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Genetic and Epigenetic Regulation in Age-related Macular Degeneration

Lai Wei[1,2,3,*], **Ping Chen**[1], **Joo Hyun Lee**[1], and **Robert B. Nussenblatt**[1,2,*]

[1]Laboratory of Immunology, National Eye Institute, Bethesda, MD 20892, USA

[2]National Center for Complementary and Alternative Medicine, National Institutes of Health, Bethesda, MD 20892, USA

[3]Zhongshan Ophthalmic Center, Guangzhou, China

Abstract

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the older population worldwide. While strong genetic risk factors have been associated with AMD etiology, environmental influences through epigenetic regulation are also likely to play a role. Recent advances in epigenetic studies have resulted in the development of numerous epigenetic drugs for the treatment of cancer and inflammation. Here, we review the current literature on the genetic and epigenetic mechanisms of AMD and suggest that understanding the cooperation of epigenetic and genetic mechanisms will greatly advance the clinical management of AMD.

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the older population worldwide.[1] The prevalence of early and late AMD is estimated to be 6.8% and 1.5% in white people aged 40 years and older.[2] These rates are largely similar in Asian countries,[3] while the Baltimore Eye Study[4] shows that the prevalence of late AMD is significantly lower in African Americans than in white or Asian populations.

AMD leads to progressive loss of central vision due to geographic atrophy or choroidal neovascularisation. Gradually progressed visual impairment can also lead to significantly compromised quality of life and depression. In the early stage, lipofuscin (A2E) accumulation, decrease of retinal pigment epithelium (RPE) cell number in the macular region, increased thickness of Bruch's membrane, and drusen formation are the major hallmarks of AMD pathology. Patients can progress to one or both advanced forms of AMD at the late stage called geographic atrophy, characterized by RPE atrophy and photoreceptor degeneration (dry AMD), as well as choroidal neovascularisation (CNV) characterized by pathological angiogenesis and/or haemorrhage in the choroidal macular regions (wet AMD).[5] Currently, no medical or surgical treatment is available for central geographic atrophy, while photodynamic therapy (PDT)[6] as well as anti-vascular endothelial growth factor (anti-VEGF) drugs including ranibizumab (Lucentis) and bevacizumab (Avastin)[7] have been used to treat choroidal neovascular AMD (CNV). The later two drugs present similar efficacy in controlling the loss of visual acuity in patients.[8]

*To whom correspondence should be addressed. lai.wei@nih.gov, drbob@nei.nih.gov.

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Genetics of AMD

Combination of multiple genetic and environmental risk factors has been suggested for the onset of AMD.[2] Powerful genetic and genomic approaches have provided important insights into the pathogenesis of AMD.[9, 10] Using family linkage and Genome-wide Association Study (GWAS), DNA variants in a growing list of immunological genes have been identified as strong genetic contributors to the etiology of AMD,[9] including complement factor H (*CFH*) gene,[11-15] *CFB*,[16] complement 2,[16] complement 3,[17,18] and complement factor I (*CFI*),[19] the *ARMS2*/*HTRA1* region,[20-25] tissue inhibitor of metalloproteinase 3 (*TIMP3*),[26] HLA,[27] and interleukin 8 (*IL8*).[28] In addition to genes involved in immune responses, apolipoprotein E (*APOE*),[29, 30] cholesteryl ester transfer protein (*CETP*),[26] ATP-binding cassette sub-family A member 4 (*ABCA4*),[31] and hepatic lipase gene (*LIPC*)[32] have also been associated with AMD. These studies have strongly suggested that AMD is a complex disease associated with multiple genetic risk factors regulating immune responses, oxidative stress, as well as lipid synthesis.

Animal Studies

A great deal has been learned about genetic factors contributing AMD risk. However, the detailed molecular mechanisms by which genetic and environmental factors lead to AMD pathology are largely unknown. Multiple animal models, especially mouse models, have been generated in order to understand the disease mechanism of AMD. Mice with deletions or mutations of key genetic factors identified by the above mentioned studies, as well as laser-induced or surgically-induced choroidal neovascularisation (CNV) models mimicking wet AMD have been developed.[33, 34, 35]

Recent studies have suggested that both innate and adaptive immune responses play important roles in AMD pathogenesis. A few transgenic and knockout mouse models of dry AMD carrying mutations or deletions in immunological genes have been developed.[33, 36] For example, *Cfh* deletion in mouse leads to traceable photoreceptor degeneration and reduction in visual acuity in two year old mice[37]; similarly, deletion of *Ccl2* or *Ccr2* leads to disregulated macrophage recruitment in the eye and results in geographic atrophy and progressive retinal degeneration in old mice[38, 39]; *Cx3cr1* deficiency in mouse also leads to drusen-like lesions associated with microglia accumulation in the subretinal space[40]; furthermore, deletion of both *Ccl2* and *Cx3cr1* in mouse significantly accelerated drusen formation and onsite of retinal degeneration.[41] On the other hand, Takeda et al. shows that the blockade of the eosinophil/mast cell chemokine receptor CCR3 reduced formation of choroidal neovascularisation followed by laser injury.[42] Taken together, these murine models have helped our understanding of the individual genes in AMD associated pathogenesis.

In addition to mouse models with genetically engineered immunological genes, addition mouse models with deletion or mutation in genes functioning in RPE physiology, oxidative stress and lipid metabolism are also developed.[33, 36] Deletion/knockdown of oxidative stress associated gene *Sod1* and *Sod2*, transgenic overexpression of lipid metabolism gene ApoEε4 and ApoB100, mutation of Cathepsin D (*Catd*), as well as immunization with carboxyethylpyrrole (CEP) all lead to varied retinal phenotype similar to dry or wet AMD.[36] Importantly, the laser induced CNV model, in which laser burns/damage from a photocoagulation laser on the murine outer retina and RPE induced subsequent vascular leakage and choroidal neovascularisation, has been extensively used to study the development of wet AMD and to test anti-angiogenesis drugs.[33, 43]

In spite of success in advancing our understanding in the roles of individual genes in AMD pathogenesis, there are apparent limitation in using these animal models to study disease

mechanism and treatment of AMD. First of all, the mouse does not have a macula, differs in lipid transport across the RPE from human, has fewer cones as compared with human, has generally more double-nucleus RPEs than human, and has a short life expectancy. Moreover, very few cases of AMD-like phenotype are found in large experimental animals such as cats or dogs.[33] Therefore, careful evaluation will be necessary when discoveries found in animal models are translated to clinical management of AMD patients.

AMD Twin studies

Twin design is broadly used in studies dissecting the genetic versus environmental contributions to various complex diseases. Therefore, there have been numerous studies on AMD that investigated identical or non-identical twin subjects. In 1988, a case of a twin pair who both had AMD with different disease manifestations was identified.[44] Earlier studies using small patient cohorts indicated a significantly higher concordance rate of AMD in monozygotic than in dizygotic twins or families[45-48] strongly suggesting a genetic predisposition to AMD. In two recent twin studies, Hammond et al.[49] showed that the concordance for AMD in monozygotic twins was 0.37 compared with 0.19 in dizygotic twins, while Seddon et al.[50] suggested that genetic factors can explain 46% to 71% of the variation in the overall severity of AMD. However, the latter study also elucidated that for specific macular drusen and retinal pigment epithelial characteristics, both significant genetic (0.26-0.71) and unique environmental (0.28-0.64) proportions of variance were detected.[50] In addition, Keilhauer CN et al.[51] also suggested that the course and visual outcome of AMD appear to be influenced by environmental factors rather than genetic determinants. Interestingly, Gottfredsdottir MS et al.[46] reported that the concordance of AMD was 70.2% between 47 pairs of spouses. Thus, all these twin studies suggested that both genetic and environmental factors play important roles in AMD etiology.

Considerable effort has been spent to assess the role of several behavioural and nutritional factors contributing to AMD pathology, using twin studies. Heavier smoking is associated with higher risk of AMD, especially more advanced stage of AMD and larger drusen size, while higher intake of fish, omega-3 fatty acid, dietary vitamin D, betaine, and methionine is associated with earlier stage of AMD and smaller drusen size.[52, 53] Further studies are needed to identify a full spectrum of environmental (epigenetic, as described below) factors associated with AMD etiology.

Epigenetics and Epigenomics

While identical twins are often concordant for AMD, some twin pairs present a discordant phenotype. This argues that non-genetic factors also play a potentially crucial role in the pathogenesis of AMD. Studies investigating inheritable and non-inheritable non-genetic environmental influences beyond DNA sequence (genetic) changes are defined as epigenetics.[54] A collection of all genome-wide epigenetic changes is referred to as the epigenome.[55] Although most cells, with the exception of B and T cells or cells with somatic mutations, in an organism share the identical genome, their cellular morphology and function can be significantly different. In addition, most current therapeutic drugs target to adjust the epigenome instead of changing the underlining DNA sequences in patients.[56, 57] Therefore, much attention has been given to studies of epigenetic regulations in AMD.

Currently, molecular epigenetics studies the modifications of DNA and associated chromatin structures that can be adjusted according to three types of interrelated alterations: DNA methylation, histone modifications, and genomic imprinting. A number of proteins such as DNA methyltransferases and demethylases, histone acetyltransferases and deacetylase function to coordinate the machinery of epigenetic regulation that is ultimately responsible for the gene expression regulation.

The most common DNA methylation form is the 5' methylcytosine. It occurs predominantly in the symmetric CG context. About 70–80% of CG dinucleotides of the genome is normally methylated and called CpG. In vertebrates, CpG dinucleotides tend to cluster together and form CpG islands. Approximately 70% of gene promoters are associated with CpG islands, indicating the important role of CpG in regulation of vertebrate gene expression. A small amount of non-CG 5' methylcytosine occurs in embryonic stem cells and also regulates gene expression.[58] The 5' methylated cytosine located in promoter regions is generally associated with gene silencing. However, it can also be found in the gene bodies of actively transcribed genes in both plant and mammals.[59] The mammalian DNA methylation machinery is composed of several components, including DNA methyltransferase 1 (DNMT1) that maintains heritable DNA methylation patterns, DNMT3a/3b and their cofactors DNMT3L that establish de novo DNA methylation marks, and the methyl-CpG binding proteins (MBDs) and other transcription factors that are involved in 'reading' methylation marks.[60] Recent studies revealed more forms of DNA methylation in addition to 5' methylcytosine.[61] In mammalian cells, the ten-eleven translocation proteins (TET1, TET2, TET3), a family of iron-dependant oxygenases, can oxidize the methyl group at position 5 of 5-methylcytosine to form 5-hydroxymethylcytosine,[62, 63] which can be further oxidized by TET1 to generate 5-formylcytosine and 5-carboxylcytosine.[64, 65] The oxidation process of 5' methylcytosine may serve as the natural way of DNA demethylation because in spite of intense search no convincing evidence suggested the exist of DNA demethylases.[66]

In addition to DNA methylation, post-translational covalent modifications of histones play major roles in epigenetic regulation of various cellular processes, and are often referred to as the histone code.[67] The most abundant modifications on histone tails are acetylations and methylations, including major active marks H3K56Ac, H4K16Ac, and H3K4me3, as well as repressive marks H3K27me3 and H3K9me3 et al. Ubiquitination, ADP-ribosylation, and sumolation of lysines as well as phosphorylation of serines and threonines also exist as infrequent histone marks.[68] The combination of all DNA methylation and histone modifications decides the chromatin structure and DNA accessibility for factors involved in transcription regulation, therefore controlling gene activation or repression. Multiple families of proteins are involved in the addition, removal, and binding of the histone modifications. In particular, enzymes and proteins mediating histone acetylation and methylation are extensively studied, including histone acetyltransferases (HAT) and their inhibitors (HDACs), as well as histone methyltransferases and demethylases.[69]

Recent studies strongly suggest a crosstalk between DNA methylation and histone modifications in coordinating the epigenetic regulation of various cellular functions.[70] For example, during early development, binding of RNA polymerase II recruits H3K4 methyltransferases onto the gene promoters with unmethylated CpG islands first. By interrupting the necessary interaction between DNMT3L and H3 tails, the methylated H3K4 blocks de novo DNA methylation mediated by DNMT3A, DNMT3B, and DNMT3L complex, resulting in DNA methylation only at the regions of H3K4me desert in the early embryo.[71] Moreover, histone methyltransferase EZH2,[72] SUV39H1,[73] and SETDB1[74] are found directly interacting with DNA methyltransferase DNMT3A and DNA methylation machinery in addition to transcriptional activators and repressors. Therefore, the physiological and pathological processes are controlled by cooperation of both DNA methylation and histone modifications.

Epigenetic Mechanism and Therapy of Human Diseases

Properly establishing, maintaining, and regulating the epigenome is essential for development and normal function of the organism. Importantly, organisms dynamically adjust their epigenomes in response to environmental influences. A number of epigenetic

abnormalities have been found to contribute to the development of human diseases. Increasing understanding of epigenetic regulation of human disease has led to potential therapies for diseases such as cancer, inflammatory diseases, neuropsychiatric and metabolic disorders.[56, 69]

Cancer has been defined as much an epigenetic disease as it is a genetic disease.[75] In the 1980s, cancer cells were found to have hypomethylated genomes relative to their normal counterparts, resulting in genomic instability.[76-79] Later, much gene-specific aberrant hyper- or hypo-methylation was discovered on oncogenes and tumor suppressor genes that served as either the cause or consequence of tumorgenesis.[80, 81] Recently, exploration of the broader cancer epigenome including the histone modifications revealed new insights into the complete view of abnormal heritable epigenetic alterations that can lead to the initiation and progression of cancer.[82] These aberrant epigenetics marks found in cancer cells not only serve as biomarkers for diagnosis and prognosis of many cancers, they can also be targeted for correction as a new generation of cancer therapies.[83] Multiple DNA methyltransferases inhibitors including 5-azanucleosides, azacitidine and decitabine, as well as histone deacetylase inhibitors including Vorinostat and Romidepsin have been approved by FDA for cancer therapy. Many small molecules targeting the HDAC and histone methyltransferases are under development and clinical test for controlling cancer, although more target specific suppression will be needed to address the safety concerns associated with these new therapies.[69]

A growing body of evidence indicates critical role of epigenetic regulation in the immune system. Therefore, targeting the epigenetic regulators is currently extensively investigated as powerful new approaches for treating autoimmune and inflammatory diseases.

Epigenetics in AMD

Despite the significant advances that have been made in understanding the epigenetic regulation in cancer and inflammation, inheritable and dynamic epigenetic changes characterizing ocular diseases are largely unknown.[84, 85] Recently, studies have started to reveal the environmental epigenetic factors for AMD, such as smoking and dietary intake.[52, 53, 86, 87] However, the molecular epigenetic mechanism underlying the disease pathogenesis is not clear.[88]

Our recent genome-wide DNA methylation analysis identified differences in ~1.5% of the total CpG sites within 231 gene promoters between 3 pairs of twins with discordant advanced AMD phenotype. Among these genes, interestingly, we are able to confirm that a hypomethylated *IL17RC* promoter is associated with AMD disease. The hypomethylation of *IL17RC* promoter results in the elevated expression of the IL-17RC protein on selected cells in peripheral blood and retinal tissues of AMD patients, suggesting that epigenetic regulation of inflammatory gene *IL17RC* may play an important role in AMD.[89]

The 231 differentially methylated genes identified in discordant AMD twins belong to different functional categories. Among these categories, “Immunological Disease” is one of the 5 most significantly enriched one, reconfirming a speculation that AMD is an immunological disease.[89]

Intriguingly, none of the 231 genes with differential epigenetic regulation overlap with these “top hits” identified by Genome-wide Association Studies (GWAS) in the AMD Gene Consortium,[90] providing no direct link between genetic and epigenetic mechanisms in AMD etiology. It is still unclear how genetic factors, such as *CFH* and *HTRA1*/*ARMS2* SNPs, control AMD progress. It is reasonable to hypothesize that genetic factors may function through epigenetic regulation to manipulate cellular functions that resulted in the

development of AMD disease. We recently found that an elevated level of interleukin 17 (IL-17) and IL-22 produced by Th17 cells, a subset of $CD4^+$ helper T cells causing tissue inflammation, in the serum of AMD patients, which could be resulted from disregulated complement system (potentially CFH).[91] Both IL-17 and IL-22 can induce demethylation of the *IL17RC* promoter and promote *IL17RC* expression in peripheral blood and retinal tissues of AMD patients,[89] therefore indicating a potential molecular cascade connecting genetic risk factor of *CFH* SNPs with epigenetic disregulation of *IL17RC*, both found in AMD patients.

Conclusion

It is clear that both genetic susceptibility and environmental influences control the risk of AMD. Thus, the incorporation of epigenetic and epigenomic status with genetic study will provide a basis for better understanding of the complexity of AMD pathogenesis and accelerate the development of novel therapeutic agents targeting both human genome and epigenome. With the advent of next generation high-throughput sequencing technologies, it is now feasible to quickly and robustly map the details of human genome and epigenome in the clinic.[92-94] Therefore, understanding the cooperation of epigenetic and genetic mechanisms will greatly advance the clinical management of AMD.

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REFERENCES

1. de Jong PT. Age-related macular degeneration. N Engl J Med. 2006; 355:1474–85. [PubMed: 17021323]
2. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY. Age-related macular degeneration. Lancet. 2012; 379:1728–38. [PubMed: 22559899]
3. Kawasaki R, Wang JJ, Aung T, et al. Prevalence of age-related macular degeneration in a Malay population: the Singapore Malay Eye Study. Ophthalmology. 2008; 115:1735–41. [PubMed: 18439679]
4. Friedman DS, Katz J, Bressler NM, Rahmani B, Tielsch JM. Racial differences in the prevalence of age-related macular degeneration: the Baltimore Eye Survey. Ophthalmology. 1999; 106:1049–55. [PubMed: 10366070]
5. Patel M, Chan CC. Immunopathological aspects of age-related macular degeneration. Semin Immunopathol. 2008; 30:97–110. [PubMed: 18299834]
6. Donati G, Kapetanios AD, Pournaras CJ. Principles of treatment of choroidal neovascularization with photodynamic therapy in age-related macular degeneration. Semin Ophthalmol. 1999; 14:2–10. [PubMed: 10790570]
7. Campa C, Harding SP. Anti-VEGF Compounds in the Treatment of Neovascular Age Related Macular Degeneration. Curr Drug Targets. 2010; 2010:1.
8. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. N Engl J Med. 1897; 364:1897–908. [PubMed: 21526923]
9. Swaroop A, Chew EY, Rickman CB, Abecasis GR. Unraveling a multifactorial late-onset disease: from genetic susceptibility to disease mechanisms for age-related macular degeneration. Annu Rev Genomics Hum Genet. 2009; 10:19–43. [PubMed: 19405847]
10. Tuo J, Grob S, Zhang K, Chan CC. Genetics of immunological and inflammatory components in age-related macular degeneration. Ocul Immunol Inflamm. 2012; 20:27–36. [PubMed: 22324898]
11. Edwards AO, Ritter R 3rd, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005; 308:421–4. [PubMed: 15761121]

12. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005; 308:419–21. [PubMed: 15761120]
13. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. Science. 2005; 308:385–9. [PubMed: 15761122]
14. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci U S A. 2005; 102:7227–32. [PubMed: 15870199]
15. Maller J, George S, Purcell S, et al. Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. Nat Genet. 2006; 38:1055–9. [PubMed: 16936732]
16. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat Genet. 2006; 38:458–62. [PubMed: 16518403]
17. Yates JR, Sepp T, Matharu BK, et al. Complement C3 variant and the risk of age-related macular degeneration. N Engl J Med. 2007; 357:553–61. [PubMed: 17634448]
18. Maller JB, Fagerness JA, Reynolds RC, Neale BM, Daly MJ, Seddon JM. Variation in complement factor 3 is associated with risk of age-related macular degeneration. Nat Genet. 2007; 39:1200–1. [PubMed: 17767156]
19. Fagerness JA, Maller JB, Neale BM, Reynolds RC, Daly MJ, Seddon JM. Variation near complement factor I is associated with risk of advanced AMD. Eur J Hum Genet. 2009; 17:100–4. [PubMed: 18685559]
20. Dewan A, Liu M, Hartman S, et al. HTRA1 promoter polymorphism in wet age-related macular degeneration. Science. 2006; 314:989–92. [PubMed: 17053108]
21. Yang Z, Camp NJ, Sun H, et al. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science. 2006; 314:992–3. [PubMed: 17053109]
22. Fritsche LG, Loenhardt T, Janssen A, et al. Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. Nat Genet. 2008; 40:892–6. [PubMed: 18511946]
23. Kanda A, Chen W, Othman M, et al. A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci U S A. 2007; 104:16227–32. [PubMed: 17884985]
24. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. Am J Hum Genet. 2005; 77:389–407. [PubMed: 16080115]
25. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. Hum Mol Genet. 2005; 14:3227–36. [PubMed: 16174643]
26. Chen W, Stambolian D, Edwards AO, et al. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to age-related macular degeneration. Proc Natl Acad Sci U S A. 2010; 107:7401–6. [PubMed: 20385819]
27. Goverdhan SV, Howell MW, Mullins RF, et al. Association of HLA class I and class II polymorphisms with age-related macular degeneration. Invest Ophthalmol Vis Sci. 2005; 46:1726–34. [PubMed: 15851575]
28. Goverdhan SV, Ennis S, Hannan SR, et al. Interleukin-8 promoter polymorphism −251A/T is a risk factor for age-related macular degeneration. Br J Ophthalmol. 2008; 92:537–40. [PubMed: 18310311]
29. Klaver CC, Kliffen M, van Duijn CM, et al. Genetic association of apolipoprotein E with age-related macular degeneration. Am J Hum Genet. 1998; 63:200–6. [PubMed: 9634502]
30. Baird PN, Richardson AJ, Robman LD, et al. Apolipoprotein (APOE) gene is associated with progression of age-related macular degeneration (AMD). Hum Mutat. 2006; 27:337–42. [PubMed: 16453339]
31. Allikmets R, Shroyer NF, Singh N, et al. Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. Science. 1997; 277:1805–7. [PubMed: 9295268]

32. Neale BM, Fagerness J, Reynolds R, et al. Genome-wide association study of advanced age-related macular degeneration identifies a role of the hepatic lipase gene (LIPC). Proc Natl Acad Sci U S A. 2010; 107:7395–400. [PubMed: 20385826]
33. Fletcher EL, Jobling AI, Vessey KA, Luu C, Guymer RH, Baird PN. Animal models of retinal disease. Prog Mol Biol Transl Sci. 2011; 100:211–86. [PubMed: 21377628]
34. Grossniklaus HE, Kang SJ, Berglin L. Animal models of choroidal and retinal neovascularization. Prog Retin Eye Res. 2010; 29:500–19. [PubMed: 20488255]
35. Li Y, Huang D, Xia X, Wang Z, Luo L, Wen R. CCR3 and choroidal neovascularization. PLoS One. 2011; 6:e17106. [PubMed: 21358803]
36. Ramkumar HL, Zhang J, Chan CC. Retinal ultrastructure of murine models of dry age-related macular degeneration (AMD). Prog Retin Eye Res. 2010; 29:169–90. [PubMed: 20206286]
37. Coffey PJ, Gias C, McDermott CJ, et al. Complement factor H deficiency in aged mice causes retinal abnormalities and visual dysfunction. Proc Natl Acad Sci U S A. 2007; 104:16651–6. [PubMed: 17921253]
38. Ambati J, Anand A, Fernandez S, et al. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. Nat Med. 2003; 9:1390–7. [PubMed: 14566334]
39. Tsutsumi C, Sonoda KH, Egashira K, et al. The critical role of ocular-infiltrating macrophages in the development of choroidal neovascularization. J Leukoc Biol. 2003; 74:25–32. [PubMed: 12832439]
40. Combadiere C, Feumi C, Raoul W, et al. CX3CR1-dependent subretinal microglia cell accumulation is associated with cardinal features of age-related macular degeneration. J Clin Invest. 2007; 117:2920–8. [PubMed: 17909628]
41. Tuo J, Bojanowski CM, Zhou M, et al. Murine ccl2/cx3cr1 deficiency results in retinal lesions mimicking human age-related macular degeneration. Invest Ophthalmol Vis Sci. 2007; 48:3827–36. [PubMed: 17652758]
42. Takeda A, Baffi JZ, Kleinman ME, et al. CCR3 is a target for age-related macular degeneration diagnosis and therapy. Nature. 2009; 460:225–30. [PubMed: 19525930]
43. Doyle SL, Campbell M, Ozaki E, et al. NLRP3 has a protective role in age-related macular degeneration through the induction of IL-18 by drusen components. Nat Med. 2012; 18:791–8. [PubMed: 22484808]
44. Meyers SM, Zachary AA. Monozygotic twins with age-related macular degeneration. Arch Ophthalmol. 1988; 106:651–3. [PubMed: 3358731]
45. Meyers SM. A twin study on age-related macular degeneration. Trans Am Ophthalmol Soc. 1994; 92:775–843. [PubMed: 7886884]
46. Gottfredsdottir MS, Sverrisson T, Musch DC, Stefansson E. Age related macular degeneration in monozygotic twins and their spouses in Iceland. Acta Ophthalmol Scand. 1999; 77:422–5. [PubMed: 10463414]
47. Klein ML, Mauldin WM, Stoumbos VD. Heredity and age-related macular degeneration. Observations in monozygotic twins. Arch Ophthalmol. 1994; 112:932–7. [PubMed: 8031273]
48. Heiba IM, Elston RC, Klein BE, Klein R. Sibling correlations and segregation analysis of age-related maculopathy: the Beaver Dam Eye Study. Genet Epidemiol. 1994; 11:51–67. [PubMed: 8013888]
49. Hammond CJ, Webster AR, Snieder H, Bird AC, Gilbert CE, Spector TD. Genetic influence on early age-related maculopathy: a twin study. Ophthalmology. 2002; 109:730–6. [PubMed: 11927430]
50. Seddon JM, Cote J, Page WF, Aggen SH, Neale MC. The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences. Arch Ophthalmol. 2005; 123:321–7. [PubMed: 15767473]
51. Keilhauer CN, Fritsche LG, Weber BH. Age-related macular degeneration with discordant late stage phenotypes in monozygotic twins. Ophthalmic Genet. 2011; 32:237–44. [PubMed: 21740222]
52. Seddon JM, George S, Rosner B. Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration. Arch Ophthalmol. 2006; 124:995–1001. [PubMed: 16832023]

53. Seddon JM, Reynolds R, Shah HR, Rosner B. Smoking, dietary betaine, methionine, and vitamin D in monozygotic twins with discordant macular degeneration: epigenetic implications. Ophthalmology. 2011; 118:1386–94. [PubMed: 21620475]
54. Bernstein BE, Meissner A, Lander ES. The mammalian epigenome. Cell. 2007; 128:669–81. [PubMed: 17320505]
55. Fazzari MJ, Greally JM. Introduction to epigenomics and epigenome-wide analysis. Methods Mol Biol. 2010; 620:243–65. [PubMed: 20652507]
56. Feinberg AP. Phenotypic plasticity and the epigenetics of human disease. Nature. 2007; 447:433–40. [PubMed: 17522677]
57. Kaiser J. Epigenetic drugs take on cancer. Science. 2010; 330:576–8. [PubMed: 21030620]
58. Deaton AM, Bird A. CpG islands and the regulation of transcription. Genes Dev. 2011; 25:1010–22. [PubMed: 21576262]
59. Laird PW. Principles and challenges of genomewide DNA methylation analysis. Nat Rev Genet. 2010; 11:191–203. [PubMed: 20125086]
60. Law JA, Jacobsen SE. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat Rev Genet. 2010; 11:204–20. [PubMed: 20142834]
61. Wu H, Zhang Y. Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. Genes Dev. 2011; 25:2436–52. [PubMed: 22156206]
62. Kriaucionis S, Heintz N. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science. 2009; 324:929–30. [PubMed: 19372393]
63. Tahiliani M, Koh KP, Shen Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009; 324:930–5. [PubMed: 19372391]
64. He YF, Li BZ, Li Z, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science. 2011; 333:1303–7. [PubMed: 21817016]
65. Ito S, Shen L, Dai Q, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science. 2011; 333:1300–3. [PubMed: 21778364]
66. Nabel CS, Kohli RM. Molecular biology. Demystifying DNA demethylation. Science. 2011; 333:1229–30. [PubMed: 21885763]
67. Jenuwein T, Allis CD. Translating the histone code. Science. 2001; 293:1074–80. [PubMed: 11498575]
68. Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell. 2007; 128:707–19. [PubMed: 17320508]
69. Arrowsmith CH, Bountra C, Fish PV, Lee K, Schapira M. Epigenetic protein families: a new frontier for drug discovery. Nat Rev Drug Discov. 2012; 11:384–400. [PubMed: 22498752]
70. Cedar H, Bergman Y. Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet. 2009; 10:295–304. [PubMed: 19308066]
71. Ooi SK, Qiu C, Bernstein E, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature. 2007; 448:714–7. [PubMed: 17687327]
72. Vire E, Brenner C, Deplus R, et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature. 2006; 439:871–4. [PubMed: 16357870]
73. Fuks F, Hurd PJ, Deplus R, Kouzarides T. The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. Nucleic Acids Res. 2003; 31:2305–12. [PubMed: 12711675]
74. Li H, Rauch T, Chen ZX, Szabo PE, Riggs AD, Pfeifer GP. The histone methyltransferase SETDB1 and the DNA methyltransferase DNMT3A interact directly and localize to promoters silenced in cancer cells. J Biol Chem. 2006; 281:19489–500. [PubMed: 16682412]
75. Iacobuzio-Donahue CA. Epigenetic changes in cancer. Annu Rev Pathol. 2009; 4:229–49. [PubMed: 18840073]
76. Feinberg AP, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. Nature. 1983; 301:89–92. [PubMed: 6185846]
77. Gama-Sosa MA, Slagel VA, Trewyn RW, et al. The 5-methylcytosine content of DNA from human tumors. Nucleic Acids Res. 1983; 11:6883–94. [PubMed: 6314264]

78. Goelz SE, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. Science. 1985; 228:187–90. [PubMed: 2579435]
79. Feinberg AP, Gehrke CW, Kuo KC, Ehrlich M. Reduced genomic 5-methylcytosine content in human colonic neoplasia. Cancer Res. 1988; 48:1159–61. [PubMed: 3342396]
80. Yu W, Gius D, Onyango P, et al. Epigenetic silencing of tumour suppressor gene p15 by its antisense RNA. Nature. 2008; 451:202–6. [PubMed: 18185590]
81. Feinberg AP, Tycko B. The history of cancer epigenetics. Nat Rev Cancer. 2004; 4:143–53. [PubMed: 14732866]
82. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer. 2011; 11:726–34. [PubMed: 21941284]
83. Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nat Biotechnol. 2010; 28:1069–78. [PubMed: 20944599]
84. Nickells RW, Merbs SL. The potential role of epigenetics in ocular diseases. Arch Ophthalmol. 2012; 130:508–9. [PubMed: 22491920]
85. Cvekl A, Mitton KP. Epigenetic regulatory mechanisms in vertebrate eye development and disease. Heredity (Edinb). 2010; 105:135–51. [PubMed: 20179734]
86. Group A-REDSR. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 2001; 119:1417–36. [PubMed: 11594942]
87. Hunter A, Spechler PA, Cwanger A, et al. DNA methylation is associated with altered gene expression in AMD. Invest Ophthalmol Vis Sci. 2012; 53:2089–105. [PubMed: 22410570]
88. Hjelmeland LM. Dark matters in AMD genetics: epigenetics and stochasticity. Invest Ophthalmol Vis Sci. 2011; 52:1622–31. [PubMed: 21429863]
89. Wei L, Liu B, Tuo J, et al. Hypomethylation of the IL17RC promoter associates with age-related macular degeneration. Cell Rep. 2012; 2:1151–8. [PubMed: 23177625]
90. Fritsche LG, Chen W, Schu M, et al. Seven new loci associated with age-related macular degeneration. Nat Genet. 2013; 2013:2578.
91. Liu B, Wei L, Meyerle C, et al. Complement component C5a promotes expression of IL-22 and IL-17 from human T cells and its implication in age-related macular degeneration. J Transl Med. 2011; 9:1–12. [PubMed: 21762495]
92. Chen R, Mias GI, Li-Pook-Than J, et al. Personal omics profiling reveals dynamic molecular and medical phenotypes. Cell. 2012; 148:1293–307. [PubMed: 22424236]
93. Ashley EA, Butte AJ, Wheeler MT, et al. Clinical assessment incorporating a personal genome. Lancet. 2010; 375:1525–35. [PubMed: 20435227]
94. Baranzini SE, Mudge J, van Velkinburgh JC, et al. Genome, epigenome and RNA sequences of monozygotic twins discordant for multiple sclerosis. Nature. 2010; 464:1351–6. [PubMed: 20428171]