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Genome-wide analysis of copy number variants in age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is a complex genetic disease, with many loci demonstrating appreciable attributable disease risk. Despite significant progress toward understanding the genetic and environmental etiology of AMD, identification of additional risk factors is necessary to fully appreciate and treat AMD pathology. In this study, we investigated copy number variants (CNVs) as potential AMD risk variants in a cohort of 400 AMD patients and 500 AMD-free controls ascertained at the University of Iowa. We used three publicly available copy number programs to analyze signal intensity data from Affymetrix[®] GeneChip SNP Microarrays. CNVs were ranked based on prevalence in the disease cohort and absence from the control group; high interest CNVs were subsequently confirmed by qPCR. While we did not observe a single-locus “risk CNV” that could account for a major fraction of AMD, we identified several rare and overlapping CNVs containing or flanking compelling candidate genes such as NPHP1 and EFEMP1. These and other candidate genes highlighted by this study deserve further scrutiny as sources of genetic risk for AMD.

Introduction

Age-related macular degeneration (AMD) is estimated to affect nearly 2 million people in the United States, or roughly 1.5% of the population over the age of 40 years (Friedman et al. 2004; Moshfeghi and Blumenkranz 2007). The prevalence of AMD rises to over 13% in individuals over 84 years (Smith et al. 2001). The disease is characterized by progressive vision loss of variable severity accompanied by distinct changes in the structure of the retina, retinal pigment epithelium, Bruch’s membrane, and choriocapillaris. The patient phenotype can be classified as “wet” or “dry” based on the presence or absence, respectively, of choroidal neovascularization, with wet AMD comprising 1–10% of cases. Ocular injections of anti-vascular-endothelial cell growth factor (VEGF) antibodies have been successful in preserving or even restoring vision in neovascular AMD, but there is no effective treatment for the atrophic form of the disease.

Significant progress toward understanding the etiology of AMD has been made in the past 15 years. Epidemiological studies have linked the disease to environmental factors such as smoking (Age-Related Eye Disease Study Research Group 2000; McCarty et al. 2001) and diet (Flood et al. 2002). In addition, heritability for AMD is estimated to be 46–71% (Seddon et al. 2005), and genome-wide association studies (GWAS) using SNP genotyping have identified several loci associated with disease. Most notably, variation in the Complement Factor H (CFH) gene at 1q31 accounts for 25–50% of the attributable risk for AMD (Edwards et al. 2005; Hageman et al. 2005; Haines et al. 2005; Klein et al. 2005; Li et al. 2006; Maller et al. 2006). Complement Factor H is a potent inhibitor of the alternative complement pathway. Candidate gene studies have revealed associations with additional complement pathway genes, including BF (Gold et al. 2006), C2 (Gold et al. 2006; Maller et al. 2006), C3 (Maller et al. 2007; Yates et al. 2007), CFI (Fagerness et al. 2009), CFHR1 (Hughes et al. 2006), CFHR3 (Hughes et al. 2006), and SERPING1 (Ennis et al. 2008).

Significant associations have also been reported for variants in the ARMS2/HTRA1 locus on chromosome 10 (Dewan et al. 2006; Rivera et al. 2005; Weeks et al. 2000; Yang et al. 2006) and LIPC on chromosome 15 (Neale et al. 2010).

Beyond genotyping, SNP microarrays have been found to be useful for identifying a type of genomic variability called copy number variations (CNVs). CNVs are currently defined as chromosomal insertions and deletions ranging in size from 1,000 bases to several megabases (though the size limit is somewhat arbitrary) (Feuk et al. 2006). Previously thought to be rare, numerous microarray experiments have revealed that CNVs in fact account for more human genetic variation than all other sources combined, with recent data showing that up to 29.74% of the human genome may vary in copy number (Zhang et al. 2009). While most CNVs are benign, a small subset was already known to cause disorders such as Williams–Beuren syndrome (Ewart et al. 1993) and thalassemia (Orkin et al. 1979; Vandenplas et al. 1987) and to confer risk to develop genetically complex diseases such as autism (Szatmari et al. 2007) and schizophrenia (Kirov et al. 2008). Of relevance to AMD, an 84-kb deletion of two CFH-related genes near the 1q25–q31 AMD linkage peak has been associated with protection against development of AMD (Hageman et al. 2006; Hughes et al. 2006; Spencer et al. 2008). Our study is a natural extension of first generation SNP-based genome-wide association studies (GWAS) and the isolated finding of a CNV associated with AMD disease protection. Using multiple microarray platforms and copy number analysis programs, we performed a genome-wide investigation of the potential role of copy number variations as risk factors for AMD.

Methods

Patient ascertainment

Four hundred unrelated patients with AMD and 500 AMD-free controls were ascertained at the University of Iowa, and all patients provided written informed consent to a protocol approved by the University of Iowa IRB. DNA was extracted from whole blood using a salt precipitation technique (Grimberg et al. 1989). Patients with AMD and the non-AMD controls were of similar age and gender distribution (see Online Resource 1). Admixture analysis did not identify any significant difference in ancestral stratification between the case and control populations.

AMD patients

Candidates for this project were selected from a pool of patients diagnosed with AMD by faculty ophthalmologists at the University of Iowa. Retinal experts with extensive experience in AMD and AMD trials reviewed patient charts and photo files. For inclusion in this study, a patient had to have either Category 3 or 4 AMD in both eyes as defined by the age-related treatment trial (Age-Related Eye Disease Study Research Group 2000, 2001). For an eye to be classified as Category 3 it must have had at least one large druse ($\geq 125 \mu$) or enough intermediate size drusen ($63\text{--}125 \mu$) to occupy at least half of a disc area. To be Category 4, an eye must have had advanced AMD defined as geographic atrophy of the retinal pigment epithelium (RPE) in the center of the fovea or choroidal neovascularization. Geographic atrophy of the RPE is defined in the AREDS as the presence of at least two of the three following characteristics: a circular area, sharply defined margins, and visible choroidal vessels. Signs of choroidal neovascularization include elevation of the retinal pigment epithelium, sub-retinal hemorrhage or fibrosis, serous retinal detachment, hard exudation, and the presence of new vessels on fluorescein angiography. If a patient had Category 4 AMD in both eyes, at least one eye had to have at least one large druse or half a disc area of intermediate drusen.

Patients with evidence of myopic degeneration, chorioretinal scars in the macula, angioid streaks, or diabetic retinopathy consisting of more than five microaneurysms or hemorrhages were excluded from the study. The few patients who had equivocal findings, no photos, or poor quality photos that could not be evaluated were excluded from the study.

AMD-free controls

As controls for this study we used 100 individuals that were shown to be free of any eye disorder. These control individuals were over the age of 59 years at the time of ascertainment and were judged to have no signs of AMD after a complete eye examination by a board-certified ophthalmologist and/or review of eye clinic charts. In addition, 400 individuals over the age of 59 years ascertained from the University of Iowa Glaucoma Clinic were also utilized as controls. These individual were shown to be free of AMD on ophthalmologic exam. Current literature supports a distinct genetic etiology for AMD from that of glaucoma (Fan et al. 2006). In addition, GWAS data from the cohort described in this manuscript was able to identify previously reported AMD genetic associations, supporting the use of AMD-free glaucoma patients as controls for CNV discovery in the AMD patient cohort (Scheetz TE et al., in review).

Array hybridization

The experimental protocol employed two different arrays because a more advanced array became available midway through the experiment. DNA from 200 patients and 200 controls was thus hybridized to the Affymetrix GeneChip® Human Mapping 500K Array Set, and DNA from 200 patients and 300 controls was hybridized to the Affymetrix Genome-Wide Human SNP Array 5.0.

For the 500K Array Set, 250 ng of DNA was digested with either NspI or StyI and ligated to adaptors that allow PCR amplification of DNA fragments ranging in size of 200–1,100 bp. The PCR products were then purified, and subsequently, a 90-μg aliquot was fragmented with DNaseI. Fully fragmented samples were labeled with biotin and hybridized to the appropriate array at the University of Iowa DNA Core Facility. A mixture of cases and controls were included in each hybridization batch. Arrays were washed and stained using an Affymetrix Fluidics Station 450 and scanned with an Affymetrix GCS3000.

The Array 5.0 includes the same SNPs as the 500K Array Set as well as additional non-polymorphic probes used for copy number detection. The hybridization protocol is the same as for the 500K Array Set except that the NspI and StyI PCR products are pooled (instead of being kept separate) prior to fragmentation.

CNV analysis

The raw intensity data from the arrays were analyzed for copy number changes using three publicly available programs: CNAG 2.0, PennCNV, and dChip. We analyzed all of the arrays with PennCNV, the 500K Array Set arrays with CNAG, and the SNP Array 5.0 arrays with dChip. Thus, each array was analyzed with two CNV detection programs.

A useful measure of array quality is the number of CNVs called, with a high number of calls indicating poor DNA quality or a problematic hybridization. Thus, samples generating more than 30 called CNVs were either rerun or removed from analysis. To further minimize false-positive calls CNVs containing fewer than five probes or smaller than 1 kb in size were removed from the dataset. Additionally, a minimum SNP call rate of 85% was required of each array.

CNV analysis with PennCNV

PennCNV (Wang et al. 2007) was used to analyze probe signal intensity data for 1,300 arrays from all 900 patient samples. PennCNV applies a hidden Markov model to the signal intensity data while also incorporating genotyping data to infer copy number. Additional quality exclusion criteria specific to PennCNV, as recommended by the program's author, Kai Wang, included: a LogR ratio C0.35, a B-allele frequency (BAF) drift C0.05, and a wave factor (WF) threshold C0.10.

CNV analysis with CNAG 2.0

CNAG 2.0 (Nannya et al. 2005) was used to analyze data from the 400 samples hybridized to the 500K Array Set. Each array was compared to a reference panel of the appropriate array type drawn from the entire pool of 400 NspI and 400 Styl arrays. The reference panel is automatically chosen for each test array by CNAG based on the standard deviation of the signal intensities in the test array and comprising at least five arrays with standard deviations most similar to the test array. Samples with high standard deviations can generally not be referenced to at least five other arrays and were removed from the study. CNAG uses a hidden Markov model to detect CNVs with the graphical output also being visually inspected for CNV's and Styl arrays for the same patient were scored separately so that the reader was blinded to the results of the complementary array.

CNV analysis with dChip

dChip (Li and Wong 2001; Lin et al. 2004) was used to analyze probe signal intensity data from the 5.0 arrays. The arrays were analyzed in batches of 50. dChip normalizes signal intensity data with an invariant set normalization method. A perfect match only model was used to calculate corrected signal intensities for each probe, 10% of the sample was trimmed, and a hidden Markov model was implemented with a maximum of 1,000 probes to infer copy number.

Identifying CNVs of interest

The flowchart used to identify CNVs of interest for AMD can be visualized in Fig. 1. The total pool of all called CNVs was named the complete data set. Stringent criteria CNVs were those called by more than one algorithm. CNVs were considered to be of high interest with respect to AMD if they met one of the following criteria: (1) Identified in the complete data set and present in at least four AMD patients while being absent from controls and/or (2) Identified in the stringent criteria set and present at least twice in the AMD patient group and absent from the controls. A final list of high interest AMD CNVs was generated by separating CNV calls based on CNV state (gain vs. loss) and reanalyzing the data as described above. Individuals with overlapping high interest CNVs were compared across all CNVs to ensure that they were not identical or related.

Confirmation of CNVs by qPCR

All high interest CNVs were validated by qPCR (Fig. 2; Online Resource 2). All qPCR primers were picked from genomic DNA sequence obtained from the UCSC Genome Browser using Primer3 and their specificity was checked using the BLAT tool. The qPCR reaction contained 12.5 μ l of 29 QuantiTect SYBR Green PCR Master Mix (QIA-GEN), 12 μ l genomic DNA (1 ng/ μ l), and 0.25 μ l of each primer (10 pmol/ μ l) in a total volume of 25 μ l, and real-time PCR was run using an Applied Biosystems 7500 Real-Time PCR System. Each sample was amplified in triplicate with primers designed to assay controls at GAPDH and G6PD (gene dosage control) as well as the putative CNV. qPCR results were analyzed using the DDCT method, and the data were normalized by setting a pooled genomic DNA reference (Promega) to a fold change of 1.0. For each CNV that required validation we

began with a single qPCR assay. If that initial assay was in agreement with the CNV call from the array analysis, we regarded the result as confirmation. If the first qPCR assay was in conflict with the results from the array, we used the second qPCR assay to reconfirm. The third qPCR assay was used if results from either the first or second qPCR assay were inconclusive.

Results

Total number of called CNVs and array-specific data

A total of 11,671 CNVs were called after dataset-based quality control measures were applied (Online Resource 3). From the 500K Array Sets, 373 AMD arrays (Table 1) and 376 control arrays (Table 2) passed quality measures. We detected an average of 6.4 CNVs per AMD array, and there was no significant difference in copy number load or size distribution between the AMD diagnosis group and the control group. From the Array 5.0 group, 197 AMD arrays (Table 1) and 294 control arrays (Table 2) passed quality measures. We observed an average of 16.1 CNVs per AMD array, more than twice the number we detected using the Affymetrix GeneChip® Human Mapping 500K Array Set. In addition, the average size of duplications and deletions detected by the 5.0 array in both the disease and control groups was significantly smaller than that detected by the 500K two-chip array. These observations reflect the increased SNP density as well as the presence of copy number probes on the 5.0 array. In agreement with data from the 500K Array Set, there was no significant difference in copy number load or size between the AMD diagnosis group and the control group.

High interest AMD CNVs

Five CNVs met criteria for being high interest for AMD (Table 3). Two-thousand eight CNVs were entered into our stringent set. Of these, based on our filtering criteria, a deletion on chromosome 10p12.1 that contained the gene *PTCHD3* was considered to be high interest for AMD. From the complete data set of 11,671 CNVs, a CNV on 15q15.3 that contained the genes *STRC* and *CATSPER2* was placed in the high interest group. Finally, when examining the data based on specific copy number state, we identified three more CNVs of high interest: a deletion on 2q13 containing *MALL* and *NPHP1* (Fig. 2a, c), a non-genic duplication on 2p16.1 upstream of *EFEMP1* (Fig. 2b, d), and a duplication on 6q26 containing *PARK2*. All high interest CNVs were confirmed by qPCR (Fig. 2e, f; Online Resource 2). Phenotype data from patients harboring high interest CNVs are provided in Table 4.

Discussion

We analyzed genome-wide SNP microarray data from 400 AMD patients and 500 AMD-free controls in an effort to identify CNVs that may play a role in the etiology of AMD. While there was no difference in copy number load between patients with AMD and controls, when we applied rigorous prioritization criteria to the more than 11,000 CNVs that were called, we identified five that we consider to be of high interest in AMD based upon enrichment in the AMD patient cohort.

The CNV most strongly implicated by our study is the 2q13 deletion containing the genes *MALL* and *NPHP1* that was found in four patients diagnosed with AMD and no controls (Fig. 2a, c). *NPHP1*, or Nephrocystin 1, is an evolutionarily conserved gene that, when mutated, causes the autosomal recessive disorder juvenile nephronophthisis (MIM 256100), a severe and progressive disease resulting in kidney failure (Konrad et al. 1996). *NPHP1* mutations have also been identified in patients with Senior-Loken syndrome (MIM 266900)

(Caridi et al. 1998) and Joubert syndrome (MIM 213300) (Parisi et al. 2004), both autosomal recessive diseases. Senior-Loken syndrome is the co-occurrence of nephronophthisis with Leber congenital amaurosis (MIM 204000), an important cause of childhood blindness. Joubert syndrome is a genetically heterogeneous disorder characterized by structural changes in the cerebellar vermis accompanied by neurological symptoms and developmental delay. Patients with Joubert syndrome may also develop nephronophthisis and/or retinal dystrophy. Knockout mice for *Nphp1* exhibit general disorganization of the inner and outer segments of the retina along with remarkable retinal degeneration (Jiang et al. 2009). The function of *MALL* is less understood, with current data suggesting that it is a proteolipid involved in cholesterol homeostasis (de Marco et al. 2001).

According to the database of genomic variants (DGV) (Iafrate et al. 2004), copy number variation is common in *MALL* and *NPHP1*. Thus, the variants we have detected may be phenotypically benign. Conversely, the DGV may contain AMD risk variants from control populations unscreened for AMD due to the disorder's late age of onset and relatively high prevalence. Our control sample, with an older age and AMD excluded by an ophthalmology examination, is more suited to our study making *NPHP1*, in combination with the functional data reported in the literature, a compelling candidate for AMD.

Connections between the other high interest CNVs and AMD are less obvious. *PTCHD3* is thought to have Hedgehog receptor activity (Fan et al. 2007), and the Sonic hedgehog pathway has been implicated in animal models of retinal and choroidal neovascularization (Surace et al. 2006). Expression of *PTCHD3* in the mouse, however, appears to be confined to male germ cells although expression in the eye has not been tested (Fan et al. 2007). Mutations in *STRC* and *PARK2* are known to cause autosomal recessive non-syndromic deafness (MIM 603720) (Verpy et al. 2001) and autosomal recessive juvenile Parkinson disease (MIM 600116) (Matsumine et al. 1997; Kitada et al. 2000), respectively, with neither disorder having an eye phenotype.

Finally, we identified a non-genic region of chromosome 2p16.1 in three AMD patients with a duplication (Fig. 2b, d). Upon closer examination, this duplication is less than 1.5 Mb upstream of *EFEMP1*, a gene that when mutated causes an autosomal dominant disease called Doyme Honeycomb Retinal Dystrophy (DHRD, MIM 126600) (Stone et al. 1999). DHRD is characterized by drusen in the macula of the eye in a pattern described as a honeycomb appearance (Doyme 1899). Thus, this CNV may affect transcriptional regulation of *EFEMP1*, but until functional testing has been completed, the effect of this duplication is unclear.

Interestingly, we did not identify any CNVs overlapping the *CFH* gene. Although SNP variation within *CFH* may account for 25–50% of the attributable risk for AMD (Hageman et al. 2005; Haines et al. 2005; Klein et al. 2005), based upon our study, CNV in *CFH* is not likely to be a contributing factor. In agreement with previous reports (Hageman et al. 2006; Hughes et al. 2006; Spencer et al. 2008), we identified a deletion encompassing *CFHR1* and *CFHR3* in two controls that was absent from individuals diagnosed with AMD, suggesting a protective role for this deletion in the development of AMD. Of note, we did detect a duplication of the same region in one individual with AMD.

In conclusion, this study was designed to detect rare CNVs that cause AMD with high penetrance. Our data does not support a model in which a single-locus CNV with high penetrance could account for a major proportion of AMD. However, we did identify several rare and over-lapping CNVs, most notably in *NPHP1* and upstream of *EFEMP1*, that may contribute to AMD disease risk. The possibility remains that common CNVs of reduced penetrance may confer risk to AMD and should be tested in future studies.

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Fig. 1.

Study design flow chart. DNA samples from a total of 400 AMD patients and 500 AMD-free controls of similar age and gender distribution were processed on either the Affymetrix 500K SNP Array Set or the Affymetrix 5.0 SNP Array. Two programs were used to analyze signal intensity data from each platform, and the NspI and StyI arrays of the 500K SNP Array Set were analyzed separately. After implementing measures of quality control, the complete data set contained 11,671 copy number variant (CNV) calls. A stringent criteria set derived from the complete data set is composed of 2,008 CNVs that were called by two or more independent tests. CNVs were classified as high interest by identifying those that were most prevalent in the AMD patient cohort and absent from controls

Fig. 2. Chromosome 2q13 and 2p16.1 copy number variant (CNV) coordinates and validation. The coordinates used for the purpose of parts A and B yield the largest possible CNV size for each patient. a A deletion of chromosome 2q13 was identified in four patients diagnosed with AMD, but not in controls, and the coordinates of the deletion for each patient is displayed on a UCSC custom track. Deletions for all four patients overlap the genes MALL and NPHP1 and occur in a region of common copy number variation as seen in the Database of Genomic Variants track. b A non-genic duplication of 2p16.1 was identified in three individuals diagnosed with AMD, but not in controls, and the coordinates of the duplication for each patient are displayed on a UCSC custom track. The duplications are located less than 1.5 Mb upstream of EFEMP1, a gene involved in the pathogenesis of Doyme Honeycomb Retinal Dystrophy. c, d Gray shading indicates that the CNV was called by multiple independent tests within the same patient. c CNV coordinates, CNV size, CNV state, and array type for each patient identified with overlapping CNVs on chromosome 2q13. d CNV coordinates, CNV size, CNV state, and array type for each patient identified with overlapping CNVs on chromosome 2p16.1. e, f qPCR results were analyzed using the DDCt method, and the data were normalized to a pooled genomic DNA reference. e qPCR validation of NPHP1 copy number loss. The qPCR assay was designed within the NPHP1 gene. f qPCR validation of 2p16.1 non-genic copy number gain

Table 1
Descriptive statistics for copy number variants identified in the AMD patient cohort

	Two chip mapping 500K SNP array Set (CNAG and PennCNV)	5.0 SNP array (dChip and PennCNV)
Number of arrays (QC pass)	400 (373)	200 (197)
Number of deletions	1,361	1,723
Number of duplications	1,011	1,445
Average number of CNVs per array	6.4	16.1
Average size of deletions (SD, bp)	259,409 (565,719)	104,473 (231,457)
Average size of duplications (SD, bp)	539,026 (858,883)	254,668 (354,432)

Averages for the Affymetrix 500K SNP Array Set are derived by averaging data between the NspI and StyI arrays (analyzed separately) as well as between CNAG and PennCNV, the two programs used to call CNVs from this platform. The results for the Affymetrix 5.0 SNP Array platform reflect averages of CNV data generated by dChip and PennCNV

CNV copy number variant, QC quality control, SD standard deviation, bp base pairs

Table 2

Descriptive statistics for copy number variants identified in the non-AMD controls

	Two chip mapping 500K SNP array Set (CNAG and PennCNV)	5.0 SNP array (dChip and PennCNV)
Number of arrays (QC pass)	400 (376)	300 (294)
Number of deletions	1,061	1,512
Average number of CNVs per array	6.3	12.8
Average size of deletions (SD, bp)	271,285 (587,856)	107,127 (222,583)
Average size of duplications (SD, bp)	594,269 (871,414)	275,239 (377,508)

Averages for the Affymetrix 500K SNP Array Set are derived by averaging data between the NspI and SstI arrays (analyzed separately) as well as between CNAG and PennCNV, the two programs used to call CNVs from this platform. The results for the Affymetrix 5.0 SNP Array platform reflect averages of CNV data generated by dChip and PennCNV

CNV copy number variant, QC quality control, SD standard deviation, bp base pairs

Table 3

Copy number variants of interest with respect to AMD causation

Cytoband	Genes in CNV	Size (kb)	CNV state	Number of Individuals	Gene function
2p16.1	No RefSeq genes, upstream of EFEMP1	47.2	Gain	3	EFEMP1: calcium ion binding; visual perception; mutations cause Doyme Honeycomb Retinal Dystrophy
2q13	MALL, NPHP1	272.3	Loss	4	MALL: protein binding; cholesterol homeostasis. NPHP1: signal transduction; cell-cell adhesion; actin cytoskeleton organization; visual behavior; mutations cause juvenile nephronophthisis, Joubert syndrome, and Senior-Loken syndrome
6q26	PARK2	219.3	Gain	2	Ubiquitin ligase activity; central nervous system development; dopamine metabolism; mutations cause Parkinson disease
10p12.1	PTCHD3	75.3	Loss	2	Hedgehog receptor activity
15q15.3	STRC, CATSPER2	59.2	Both	5	STRC: sensory perception of sound, mutations cause autosomal recessive deafness. CATSPER2: calcium ion transport, spermatogenesis

Table contains CNV cytoband location, genes within the CNV of interest, approximate CNV size averaged between programs and individuals, CNV state, the number of individuals with the CNV of interest, and gene functions. Exact CNV breakpoints for each individual called by each program are provided in Online Resource 4

CNV copy number variant, kb kilobases

Table 4

Phenotype information of individuals with copy number variants of interest

Patient ID	Cytoband	Genes in CNV	CNV State	Sex	Age at diagnosis (years)	Age at most recent exam (years)	VA at most recent exam	GA	CN	Family history of AMD
MDI-451-1	2p16.1	None (near EFEMP1)	Gain	M	81	81	20/200 OD ^a , 20/40 OS ^b	NA	Y	+ ^c
MDI-565-1	2p16.1	None (near EFEMP1)	Gain	F	68	72	20/20 OD, 20/25 OS	N	N	- ^d
MDI-767-1	2p16.1	None (near EFEMP1)	Gain	F	75	82	20/250 OD, 20/200 OS	NA	Y	-
MDI-376-1	2q13	MALL, NPHP1	Loss	F	75	79	20/250 OD, CF @ 2' OS	NA	Y	+
MDI-511-1	2q13	MALL, NPHP1	Loss	F	78	84	20/30 OD, CF @ 3' OS	NA	Y	ND
MDI-651-1	2q13	MALL, NPHP1	Loss	F	81	87	20/160 OD, 20/30 OS	NA	Y	-
MDI-776-1	2q13	MALL, NPHP1	Loss	M	65	83	CF @ 1' OD, CF @ 4' OS	Y	N	+
MDI-414-1	6q26	PARK2	Gain	F	79	82	20/40 OD, 20/400 OS	NA	Y	ND
MDI-742-1	6q26	PARK2	Gain	F	90	93	20/50 OD, CF @ 2' OS	Y	Y	-
MDI-848-1	10p12.1	PTCHD3	Loss	F	67	76	20/25 OD, 20/200 OS	NA	Y	-
MDI-939-1	10p12.1	PTCHD3	Loss	M	68	76	20/30 OD, 20/40 OS	NA	Y	-
MDI-393-1	15q15.3	STRC, CATSPER2	Gain	F	64	78	20/250 OD, 20/160 OS	Y	Y	-
MDI-734-1	15q15.3	STRC, CATSPER2	Gain	M	80	85	20/125 OD, 20/250 OS	Y	Y	-
MDI-923-1	15q15.3	STRC, CATSPER2	Loss	F	80	87	20/70 OD, 20/50 OS	Y	Y	-
ZAMD-168-1	15q15.3	STRC, CATSPER2	Loss	F	ND	82	20/2000 OD, 3/350 OS	NA	Y	ND

Genes included in the CNV region and the type of CNV (gain or loss) is indicated for each individual. Clinical variables including identification number, sex, age at diagnosis, age at most recent examination, visual acuity at most recent exam, presence or absence of geographic atrophy, presence or absence of choroidal neovascularization, and family history of AMD are reported. The presence or absence of geographic atrophy cannot be evaluated in the presence of choroidal neovascularization, and therefore, if a patient is noted to have both geographic atrophy and choroidal neovascularization, the geographic atrophy must have been observed prior to the onset of neovascularization

CNV copy number variant, ID identification number, VA visual acuity, GA geographic atrophy, CN choroidal neovascularization, M male, F female, CF count fingers, NA not applicable, Y yes, N no, ND data not available

^aRight eye

^bLeft eye

^cFamily history of AMD

^dNo family history of AMD