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Hypomethylation of the *IL17RC* Promoter Associates with Age-Related Macular Degeneration

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SUMMARY

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the elderly population worldwide. Although recent studies have demonstrated strong genetic associations between AMD and SNPs in a number of genes, other modes of regulation are also likely to play a role in the etiology of this disease. We identified a significantly decreased level of methylation on the *IL17RC* promoter in AMD patients. Furthermore, we showed that hypomethylation of the *IL17RC* promoter in AMD patients led to an elevated expression of its protein and messenger RNA in peripheral blood as well as in the affected retina and choroid, suggesting that the DNA methylation pattern and expression of *IL17RC* may potentially serve as a biomarker for the diagnosis of AMD and likely plays a role in disease pathogenesis.

INTRODUCTION

Age-related macular degeneration (AMD) is the most common cause of irreversible central blindness in the older population worldwide. It currently affects more than 1.75 million individuals in the United States alone, and it has been estimated that this number will increase to almost 3 million in 10 years (Friedman et al., 2004). AMD leads to progressive loss of central vision due to macular atrophy or choroidal neovascularization (CNV). Currently, no medical or surgical treatment is available for central geographic atrophy (GA), the "dry" form of advanced AMD; however, antivascular endothelial growth factor (anti-VEGF) drugs such as ranibizumab (Lucentis) and bevacizumab (Avastin) have been used to treat CNV AMD, the "wet" form of advanced AMD (Campa and Harding, 2010).

In the past two decades, genetic susceptibility factors for AMD have been extensively studied and documented. DNA variants in a growing list of genes have been identified as strong genetic contributors to the etiology of AMD (Swaroop et al., 2009), including complement factor H (*CFH*) gene, *CFB*, complement 2, complement 3 (*C3*), and complement factor I (*CFI*). In addition to genes within the complement pathway, the *ARMS2*/*HTRA1* region, apolipoprotein E (*APOE*), tissue inhibitor of metalloproteinase 3 (*TIMP3*), cholesteryl ester transfer protein (*CETP*), ATP-binding cassette subfamily A member 1 (*ABCA1*), and hepatic lipase gene (*LIPC*) have also been associated with AMD (Tuo et al., 2012). Although investigators have achieved considerable success in deciphering the genetic risk factors associated with the etiology of AMD in various studies, such studies have been limited by the lack of direct functional links between genes and the pathogenesis of the disease, as well as an inconsistency among genetic studies (Peter and Seddon, 2010). In addition, several major nongenetic risk factors for AMD, including aging, smoking, diet, and inflammation (de Jong, 2006, Swaroop et al., 2009, Baird et al., 2006), have been reported. However, the mechanism by which these environmental factors affect the retina during the pathogenesis of AMD is largely unknown.

Epigenetic regulation, which includes DNA methylation and histone modifications, is the main mechanism by which gene expression patterns can be altered depending on the environmental stimulus, without a change in DNA sequence. In vertebrates, DNA can be methylated by DNA methyltransferase, and this modification occurs mostly in the context of CpG dinucleotides. DNA methylation has a substantial influence on chromatin structure and mediates silencing of gene expression (Bernstein et al., 2007). Recent studies have indicated that dynamic regulation of DNA methylation plays an important role in pluripotent stem cell differentiation and tumorigenesis (Feinberg, 2007). A number of therapeutic agents that modulate

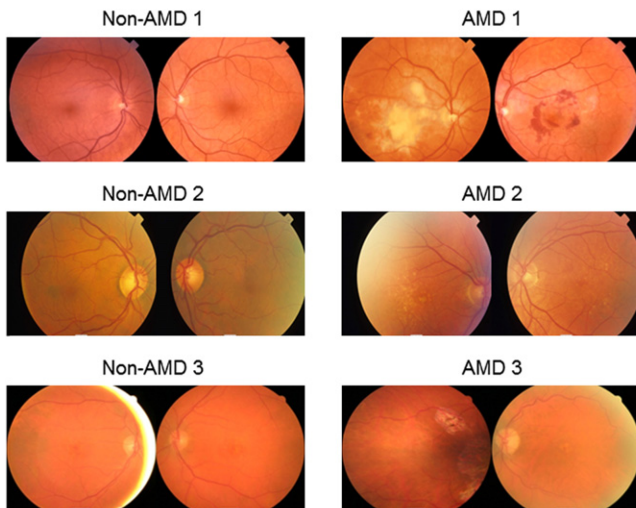

Figure 1. Fundus Photographs of Twins with Discordant AMD
Twins with AMD were named AMD 1, AMD 2, and AMD3; twins without AMD were named Non-AMD 1, Non-AMD2, and Non-AMD 3, respectively.

DNA methylation patterns have been tested in clinics for cancer treatment (Cedar and Bergman, 2009).

We were interested in assessing whether methylation changes could be identified through the analysis of twin pairs, because such analyses provide a means of dissecting the genetic and environmental components of disease. Although the majority of identical twins are concordant for end-stage AMD, a small minority present with a discordant phenotype. We recently identified twins and siblings who display such a discordant disease phenotype for AMD. This argues that nongenetic factors also play a potentially crucial role in the pathogenesis of AMD. Recent studies of genome-wide DNA methylation and histone modifications in identical twins with discordant disease phenotypes support the notion that environmentally driven epigenetic changes contribute to the etiology of diseases such as systemic lupus erythematosus and multiple sclerosis (Javierre et al., 2010, Baranzini et al., 2010, Kaminsky et al., 2009, Fraga et al., 2005). In this study, we initially investigated genome-wide differences in DNA methylation patterns between twins (both monozygotic and dizygotic) with discordant AMD, and further validated methylation changes identified at the *IL17RC* promoter in siblings with discordant AMD as well as in an AMD case control cohort of patients with either the dry or wet form of AMD. Furthermore, we evaluated IL17RC expression in the eyes and blood of AMD patients.

RESULTS

Difference in DNA Methylation Patterns between Twins and Siblings with Discordant AMD

Three pairs of twins (one monozygotic and two dizygotic) with phenotypically discordant AMD were identified from our twin patient cohort at the Centre for Eye Research Australia (CERA). Age and gender information regarding these subjects is provided in Table S1. As shown in Figure 1, the fundus photographs of the patients (named non-AMD 1, non-AMD 2, and non-AMD 3, respectively) show a normal-appearing retina and no evidence of drusen in any of the eyes. In contrast, the photographs of their monozygotic (AMD 1) and dizygotic (AMD 2 and AMD 3) twins show evidence of macular hemorrhage that was secondary to CNV (AMD 1), multiple large drusen (AMD 2), and GA (AMD 3). Monozygotic and dizygotic twins have identical or very similar genetic backgrounds; therefore, we hypothesized that the phenotypic difference between the AMD twins could be due to epigenetic diversity, in addition to the difference in genetic information between twin pairs.

Although traditionally AMD has been considered a neurodegenerative disease, the recently reported association between AMD and SNPs in genes involving the immune response, such as *CFH*, *CFB*, *CFI*, *C3*, *ARMS2/HTRA1*, *CX3CR1*, and *TIMP3*, argues that a dysregulated immune system may play an essential role during the onset and duration of AMD (Swaroop et al., 2009). In addition, in a recent small, randomized study, we found that immunotherapy positively altered the clinical course of late-stage neovascular AMD (Nussenblatt et al., 2010). Therefore, we next obtained genomic DNA from peripheral blood mononuclear cells (PBMCs), which could reflect the status of the immune system, to investigate the DNA methylome difference between AMD twins. We subjected the DNA to DNA methylation microarray (MeDIP-chip) analysis using the NimbleGen Human DNA Methylation 2.1M Deluxe Promoter Array. A total of 231 genes showed consistent differential methylation patterns on their promoters between AMD twins and their non-AMD cotwins (Table S2). Consistent with the speculation that AMD may be an immunological disease, our gene ontology enrichment analysis using Ingenuity Systems (http://www.ingenuity.com/) identified ‘‘immunological disease’’ as one of the five most significantly enriched gene categories among the list of genes with differential DNA methylation patterns between twins (Table S3). However, we did not find consistent DNA methylation changes in AMD patients on the promoters of *CFH*, *HTRA1*, *ARMS2* (Figure S1A), *CFB*, *CFI*, *C3*, *TIMP3*, *TLR3*, and *TLR4* (data not shown).

In a recent study, we demonstrated elevated serum levels of the Th17 cytokines interleukin (IL)-17A and IL-22 in AMD patients (Liu et al., 2011). Previous studies also indicated the induction of IL-17A by complement C5a (Hashimoto et al., 2010, Lajoie et al., 2010). Importantly, our microarray analysis identified IL22, IL17A, and IL17F as the top three most differentially induced genes by C5A in $CD4^+$ T cells between non-AMD controls and AMD patients (Figure S1B). Therefore, among our list of 231 genes with differential methylation patterns between twin pairs, we first focused on molecules that contribute to Th17 immunity. Intriguingly, as shown in Figure 2A, the MeDIP-chip data suggested that methylated CpG sites were found only in the twins without AMD, and not in their AMD cotwins, in the promoter regions of *IL17RC*, which encodes the receptor for IL-17A and IL-17F dimers, as well as *CCL22*, which is the chemokine that mediates trafficking of activated T lymphocytes to inflammatory sites. In contrast, we did not find consistent DNA methylation pattern differences on the promoters of the Th17 or Th1 cytokines themselves, including *IL17A*, *IL17F*, *IL22*, *IL26*, and *IFNG* (Figure S1C).

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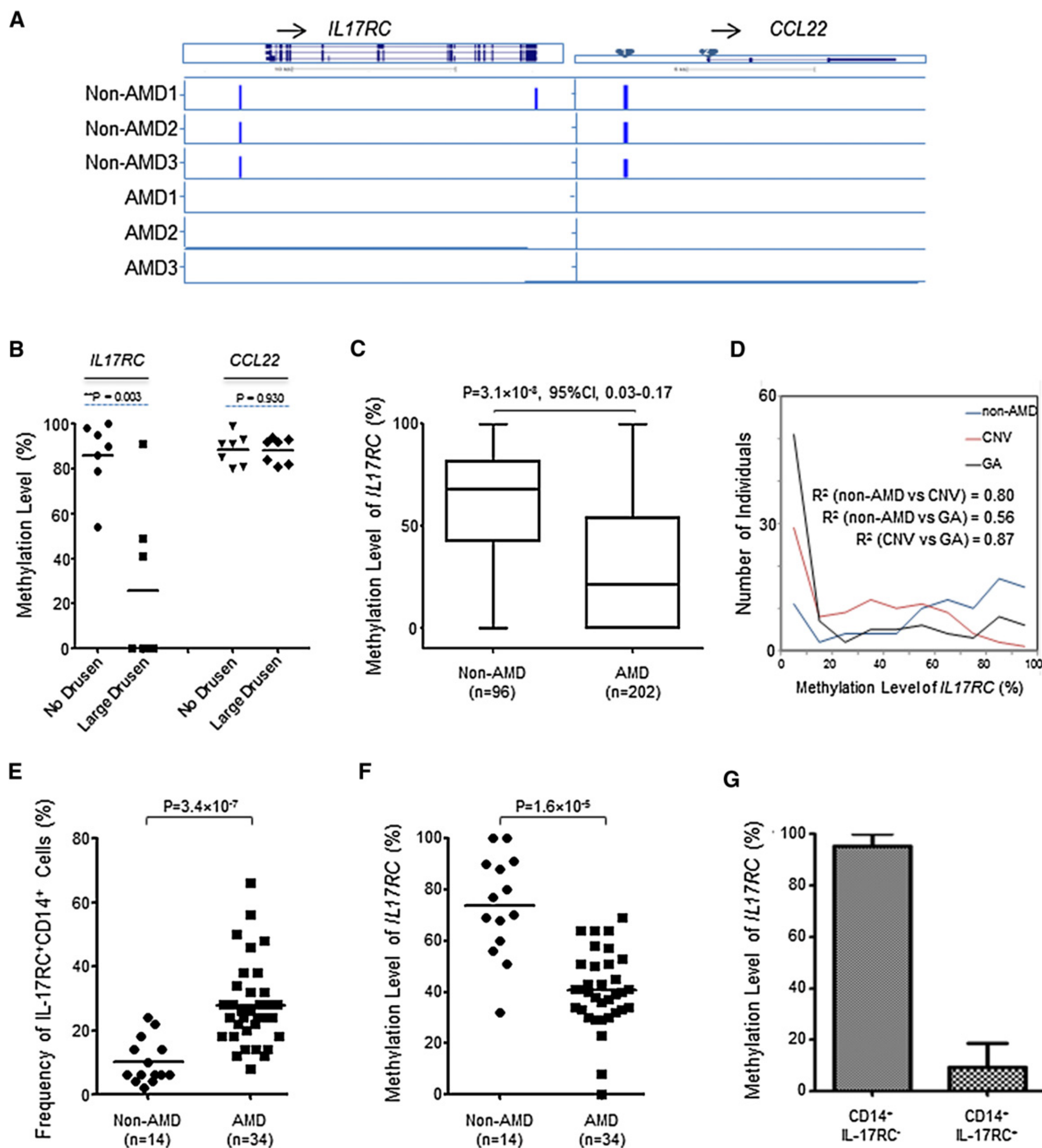

Figure 2. Hypomethylated IL17RC Promoters in AMD Patients

(A) Genome browser view of DNA methylation peaks, identified by MeDIP-chip analysis, on the loci of *IL17RC* and *CCL22*.

(B) Promoter methylation levels of *IL17RC* and *CCL22* in seven pairs of siblings with discordant AMD phenotype.

(C) Promoter methylation level of *IL17RC* in 96 non-AMD controls and 202 AMD patients.

(D) Methylation levels of *IL17RC* promoters and their distribution in CNV and GA patients.

(E) Summary of the frequency of IL-17RC$^+$ monocytes in the peripheral blood of 14 non-AMD controls and 34 AMD patients (NEI patient cohort).

(F) Promoter methylation level of *IL17RC* in the NEI patient cohort of 14 non-AMD controls and 34 AMD patients.

(G) Promoter methylation level of *IL17RC* in CD14$^+$IL-17RC$^-$ and CD14$^+$IL-17RC$^+$ monocytes isolated from the peripheral blood of AMD patients. Data are expressed as the means ± SE from three AMD patients.

See also Figures S1 and S2.

To test whether a similar DNA methylation pattern could be found in other AMD patients with more diverse genetic backgrounds, we identified seven pairs of siblings from our patient cohort at the Casey Eye Institute (CEI; Oregon Health & Science University, Portland, OR) who had discordant AMD phenotypes (for all sibling pairs, one of the two had large drusen >125 μg in diameter in at least one eye, and the other had no drusen). We next carried out Methyl-Profiler assays to detect the

methylation status on the promoters of *IL17RC* and *CCL22* (Figure 2B). Intriguingly, a significant differential methylation status between siblings with discordant AMD was found on the promoter of *IL17RC* ($p = 0.003$, Mann-Whitney test). A hypomethylated *IL17RC* promoter was found only in the DNA from the siblings with AMD, similar to what was observed in the discordant twins. We also noted that no difference in methylation status was found on the promoters of *CCL22* in the sibling pairs from Oregon, which was different from the promoter methylation patterns found in the twin pairs from Australia. To rule out the possibility that the methylation pattern difference in *CCL22* promoters between these two patient cohorts was CpG-site dependent, we designed two more primer pairs that specifically targeted two other CpG sites as indicated in Figure 2A (sites 1 and 2). Using PBMC DNA samples from 34 AMD patients and 14 non-AMD controls collected in our clinics at the National Eye Institute (NEI, National Institutes of Health [NIH]), we performed Methyl-Profiler assays on the *CCL22* promoter targeting CpG sites 1 and 2. As shown in Figure S2A, no difference in methylation status was found on these two CpG sites. In addition to the *IL17RC* and *CCL22* promoters, we performed Methyl-Profiler assays on the promoters of *IL17F*, *IFNG*, *IL26*, *CFH*, and *HTRA1*. Consistent with our MeDIP results, we did not find a consistent difference in methylation status on these promoters in our NEI collected patient cohorts (34 AMD patients and 14 non-AMD controls; Figure S2B). Taken together, these data suggest that a hypomethylated *IL17RC* promoter is associated with AMD disease not only in twins but also in siblings.

A Hypomethylated *IL17RC* Promoter Was Found in 202 AMD Patients but Not in 96 Non-AMD Controls

It is well known that microarray-based methylation detection technologies such as MeDIP-chip are prone to low specificity (Fazzari and Greally, 2010). In addition, the NimbleGen MeDIP-chip analysis provides only binary and fragment-level measurements of DNA methylation on gene promoters. The genetic similarity within a twin pair and within sibling pairs could also lead to bias. Therefore, to investigate the methylation status of *IL17RC* promoters in patients and healthy controls who did not share known genetic similarities, we collected genomic DNA from the PBMCs of 202 AMD patients (95 CNV and 107 GA) as well as 96 healthy individuals without AMD (non-AMD) from our clinics at the NEI and CEI. The average ages of the AMD patients and non-AMD controls were 80.5 and 70.2 years, respectively (Table S1, Case-Control Patient Cohort). In similarity to our findings with both twins and siblings, we found a hypomethylated *IL17RC* promoter in AMD patients and a hypermethylated *IL17RC* promoter in non-AMD controls (95% confidence interval [CI], 0.03–0.17; $p = 3.1 \times 10^{-8}$, multivariable logistic regression adjusted for age and gender; Figure 2C; Table S4). Interestingly, the methylation status of the *IL17RC* promoters did not differ significantly between patients with wet AMD and those with dry AMD ($p = 0.41$, multivariable logistic regression adjusted for age and gender; Figure S2C). However, the distribution of these promoters was not identical between CNV and GA patients (Figure 2D). Collectively, these results show an association between AMD and a hypomethylated *IL17RC* promoter.

Elevated Expression of IL-17RC in the Peripheral Blood of AMD Patients

DNA methylation is generally associated with gene silencing. To test whether DNA hypomethylation found on the promoter of *IL17RC* results in gene desilencing and hence increased expression in only AMD patients, we performed a fluorescence-activated cell sorting (FACS) analysis of whole-blood samples, together with other cell-surface markers that identify peripheral hematopoietic cell lineages as well as the isotype control for IL-17RC staining. Intriguingly, in the NEI patient cohort with 14 non-AMD controls and 34 AMD patients, we found a significantly elevated frequency of IL-17RC$^+$CD14$^+$ monocytes in the peripheral blood of the AMD patients (Figures 3E and S3A). We consistently found hypomethylated *IL17RC* promoters in these AMD patients (Figure 3F). Importantly, we found that the methylation level of the *IL17RC* promoter was significantly lower in the CD14$^+$IL-17RC$^+$ cells than in the CD14$^+$IL-17RC$^-$ cells (Figure 3G), indicating that the elevated IL-17RC expression was the result of demethylation of its promoter. In addition, we found an increased frequency of IL-17RC$^+$ cells among other types of cells, including CD3$^+$CD4$^-$CD8$^-$ and CD8$^+$ T cells (but not CD4$^+$ T cells), from AMD patients as compared with age-matched non-AMD controls (Figure S3B). These data suggest that hypomethylation of the *IL17RC* promoter results in an elevated expression of the IL-17RC protein on selected cells in the peripheral blood of AMD patients.

Induction of IL-17RC Expression by IL-17A and IL-17F

Previous studies demonstrated that IL-17RC mediates both IL-17A and IL-17F signaling (Ho and Gaffen, 2010). However, it is not clear which factors regulate the expression of IL17RC. As shown in Figures 4A and 4B, we found that both IL-17A and IL-17F, through activation of NF-κB (Figure 4C), induced the expression of IL-17RC in THP1 cells (a human acute monocytic leukemia cell line) as well as in primary CD14$^+$ monocytes. Our microarray analysis also indicated a great difference in genome-wide gene expression profiles between IL-17RC$^+$ and IL-17RC$^-$ THP1 cells (Figure 4D). Using semiquantitative real-time PCR, we confirmed that IL-17RC$^+$ THP1 cells expressed higher levels of the chemokine receptors *CXCR1*, *CXCR2*, *CXCR4*, and *CXCR7* (Figure 4E). Similarly, we also found elevated expression of *CXCR1*, *CXCR2*, and *CXCR4*, but not *CXCR7*, in CD14$^+$IL-17RC$^+$ cells from AMD patients as compared with CD14$^+$IL-17RC$^-$ cells (Figure 4F). Moreover, we found that IL-17A induced the expression of CXCR2 and CXCR4 (Figure 4G). Therefore, our results suggest that IL-17RC can be induced by both IL-17A and IL-17F, and IL-17RC$^+$ monocytes highly express the chemokine receptors *CXCR2* and *CXCR4*.

Elevated Expression of IL17RC in the Eyes of AMD Patients

Previous studies suggested the involvement of monocytes/macrophages in AMD pathogenesis (Nussenblatt et al., 2009, Cao et al., 2011). Chemokine receptors expressed on the cell surface play a critical role in enabling monocytes to exit the vasculature through endothelial cells in the choroid or retina. Because we found that IL-17RC$^+$ monocytes highly

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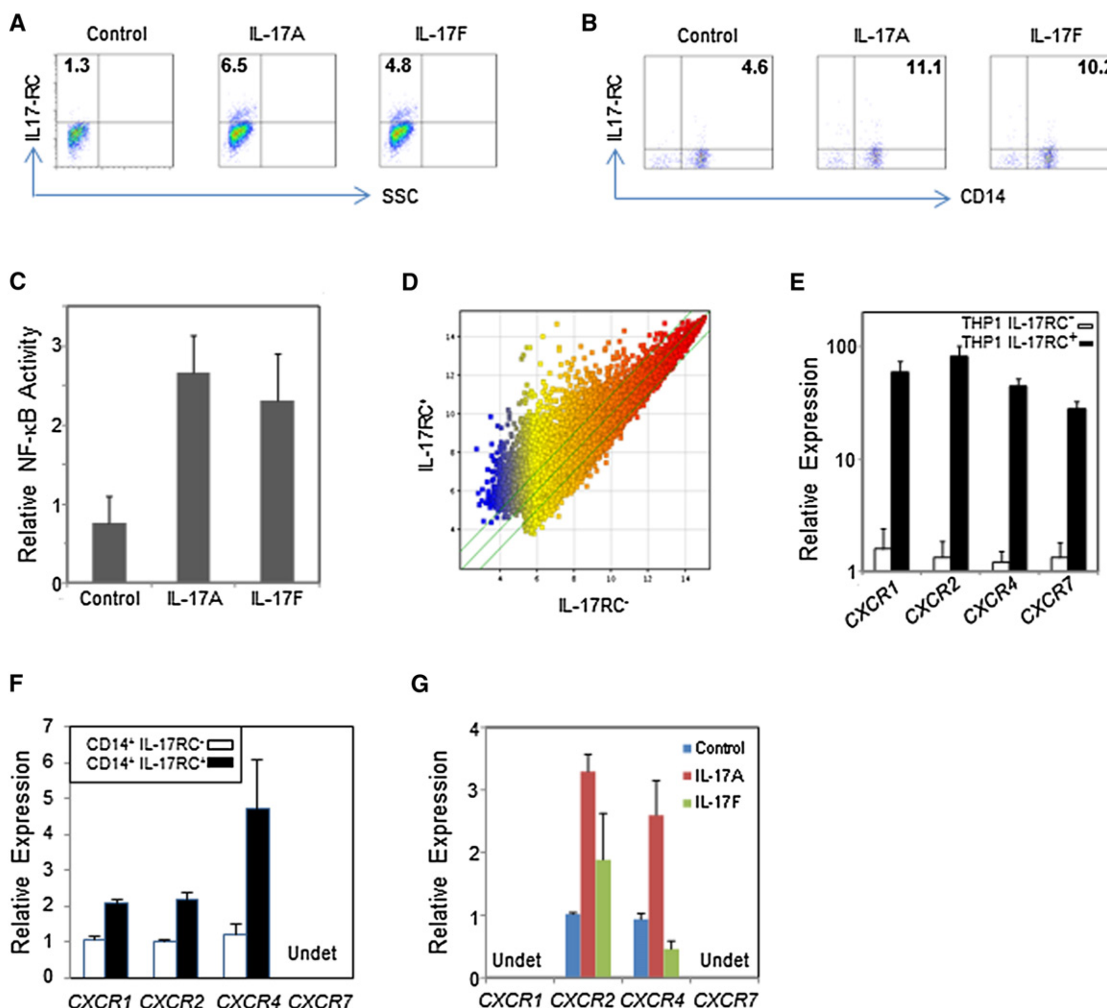

Figure 3. Induction of IL-17RC by IL-17A and IL-17F in Monocytes, and Characteristics of IL-17RC$^+$ Monocytes

(A) FACS staining of IL-17RC induced by IL-17A and IL-17F in THP1 cells.

(B) FACS staining of IL-17RC induced by IL-17A and IL-17F in primary CD14$^+$ monocytes. See also Figure S3.

(C) Relative activity of NF-κB measured by TransAM NF-κB p65 transcription factor ELISA kit.

(D) Scatter plot showing the genome-wide expression difference between sorted IL-17RC$^+$ and IL-17RC$^-$ THP1 cells.

(E) Relative expression of *CXCR1*, *CXCR2*, *CXCR4*, and *CXCR7* mRNA in sorted IL17RC$^+$ and IL17RC$^-$ THP1 cells.

(F) Relative expression of *CXCR1*, *CXCR2*, *CXCR4*, and *CXCR7* mRNA in sorted IL17RC$^+$CD14$^+$ and IL17RC$^-$CD14$^+$ monocytes from two AMD patients. Undet, undetectable.

(G) Relative expression of *CXCR1*, *CXCR2*, *CXCR4*, and *CXCR7* mRNA in primary CD14$^+$ monocytes stimulated by IL-17A or IL-17F overnight.

Data are expressed as the means ± SE.

expressed the chemokine receptors *CXCR2* and *CXCR4*, we hypothesized that they might be able to migrate to the macular regions of the eye. To test whether IL17RC expression could be found in the macular tissues of AMD patients, we obtained formalin-fixed, paraffin-embedded (FFPE) archived slides from 26 eyes with either GA (five eyes) or CNV (21 eyes) AMD, as well as six eyes from age-matched non-AMD controls. We microdissected the macular tissue into RNA lysis buffer (Ambion) and extracted total RNA using an RNA extraction kit (Ambion). We then performed quantitative real-time PCR to detect the expression of *IL17RC* mRNA. Interestingly, *IL17RC* was highly expressed in the macular tissues of the AMD patients, but not in those of the non-AMD controls (Figure 4A). Moreover, immunohistochemistry assays were carried out to detect the expression of IL-17RC protein in the macular regions of both AMD and control eyes. As shown in Figures 4B–4E, the immunoreactivity against IL-17RC was stronger in the macular tissues from patients with GA AMD (Figures 4D and S4A) and CNV AMD (Figures 4E and S4B) than in the macular tissues from non-AMD controls (Figure 4C) or in the macular tissues stained with nonspecific antibody (Figure 4B). Thus, elevated IL17RC expression was found not only in the peripheral blood but also in the chorioretinal tissues with AMD lesions.

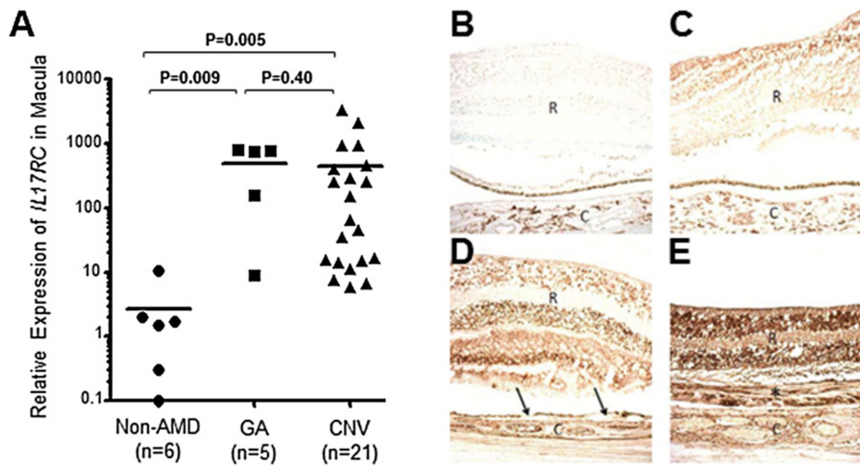

Figure 4. Expression of IL17RC in the Macular Tissues of AMD Patients

(A) Relative expression of *IL17RC* mRNA in archived microdissected macular tissues from five eyes with GA, 21 eyes with CNV, and six eyes from non-AMD controls.

(B–E) Immunohistochemistry staining using nonspecific serum in AMD eyes. Immunohistochemistry staining of IL-17RC protein in non-AMD control (C), GA (D), and CNV (E) eyes.

Arrows, drusen; asterisk, chorioretinal neovascular fibrous tissue; R, retina; C, choroid. See also Figures S4A and S4B.

DISCUSSION

Twin and sibling designs are broadly used in studies that aim to dissect the genetic and environmental contributions to various diseases. Earlier studies using small patient cohorts indicated a significantly higher concordance rate of AMD in monozygotic than in dizygotic twins or families, strongly suggesting a genetic predisposition to AMD (Meyers, 1994, Gottfredsdottir et al., 1999, Klein et al., 1994, Heiba et al., 1994). In addition, Hammond et al. (2002) showed that the concordance for AMD in monozygotic twins was 0.37 compared with 0.19 in dizygotic twins. Seddon et al. (2005) suggested that genetic factors can explain 46%–71% of the variation in the overall severity of AMD; however, they also detected both significant genetic (0.26–0.71) and unique environmental (0.28–0.64) proportions of variance for specific macular drusen and retinal pigment epithelial characteristics. In a later study, Seddon et al. (2011) suggested that in addition to genetic susceptibility, smoking and diet associated with epigenetic mechanisms are involved in the etiology of AMD. We were able to identify three pairs of twins (one monozygotic and two dizygotic) in our patient cohort who displayed a significant discordance for end-stage disease, which gave us a unique opportunity to study the potential epigenetic differences between them. Our MeDIP-chip analysis identified differences between AMD twins in ~1.5% of the total CpG sites within 231 gene promoters. This study attempts to address genome-wide epigenetic regulation in AMD patients. It only confirms the association between a hypomethylated *IL17RC* promoter and the onset of AMD; however, we are in the process of investigating whether epigenetic regulation of the other 230 genes is also associated with AMD. Our results also suggest that epigenetic modifications of DNA may be crucial for understanding the molecular basis of AMD.

IL-17RC is found highly expressed on the endothelium and epithelium of the lung, intestine, kidney, prostate, and joint (Ge and You, 2008). However, it was not clear which types of cells normally express IL-17RC in the human peripheral blood or eyes. IL-17RC serves as an essential subunit of the IL-17 receptor complex, which mediates the signal transduction and proinflammatory activities of IL-17A and IL-17F. Its binding affinity, ligand preference, and tissue-specific distribution differ slightly between murine and human systems. Recent studies have suggested that high levels of IL-17RC expression are found in prostate cancer cells, as well as in synoviocytes and PBMCs from patients with rheumatoid arthritis (Gaffen, 2009). In our study, we found hypomethylated *IL17RC* promoters in both GA and CNV AMD patients as compared with non-AMD controls, which resulted in the increased expression of its protein in $CD14^+$ monocytes. Moreover, we demonstrated that IL17A, which was found elevated only in AMD patients' serum and macular tissues, induced IL-17RC expression in primary $CD14^+$ monocyte and human RPE cells, as well as in THP1 and ARPE19 cell lines, suggesting a potentially crucial role for IL-17A and IL-17RC in the pathogenesis of AMD. Intriguingly, our results suggested that $CD14^+$IL-17RC$^+$ monocytes expressed elevated CXCR4 and CXCR2, which serve as the receptors for chemokines such as IL8, CXCL1, CXCL5, and CXCL6. These chemokines were also highly induced in RPE cells by both IL-17A and IL-17F. Therefore, Th17-cytokine-induced intraocular inflammation may promote monocyte trafficking into inflammatory sites within the macular tissues of AMD patients and eventually cause the pathology of AMD. Therefore, our results suggest a potential mechanism by which proinflammatory monocytes could promote AMD pathology.

IL-17-producing Th17 cells are essential for clearing pathogens during host defense and for inducing inflammation in autoimmune disease (Korn et al., 2009). Genetic mutations in genes involved in IL-17 signaling have been associated with multiple diseases (Puel et al., 2011, Milner et al., 2008). Our study demonstrates that epigenetic regulation of *IL17RC* may also play an important role in the pathogenesis of AMD, one of the best genetically characterized complex disorders. More importantly, promoter DNA methylation patterns and expression of *IL17RC* may serve as potential biomarkers for AMD diagnosis and targets for AMD therapy.

EXPERIMENTAL PROCEDURES

Patients

All protocols were approved by institutional review boards, and written informed consent for epigenetic and genetic testing and participation was provided by the patients to the NIH, CEI, or CERA through the Human Research and Ethics Committee of the Royal Victorian Eye and Ear Hospital.

The Australian Twin Registry (ATR) approved the project. Twin volunteers, both identical and nonidentical, with or without known eye disease, of either gender, and ≥18 years of age, were invited to participate through the ATR. The non-AMD controls were recruited from the NIH eye clinics and were screened for AMD. All patients and non-AMD controls were Caucasian. Age and gender information regarding the subjects is provided in Table S1.

MeDIP-chip and Data Analysis

DNA sample preparation and hybridization to MeDIP-chip were performed according to protocols provided by Roche-NimbleGen. Standard data analysis service was provided by NimbleGen. All raw data and processed bed files were deposited in the Gene Expression Omnibus with accession number GSE28033.

Detection of DNA Methylation on Selected Gene Promoters

Genomic DNA was extracted from PBMCs of AMD patients or healthy controls, as well as from THP1 cells using the DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's protocol. Then, 1 μg total genomic DNA from PBMCs was digested for 6 hr at 37°C using the Methyl-Profiler DNA Methylation Enzyme Kit (SABiosciences) according to the manufacturer's instructions. Quantitative real-time PCR was performed with the use of the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The percentage of methylated DNA was calculated by the difference in threshold cycle (CT) numbers between samples with total undigested DNA and methylation-sensitive restriction enzyme-digested DNA.

Cell Culture

PBMCs from healthy controls or AMD patients were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1X penicillin-streptomycin antibiotics (Invitrogen). They were treated with or without C5A (50 ng/ml), IL-17A (20 ng/ml), or IL-17F (20 ng/ml) overnight, followed by FACS sorting for $CD3^{+}CD4^{+}$ T cells or $CD14^{+}$ monocytes using FACSAria II (BD). THP1 cells (ATCC) were cultured in RPMI-1640 medium supplemented with 10% FBS, 2 mM L-glutamine, and 1X penicillin-streptomycin antibiotics (Invitrogen). ARPE-19 cells and primary human RPE cells (ATCC) were cultured in complete Dulbecco's modified Eagle's medium supplemented with 10% FBS, 2 mM L-glutamine, and 1X penicillin-streptomycin antibiotics (Invitrogen) with or without IL-17A (20 ng/ml), or IL-17F (20 ng/ml) overnight.

Detection of RNA Expression in THP1 cells, RPE Cells, and $CD14^{+}$ Monocytes

Total RNA was isolated with the use of a mirVana miRNA isolation kit (Ambion). Reverse transcription of RNA and quantitative real-time PCR were performed according to the manufacturer's protocols, using the primers and probes for *18 s rRNA* (Hs03928990_g1), *CXCR1* (Hs00174146_m1), *CXCR2* (Hs01011557_m1), *CXCR4* (Hs00607978_s1), *CXCR7* (Hs00604567_m1), *IL6* (Hs00174131), *IL8* (Hs00174103), *CXCL1* (Hs00236937), *CXCL5* (Hs00607029), and *CXCL6* (Hs00605742) from Applied Biosystems. The comparative CT method and *18 s rRNA* was used to normalize the expression.

Detection of IL17A and IL17RC Expression in Human Retinal and Choroidal Tissues

Archived FFPE ocular sections were first deparaffinized in 100% Xylene ten times, followed by sequential washes in 100% ethanol, 95% ethanol, 75% ethanol, and water (ten times in each solution). Total RNA was isolated from the microdissected macular regions of five eyes with GA, 21 eyes with CNV, as well as six age-matched eyes without AMD pathology, using mirVana miRNA kit (Ambion). Reverse transcription of RNA was performed for 12 hr at 37°C, followed by quantitative real-time PCR, using the primers and probes for *18 s rRNA* (Hs03928990_g1), *IL17A* (Hs00936345), and *IL17RC* (Hs00994305_m1) from Applied Biosystems. The comparative CT method and *18 s rRNA* were used to normalize the expression of *IL17A* and *IL17RC*.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5.0 software (GraphPad). The Mann-Whitney test was used to compare differences between two groups and the significance level was set at $p < 0.05$. Multivariable logistic regression analysis was used to detect the difference of the *IL17RC* promoter methylation status, adjusted by age, gender, and genotypes of *CFH*, *ARMS2*, or *HTRA1*, using SAS 9.2. The significance level was set at $p < 0.05$.

ACCESSION NUMBERS

Microarray data are available at the NCBI Gene Expression Omnibus database under the series accession number GSE28033.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Results, Extended Experimental Procedures, four figures, and four tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.10.013.

LICENSING INFORMATION

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