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厚生労働科学研究費補助金　難治性疾患克服研究事業

加齢黄斑変性症、ポリープ状脈絡膜血管症の生体試料バンク及び情報デーベースの構築

平成２１年度 総括研究報告書

平成２２年５月

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平成２１年度 総括研究報告書

平成２２年５月

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加齢黄斑変性症、ポリープ状脈絡膜血管症の生体試料バンク及び情報デーベースの構築

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Ⅰ. 総括研究報告

平成２１年度　厚生省科学研究費補助金（難治性疾患克服研究事業）総括研究報告書

加齢黄斑変性症、ポリープ状脈絡膜血管症の生体試料バンク及び情報デーベースの構築に関する研究

研究代表者　岩田　岳　国立病院機構東京医療センター臨床研究センター部長

研究要旨：本研究は中心視野を障害する難治性眼疾患である加齢黄斑変性とポリープ状脈絡膜血管症について、難病研究資源バンクへの検体提供を前提に検体の収集、症例情報のデータベース化、全ゲノム相関解析と血漿プロテオーム解析から得られた疾患バイオマーカーを解析し、これらの情報を付加して検体を提供する体制を構築した。

分担研究者：野田徹・国立病院機構東京医療センター・医長、白神史雄・香川大学医学部眼科・教授、村上晶・順天堂大学医学部眼科・教授、溝田淳・帝京大学医学部眼科・教授、安川力・名古屋市立大学医学部眼科・准教授

A．研究目的

加齢黄斑変性症と広義加齢黄斑変性症に含まれるポリープ状脈絡膜血管症は中心視野が障害される難治性眼疾患である。現在の治療法は主に病態末期の血管新生を抑制する抗VEGF治療（抗VEGFアプタマー、抗VEGF抗体）が主流であるが、視力の回復には至らない場合が多い。加齢性の疾患であることから早期診断と早期介入に必要な十分な時間が存在し、予防あるいは発症を遅延させる可能性は高い。病態早期に網膜色素上皮細胞内に蓄積される蛍光分子群のリポフスチン(Zhou et al, IOVS 2009)や網膜色素上皮細胞とブルッフ膜の間に蓄積する補体活性化分子群(Johnson et al, PNAS 2002、Klein et al, Science 2005)のドルーゼンを抑制するために我々はこれまでに霊長類モデルを用いてリン脂質機能性食品や補体抑制薬による食品試験、薬効試験を行ってきた。米国ではすでに米国眼研究所(National Eye Institute, NIH)や製薬企業１１社がこの方向で動いており、日本でも早急に加齢黄斑変性症、ポリープ状脈絡膜血管症に対する早期診断、早期予防に向けた研究が必要である。

我々はこれまでにオンライン症例登録システムを構築し、日本人を対象にした全ゲノム相関解析(Genome Wide Association Study)を行った結果、日本人は欧米人やインド人とも病気と相関する遺伝子多型について種類や相対比が異なることを発見している。本研究はこのような状況を踏まえ、欧米の眼科研究所に迫る検体数を収集し、さらにこれまで蓄積してきた日本人を対象にした全ゲノム相関解析や血漿プロテオーム解析の結果を加えてデータベースを構築し、これを生体試料バンクと統合して広く国内の研究者が利用できる体制を整えることを目的とする。

B．研究方法

（１）加齢黄斑変性症、ポリープ状脈絡膜血管症の症例情報登録システムの構築：本研究は国立病院機構病院（東京医療センター、大阪医療センター、九州医療センターなど）に加え、大学病院として香川大学、順天堂大学、慈恵医科大学、名古屋市立大学がすでに参加しており、さらに全国の施設に広げる予定である。各病院間の診断基準を統一し、眼底撮影、インドシアニングリーン造影、フルオレセインナトリウム造影、抗ＶＥＧＦ療法の効果、などについて情報を収集する。加齢黄斑変性症とポリープ状脈絡膜血管症についてはすでに遺伝子解析用にオンライン症例登録システムを構築している。本研究ではこのシステムを改良し、臨床情報、遺伝子情報、血漿蛋白情報に加えて、生体試生体試料バンクの在庫管理についての機能を盛り込み、情報データベースとも連動するようにする。

（２）加齢黄斑変性症、ポリープ状脈絡膜血管症の生体試料バンクの構築：書面による同意が得られた患者について採血を行い、冷蔵状態で３０分以内に血漿を分離し、白血球からＤＮＡを抽出する。検体は全て匿名化状態でバーコード化する。皮膚上皮細胞あるいは口内粘膜細胞を採取し、すでに報告されている実験手法によってiPS細胞を作製し、さらに網膜色素上皮細胞へと分化させる。ＤＮＡ，血漿、網膜色素上皮細胞を生体試料として分注し、保存する。東京医療センターに生体試

料受付のための研究者登録サーバーを設置する。

（３）加齢黄斑変性症、ポリープ状脈絡膜血管症の情報データベースの構築：生体試料バンクで提供する検体についてより多くの情報を付加させるために、症例情報、治療情報、遺伝情報、血漿タンパク質情報、網膜色素上皮細胞の薬剤応答性などを随時蓄積していく。これらの複数のデータは研究室内で統計処理を行い、優位な結果についてはウェブサイト(www.kankakuki.go.jp/amd)で情報公開する予定である。日本人独自の情報を活用することにより、将来の加齢黄斑変性症、ポリープ状脈絡膜血管症のテーラーメード医療の端緒を開くことができると考えられる。書面による同意が得られた患者について採血を行い、冷蔵状態で３０分以内に血漿を分離し、白血球からＤＮＡを抽出する。検体は全て匿名化状態でバーコード化する。ＤＮＡ，血漿、網膜色素上皮細胞を生体試料として分注し、保存する。

（倫理面への配慮）
検体は連結可能匿名化され、検査に携わる人員は個人情報を知り得ないしくみにする。「ヒトゲノム・遺伝子解析研究に関する倫理指針」に即して、研究計画、研究説明書、同意書、を作成している。また、当該遺伝子診断については、国立病院機構東京医療センターとそれぞれの施設の倫理委員会の承認を得ている。加齢黄斑変性症外来において患者に対し研究目的、人権擁護上の配慮、不利益・危険性の排除などの説明を十分に行う。書面による同意が得られた患者についてのみ生体検体収集及び臨床情報の収集を行う。患者が検体の破棄を希望した場合には直ちに検体を焼却処分する。

Ｃ．研究結果

（１）加齢黄斑変性症、ポリープ状脈絡膜血管症の症例情報登録システムの構築及び難病研究資源バンクへの検体提供：本研究は国立病院機構病院、香川大学、順天堂大学、慈恵医科大学、名古屋市立大学を中心に全国の施設に広げる予定であったが、本研究の交付時期が１０月にずれ込み、「生体試料等の収集に関する研究（仮称）」が平成２１年度で終了したために、検体総数は２００検体ほどにとどまっている。しかし、各病院間の診断基準を統一し、眼底撮影、インドシアニングリーン造影、フルオレセインナトリウム造影、抗ＶＥＧＦ療法の効果などについての情報は収集され、症例登録データベース上に保存されている。臨床情報に加え、遺伝子情報と血漿蛋白情報を加えて、独立行政法人医薬基盤研究所で進められている「難病研究資源バンク」への検体提供を準備中である。

（２）加齢黄斑変性症、ポリープ状脈絡膜血管症の遺伝子多型と血漿バイオマーカー：
検体収集については書面による同意が得られた患者について採血を行い、冷蔵状態で３０分以内に血漿を分離し、白血球からDNAを抽出し遺伝子解析を、そして血漿についてはプロテオーム解析を行った。遺伝子解析についてはAffymetrix社の５０万SNP(遺伝子多型)チップを用いて我々が独自に行った全ゲノム相関解析 (Genome Wide Association Study)の結果(Goto, Akahori, Iwata et al, JOBDI 2009)で強い相関が得られた遺伝子多型について(特許出願)、患者の選別を行った。また、血漿成分によって疾患の早期発見が可能か検討を行った。東レ株式会社と共同開発した低分子量蛋白分画装置を用いてアルブミンやグロブリンなどの主要高分子量タンパク質を除き、その残りの分画について逆相クロマトグラフィーを行った結果、加齢黄斑変性患者と白内障患者のクロマトグラムには大きな差が観察された。分画別にトリプシン処理を行い、低分子化された後に２次元液体クロマトグラフィーでさらに分画して、イオントラップ型質量分析計によってプロテオーム解析が行われた。この結果、加齢黄斑変性の患者のみで検出されるタンパク質を複数発見し（特許出願)、現在これらのタンパク質について抗体を用いて、患者・健常者の血漿中での濃度及び霊長類の眼球切片による免疫染色法による解析を行っている。臨床情報に加えて遺伝子情報及び血漿プロテオーム情報を加えたデータベースを構築中である。また、これらのデータを付加して「難病研究資源バンク」への試料提供を検討中である。

Ｄ．考察

本研究は交付時期が 10月にずれ込み、また、「生体試料等の収集に関する研究（仮称）」が平成２１年度で終了するために、当初の予定よりも検体数が集まっていない。しかしながら、検体収集とその解析体制は構築され、難病研究資源バンクへの検体提供のための基礎情報を各検体に付加できる体制にある。

加齢黄斑変性、ポリープ状脈絡膜血管症は多因子疾患と考えられており、複数の因子が関与して発症すると考えられている。すなわち、遺伝子因子に加えて習慣因子（喫煙、食事内容）や環境因子（仕事、住居）についても詳細に記録する必要ある。本研究で整備されたデータベースは今後の情報収集の基礎となると考えられる。

データベースの登録数が1千検体を超えた時点で匿名化の状態でデータベースを公開する予定である。加齢黄斑変性とポリープ状脈絡膜血管症の個々の患者についての症例情報、遺伝子、血漿タンパク質情報に加え、全体の傾向を統計的に整理した情報が閲覧できるようにしたい。遺伝子情報や血漿プロテオーム情報から将来、重篤な病態へと進行すると考えられる患者候補者を選別できるか検討したい。現在、補体抑制薬などの予防薬や機能性食品が計画されたおり、これを評価する上でもデータベースが有効に利用できると考えられる。

限られた時間ではあったが、目的とする生体試料の収集とその情報付加に関して、収集体制の基礎的な部分を構築できたと思う。
我々は症例情報と血液検体の収集に特化し、我々が独自に明らかにした疾患バイオマーカーを加えて評価することによって、効率的に個別化医療を実現するためのデータベースが構築できると考えられる。

E．結論

本研究は中心視野を障害する難治性眼疾患である加齢黄斑変性とポリープ状脈絡膜血管症について、難病研究資源バンクへの検体提供を前提に検体の収集、症例情報のデータベース化、全ゲノム相関解析と血漿プロテオーム解析から得られた疾患バイオマーカーを解析し、これらの情報を付加して検体を提供する体制を構築した。

F．健康危険情報

なし

G．研究発表

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H．知的財産権の出願・登録状況（予定を含む）

１．特許取得
　なし

２．実用新案登録（平成２０－２１年度）

岩田岳
特願2008-091522　神経障害の検定のための組成物、キットおよび方法

岩田岳
特願2008-092021　代謝障害を伴う疾患の検定のための組成物、キットおよび方法

岩田岳
特願2008-092245　老化、および血管障害を伴う疾患の検定のための組成物、キットおよび方法

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Genetic analysis of typical wet-type age-related macular degeneration and polypoidal choroidal vasculopathy in Japanese population

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Abstract Age-related macular degeneration (AMD) is a common cause of blindness in the elderly. Caucasian patients are predominantly affected by the dry form of AMD, whereas Japanese patients have predominantly the wet form of AMD and/or polypoidal choroidal vasculopathy (PCV). Although genetic association in the 10q26 (*ARMS2/HTRA1*) region has been established in many ethnic groups for dry-type AMD, typical wet-type AMD, and PCV, the contribution of the 1q32 (*CFH*) region seem to differ among these groups. Here we show a single nucleotide polymorphism (SNP) in the *ARMS2/HTRA1* locus is associated in the whole genome for Japanese typical wet-type AMD (rs10490924: $p = 4.1 \times 10^{-14}$, OR=4.16) and PCV (rs10490924: $p = 3.7 \times 10^{-8}$, OR=2.72) followed by *CFH* (rs800292: $p = 7.4 \times 10^{-5}$, OR=2.08; $p = 2.6 \times 10^{-4}$, OR=2.00), which differs from previous studies in Caucasian populations. Moreover, a SNP (rs2241394) in complement component *C3* gene showed significant association with PCV ($p = 2.5 \times 10^{-3}$, OR=3.47). We conclude that dry-type AMD, typical wet-type AMD, and PCV have both common

The first two authors Goto and Akahori have contributed equally to this work.

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and distinct genetic risks that become apparent when comparing Japanese versus Caucasian populations.

Keywords Age-related macular degeneration · Polypoidal choroidal vasculopathy · ARMS2 · HTRA1 · CFH · C3

Introduction

Age-related macular degeneration (AMD) is the most common cause of blindness in elderly people of European descent and affects more than 1.75 million individuals in the USA alone [1]. In Japan, the prevalence of AMD has risen from 0.87% in 1988 to 1.3% in 2003 (Japan Ophthalmologists Association Study Group Report 2006–2008). Steady increase of AMD due to longer life span and improvement of diagnostic methods has been observed around the country. The most recent report by Kawasaki et al. describes the prevalence of early stage AMD in the Funagata study (Funagata-cho, Yamagata Prefecture, Japan) as 4.4% while the prevalence of late stage AMD was 1.1%, showing no difference from a Caucasian study in Australia [2].

AMD is considered a multifactorial disease with involvement of genetic, behavior, and environmental factors, and primarily affects the macular region of the retina [3]. Clinical phenotypes of AMD are manifold. Small and hard drusen often appear in normal-aged eyes and do not necessarily cause AMD [4]. In Caucasians, the early stage of AMD is associated with an increase in the number of large soft drusen and progresses to either the dry form or wet form of the disease [3]. In contrast, Japanese patients predominantly exhibit wet-type AMD with choroidal neovascularization and few or no drusen [5]. Maruko et al. have classified wet-type AMD patients into three subgroups, namely typical wet-type AMD, polypoidal choroidal vasculopathy (PCV), and retinal angiomatous proliferation (RAP) [5]. From 289 Japanese patients examined with wet-type AMD, 35.3%, 54.7%, and 4.5% were diagnosed with typical AMD, PCV, and RAP, respectively. In the remaining 5.5% patients, one eye had PCV and the other eye had typical AMD. Thus, PCV is the predominant subgroup of wet-type AMD in the Japanese population. PCV is characterized by branching of the choroidal vasculature basal to the RPE comprising various sized polypoidal structures connected to the branching vascular network, which can be clearly seen by indocyanine green angiography [6]. PCV can be misdiagnosed as typical AMD if only fluorescein angiography is performed. The recent increase in prevalence of PCV is mainly due to the improvement of diagnostic methods. In most Japanese patients, typical wet-type AMD and PCV occur unilaterally (94.1% and 81.6%, respectively) and show a male preponderance (71.6% and 77.8%, respectively) [5] consistent with studies of Eastern Asian patients in China and Korea [7, 8]. However, in Caucasians, PCV predominantly affects women and occurs bilaterally with a prevalence ranging from 4% to 14%, which is comparably lower than the Eastern Asian populations [6]. On the other hand, it has been reported that the incidence of PCV in black individuals exceeds that of Asians [6]. Although the phenotypic diversity of AMD has been speculated to be associated with differences in genetic background, this has not been clearly established.

Initial efforts to investigate the genetic basis of AMD utilized family studies. A concordance for AMD phenotypes in twins, and a higher risk of siblings of individuals with AMD have been reported [9–14]. These early studies lead to genome-wide linkage analyses using microsatellite markers to search for chromosomal regions associated with affected individuals [15–24]. Several candidate regions including 1q32 and 10q26 were confirmed by a meta-analysis [25]. Progress in genotyping and sequencing technology extended detailed genetic association studies to the entire genome. Age-related eye disease studies (AREDS) of AMD case-control subjects using 100,000 SNPs resulted in the identification of four chromosomal regions significantly associated with the disease, namely *CFH* (1q32), *ARMS2/HTRA1* (10q26), complement component 2/complement factor B (*C2/BF*, 6p21), and complement component 3 (*C3*, 19p13) [26], which is consistent with another genome wide association study recently reported by Swaroop et al. (*IOVS* 2009; 50:ARVO E-Abstract 1614). It should be noted that these genome-wide scans have been conducted on subjects with both the dry and wet form of AMD, with the majority of cases representing the dry form of the disease. However, Zang et al. have identified 34 SNPs which were associated with AMD at p value of less than 10^{-6} in the AREDS Caucasian cohort having typical wet-type AMD [27]. They showed that 1q32 and 10q26 were also significantly associated with typical wet-type AMD. To date, there are no genome-wide genetic studies reported for PCV.

Direct examinations of SNPs in chromosomal regions identified by genome-wide linkage analysis showed that two genomic loci 1q32 and 10q26 including the *CFH*, *ARMS2*, and *HTRA1* genes were associated with AMD in Caucasians and individuals of Hong Kong [28–32]. The association between AMD and three SNPs in these gene regions, namely rs1061170 (*CFH*), rs10490924 (*ARMS2*), and rs11200638 (*HTRA1*), were verified by a number of research groups around the world. We also confirmed the association of rs10490924 and rs11200638 with typical wet-type AMD in the Japanese cohort [33]. From our results and others, rs10490924 and rs11200638 have been shown to strongly associate with dry-type AMD [34], typical wet-type AMD [31, 32, 34], and PCV [35]. It is still unclear how these SNPs contribute to the development of

different types of AMD. On the other hand, no association was confirmed for rs1061170 in Japanese AMD [36–40]. This is probably due to the lower allele frequency of 0.07±0.02 for this variant in Eastern Asian population compared to the higher frequency of 0.34±0.03 in Caucasians [41]. However, rs800292, another coding SNP in the *CFH* gene region originally associated with AMD in Caucasians [28], has been shown to associate with typical wet-type AMD and PCV in Japanese and Chinese populations [39, 42–44]. Thus, there is a clear difference in genetic risk for AMD based on ethnicity.

Since the association between the *CFH* gene and AMD has been established, other components of the complement pathway have been thoroughly examined in Caucasian populations. Among them, the 19p13, 6p21 and 4q25 loci, including the *C2*, *BF*, *C3*, and complement factor I (*CFI*) genes, show strong association with AMD [45–49]. However, it still unclear whether *CFI* or the nearby *PLA2G12A* gene is associated with the disease. Lee et al. analyzed the same AMD associated SNPs in the C2/BF gene region and reported that there were no differences between Chinese PCV patients and control groups [43]. Similar results were obtained for PCV in Japanese population for SNPs in the *C2/BF* gene region [50]. However, in this study, significant association of disease-protective haplotype was observed. There is currently no evidence that the *C2/BF* gene region is a risk for wet-type AMD or the *C3* gene region a risk for typical wet-type AMD and PCV for Eastern Asians. With the exception of 1q32, 10q26, 19p13, 6p21, and 4q25 regions, association of other loci with AMD pathogenesis reported by some genome-wide genetic studies remains unclear [15–24]. These SNP variants may be ethnic specific as is the case for *CFH*.

To investigate the involvement of genetic factors in Japanese patients who progressed to typical wet-type AMD and PCV, over 500,568 SNPs covering *ARMS2/HTRA1*, *CFH*, *C2/BF*, *C3*, and *CFI* and other regions were genotyped using Affymetrix Human Mapping 500 K Arrays and *Taq*Man assay.

Materials and methods

Subjects

One hundred Japanese patients with typical wet-type AMD but without PCV (average age 74.56±8.83 years), classified as 5b by Seddon et al. [51], 100 Japanese patients with PCV (average age, 72.71±8.25 years), and 190 age-matched Japanese controls (average age, 72.22±8.51 years) were recruited for this study (Table 1). All patients were diagnosed by ophthalmoscopic, fluorescein, and indocyanine green angiographic findings. In controls, no signs of early AMD, such as soft drusen or alterations of the retinal pigment epithelium in the macula area, were observed ophthalmoscopically. Informed consent was obtained from all participants and the procedures used conformed to the tenets of the Declaration of Helsinki.

Table 1 Summary of study populations

	AMD	PCV	Control	*p* value
No. of subjects	100	100	190	
Male/female	73:27	81:19	86:104	5.0×10^{-10}
Mean ± SD	74.56±8.83	72.71±8.25	72.22±8.51	0.08
Range	51–90	52–89	50–89	

p values were obtained by Pearson's chi-square test (5.0×10^{-10}) and one-way ANOVA (0.08) among three groups

AMD typical wet-type AMD

Genotyping

DNA was extracted from blood samples using the Magtration System 8Lx (Precision System Science Co., Ltd., Tokyo, Japan), and 50 ng/μl samples were evaluated by gel electrophoresis. Genotyping was performed on the Affymetrix GeneChip® Human Mapping 500 K Array Set (Affymetrix Japan, Tokyo, Japan), according to the manufacturer's instructions. To check the quality of each array, the SNPs were initially genotyped by a DM algorithm using Genotyping Console (Affymetrix). All arrays passed a call rate of 93% at a confidence threshold of 0.33. For the association analysis, we genotyped the SNPs by the Bayesian robust linear model with a Mahalanobis distance classifier (BRLMM) algorithm using Affymetrix Power Tools ver.1.10.0 (Affymetrix). Genotyping using Taqman® SNP Genotyping Assays (Applied Biosystems, Foster City, CA) on a StepOnePlus™ real-time PCR system (Applied Biosystems) was performed for the following SNPs in accordance with the manufacturer's instructions: rs800292, rs547154, rs2230199, rs10033900 (Assay ID: C_2530382_10, C_940286_10, C_26330755_10, C_34681305_10).

Statistical analyses

Statistical analyses were carried out with the R version of 2.7.0 [52]. Deviation of the genotype frequencies in the controls from those expected under Hardy-Weinberg Equilibrium (HWE) was assessed by chi-square test. SNPs with call rate <90% for case and control samples, respectively, a Hardy–Weinberg $p<0.0001$ for control samples, a minor allele frequency <0.05 for all samples, or those that exhibited poorly defined genotype clusters were disqualified from association analysis. For each case-control study, Fisher's exact test was used to compare allelic or genotypic

frequencies in three different models (genotype, dominant, and recessive forms) of each case group with controls. In the allelic model, the allelic frequencies were compared between cases and controls using a 2×2 contingency table. In the genotype model, the frequencies of the three genotypes were compared using a 2×3 contingency table, in the dominant model, the frequencies of the homozygote for the non-risk allele were compared using a 2×2 contingency table, and in the recessive model, the frequencies of the homozygote for the risk allele were compared using a 2×2 contingency table. Also, association analysis was performed with the use of the Cochran–Armitage trend test. After statistical analysis in three different models (genotype, recessive, and dominant model) and trend test, the minimum p value of each SNPs were obtained from lowest p value of these tests. The minimum p values were used to test whether they were lower than the significance levels. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated for the effects of risk allele and also for both the dominant and recessive forms of the genotypes. To account for multiple statistical testing, the false discovery rate (FDR) was calculated based on the minimum p values from each typical type of AMD and PCV case-control study by the method of Benjamini and Hochberg [53], and it was employed at a significance level of 0.05. Pair wise SNP linkage disequilibrium (LD) values were calculated from the genotype data using Haploview ver.4.0.

Logistic regression analysis and calculation of joint OR

Logistic regression analysis was performed to assess the joint contributions of the three candidate SNPs (rs800292, rs10490924, and rs2241394) to the risk of typical wet-type AMD or PCV using R software. To model the genetic effects, we adopted the following three genetic models with classification variables: the 2-genotype model (AA+AB and BB or AA and AB+BB) and the 3-genotype model (AA, AB, and BB). The logistic regression models were compared by the Akaike information criterion (AIC) to obtain the best-fitting model with the lowest AIC. Univariate logistic regression analysis was initially carried out for three genotype models on each SNPs, and multivariate analysis was then performed. The logistic regression models for all the possible combinations of SNPs were compared by the AIC to obtain the best-fitting model with the lowest AIC. Joint ORs for pairs of loci (rs10490924 and rs800292; rs10490924 and rs2241394; rs10490924 and rs2241394) were calculated for each 2-locus genotype separately, using the non-risk double homozygote genotype as a reference. The estimation of joint ORs was carried out with the R package Epitools. This analysis added 0.5 to each cell in a case including 0 in contingency table [54].

Results

Genotyping of typical wet-type AMD and PCV

We conducted genotyping on DNA samples obtained from Japanese patients with typical wet-type AMD and PCV. To increase the power of detecting genetic difference, we selected samples from AMD cases at stage 5b, at least in one eye without PCV, for typical wet-type AMD analysis. Stage 5 is defined as exudative AMD, including nondrusenoid pigment epithelial detachments, serious or hemorrhage retinal detachments, choroidal neovascular membrane with subretinal or sub-RPE hemorrhages or fibrosis, or scars consistent with treatment of AMD. AMD with choroidal neovascular membrane or disciform scar is defined as stage 5b [51]. Although the controls were age-matched (Table 1), the gender of the population was biased because of the characteristically higher incidence of men in Japanese AMD population [6]. One hundred cases versus 190 controls were genotyped for 500,568 tag-SNPs using the Affymetrix Human Mapping 500 K Array Set. For typical AMD, statistical analysis was performed for a selected 314,950 tag-SNPs through quality control filters (HWE $p<0.0001$, minor allele frequency <5%, and call rate <90%) to reduce low-quality signal. These SNPs are shown in Fig. 1a and b, and the 77 SNPs associated with typical wet-type AMD with genotypic p values<0.0001 are listed in the Supplementary Table S1. Our study identified several genomic locations as being potentially associated with AMD risk. Among these SNPs, rs10490924, rs3750848, and rs2672587 with false discovery rate <0.05 (Benjamini–Hochberg method) were significantly associated with typical wet-type AMD in Japanese patients with a minimum p value of 4.1×10^{-14}, 4.6×10^{-14} and 2.2×10^{-9}, respectively (Table 2). For PCV, statistical analysis was performed for 313,772 quality controlled filtered SNPs. These SNPs are shown in Fig. 1c, d, and the 98 SNPs associated with PCV with genotypic p values$<$ 0.0001 are listed in Supplementary Table S2. Two SNPs: rs10490924 and rs3750848 are statistically associated with PCV with a minimum p value of 3.7×10^{-8} and 2.4×10^{-8}, respectively. These results confirm a strong association of the *AMRS2/HTRA1* gene region with both typical wet-type AMD and PCV in Japanese, however, we could not comprehensively evaluate the association between Japanese AMD and complement related genes probably due to the low statistical power by this method.

Analysis of the SNPs in *ARMS2/HTRA1*, *CFH*, *C2/BF*, *C3*, and *CFI* gene regions

Despite the success of many genome wide association study published, not all the genome wide screening data are analyzed to their full potential. Thus, recently the advantage

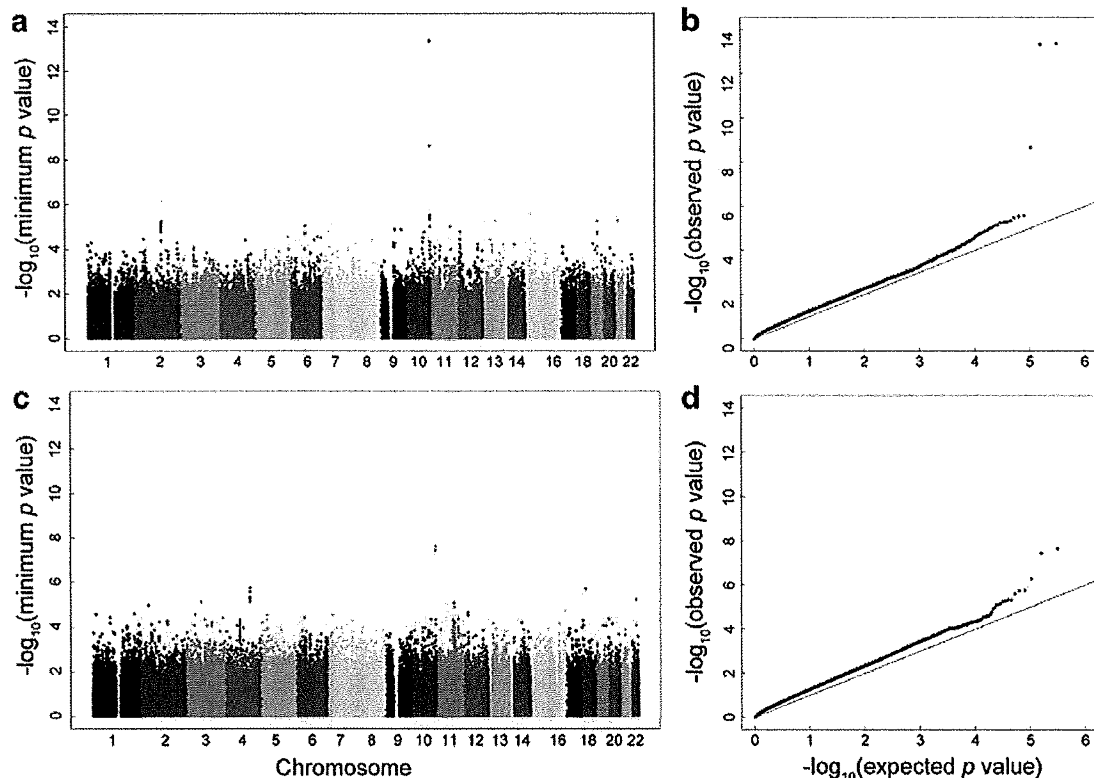

Fig. 1 Genome wide screening for Japanese typical wet-type AMD and PCV. Whole-genome association analaysis of typical wet-type AMD (**a**) and PCV (**c**). The scatter plots were drawn for $-\log_{10}$ (minimum p value) against SNP position in the chromosomes. Quantile-quantile plot for association results of typical wet-type AMD (**b**) and PCV (**d**). The distribution of observed p values versus expected p values. *Dots* represent all the 314950 SNPs and 313772 SNPs analyzed for association studies for typical wet-type AMD and PCV, respectively

of association analysis on candidate genes selected by certain criteria has been proposed. We extracted genotyping data in gene regions, which were associated with typical wet-type AMD in Caucasian populations, namely *ARMS2/HTRA1*, *CFH*, *C2/BF*, *C3*, and *CFI*. Using *Taq*Man analysis, we additionally genotyped four SNPs; rs800292 (*CFH*), rs547154 (*C2*), rs2230199 (*C3*), and rs10033900 (*CFI*), which were significantly associated with Caucasian typical wet-type AMD but were not covered in the Affymetrix Human Mapping 500 K Array. rs800292 shows association with both typical wet-type AMD and PCV ($10^{-4}<p<10^{-3}$, Table 2) as reported previously [39, 44]. No difference in genetic frequency between case and control was detected for rs547154, rs10033900, and rs2230199. After Benjamini–Hochberg correction, 12 and seven SNPs showing significant association with typical wet-type AMD and PCV, respectively (Table 2, Fig. 2), were found at the threshold FDR <0.05. For typical wet-type AMD, four SNPs in the *CFH* gene region including rs800292 appeared to be significantly associated following the *ARMS2/HTRA1* gene region. While in PCV, rs800292 in the *CFH* gene region and rs2241394 in the *C3* gene region were significantly associated with the *ARMS2/HTRA1* gene region. These results indicate common and distinctive aspects of genetic background between typical wet-type AMD and PCV in Japanese population. We also used logistic regression to further compare the genetic risk properties of typical wet-type AMD and PCV. Table 3 summarizes the best-fit and most parsimonious model analyzed by logistic regression, which was used to explore the joint contributions of the three candidate SNPs (rs800292, rs10490924, and rs2241394) to the risk of typical wet-type AMD and PCV. The AIC for three genetic models of the candidate SNPs were compared, followed by logistic regression analysis to assess the joint contributions of the genetic models with the lowest AIC. These results suggest that the joint effect of rs800292, rs10490924, and rs2241394 best described the risk for development of PCV. However, an effect of rs2241394 was not observed for typical wet-type AMD. Independent and joint ORs of typical wet-type AMD combining the genotypes from the rs800292 and rs10490924 or PCV for the two variants among rs800292, rs10490924 and rs2241394 were estimated (Table 4). When three SNPs were combined, some values

Table 2 Statistically significant SNPs associated with typical wet-type AMD and PCV from analysis of candidate gene

dbSNP ID (A/B)	Chr.	Position	Associated gene	SNP type	Case			Control			*p* value			Odds ratio (95% CI)		
					AA	AB	BB	AA	AB	BB	Allele	Minimum	Model	Allele	Recessive	Dominant
AMD																
rs800292 (G/A)	1	194,908,856	*CFH*	Exon	47	46	3	60	92	36	1.2×10^{-4}	7.4×10^{-5}	Genotype	2.08 (1.43 –3.04)	7.34 (2.20 –24.52)	2.05 (1.24–3.39)
rs1329423 (G/A)	1	194,913,010	*CFH*	Intron	35	50	15	47	87	55	0.0066	0.0049	Trend	1.63 (1.15 –2.31)	2.33 (1.24 –4.38)	1.63 (0.96–2.76)
rs10737680 (T/G)	1	194,946,078	*CFH*	Intron	43	49	8	58	88	44	0.0014	0.0012	Dominant	1.79 (1.25 –2.56)	3.47 (1.56 –7.69)	1.72 (1.04–2.84)
rs742855 (G/A	1	194,972,143	*CFH*	Intron	30	54	16	46	84	59	0.0183	0.0059	Dominant	1.52 (1.08 –2.15)	2.38 (1.29 –4.42)	1.33 (0.78–2.29)
rs2736911 (G/A)	10	124,204,345	*HTRA1*	−6,686	80	20	0	108	74	6	1.1×10^{-4}	4.8×110^{-5}	Trend	2.67 (1.59 –4.49)	NA	2.96 (1.68 –5.23)
rs10490924 (A/C)	10	124,204,438	*HTRA1*	−6,593	52	30	18	20	84	85	4.5×10^{-15}	4.1×10^{-14}	Recessive	4.16 (2.89 –5.99)	3.72 (2.07 –6.68)	9.15 (4.99 –16.80)
rs3750848 (C/A)	10	124,205,305	*HTRA1*	−5,726	52	30	18	20	84	84	5.1×10^{-15}	4.6×10^{-14}	Recessive	4.13 (2.86 –5.94)	3.68 (2.05 –6.61)	9.10 (4.96 –16.70)
rs11200644 (A/G)	10	124,220,931	*HTRA1*	Intron	76	23	0	105	79	6	3.9×10^{-4}	1.7×10^{-4}	Trend	2.40 (1.46 –3.93)	NA	2.67 (1.55 –4.62)
rs7093894 (C/A)	10	124,224,870	*HTRA1*	Intron	73	27	0	88	86	15	4.2×10^{-6}	3.0×10^{-6}	Trend	2.84 (1.79 –4.50)	NA	3.10 (1.83 –5.25)
rs2672587 (C/G)	10	124,225,345	*HTRA1*	Intron	47	38	15	29	85	74	2.0×10^{-10}	2.2×10^{-9}	Trend	3.16 (2.21 –4.53)	3.68 (1.97 –6.85)	4.86 (2.78 –8.49)
rs2250804 (G/A)	10	124,254,868	*HTRA1*	Intron	27	48	25	16	97	76	1.2×10^{-4}	7.1×10^{-5}	Recessive	2.01 (1.42 –2.85)	2.02 (1.18 –3.46)	4.00 (2.03 –7.86)
rs2268356 (G/A)	10	124,255,316	*HTRA1*	Intron	47	40	12	59	104	24	0.057	0.0068	Recessive	1.43 (1.00 –2.06)	1.07 (0.51 –2.24)	1.96 (1.19 –3.24)
PCV																
rs800292 (G/A)	1	194,908,856	*CFH*	Exon	5	43	47	36	92	60	3.4×10^{-4}	2.6×10^{-4}	Trend	2.00 (1.37 –2.92)	4.26 (1.61 –11.26)	2.09 (1.26 –3.46)
rs10490924 (A/C)	10	124,204,438	*HTRA1*	−6,593	32	50	18	20	84	85	3.0×10^{-8}	3.7×10^{-8}	Trend	2.72 (1.91 –3.86)	3.72 (2.07 –6.68)	3.98 (2.13 –7.43)
rs3750848 (C/A)	10	124,205,305	*HTRA1*	−5,726	17	50	32	84	84	20	1.7×10^{-8}	2.4×10^{-8}	Trend	2.76 (1.93 –3.93)	3.90 (2.15 –7.07)	4.01 (2.14 –7.51)
rs11200644 (A/G)	10	124,220,931	*HTRA1*	Intron	72	26	2	105	79	6	0.0132	0.0055	Recessive	1.78 (1.13 –2.81)	1.60 (0.32 –8.07)	2.08 (1.24 –3.51)
rs7093894 (C/A)	10	124,224,870	*HTRA1*	Intron	4	29	67	15	86	88	0.0015	0.0012	Recessive	1.95 (1.28)	2.07 (0.67 –6.41)	2.33 (1.41 –3.86)
rs2672587 (C/G)	10	124,225,345	*HTRA1*	Intron	26	55	18	29	85	74	2.8×10^{-4}	2.5×10^{-4}	Trend	1.92 (1.35 –2.71)	2.92 (1.62 –5.26)	1.95 (1.07 –9.55)
rs2241394 (G/C)	19	6,636,260	*C3*	Intron	0	6	93	2	33	154	0.0025	0.0037	Trend	3.47 (1.48 –8.38)	NA	3.52 (1.43 –8.69)

Allele *p* values were obtained from Fisher's exact test. Minimum *p* values were obtained from lowest *p* value of Fisher's exact test for three disease-risk models (genotype, dominant, and recessive model) and Cochran-Armitage trend test. Model showed the genetic model which minimum *p* value was found in. *AMD* typical wet-type AMD, *Chr.* chromosome number, *A* risk allele, *B* non-risk allele, *CI* confidence interval

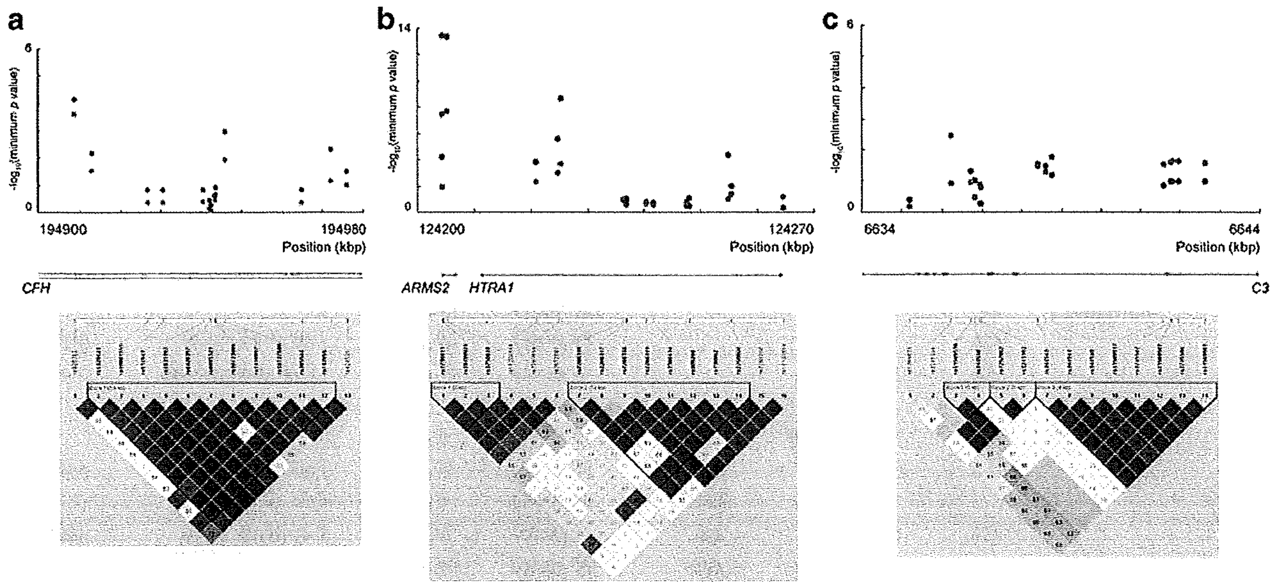

Fig. 2 Schematic view of association and LD results from association analysis for typical wet-type AMD and PCV in the significantly associated region. *Upper panel*, SNPs association results in the chromosome 1 (**a**), chromosome 10 (**b**), and chromosome 19 (**c**). This panel shows *p* values for association testing drawn from the candidate genes focused study including results using *Taq*man genotyping assay. Known genes in the area are shown. *Down panel*, LD structure of each region. Pairwise LD was calculated from all the data set in this study using the methods of Gabriel as implemented in Haploview. In each *box* are shown are the LD relationships between each SNP pair using Haploview's standard color scheme. *Blue circles*, typical wet-type AMD; *Red circles*, PCV

appeared to be unreliable because of small numbers of individuals in some of the genotype combinations. Therefore, two SNPs were analyzed. The heterozygous (CA) risk genotype of rs10490924 was detected more often in PCV cases than in controls with *p* value of 0.0008 and OR of 2.8 (95% CI=1.5–5.2), however the difference in the frequency of the heterozygous (CA) at the same loci between typical wet-type AMD cases and controls did not reach statistical significance. Both typical wet-type AMD and PCV cases carried the rs800292 heterozygous (AG) and homozygous

Table 3 Calculation of AIC by logistic regression analysis in three genotype models

dbSNP ID	Model	AMD		PCV	
		AIC	AIC difference	AIC	AIC difference
Single					
rs800292	Gen	349.6		352.1	
	Rec	350.4		353.8	
	Dom	359.6		357	
rs10490924	Gen	317.9		347.8	
	Rec	354.7		354.7	
	Dom	318.4		357.4	
rs2241394	Gen	375.4		366.7	
	Rec	345.1		373	
	Dom	374.7		365.3	
Joint					
rs800292gen + rs10490924gen		291.8	0	325.9	5.5
rs800292gen + rs2241394dom		349.2	57.4	344	23.6
rs10490924gen + rs2241394dom		319.2	27.4	344	23.6
rs800292gen + rs10490924gen + rs2241394dom		293.4	1.6	320.4	0

AMD typical wet-type AMD, *Gen* genotype model, *Rec* recessive model, *Dom* dominant model, *AIC* Akaike information criterion (the AIC difference is the difference from the AIC of the best fitting model)

Table 4 Two-locus odds ratios and p values for different genotypic combinations of the rs800292, rs10490924, and rs2241394 polymorphisms

Genotype combination					Number (%)			
					Control	Case	Odds ratio (95%CI)	p value
AMD	Single							
	rs800292	AA			36 (0.19)	3 (0.03)	1	
		AG			92 (0.49)	46 (0.48)	6.00 (1.75–20.52)	0.0011
		GG			60 (0.32)	47 (0.49)	9.40 (2.73–32.42)	2.3×10^{-5}
	rs10490924	CC			85 (0.45)	18 (0.18)	1	
		CA			84 (0.44)	30 (0.3)	1.69 (0.87–3.26)	0.1411
		AA			20 (0.11)	52 (0.52)	12.28 (5.95–25.33)	2.9×10^{-13}
	Joint							
	rs10490924	CC	rs800292	AA	15 (0.05)	1 (0)	1	
				AG	38 (0.13)	11 (0.04)	3.09 (0.51–18.69)	0.3234
				GG	32 (0.11)	5 (0.02)	1.75 (0.26–11.74)	1
		CA		AA	15 (0.05)	0 (0)	0.33 (0.01–8.83)	0.4866
				AG	45 (0.16)	15 (0.05)	3.52 (0.6–20.71)	0.3353
				GG	23 (0.08)	14 (0.05)	6.38 (1.05–38.54)	0.061
		AA		AA	6 (0.02)	2 (0.01)	3.97 (0.43–36.66)	0.5633
				AG	8 (0.03)	20 (0.07)	24.92 (3.89–159.6)	7.1×10^{-5}
				GG	5 (0.02)	28 (0.1)	53.55 (7.94–361.02)	
PCV	Single							
	rs800292	GG			36 (0.19)	5 (0.05)	1	
		GA			92 (0.49)	43 (0.45)	3.37 (1.23–9.18)	0.0156
		AA			60 (0.32)	47 (0.49)	5.64 (2.05–15.49)	
	rs10490924	CC			85 (0.45)	18 (0.18)	1	
		CA			84 (0.44)	50 (0.5)	2.81 (1.52–5.21)	0.0008
		AA			20 (0.11)	32 (0.32)	7.56 (3.55–16.08)	6.0×10^{-8}
	rs2241394	CC + CG			35 (0.19)	6 (0.06)	1	
		GG			154 (0.81)	93 (0.94)	3.52 (1.43–8.69)	0.0041
	Joint							
	rs10490924	CC	rs80092	AA	15 (0.05)	0 (0)	1	
				AG	38 (0.13)	7 (0.02)	6.04 (0.32–112.28)	0.0994
				GG	32 (0.11)	11 (0.04)	10.97 (0.61–198.43)	0.0253
		CA		AA	15 (0.05)	4 (0.01)	9.00 (0.45–181.74)	0.1131
				AG	45 (0.16)	24 (0.09)	16.69 (0.96–291.08)	0.0043
				GG	23 (0.08)	21 (0.07)	28.36 (1.6–503.36)	0.0005
		AA		AA	6 (0.02)	1 (0)	7.15 (0.26–199.68)	0.1014
				AG	8 (0.03)	12 (0.04)	45.59 (2.39–869.16)	0.0002
				GG	5 (0.05)	15 (0.05)	87.36 (4.44–1,719.03)	4.6×10^{-6}
	rs10490924	CC	rs2241394	CC+CG	15 (0.08)	2 (0.02)	1	
				GG	70 (0.37)	16 (0.16)	1.71 (0.36–8.26)	0.7302
		CA		CC+CG	17 (0.09)	2 (0.02)	0.88 (0.11–7.06)	1
				GG	67 (0.35)	48 (0.48)	5.37 (1.17–24.6)	0.0172
		AA		CC+CG	3 (0.02)	2 (0.02)	5.00 (0.49–50.83)	0.2098
				GG	17 (0.09)	29. (0.29)	12.79 (2.6–62.88)	0.0004

AMD, typical wet-type AMD; 95%CI, 95% confidence intervals; p values were obtained from Fisher's exact text

(GG) risk genotype more often than the non-risk genotype (AA) (typical wet-type AMD cases: p=0.001, OR=6.0; AG and $p=2.2\times10^{-5}$, OR=9.4; GG, PCV cases: p=0.016, OR=3.4; AG and p=0.0002, OR=5.6; GG). A joint OR of 53.5 for typical wet-type AMD in individuals with homozygous (AA and GG) risk alleles at both loci was observed when compared with the non-risk genotype (CC and AA), with a wide range of calculated 95% CI from 7.9 to 361.0. Similarly, a joint OR of 87.4 for PCV in individuals with homozygous (AA and GG) risk alleles at the same loci was observed. A joint analysis of ORs for the rs10490924 and rs2241394 showed that the risk of PCV was 5.4-fold (p=0.017) and 12.8-fold (p=0.0004) if individuals had homozygous (GG) risk genotype of rs2241394 and heterozygous (CA) and homozygous (AA) risk genotype of rs10490924, respectively, compared with the non-risk genotype. A plot of the two-locus genotype specific for typical wet-type AMD and PCV risks further illustrates the stronger impact of rs10490924 on these diseases (Fig. 3).

LD block analyses

rs800292 and rs1061170 are located within exon 2 and exon 9, respectively, of the *CFH* gene on chromosome 1. rs800292 was included in LD block 1 indicated in Fig. 2a. rs1061170 was located in a different LD block from rs800292 (data not shown). The leading two SNPs rs10490924 and rs3750848, which have been reported to associate with AMD [30], maps to a small LD block 1 on chromosome 10 (Fig. 2b). rs10490924 and rs3750848 were located within exon 1 and intron 1 of *ARMS2*. Another SNP, rs2672587, included in the intronic region of *HTRA1* is located downstream of the leading two SNPs between block 1 and 2 (Fig. 2b). rs2230199 and rs2241394 were located within exon3 and intron 13 of the *C3* gene, respectively on chromosome 19. rs2241394 was not included in LD blocks indicated in Fig. 2c.

Discussion

We performed a first genome wide genotyping of Japanese patients with typical wet-type AMD and PCV using Affymetrix GeneChip® Human Mapping 500 K Array and *Taq*Man assay. Previous genome wide scans on individuals with the dry and wet-type AMD in Caucasian populations indicated that *CFH* and *ARMS2/HTRA1* are the two most significantly associated gene regions for AMD

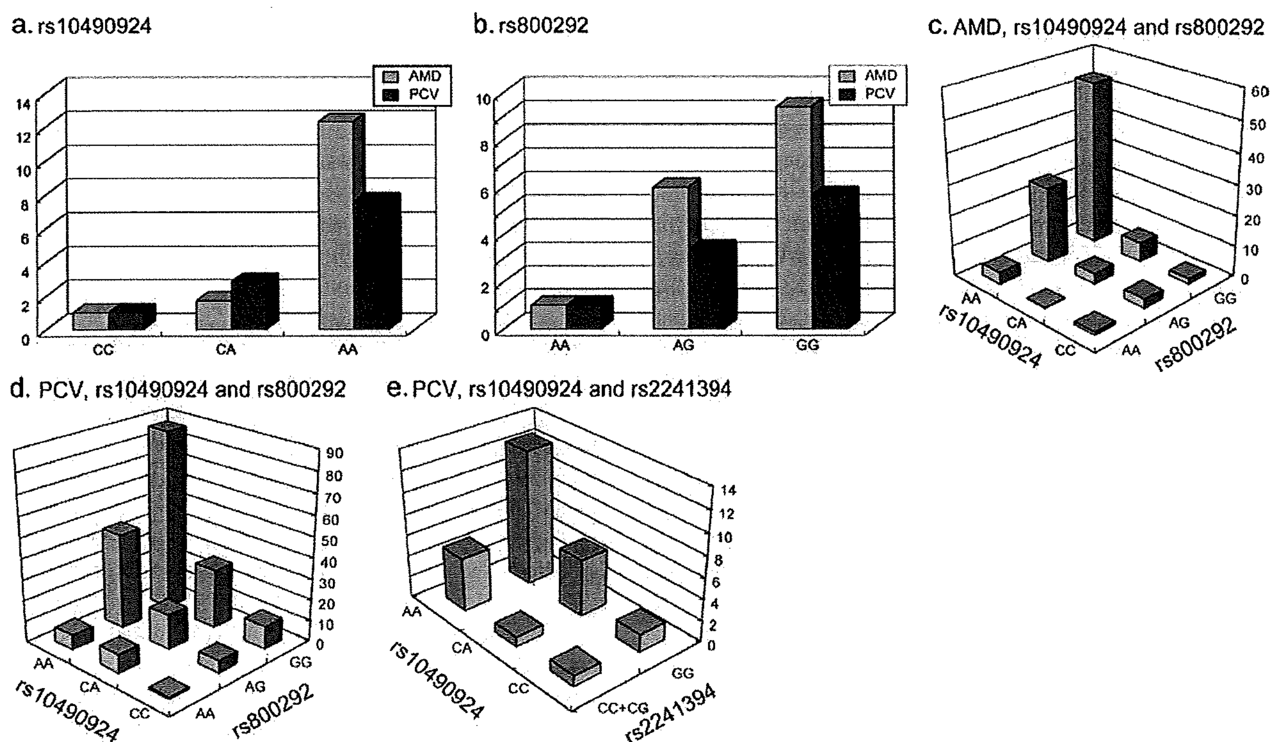

Fig. 3 Schematic view of two-locus odds ratios. *Upper panel*, independently genetic effect of rs10490924 (**a**) and rs800292 (**b**). *Blue boxes*, typical wet-type AMD; *Purple boxes*, PCV. *Down panel*, joint effect of indicated SNPs to typical wet-type AMD and PCV. Joint odds ratios for the combination of rs10490924 and rs800292 for typical wet-type AMD (**c**), rs10490924 and rs800292 for PCV (**d**), and rs10490924 and rs2241394 (**e**) are shown. The combinations had statistically significant odds ratio are *colored*. *AMD* typical wet-type AMD

followed by *C2/BF*, *C3*, and *CFI* [15–18, 26] (Swaroop A, et al. *IOVS* 2009; 50: ARVO E-Abstract 1614). Interestingly, Swaroop et al., reported that the most significantly associated gene locus was *CFH* at *p* values $<10^{-74}$ followed by *ARMS2* at *p* values $<10^{-57}$. In contrast, the Japanese typical wet-type AMD patients showed stronger association with *AMRS2/HTRA1* compared to *CFH* (Table 2). Affymetrix GeneChip® Human Mapping 500 K Array used in this study contained 19 SNPs covering the *CFH* gene region (13 SNPs passed the quality control filters). The LD blocks in this region are notably large and with the additional genotyping of rs800292 by *Taq*Man assay, the whole *CFH* gene region has been covered. Thus, the association of rs800292 with typical wet-type AMD and PCV has been confirmed, and the results in this study clearly indicate a lower contribution of *CFH* compared to *AMRS2/HTRA1* on typical wet-type AMD and PCV in the Japanese AMD population. This is consistent with our earlier findings together with others, which have shown lower association of *CFH* compared to *ARMS2/HTRA1* for AMD in Japanese individuals [33, 38–41]. Furthermore, recent studies demonstrated that 10q26 is more strongly associated than 1q32 in wet-type AMD in Caucasian populations [27, 31, 32]. Combining the results of our study with previous studies suggests that *CFH* and *ARMS2/HTRA1* independently influence the progression of both dry-type and wet-type AMD. Taking into consideration, the lower association of rs1061170 and other variations in *CFH* gene reported in Chinese [42, 55, 56] and Korean populations [57], our results may have revealed some of the common features of AMD patients among East Asian. However, it should be noted that despite the low frequency of the risk allele, a significant association between the rs1061170 variant and typical wet-type AMD have been demonstrated in some Chinese population [58, 59]. As Chu et al. have suggested in their paper, the existence of ethnic group variation within the East Asian population is possible [59]. We also performed additional genotyping of SNPs in the *C2/BF*, *C3*, and *CFI* gene regions, with SNPs not included on the SNP array. rs547154 (*C2*) is one of the protective haplotype-tagging SNPs originally reported by Gold et al. [45] who discovered a risk haplotype and two protective haplotypes. Kondo et al. have examined the effects of 11 SNPs in the above gene region in Japanese PCV cases [50]. Although individual SNPs were not associated, statistically significant association of a protective haplotype was found. This protective haplotype was different from the two haplotypes described by Gold et al. It is unclear whether this difference is due to the difference in pathogenesis between dry-type AMD and PCV or ethnicity. As for rs2230199 (*C3*), the risk allele frequency was 0 in subjects analyzed in this study, which was consistent with the data from the Hap Map project. The risk allele for rs2230199 was 0.175 in European population (Hap Map project). In our study, rs2241394 in intron 13 of *C3* gene showed significant association with PCV but not with typical wet-type AMD (Table 2).

The risk of typical wet-type AMD associated with rs10490924 was very high (OR=12.3; 95% CI=6.0–25.3), whereas the risk of PCV conferred by rs10490924 (OR=7.6; 95% CI=3.5–16.1) was similar to the risk of rs800292 (OR=5.6; 95% CI=2.1–15.5) (Table 4). Tam et al. reported that combined rs11200638 and rs800292 caused a 23.3-fold increased risk for exudative AMD [60]. rs11200638 is a SNP shown to be in LD with rs10490924 [29–31]. Similarly, our results showed that the joint risk of rs10490924 and rs800292 for PCV was 87.4-fold (Table 4, Fig. 3).

Structures of CFH and C3 have been reported previously [61, 62]. CFH consists of twenty short consensus repeats, each presumed to fold into a distinct structure termed the complement control protein module (CCP) [61]. Y402H (rs1061170) and I62V (rs800292) are located in the CCP1 and CCP7, which are implicated in C3/C3b binding and polyanion binding, respectively [61]. Accordingly, rs1061170 and rs800292 polymorphisms may differentially influence the CFH activity. C3 consist of multiple domains [62]. R80G (rs2230199) is included in the macroglobulin (MG) 1 domain which is located in the electropositive patch essential for binding target surfaces such as bacterial cells or apoptotic host cells [62]. rs2241394, which is located in intron 13 of the *C3* gene, showed strong associated with PCV in this study (Fig. 2c). Interestingly, exons 13 and 14 adjacent to rs2241394 encode the MG5 domain, which is also located within the electropositive patch together with MG1 domain. To investigate the molecular mechanism of PCV pathogenesis related to rs2241394, further exploration of SNPs within this region is needed.

The LD block including *ARMS2* contains two of the significantly associated SNPs, rs10490924 and rs3750848 (Fig. 2b, Table 2). Another significantly associated SNP, rs2672587, was in the intronic region of *HTRA1*, located approximately 6 Kbp downstream of *ARMS2* (Table 2, Fig. 2b). The rs11200638 located 512 bp upstream of the transcription initiation point is absent in the Affymetrix Human Mapping 500 K Array but has been reported to associate with AMD at a level comparable to rs10490924 [31, 34, 37]. Our genotyping of rs11200638 by sequencing also confirmed this result [33]. Our data indicate that both rs11200638 and rs10490924 share the same LD block which contains *ARMS2* and *HTRA1* but different from rs2672587 (data not shown). This result is consistent with previous Caucasian studies [29–31]. Some of the candidate loci appeared in this study may be specific to the Japanese AMD population or critical for the development of AMD in combination with *ARMS2* (rs10490924) and/or *HTRA1*