susceptibility locus[3]. Since then, multiple studies have uniformly corroborated the association of genetic variants of *LOXL1* with XFS[4-21]. However, data from these studies showed that associated alleles for *LOXL1* SNPs frequently undergo allelic reversal depending on ancestry group[22]. These findings suggest that complex genetic mechanisms are present in XFS pathogenesis and that additional susceptibility loci for XFS remain to be identified. We assembled an international, multi-institutional collaborative effort across 6 continents and 17 countries to conduct a GWAS discovery and 2-stage replication study of XFS (Online Methods, **Supplementary Fig. 1** and **Supplementary Table 1**). Participating subjects provided written informed consent under the oversight of all local institutional review boards in accordance with the tenets of the Declaration of Helsinki.

For the GWAS discovery stage, we genotyped 717,991 SNP markers in 1,578 Japanese subjects with XFS (cases) and 1,215 controls using the Illumina HumanOmniExpress-12 v1.0 DNA analysis BeadChip microarray. Control subjects were drawn from the same hospital where the XFS cases were first identified. A total of 1,484 cases and 1,188 controls passed quality control filters for call rate, relatedness, heterozygosity and ancestry (see the Online Methods for details on quality control) and were included for downstream association analysis. Multiple markers in strong linkage disequilibrium (LD) at the *LOXL1* locus showed strong evidence of association with XFS (**Supplementary Fig. 2a**), with rs4886776 ($P = 7.37 \times 10^{-137}$) serving as the sentinel SNP.

A total of 66 SNPs outside of *LOXL1* showed evidence of association with XFS surpassing $P < 1 \times 10^{-4}$ at the GWAS discovery stage. We thus designed validation assays for these 66 SNP markers, together with *LOXL1* rs4886776, and genotyped them in a follow-up collection of 2,628 XFS cases and 8,947 controls drawn from 9 countries (stage 1 validation; **Supplementary Table 1**). For each SNP examined, we conducted a fixed-effects meta-analysis to summarize the observations across the nine studies. One SNP marker (rs4926244) mapping within the *CACNA1A* gene was associated in the GWAS discovery stage at $P = 5.50 \times 10^{-5}$ ($OR_{G\ allele} = 1.29$) and was also significantly associated in the validation stage ($OR_{G\ allele} = 1.17$, $P = 4.17 \times 10^{-5}$). For rs4926244, meta-analysis of both the discovery and validation stages showed a genome-wide significant association ($OR_{G\ allele} = 1.20$, $P = 2.45 \times 10^{-8}$) (**Fig. 1, Supplementary Fig. 2b** and **Supplementary Table 2**). Results for all 67 SNP markers from the GWAS discovery and stage 1 replication are shown in **Supplementary Table 2**. We did not observe consistent evidence of association at *CNTNAP2*, a locus previously reported to associate with XFS in a pooled GWAS analysis[23], or at other previously reported candidate genes (**Supplementary Table 3**).

We subjected *CACNA1A* rs4926244 to further technical scrutiny in a third, independent data set consisting of 4,273 XFS cases and 11,780 controls drawn from 8 additional countries (stage 2 replication; **Supplementary Table 1**). The association maintained significance, consistent with the findings from the two previous stages ($OR_{G\ allele} = 1.13$, $P = 1.14 \times 10^{-4}$). Together, the combined discovery and 2-stage replication collections consisting of 8,385 XFS cases and 21,915 controls provided evidence for association of the minor G allele at rs4926244 with XFS ($P = 3.36 \times 10^{-11}$). These data suggest that risk for XFS increases by approximately 1.16-fold for each copy of the minor G allele (**Fig. 1** and **Supplementary Table 4**). This association appeared to be consistent, with minimal heterogeneity

Figure 1 Forest plot for the associations between *CACNA1A* rs4926244 and XFS in discovery and follow-up case-control collections. Black lines denote the 95% confidence intervals of the OR estimates for each collection. Diamonds denote summary results for the GWAS, validation and replication stages (blue), as well as for meta-analysis of the GWAS and validation stages and meta-analysis of data from all collections (red). Asian- and European-ancestry summary results are represented by black diamonds.
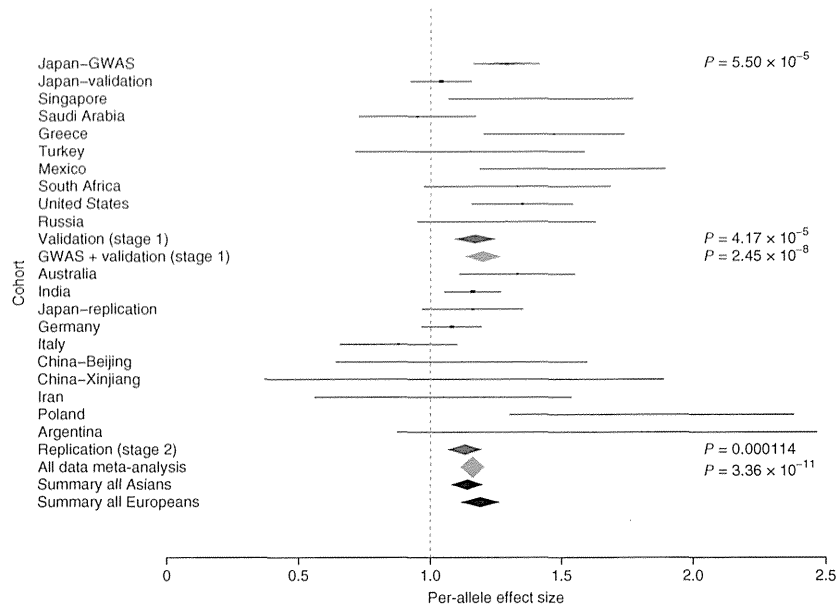
with stratification for Asian ($OR_{G\ allele} = 1.14$, $P = 7.46 \times 10^{-6}$), European ($OR_{G\ allele} = 1.19$, $P = 1.90 \times 10^{-6}$) or South African ($OR_{G\ allele} = 1.33$, $P = 0.11$) ancestry groups (P value for heterogeneity (P_{het}) = 0.5, I^2 index for heterogeneity = 0%) (**Fig. 1**).

SNP rs4926244 resides within an intronic region near the 3' end of *CACNA1A*. It is closely flanked by recombination events (**Fig. 2**) and is confined to its own LD block (**Supplementary Fig. 3**). We did not observe association with any genetic marker surpassing the nominal threshold of $P < 0.001$ outside of this region (**Fig. 2**)[24]. We next performed imputation for ungenotyped SNPs at the *CACNA1A* locus on the basis of 1000 Genomes Project cosmopolitan data using the Phase 3 release (June 2014; Online Methods) across the GWAS discovery collection. We were able to successfully impute 5,602 SNPs across the *CACNA1A* locus. However, subsequent association analysis using the imputed SNPs did not identify additional genetic associations that surpassed the statistical significance of rs4926244 (**Supplementary Fig. 4**). Notably, the most significant SNPs emerging from the cosmopolitan imputation analysis were intronic and all showed moderate-to-high correlation with rs4926244 (**Supplementary Table 5**). None of these correlated SNP markers were located in strong motifs for transcription factor binding sites as identified by the Encyclopedia of DNA Elements (ENCODE). They also did not tag any common nonsynonymous variants in *CACNA1A* (**Supplementary Table 6**). Haplotype association analysis assessing SNPs in a two-, three- or four-marker sliding window did not find evidence of an association surpassing that observed for rs4926244 (lowest haplotype $P = 0.00021$; **Supplementary Table 7**), and we further note that all but one haplotype showing evidence of association exceeding $P < 0.0005$ in the GWAS data set contained SNP rs4926244 (**Supplementary Table 7**). These findings suggest that rs4926244 is likely driving the

common-variant haplotype association results and that detailed fine mapping of this locus using deep resequencing may be required. Examination of a recently available large-scale expression quantitative trait locus (eQTL) mapping database indicated that the G risk allele at rs4926244 is modestly correlated with lower *CACNA1A* mRNA levels in peripheral blood cells ($z = -3.00$, $P = 0.0027$), suggesting that it may influence XFS risk through an effect on *CACNA1A* expression[25]. Further work will be needed to evaluate its effect in human ocular tissues.

Initial analysis of the *LOXL1* locus in the GWAS discovery data set comprising individuals of Japanese descent demonstrated strong association at rs4886776 ($OR_{A\ allele} = 8.31$, $P = 7.37 \times 10^{-137}$). The strength of this association vastly exceeded that of marker rs3825942 (responsible for a p.Gly153Asp substitution encoded in exon 1 of *LOXL1*), which has been the most widely tested and reported SNP association before this analysis[22]. Performing the analysis after conditioning for the allele dosage at rs4886776 extinguished the signal of association for every other genetic marker within the *LOXL1* locus. Conversely, conditioning the analysis for allele dosage at rs3825942 still resulted in genome-wide significant association at many of the other *LOXL1* SNPs, including rs4886776 (**Supplementary Table 8**). These data suggest that, within the Japanese GWAS discovery set, the observed association at *LOXL1* can be attributed to rs4886776 alone. We note that rs4886776 is in high LD with rs1048661 ($r^2 = 0.98$ in 1000 Genomes Project Asians), a SNP that is responsible for another nonsynonymous substitution in *LOXL1* (encoding p.Arg141Leu) but that was not directly genotyped in our data set. However, we were able to successfully impute rs1048661 in our GWAS discovery data set, and we confirmed its strong association with XFS ($OR_{T\ allele} = 8.13$, $P = 1.32 \times 10^{-126}$). SNP rs1048661 has previously been reported to show strong association with XFS in multiple populations, although
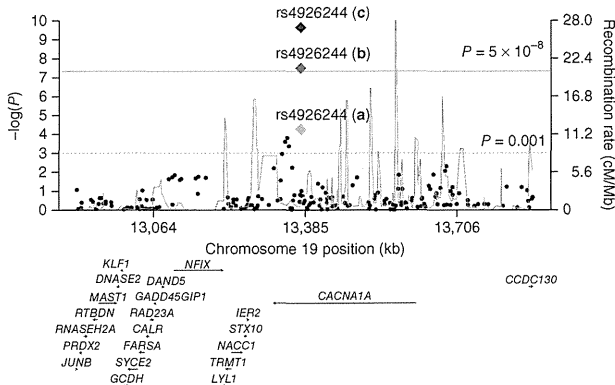

Figure 2 Regional association and recombination rate plot for the *CACNA1A* rs4926244 locus. The left y axis represents $-\log_{10}$ (P values) for association with XFS, and the right y axis represents the recombination rate. The x axis represents base-pair positions along the chromosome (human genome Build 37). Diamonds denote the summary results for each experimental stage. (**a**) GWAS discovery. (**b**) Meta-analysis between the GWAS discovery and validation stages. (**c**) Meta-analysis between the GWAS discovery, validation and replication stages.

Figure 3 CACNA1A and LOXL1 protein expression and light-microscopy analysis in XFS and non-XFS control eyes. (a–d) Immunolocalization of CACNA1A in human non-XFS globes (a,d) and in XFS1 (b) and XFS2 (c) globes with XFS shows CACNA1A-positive immunoreactivity in the smooth musculature of the ciliary body (CB) and pigmented and non-pigmented ciliary process (CP) epithelium, with variable staining in the zonules (white and black arrows; exfoliated material, green asterisks). In contrast, LOXL1 immunoreactivity is present only in the exfoliated material and the ciliary process epithelium (zonules, white arrows). Double-immunofluorescence analysis (overlay) shows colocalization of CACNA1A and LOXL1 in the non-pigmented and pigmented epithelium of the ciliary process but not in the ciliary body smooth musculature or the zonules (white arrows). Light-microscopy comparison of non-XFS and XFS irides identifies the typical XFS findings of exfoliated material (green asterisks) on the posterior iris and atrophic iris pigment epithelium with possible atrophy of the iris dilator muscle (IDM; blue arrowheads) in XFS irides. The sphincter pupillae in non-XFS and XFS eyes show negligible differences. (d,e) CACNA1A-positive immunoreactivity is also seen in the anterior iris border, iris stromal cells, and iris dilator (blue arrowheads) and sphincter muscles as well as in the iris pigmented epithelium in both XFS (e) and non-XFS (d,e) irides. Stromal cells are highlighted by the blue ovals in e. IHC, immunohistochemistry.
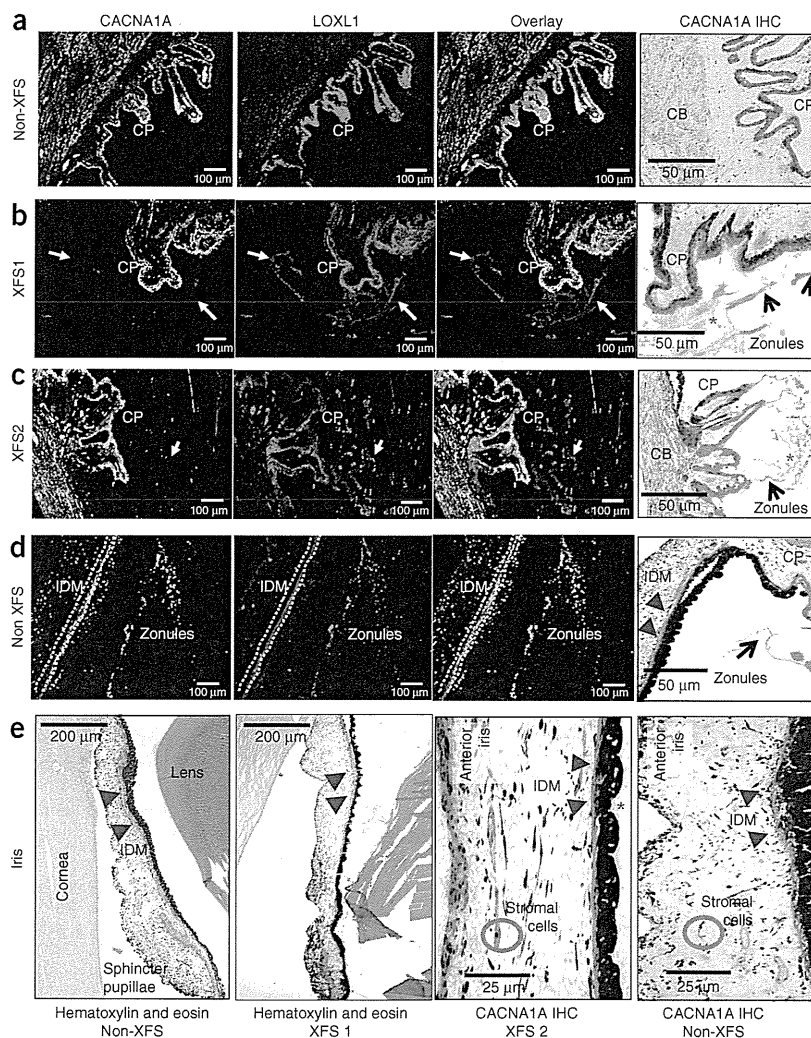
the risk allele is reversed depending on which ancestry group is being studied[11,22]. This SNP is also in LD with several other *LOXL1* SNPs located in potential transcription factor binding sites (**Supplementary Table 6**)[26,27].

SNPs rs4886776 and rs3825942 are in moderate pairwise LD ($r^2 = 0.23$). When we genotyped rs4886776 for the 2,628 XFS cases and 8,947 controls from stage 1 validation (**Supplementary Table 1**), we noted very strong evidence of consistent association for Japanese individuals ($OR_{A\ allele} = 21.7$, $P = 1.54 \times 10^{-135}$), leading to an overwhelmingly significant association in the Japanese cases and controls analyzed ($OR_{A\ allele} = 9.87$, $P = 2.13 \times 10^{-217}$). Strikingly, in non-Japanese populations, the direction of the association was opposite to that seen in the Japanese ($OR_{A\ allele} = 0.49$, $P = 2.35 \times 10^{-31}$) (**Supplementary Fig. 5**). Such a scenario echoes recently reported observations for the reversed effect of rs3825942 on XFS risk in South Africans and suggests that the genetic mechanism whereby *LOXL1* exerts its effect on individual susceptibility to XFS is complex[22]. We failed to detect any evidence of statistically significant interaction between *CACNA1A* rs4926244 and the sentinel *LOXL1* polymorphisms, suggesting that these loci affect XFS risk via distinct biological pathways.

CACNA1A encodes the α1A subunit of the type P/Q voltage-dependent calcium channel. Calcium channels are responsible for the transport of calcium ions across cell membranes and have a key role in a cell's ability to generate and transmit electrical signals. Previous electron microscopy studies on human eyes with XFS showed the presence of high calcium concentrations in direct association with aggregating XFS fibrils[28]. In addition, it is well known that fibrillin

uses calcium to form stable aggregates[29]. Thus, it can be hypothesized that the altered function of a calcium channel could lead to alterations in calcium concentrations that might facilitate the formation of XFS aggregates.

As there is a paucity of information on CACNA1A expression in the eye, we examined the mRNA expression profile of *CACNA1A* and protein expression of CACNA1A in a variety of human ocular tissues and cell lines, respectively (**Supplementary Fig. 6**). We detected *CACNA1A* mRNA expression in all of the ocular tissues we studied, with the exception of the optic nerve head (**Supplementary Fig. 6a**). Expression of different CACNA1A isoforms appears to be higher in human ocular tissue–derived cells than in cells of non-ocular origin (**Supplementary Fig. 6b**). We also performed immunofluorescence and immunohistochemistry analysis on adult human eyes and observed positive immunoreactivity for CACNA1A in multiple human ocular tissues (**Fig. 3**, and **Supplementary Figs. 7** and **8**). The distribution of CACNA1A was similar in human ocular tissues from individuals with or without XFS (**Fig. 3**, and **Supplementary Figs. 8** and **9**). Positive staining and localization of CACNA1A in the human eye was further corroborated by immunofluorescence microscopy analysis in mouse eyes (**Supplementary Fig. 10**). In human eyes, we observed positive CACNA1A immunoreactivity in the ciliary body and iris (**Fig. 3**). We also found positive staining for CACNA1A in