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The role of the alternative pathway of the complement system in the development of dense deposit disease

Maria Asuncion Abrera Abeleda
University of Iowa

THE ROLE OF THE ALTERNATIVE PATHWAY OF THE COMPLEMENT SYSTEM IN THE DEVELOPMENT OF DENSE DEPOSIT DISESASE

by

Maria Asuncion Abrera Abeleda

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
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Thesis Supervisor: Professor Richard J.H. Smith

ABSTRACT

Dense Deposit Disease (DDD) causes chronic renal dysfunction which progresses to end-stage renal disease in about half of patients within 10 years of diagnosis. Deficiency of and mutations in complement Factor H (CFH) are associated with the development of DDD, suggesting that dysregulation of the alternative pathway (AP) of the complement cascade is important in disease pathophysiology. Patients with DDD were studied to determine whether specific allele variants of the genes of the alternative pathway of the complement system segregate preferentially with the DDD. Coding and intronic regions of genes of the complement system in DDD cases and controls were screened for variants using PCR, restriction digest and bidirectional sequencing. We were able to identify novel mutations, allele variants and haplotypes in several genes of the complement system, which are associated with the DDD phenotype. Possible gene-gene interactions were determined using computational analyses. A strong synergistic interaction between polymorphisms in Complement Factor H and C3 was observed. To ascertain if the associated allele variants have a functional impact in complement activity, serum samples from normal controls were genotyped for variants in CFH and C3 and AP complement activity was measured. We found a significant association between CFH and C3 variants and AP complement activity. Lastly, we generated mutant mice deficient in CFH and Factor D (CFD). CFH deficient mice develop renal pathology similar to DDD. Renal function and complement activity in mice deficient in both CFH and CFD were compared to CFH deficient and CFD deficient mice. The absence of Factor D inhibits complement activation in CFH-deficient mice. In aggregate, our data show that: 1) DDD is a complex genetic disease; 2) mutations and allele variants of genes of the AP

complement system contribute to the level of complement activity and the pathogenesis of DDD; 3) the deletion of CFD rescues the disease phenotype in a murine model of DDD. These findings should facilitate the diagnosis of DDD and provide targets that can be explored as potential therapies in affected patients.

Abstract Approved: _____
Thesis Supervisor

_____
Title and Department

_____
Date

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The University of Iowa
Iowa City, Iowa

CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

Maria Asuncion Abrera Abeleda

has been approved by the Examining Committee
for the thesis requirement for the Doctor of Philosophy
degree in Genetics at the July 2010 graduation.

Thesis Committee: _____
Richard J.H. Smith, Thesis Supervisor

_____
Curt D. Sigmund

_____
Beverly L. Davidson

_____
Mary E. Wilson

_____
David G. Motto

To God, my country and my family

Start by doing what's necessary, then do what's possible;
and suddenly you are doing the impossible.

St. Francis of Assisi

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Dense Deposit Disease (DDD) causes chronic renal dysfunction which progresses to end-stage renal disease in about half of patients within 10 years of diagnosis. Deficiency of and mutations in complement Factor H (CFH) are associated with the development of DDD, suggesting that dysregulation of the alternative pathway (AP) of the complement cascade is important in disease pathophysiology. Patients with DDD were studied to determine whether specific allele variants of the genes of the alternative pathway of the complement system segregate preferentially with the DDD. Coding and intronic regions of genes of the complement system in DDD cases and controls were screened for variants using PCR, restriction digest and bidirectional sequencing. We were able to identify novel mutations, allele variants and haplotypes in several genes of the complement system, which are associated with the DDD phenotype. Possible gene-gene interactions were determined using computational analyses. A strong synergistic interaction between polymorphisms in Complement Factor H and C3 was observed. To ascertain if the associated allele variants have a functional impact in complement activity, serum samples from normal controls were genotyped for variants in CFH and C3 and AP complement activity was measured. We found a significant association between CFH and C3 variants and AP complement activity. Lastly, we generated mutant mice deficient in CFH and Factor D (CFD). CFH deficient mice develop renal pathology similar to DDD. Renal function and complement activity in mice deficient in both CFH and CFD were compared to CFH deficient and CFD deficient mice. The absence of Factor D inhibits complement activation in CFH-deficient mice. In aggregate, our data show that: 1) DDD is a complex genetic disease; 2) mutations and allele variants of genes of the AP

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LIST OF ABBREVIATIONS

AP	Alternative Pathway
CP	Classical Pathway
MAC	Membrane Attack Complex
TCC	Terminal Complement Complex
DDD	Dense Deposit Disease
AMD	Age-related Macular Degeneration
aHUS	Atypical Hemolytic Uremic Syndrome
SLE	Systemic Lupus Erythematosus
CFH	Complement Factor H
CFD	Complement Factor D
CR1	Complement Receptor 1
LR	Logistic Regression
FITF	Focused Interaction Testing Framework
MDR	Multifactor Dimensionality Reduction

CHAPTER I

INTRODUCTION

The Complement System

In the late nineteenth century, many had been interested on the mechanisms involved in the protection of the body from microorganisms. The work of Hans Ernst Buchner (1850-1902) et.al demonstrated the existence of a heat-labile component of fresh, cell-free serum which was capable of killing microorganisms (Buchner 1889). He named this component as “alexin”, from the Greek “without a name”. In 1894, Jules Bordet (1870-1961) who was working at the Pasteur Institute, continued this work by showing that the lytic activity of serum from immune animals was lost after exposure to heat. However, lytic activity was restored upon addition of non-immune serum. He hypothesized that two factors were involved in cell lysis which one was heat-labile and the other was a heat-stable substance present in the immune serum (Bordet 1909). In his work, Paul Ehrlich (1854-1915) verified the presence of the two factors in the immune serum was required to produce cell lysis. He termed the thermostable form as “amboreceptors” or “immune bodies” (presently known as antibodies). On the other hand, the heat-labile component was termed “complement” due to the fact that it “complemented” the activity of the amboreceptors.

The complement system is an important arm of the innate immune system. The human body is constantly exposed to different pathogens and infectious agents that can cause life-threatening diseases. Protection is provided by a complex network of immune defenses, which recognize and eliminate foreign pathogens. Several infectious agents have developed various and efficient ways to inactivate host immune system (Lachmann

2002). It is, therefore, necessary for the immune system to cultivate several layers of immune defenses which include the innate immunity and adaptive immunity. The innate immunity is able to effectively reduce the number of infectious agents at the first level of the immediate immune response (Janeway and Medzhitov 2002). It is composed of cellular responses and the complement system. The complement system immediately responds within seconds while the cellular response takes about minutes to hours and is mediated by infiltrating macrophages and neutrophils.

The first line of immune defense against pathogens is the complement system. It is composed of several complement effectors and activators that create a cascade of activating events leading to the lysis of a target cell or pathogen. Functions of the activated complement system include (Kohl 2006):

i) release of anaphylatoxins and inflammatory mediators (e.g. C3a and C5a)

ii) opsonization of target surface with the activation products

iii) generation of the membrane attack complex (MAC) or terminal complement complex (TCC) which generates pores in the membranes of target cells

iv) removal of cellular debris and immune complexes

v) assistance with the adaptive immune response.

Activation products generated from the complement cascade can bind to the surface of infectious pathogens (e.g. bacteria, fungi, parasites and viruses). Binding of the activation components results in the damage and elimination of these pathogens. However, these activation products are able to bind to the host cell's surface to inflict damage to the host cells. Proper control for the complement cascade is necessary and is achieved by the action of several complement regulators that are either fluid-phase or

membrane-bound. A defective regulation of the complement system by the complement regulators can lead to host cell injury which can eventually lead to autoimmune diseases (Janeway 1992). These defects can be due to several factors including inappropriate timing of immune activation; unsuitable delivery of complement effectors on host cells; faulty detection between self or foreign surfaces; and deficient or defective complement regulators. Examples of complement-mediated disorders include atypical hemolytic uremic syndrome (aHUS), age-related macular degeneration (AMD) and dense deposit disease (DDD). In order to prevent these disorders from developing, the body's immune system is able to balance elimination target pathogens and protection of host cells from injury.

The complement cascade is initiated by three pathways: the classical, lectin and alternative pathways (Figure 1-1). The classical pathway is activated by binding of antibodies to their corresponding antigens (Law and Reid 1995). The lectin pathway is initiated by binding of mannan binding lectin (MBL) to mannose residues in the surface of pathogens (Fujita 2002; Degn, Thiel et al. 2007). The alternative pathway is constantly activated at low rates but also recognizes surface of different pathogens for its activation (Pangburn and Muller-Eberhard 1983; Pangburn and Muller-Eberhard 1984). These three pathways generate homologous variants of the C3 convertase (C4bC2b for classical and lectin pathways and C3bBb for alternative pathway), which cleaves C3 to C3a and C3b. C3b is deposited on a target surface leading to the opsonization of that cell. This creates an amplification loop in which more C3b and C3 convertases are produced (Gal, Barna et al. 2007; Gros, Milder et al. 2008). If activation persists, a new enzyme complex is produced called C5 convertase (C4bC2bC3b for classical and lectin

pathways and C3bBbC3b for the alternative pathway) which cleaves C5 to C5a and C5b (Pangburn and Rawal 2002). The latter initiates the formation of the terminal complement complex (TCC) or membrane attack complex (MAC) by binding to C6-C9 proteins, creating pores on the surface membrane leading to cell lysis (Ward 2009).

The complement system is comprised of about 60 components and activation products. These include the nine central components of the complement cascade (C1-C9); several activation products from the breakdown of C3 (e.g. C3a, C3b, iC3b, C3d and C3dg)(Lambris 1988; Gros, Milder et al. 2008); complement regulators and inhibitors (e.g. Factor H, Factor I, Factor H-like 1 (CFHL1), Factor H-related 1 (CFHR1), Complement receptor 1 (CR1)); proteases and enzymes (Factor B, Factor D, Properdin and C3 and C5 convertases); and complement receptors for effector molecules (e.g. C3aR1 and C5aR1). The complement system has also been shown to interact with other pathways including the coagulation pathway (Kaplan, Ghebrehiwet et al. 1981; Kazatchkine and Jouvin 1984; Dahlback 1985) and toll-like receptor signaling pathway (Hajishengallis and Lambris ; Hawlisch and Kohl 2006).

Alternative Pathway of the Complement System

Activation of the alternative pathway of the complement system is independent of the presence of antibodies and is the first one activated by the immune system. It is activated by different molecules and pathogens including zymosan, lipopolysaccharide (gram negative bacteria) and teichoic acid (cell wall of gram positive bacteria)(Pangburn and Muller-Eberhard 1984). It is also active at low rates in the plasma through the spontaneous hydrolysis of C3 which is also known as the “tickover” (Figure 1-2). The thioester bond in C3 is spontaneously cleaved to produce C3(H_2O) which is structurally

and biologically similar to C3b (Fishelson, Pangburn et al. 1984). Both C3(H_2O) and C3b can bind to Factor B in which the complex formed becomes a substrate for the protease, Factor D, cleaving a small fragment of Factor B. The initial convertase, C3(H_2O)Bb, is highly unstable and is able to cleave C3 to C3a and C3b. If present, C3b binds to an activating surface. Together with Factor B and Factor D, the complex C3bBb is produced and is stabilized by binding of Properdin to form the C3 convertase. This step in the complement cascade creates an amplification loop to generate more C3 convertase. C3b can bind to the C3 convertase to produce C5 convertase which can cleave C5 to C5a and C5b. C5b can bind to C6-9 to produce the TCC or MAC leading to osmotic lysis of target cell.

The regulation of the amplification loop of the complement cascade is dependent on the fate of C3b. If C3b levels persist, then Factor B can further bind to it which can generate more C3 convertase. However, C3b can be catabolized and degraded by complement regulators to downregulate complement activation. The protein responsible for the inactivation of C3b is Factor I with the assistance of fluid phase co-factor, Factor H, and membrane-bound co-factors and regulators, Complement Recpector 1 (CR1), Decay Accelerating Factor (DAF) and Membrane Cofactor Protein (MCP). Host cell surfaces are protected from the action of C3 convertases through the presence of these membrane-bound proteins like CR1 and MCP on the cell surface. Also, the presence of glycosaminoglycans and sialic acid on the surface can enhance binding of Factor H to C3b. The presence of DAF on the cell membrane can promote the dissociation of the C3 convertase.

The terminal complement complex is initiated by the cleavage of C5 to C5a and C5b by the C5 convertase. Binding of C5b to the C3b in the convertase bound to the surface triggers the exposure of a hydrophobic acceptor site for C6. Binding of C6 assists in the binding of C7 which modifies the conformation of the complex. C5b67 is discharged from the C5 convertase and this exposes the hydrophobic region of C7 which can allow the complex to penetrate into the lipid bilayer of the cell membrane. The β subunit of C8 binds to the C5b67 complex and α subunit of C8 inserts into the membrane due to conformational changes in the complex. Several C9 molecules bind to the C5b678 complex, producing the pore channel, MAC or TCC. During complement activation, some C5b67 complexes can deposit on nearby cells which can lead to cell lysis. To prevent this, a complement regulator called Clusterin binds to C5b67 which prevents insertion of the complex to the membrane (Tschopp and French 1994). Another regulatory protein, CD59, prevents the incorporation of C8 and C9 to form MAC. Once MAC forms into the cell or pathogen membrane, the most likely occurrence is cell lysis due to the formation of ion-permeable pores or channel or leaky patches (Muller-Eberhard 1986; Morgan 1999). But MAC can also elicit various cellular and metabolic pathways. Activation of these pathways can lead to the production of inflammatory mediators like prostaglandins and leukotrienes (Imagawa, Osifchin et al. 1983).

Dense Deposit Disease

Structure and Function of the Glomerulus

The kidneys are paired organs that functions in the excretion of wastes such as urea and ammonium and reabsorption of water, glucose and amino acids. They are also important in homeostatic activities including regulation of electrolytes, maintenance of

acid-base balance and regulation of blood pressure. They produce hormones including calcitriol, rennin and erythropoietin. The kidneys receive 20% of the cardiac output and during the course of the day filter 180 liters of blood. The functional unit of the kidney is the nephron. The nephron is composed of the renal corpuscles and tubules. One component of the renal corpuscle is the glomerulus which is composed of capillary vessels actively involved in the filtration of blood in the kidneys. The measure of the overall renal function is called the glomerular filtration rate (GFR) which estimates the rate of blood that passes through all the glomeruli.

The layers of the glomerulus are composed of endothelial cells, glomerular basement membrane (GBM) and podocytes (Figure 1-3). The endothelial cells have numerous pores or fenetrae in which anything smaller than an RBC will pass through these pores. The GBM is a thick (250-350 nm) membrane lining the glomerular endothelium. It is rich in negatively charged glycosaminoglycans like heparan sulfate. The negative charge of the GBM can deter negatively-charged proteins from the blood like albumin to prevent its passage into the Bowman's space. The podocytes line the GBM and form part of the lining of the Bowman's space. They form a network of foot processes which controls the filtration of proteins into the Bowman's space. The foot processes are also negatively charged to limit filtration of albumin and other negatively-charged proteins. Mesangial cells are not part of the filtration barrier but play an indirect role in the filtration process by regulating the blood flow of the glomerular capillaries by their contractile activity. Contraction of the mesangials cells and GBM can decrease the surface area of the basement membrane reducing GFR. Also, mesangial cells provide

structural support in the glomerulus and phagocytize glomerular basal lamina components and immunoglobulins..

Glomerular diseases are caused by damage or inflammation in the glomerulus. Most of these diseases are complex and have genetic causes or associated with systemic diseases like lupus or diabetes. The damage to the glomeruli can interfere with the clearance of waste products and filtration of important proteins like albumin. Signs and symptoms of glomerular diseases include proteinuria (large amounts of protein in urine), hematuria (blood in the urine), edema (swelling of different parts of the body), reduced GFR (inefficient filtration of wastes from the blood) and hypoprotenemia (low blood protein). Many patients with glomerular disease will develop renal insufficiency and eventually renal failure.

Clinical Findings

Dense Deposit Disease is a complex genetic disease of the glomerulus in the kidneys. Characteristic features include hematuria, proteinuria, acute nephritic syndrome and nephritic syndrome. It occurs slightly more in females than males (3:2 female:male)(Lu, McCarthy et al. 2007). The prevalence of DDD is about 2-3: 1,000,0000 population. About half of DDD patients who have disease for more than 10 years develop end-stage renal disease (di Belgiojoso, Tarantino et al. 1977; Droz, Noel et al. 1982; Swainson, Robson et al. 1983; McEnery 1990; Lu, McCarthy et al. 2007; Smith, Alexander et al. 2007). Several DDD patients would later develop impaired visual acuity (Colville, Guymer et al. 2003).

DDD is characterized by capillary wall thickening and mesangial proliferation in the glomerulus (Figure 1-4). There are also electron dense deposits present in the

glomerular basement membrane (GBM). The deposits contain complement components C3, C4, C5, C6, C8 and C9 and complement regulators CFHR1, CFHR5, Clusterin and Vitronectin (Crabb, Miyagi et al. 2002). Most DDD patients have an autoantibody against C3 convertase in the serum called C3 Nephritic Factor (C3NeF). C3NeF stabilizes the C3 convertase, making the enzyme less prone to inactivation by complement regulators (Daha, Fearon et al. 1976).

In the second decade of life, DDD patients may develop whitish-yellow deposits in the Bruch's membrane beneath the retinal pigment epithelium of the eye called drusen which is similar to pathologic eye phenotype found in Age-related macular degeneration (AMD). Initially, the presence of drusen in DDD patients does not cause visual impairment and is varied among these individuals (Duvall-Young, Short et al. 1989; Colville, Guymer et al. 2003; Holz, Pauleikhoff et al. 2004). However after several years, abnormal retinal function leading to visual loss may occur due to subretinal neovascular membranes, macular detachment and central serous retinopathy (Colville, Guymer et al. 2003). Patients with DDD have 10% long-term risk in developing visual problems.

DDD is also associated with acquire partial lipodystrophy (APL)(Eisinger, Shortland et al. 1972). APL, also known as Barraquer-Simons syndrome or cephalothoracic lipodystrophy, is a rare form of lipodystrophy in which fat loss is limited to the face and upper extremities. On the other hand, fat hypertrophy is observed in the lower extremities. The age of onset of APL is around 8-10 years of age and is preceded by an episode of an acute viral infection. APL occurs three times more often in females than males. About one-fifth of APL patients develop DDD which occurs more than 10

years of onset of APL. Both DDD and APL are due to the dysregulation of AP in the kidney and fat tissues respectively (Mathieson and Peters 1997). Patients with APL also develop hypocomplementemia or low levels of C3 and have C3 Nephritic factor in their blood. APL is associated with other autoimmune diseases like systemic lupus erythematosus (SLE), dermatomyositis, hypothyroidism, pernicious anemia, celiac disease, dermatitis herpetiformis, rheumatoid arthritis, temporal arteritis and leukocytoclastic vasculitis. Some APL patients even develop drusen or macular degeration later in life.

Current therapies for DDD focus on decreasing progression of renal damage by improving renal hemodynamics and controlling infiltration of leukocytes in the kidneys (Smith, Alexander et al. 2007). Non-specific treatment for DDD includes Angiotensin-converting enzyme inhibitors and angiotensin II type-1 receptor blockers and lipid lowering agents (e.g. hydroxymethylglutaryl coenzyme A reductase inhibitors) since both can reduce proteinuria and progression to renal failure (Ruggenenti, Perna et al. 1999; Brenner, Cooper et al. 2001; Nickolas, Radhakrishnan et al. 2003; Maisch and Pezzillo 2004). Some DDD patients undergo renal transplantation (Braun, Stablein et al. 2005). However, recurrence of DDD occurs in most grafts and is the most common cause of renal failure in 15-50% of recipients (Appel, Cook et al. 2005). Plasmapheresis has been useful in removing C3NeF from the circulatory system of DDD patients (Kurtz and Schlueter 2002). DDD patients with autoantibodies against CFH may benefit from treatments that inhibit function of B and T lymphocytes. A possible treatment for patients with CFH deficiency or protein defect is replacement of CFH or other fluid-phase complement regulators (e.g. soluble CR1). An antibody against human C5,

Eculizumab (Soliris), is being investigated as a therapy for DDD and other complement-mediated diseases (Gruppo and Rother 2009; Nurnberger, Philipp et al. 2009).

Genetic Causes of DDD

Mutations in complement regulator Factor H have been shown to cause DDD. Most of these mutations cause defects in protein secretion or deficiency of Factor H in the circulation (Licht, Heinen et al. 2006; Saunders, Abarrategui-Garrido et al. 2007). Variants in CFH and CFHR5 have been shown to be associated with DDD (Abrera-Abeleda, Nishimura et al. 2006; Lau, Smith et al. 2008).

Complement Factor H

Factor H is the most abundant fluid-phase complement regulator. The gene is located in human chromosome 1q32 within the Regulator of Complement Activation (RCA) cluster of genes and is encoded by 22 exons (120kb) (Figure 1-5A). The Factor H protein is composed of 1231 amino acids arranged in 20 complement control protein (CCP) or short consensus repeat (SCR) modules. An alternatively spliced form of Factor H is composed of the first 7 CCPs/SCRs and can support adhesion of epithelial and fibroblast cell line (Hellwage, Kuhn et al. 1997). The main function of Factor H is to regulate complement activity by binding to C3b in competition with Factor B and displacing Bb from the C3 convertase (Whaley and Ruddy 1976). Factor H has binding sites for C3b, heparin and C-reactive protein (CRP) (Figure 1-5B). It also serves as a co-factor for Factor I-mediated inactivation of C3b (Kazatchkine, Fearon et al. 1979). It can function as a chemotactic for monocytes, an adhesion protein for neutrophils and a secretagogue of IL-1β from monocytes (Iferroudjene, Schouft et al. 1991; Ohtsuka, Imamura et al. 1993; Nabil, Rihn et al. 1997). The primary site of synthesis for Factor H

is the liver and serum level in humans is approximately 550ug/ml (Weiler, Daha et al. 1976). Factor H synthesis in fibroblasts, monocytes, endothelial cells and cultured liver cells are upregulated by IFNγ (Brooimans, Hiemstra et al. 1989; Lappin, Guc et al. 1992; Vik 1996). Patients with deficiency in Factor H have developed aHUS or DDD.

Mutations in CFH are found in DDD patients. In two related patients with DDD, a deletion of a single lysine (ΔK224) in CFH has been observed (Licht, Heinen et al. 2006). This mutation is located in a regulatory region and binding site for C3b and causes decreased cofactor and decay accelerating activity of CFH protein. Another patient who developed both DDD and AMD has a novel missense mutation, C431Y, in SCR 7 of CFH (Montes, Goicoechea de Jorge et al. 2008). This mutation affects the three-dimensional conformation of SCR7 in the mutant CFH which can decrease its stability. In another study, four CFH-deficient patients diagnosed with DDD have novel mutations in CFH (Dragon-Durey, Fremeaux-Bacchi et al. 2004). Two related DDD patients have the mutation, R27L, in SCR2 which can affect C3b binding. One patient has the C431S mutation in SCR7 which can possibly affect the stability of the CFH protein. The last patient has the C673S mutation which is located in a CRP binding site in SCR11. The last two mutations involve cysteine (C) residues which are important in the formation of intrachain disulfide bonds in each SCR. Thus, this can affect the secondary structure of the CFH protein (Janatova, Reid et al. 1989). The Y402H common variant is associated with DDD (Abrera-Abeleda, Nishimura et al. 2006). The risk allele, H402, has a higher frequency in DDD patients compared to frequency found in the population. This variant is located in SCR 7 which is a CRP and heparin binding site. The presence of H402 decreases the binding of CFH protein to human umbilical

vein endothelial cells (HUVECs) and CRP (Laine, Jarva et al. 2007; Skerka, Lauer et al. 2007).

Complement Factor H-Related 5

Complement Factor H-related 5 (CFHR5) is one of the Factor-H related genes and is located in the RCA locus together with CFH (Figure 1-5A). The other six members of the CFH family include CFHL1, which is a splice isoform of CFH, and five Factor H-related proteins encoded by distinct genes (CFHR1–5). There is little known about the latter five proteins, although they do show varying degrees of structural similarity to CFH (Appel, Cook et al. 2005). CFHR5 has 9 SCRs and shows the highest similarity to CFH (Figure 1-5B) and has been demonstrated in renal biopsies of patients with other types of glomerulonephritis (Murphy, Georgiou et al. 2002; Appel, Cook et al. 2005). In vitro studies have also shown that CFHR5 is present on surfaces exposed to complement attack suggesting a possible role in the complement cascade. CFHR5 has complement regulatory and cofactor activities through binding to C3b, CRP and heparin (McRae, Cowan et al. 2001; McRae, Duthy et al. 2005). Allelic variants in CFHR5 are associated with DDD (Abrera-Abeleda, Nishimura et al. 2006).

Animal Models of DDD

The function of CFH has not only been studied in humans but also in pigs and mice by generating CFH deficient animal models (Jansen, Hogasen et al. 1993; Hogasen, Jansen et al. 1995; Hegasy, Manuelian et al. 2002; Pickering, Cook et al. 2002). Both the CFH-deficient pig and mouse models develop a renal phenotype similar to human DDD. Mice lacking in both CFH and Factor B (BF) proteins do not have DDD which implies that activation of the AP is necessary in the development of DDD (Pickering, Cook et al.

2002). Also, mice deficient of CFH and IF proteins do not develop DDD, but upon addition of IF, develop the characteristic DDD renal pathology (Rose, Paixao-Cavalcante et al. 2008). This shows that the regulatory function of CFH in the fluid phase to inactivate the C3 convertase is important in the development of DDD.

Other AP Complement-Mediated Diseases

Besides DDD, several human diseases are associated with deficiencies or defects in complement components or regulators. Examples include Systemic Lupus Erythematosus (SLE), Atypical Hemolytic Uremic Syndrome (aHUS) and Age-related Macular Degeneration (AMD).

Systemic Lupus Erythematosus

Deficiencies in the early complement components of the classical pathway have been highly associated with SLE (Vyse and Kotzin 1998). This autoimmune disease is characterized by collapse of immunotolerance and production of autoantibodies against different cells and tissues leading to acute and chronic inflammation. For example, patients with SLE often produce antibodies against dsDNA and develop glomerulonephritis. Although the cause of SLE is polygenic, it has been shown that homozygous deficiency of one of early complement component can lead to the development of SLE. Mutations and polymorphisms in the complement genes also have a strong association with SLE (Olsen, Goldstein et al. 1989; Davies, Snowden et al. 1995; Huang, Siminovitch et al. 1995; Chew, Chua et al. 2008). There is no cure for SLE, and most commonly, immunosuppressants and corticosteroids are used to prevent flaring of clinical symptoms.

Hemolytic Uremic Syndrome

Hemolytic uremic syndrome (HUS) is an autoimmune disease that is characterized by acute renal failure, microangiopathic anemia and thrombocytopenia (Noris and Remuzzi 2005; Atkinson and Goodship 2007). The typical form of HUS is associated with diarrhea caused by infection of *Escherichia coli* (serotype O157:H7) and is predominant in the younger age group. The atypical form (aHUS) is associated with genetic mutations of complement regulatory genes and affects both adult and juvenile populations. A third form called DEAP-HUS (Deficient for CFHR1/CFHR3 and Autoantibody Positive) is seen in younger patients and is caused by the combination of genetic and environmental causes (Skerka, Jozsi et al. 2009). An 84kb deletion is present in human chromosome 1q32 completely deleting CFHR1 and CFHR3 (Zipfel, Edey et al. 2007). Patients also have autoantibodies that bind to the C-terminal region of Factor H (Jozsi, Licht et al. 2008; Pizza, Donnelly et al. 2008). 60% of aHUS patients have mutations in the complement genes including complement components, C3 and Factor B and complement regulators, Factor H, Factor I and MCP/CD46 (Johnson and Taylor 2008). The main causes of kidney disease in HUS are microthrombi formation in kidney blood vessels and endothelial cell damage due to dysregulation of complement activity on the surface of host cells.

Age-Related Macular Degeneration

The most common cause of blindness in older populations of developed countries is Age-related Macular Degeneration (AMD) (de Jong 2006). This disease is characterized by damage to the macula (located at the center of the retina in the eye) and formation of drusen, which is the accumulation of debris materials in the retinal pigment

epithelial cells and Bruch's membrane. Protein deposits in drusen have been shown to contain complement components C3, C5, C6, C7, C8 and C9, and complement regulators clusterin and vitronectin (Crabb, Miyagi et al. 2002; Klein, Zeiss et al. 2005). The Factor H polymorphism, Y402H, is associated with AMD (Hageman, Anderson et al. 2005; Klein, Zeiss et al. 2005). Other complement genes with mutations and allele variants associated with AMD include Factor B, C3, C4 and C2. A deletion of CFHR1 and CFHR3 genes has protective effects in AMD (Hughes, Orr et al. 2006).

Possible Candidate Genes Associated with DDD

Complement Component C3

C3 is the most important acute phase component of the alternative pathway of the complement system. The C3 gene is located in human chromosome 19p13.3-13.2 and is about 41kb encoded by 41 exons. The C3 protein is synthesized as a preprotein consisting of 1663 amino acids with a 22 amino acid leader sequence and a four amino acid cleavage site (de Bruijn and Fey 1985). The mature protein contains a β chain (amino acid 23-667) and α chain (amino acid 672-1663). Several binding sites for complement proteins have been identified in C3 gene including Properdin (amino acid 1424-1456), Factor H (amino acid 749-790; 1209-1271)(Lambris, Avila et al. 1988), Factor B (amino acid 749-790) (Lambris, Lao et al. 1996), CR1 (amino acid 749-790) (Becherer and Lambris 1988), CR2 (amino acid 1227-1236) (Lambris, Ganu et al. 1985) and CR3 (amino acid 1383-1403) (Wright, Reddy et al. 1987). During the activation of the alternative complement pathway, C3 is cleaved to C3a and C3b (Figure 1-6). This creates a conformational change in C3b which can bind to cell surface carbohydrates or immune complexes via its reactive thioester domain (TED). Once the alternative

pathway is regulated, C3b is split by Factor I and its co-factor in two positions to produce iC3b (inactivated C3b) and C3f. iC3b is cleaved to form C3c and Cdg. On the other hand, C3a is a known anaphylatoxin that can mediate local inflammatory processes by binding to its receptors in the cell membrane. This can cause histamine release from mast cells and leukocytes, induce smooth muscle cell contraction and augment vascular permeability.

Most of the C3 is synthesized in the liver (Alper, Johnson et al. 1969). But other tissues produce C3 like kidneys (Sacks, Zhou et al. 1993), lungs (Strunk, Eidlen et al. 1988), intestines (Molmenti, Ziambaras et al. 1993), etc. Expression of C3 is controlled by several factors including interleukins (IL-1 and IL-6) (Beuscher, Fallon et al. 1987; Ramadori, Van Damme et al. 1988) and tumor necrosis factor (TNFα) (Perlmutter, Dinarello et al. 1986). In the endometrium, estrogen can decrease C3 expression while progesterone can increase C3 expression (Sundstrom, Komm et al. 1990). Normal range of C3 levels in the serum is 1-1.5 mg/ml (Kohler and Muller-Eberhard 1967). Individuals with deficiency in C3 due to mutations in the gene demonstrate increased susceptibility to pyogenic infections, systemic lupus erythematosus (SLE)-like symptoms and membranoproliferative glomerulonephritis (Botto, Fong et al. 1990; Botto, Fong et al. 1992; Huang and Lin 1994; Singer, Whitehead et al. 1994). There are two common C3 variants, C3S and C3F, which have been identified based on their electrophoretic mobilities. These variants are caused by a change in nucleotide 364 C to G in exon 3 leading to change in the protein sequence from arginine to glycine in amino acid 102. Another structural variant for C3 is based on its immunoblotting reaction with a

monoclonal antibody, HAV4-1. The variant is caused by a change in nucleotide 1001 C to T or amino acid 314 proline to lysine (Botto, Fong et al. 1990).

Factor B

Factor B is one of the components of the C3 convertase. The gene is located in human chromosome 6p21.1-6p21.3 and is encoded by 18 exons in 6 kb genomic DNA. The Factor B protein is composed of 764 amino acids with a leader sequence of 25 amino acids. Its structure consists of 3 domains: complement control protein (CCP) (amino acid 34-100, 101-161 and 163-220), von Willebrand factor A (vWFA) (amino acid 253-468) and serine protease (SP) (amino acid 482-764). A linker segment connects CCP and vWFA domain (amino acid 221-252) and is the site of cleavage of Factor B to Ba and Bb. During complement activation, Factor B binds to C3b to form the inactive complex, C3bB. A serine protease, Factor D, cleaves Factor B to remove Ba fragment. Factor B is also an acute phase reactant. It is primarily synthesized in the liver. The level of Factor B is upregulated during inflammatory reaction. Its expression is also affected by cytokines and growth factors (Ramadori, Sipe et al. 1985; Circolo, Pierce et al. 1990). Deficiencies of Factor B in humans or animals have not been reported. A Factor B null mouse has been generated showing no signs of any phenotype (Watanabe, Garnier et al. 2000). Mutations in Factor B are associated with the pathogenesis of aHUS (Tawadrous, Maga et al. ; Goicoechea de Jorge, Harris et al. 2007).

Factor D

The serine protease, Factor D or also known as adipsin, is one of the main components of the alternative pathway of the complement system and is the rate-limiting enzyme of that pathway. It cleaves Factor B bound to C3b to produce C3 convertase. It

is unlike any other serine protease because it does not require enzymatic cleavage or activation by serpin for its proteolytic activity (Volanakis and Narayana 1996). The Factor D gene is located in human chromosome 19p13.3 and is encoded by 5 exons. The Factor D protein is composed of 246 amino acids with a 13 amino acid signal peptide (amino acid 1-13) and a 5 amino acid potential activation peptide (amino acid 14-18). Factor D is primarily expressed in the adipose tissues and in macrophages (White, Damm et al. 1992). Deficiency in Factor D has been reported in patients who had recurrent *Neisseria meningitides* and *Neiseseria gonorrhoeae* infections (Biesma, Hannema et al. 2001; Sprong, Roos et al. 2006). Deficiency in Factor D has been shown to be due to mutations in the Factor D gene. (Sprong, Roos et al. 2006). Absence of Factor D in the system leads to no activity of the alternative complement pathway.

Properdin

Binding of Properdin to the C3 convertase increases the stability of the enzyme complex. This binding inhibits the cleavage of C3b by Factor I and increases affinity for Factor B (Fearon and Austen 1975; Farries, Lachmann et al. 1988). Its function enhances the amplification loop of the complement cascade and C3b deposition onto a target surface. The gene is located in human chromosome Xp11.3-11.23 and is composed of 10 exons (6kb). The protein is encoded by 469 amino acids with 27 amino acid leader sequence. Properdin is primarily synthesized in the monocytes, T cells and granulocytes (Whaley 1980; Schwaeble, Dippold et al. 1993; Westberg, Fredrikson et al. 1995; Wirthmueller, Dewald et al. 1997). Deficiency in Properdin results in highly impaired bactericidal activity and patients are prone to meningococcal infections (Westberg, Fredrikson et al. 1995).

Factor I

One of the complement regulators responsible for the inactivation of C3b and C4b is Factor I. It cleaves the α chains of C3b and C4b with the aid of co-factors like Factor H, MCP, CR1 and C4 binding protein (C4BP). It inactivates the complement cascade by inactivating C3b or C4b to prevent formation of C3/C5 convertases. The gene (63kb) is encoded by 13 exons yielding a protein with 583 amino acids with an 18 amino acid leader sequence (amino acid 1-18). It is primarily synthesized in the liver but is also produced in monocytes, endothelials cells, fibroblasts, etc. Expression of Factor I is upregulated by lipopolysaccharide (LPS) and interferonγ (IFNγ). Deficiencies in Factor I have been identified in individuals with increased incidence of recurrent pyogenic infection, glomerulonephritis and SLE-like symptoms. Absence of Factor I in the system leads to uncontrolled activation of the amplification of the alternative complement pathway.

Complement Factor H-Related Genes

The Factor H-related protein family consists of Factor H, Factor H-like 1 (CFHL1) and 5 Factor H-related genes (CFHR1-5). They are located in the RCA cluster of genes in human chromosome 1q32 (Figure 1-5A). CFHL1 is an alternative spliced form of Factor H in which the first 7 SCRs are present. It has been shown to be a complement regulator with co-factor and decay accelerating activity (Zipfel and Skerka 1999). CFHR1-4 are composed of 4-5 CCP/SCRs while CFHR5 has 9 CCP/SCRs. These are highly homologous to the CCP/SCRs in Factor H (Figure 1-5B). These Factor H-related genes also bind to complement proteins and have complement regulatory activity. CFHR1 can bind to C5 convertase to inhibit the terminal complement cascade

(Heinen, Hartmann et al. 2009). CFHR3 can bind to C3b and heparin (Hellwage, Jokiranta et al. 1999). CFHR5 can also bind to C3b (McRae, Cowan et al. 2001). Deletion of CFHR1 and CFHR3 as a result of chromosomal rearrangement represents a risk factor for aHUS (Zipfel, Edey et al. 2007). On the other hand, the CFHR1/CFHR3 deletion has a protective effect against AMD (Hughes, Orr et al. 2006).

Complement Receptors

Complement receptors are membrane-bound proteins that can regulate complement activity or act as complement effector receptors. There are five major complement receptors namely CR1, CR2, CR3, CR4 and CRIg/VSIG-4. These receptors develop an important interface between complement activation in the fluid-phase or on target surfaces and intracellular signaling pathways that affect cellular phenotype.

Complement receptor 1 (CR1) is an integral protein that regulates complement activity (Fearon 1979). The CR1 gene is located in human chromosome 1q32 within the RCA gene cluster. It spans about 133kb and is encoded by 39 exons. The CR1 protein has 2044 amino acids which are organized into a tandem of long homologous repeats (LHRs) of 7 CCPs or SCRs (Figure 1-7). These LHRs are binding sites for C4b, C3b and C1q (Klickstein, Wong et al. 1987; Klickstein, Bartow et al. 1988). The C-terminal transmembrane domain consists of 25 amino acids while the C-terminal cytoplasmic tail consists of 43 amino acids. There are four major structural allotypes in humans: CR1*1 (F or A), CR1*2 (B or S), CR1*3 (C or F') and CR1*4 (D) (Dykman, Cole et al. 1983; Wong, Wilson et al. 1983; Dykman, Hatch et al. 1984; Dykman, Hatch et al. 1985; Klickstein, Barbashov et al. 1997). The most common form is CR1*1 (F or A) which contains 4 LHRs. CR1*2 has 5 LHRs, CR1*3 has 3 LHRs and the most rare form,

CR1*4 has 6 SCRs. The different allotypes are caused by nonhomologous recombination and gene conversion of CR1.

CR1 is a receptor for C3b, C4b and C1q (Klickstein, Barbashov et al. 1997). It can also bind to iC3b, but not efficiently (Kalli, Ahearn et al. 1991). It is expressed on erythrocytes and facilitates the binding of opsonized immune complexes or pathogens (Arend and Mannik 1971; Cornacoff, Hebert et al. 1983; Schifferli, Ng et al. 1988; Kimberly, Edberg et al. 1989). These cells then are transported to the liver or spleen for removal. CR1 is also expressed on neutrophils and monocytes, which aids in the phagocytosis of an activated cell. In all cell types, CR1 acts as a complement regulator and a co-factor for Factor I mediated inactivation of C3b or C4b (Fearon 1979; Fearon 1980; Iida and Nussenzweig 1981). A soluble form of CR1 is present in the serum and arises from cleavage of the intracellular form of CR1 on the neutrophils by elastase and unknown metalloprotease during conditions in which the action of CR1 is necessary (e.g. complement regulation) (Danielsson, Pascual et al. 1994; Sadallah, Hess et al. 1999). Low levels of CR1 on the erythrocytes are associated with SLE (Miyakawa, Yamada et al. 1981; Iida, Mornaghi et al. 1982; Wilson, Wong et al. 1982; Walport, Ross et al. 1985). Besides the four structural allotypes of CR1, another variant is the quantitative allotype, H or L, which determines CR1 expression level on erythrocytes. Individuals homozygous for the H allotype have 4-10x more CR1 levels than those who are homozygous for L allotype (Wilson, Murphy et al. 1986).

Complement receptor 2 (CR2) is another regulator of the complement system. The CR2 gene is also located in the RCA locus and is composed of 19 exons spanning 30kb DNA (Fujisaku, Harley et al. 1989). The CR2 protein is a glycosylated type I

transmembrane protein with an extracellular domain consisting of 15-16 SCRs. There are 2 CR2 isoforms: one consisting 16 SCRs and the other 15 SCRs which is due to the alternative splicing of exon 11. The CR2 protein consists of 1092 (16 SCRs) or 1033 (15 SCRs) amino acids with a 20 amino acid signal peptide, a 22-24 amino acid transmembrane region and 34 amino acid intracellular domain. It is expressed in mature B lymphocytes (Tedder, Clement et al. 1984), follicular dendritic cells (Reynes, Aubert et al. 1985), thymocytes (Watry, Hedrick et al. 1991), T lymphocytes (Fischer, Delibrias et al. 1991; Levy, Ambrus et al. 1992), basophils (Bacon, Gauchat et al. 1993), keratinocytes (Hunyadi, Simon et al. 1991), astrocytes (Gasque, Chan et al. 1996) and epithelial cells (Levine, Pflugfelder et al. 1990).

CR2 is the receptor for C3d and iC3b (Iida, Nadler et al. 1983; Weis, Tedder et al. 1984) but binds to C3b less effectively (Cooper, Moore et al. 1988; Ahearn and Fearon 1989). It associates with CR1 on the surface membrane and plays a role in the conversion of C3b, a ligand of CR1, to complement products, C3d and iC3b. It is also a receptor for the Epstein-Barr virus (EBV) glycoprotein gp350/220 and thus, important in EBV binding, infection and immortalization of B cells (Fingeroth, Weis et al. 1984; Nemerow, Wolfert et al. 1985). Other functions of CR2 include binding with CD23 to increase IgE production and histamine release and to promote T:B cell adhesion (Bacon, Gauchat et al. 1993); and mediating antigen trapping and cell-cell interactions in germinal centers of lymphoid organs (Fang, Xu et al. 1998). Polymorphisms in CR2 gene have been shown to be associated with SLE (Douglas, Windels et al. 2009).

Complement receptor 3 and 4 (CR3 and CR4) are complement receptors that play a role in the phagocytosis of target surfaces opsonized with C3b and iC3b fragments

(Cabanas and Sanchez-Madrid 1999; Cabanas and Sanchez-Madrid 1999; Cabanas and Sanchez-Madrid 1999; Ehlers 2000). Both are made up of 2 genes that form type I membrane glycoproteins. Both CR3 and CR4 have CD18 or β_2-integrin as the β subunit. However, they differ with the α subunit which CR3 has CD11b and CR4 has CD11c. The gene for the β subunit is located in human chromosome 21q22.3 and encoded by 16 exons spanning 40kb. Both genes for the α subunit is located in a cluster in human chromosome 16p11-13.1. CD11b is composed of 30 exons spanning 55kb while CD11c has also 30 exons spanning 25kb DNA. CR3 and CR4 do not have complement regulatory properties but are important in intercellular adhesion and cyctotoxic events. When CR3 or CR4 binds to iC3b-opsonized pathogens, phagocytosis and degranulation occur. On the other hand, when they binds to iC3b-opsonized erythrocytes or cells, cell adhesion occurs. Deficiencies of the α subunit has been associated with SLE (Witte, Dumoulin et al. 1993) while deficiencies of the β subunit is associated with Leukocyte Adhesion Deficiency (LAD) which is characterized by immunodeficiency due to recurrent infection (Anderson and Springer 1987).

Complement Receptor of the Immunoglobulin superfamily (CRIg) is another regulator of the complement system. It is a membrane-bound receptor of C3b and iC3b. The CRIg gene is located in human chromosome Xq12-13.3 and is encoded by 8 exons spanning 18kb. There are two isoforms: the long (L) form consists of IgV-type of immunoglobulin domain and IgC-type immunoglobulin domain while the short form lacks IgC. The IgV domain is important in binding to C3b, iC3b and C3c (Wiesmann, Katschke et al. 2006) and in the phagocytosis of complement-opsonized cells (Helmy, Katschke et al. 2006). CRIg is highly expressed in the liver particularly the Kupffer cells

which are hepatic macrophages located in the sinusoidal lining of the liver and are important in pathogen clearance (Helmy, Katschke et al. 2006).

CRIg acts as an inhibitor of AP of the complement system by reducing the formation of MAC (Katschke, Helmy et al. 2007). It does not have decay accelerating nor cofactor activity but its regulatory function is due to its interference in the substrate binding capacity of C3b (Wiesmann, Katschke et al. 2006). Interestingly, CRIg can only inhibit the AP C5 convertases (C3bC3b) but not the CP C5 convertase (C3bC4b). Because of this characteristic, a soluble form of CRIg has been developed as a therapeutic potential for AP complement-mediated disorders like AMD and DDD. In two mouse models of arthritis, a recombinant CRIg-Fc fusion protein is administered to the mice and is shown to significantly decrease the inflammation (Katschke, Helmy et al. 2007).

Clusterin and Vitronectin

Clusterin is known as Apolipoprotein J. It functions as a complement regulator of the terminal complement cascade by binding to C5b-7 and MAC (Berge, Johnson et al. 1997). The gene spans 16.58kb and is encoded by 9 exons. The protein consists of 449 amino acids with a 22 amino acid signal peptide and forms heterodimers. It is primarily expressed in the liver, testes, epididymis and brain, but other organs like heart, kidneys and lungs also express Clusterin. The expression of Clusterin is dependent on the levels of endotoxins, cytokines and growth factors (Hardardottir, Kunitake et al. 1994; Jin and Howe 1997). It is also induced after tissue injury or apoptosis in certain organs and tissues. Clusterin has been implicated in the pathogenesis of Alzheimer's disease, retinitis pigmentosa, cystic renal diseases and aHUS (Harding, Chadwick et al. 1991;

Choi-Miura, Ihara et al. 1992; Jomary, Murphy et al. 1993; Stahl, Kristoffersson et al. 2009).

Another complement regulator of the terminal complement pathway is Vitronectin, also known as S-protein. The gene is located in human chromosome 17q11 and encoded by 8 exons spanning 3kb. Vitronectin is a 75kDa multifunctional glycoprotein with 459 amino acids organized into three domains: N-terminal Somatomedin B domain (amino acids 1-39), central domains with hemopexin homology (131-342) and C-terminal domain with hemopexin homology (347-459). The N-terminal domain is able to bind to the Plasminogen activator inhibitor-1 (PAI-1) which is a member of the coagulation pathway (Suzuki, Oldberg et al. 1985). The C-terminal domain contains a heparin binding site which is a proposed interaction site for C9 (Milis, Morris et al. 1993). Similar to Clusterin, Vitronectin inhibits complement activation by binding to C5b-7 complex to prevent its insertion to the membrane and inhibiting the formation of MAC (Podack and Muller-Eberhard 1979). C8 and C9 bind to the soluble C5b-7 forming a soluble MAC which is cleared from the circulation immediately (Greenstein, Peake et al. 1995). The heparin binding domain of Vitronectin also prevents the polymerization of C9 (Tschopp, Masson et al. 1988).

C3a and C5a Receptor 1

C3a receptor 1 (C3aR1) and C5a receptor 1 (C5aR1) are cell surface receptors for the anaphylatoxins, C3a and C5a respectively. These are transmembrane G-protein coupled receptors with seven transmembrane domains. Both C3aR1 and C5aR1 function in immune cell recruitment and inflammatory reaction (Benard, Raoult et al. 2008). The C3aR1 gene is located in human chromosome 12p13 and is encoded by 2 exons. The

C3aR1 protein consists of 482 amino acids and characterized by a large second extracellular domain. C3aR1 is distributed in peripheral tissues and central nervous system (Ames, Li et al. 1996; Crass, Raffetseder et al. 1996; Roglic, Prossnitz et al. 1996).

The C5aR1 gene is located in human chromosome 19q13.3-13.4 and is also encoded by 2 exons. The C5aR1 protein has 350 amino acids. It is mostly expressed on myeloid-derived cells and cell lines (e.g. granulocytes and moncytes) and non-myeloid cells (e.g. epithelial, endothelias and glial cells) (Chenoweth and Hugli 1978; Gerard, Hodges et al. 1989; Offermans, Schafer et al. 1990; Rollins, Siciliano et al. 1991). C3aR1 and C5aR1 has been implicated in the pathogenesis of allergy, asthma and other autoimmune diseases (Bautsch, Hoymann et al. 2000; Boos, Campbell et al. 2004; Ramos, Wohler et al. 2009).

Complement C4

C4 is the main complement protein in the classical and lectin pathways. The gene is located in human chromosome 6p21.3 within the class III region of the major histocompatibility complex (MHC). The C4 protein is composed of 1744 amino acid and has two isoforms in humans, C4A and C4B, which reflect the Acidic or Basic electrophoretic mobilities at alkaline pH (Dodds and Law 1990; Reilly and Mold 1997). Main difference between the two isoforms is that the acceptor nucleophiles for C4A are amino groups while C4B are hydroxyl groups. There is evidence that C4A binds more efficiently to CR1 than C4B (Reilly and Mold 1997). Thus, C4A has a lower hemolytic activity than C4B. It is also thought that C4A is involved in immune clearance while C4B is important in defense against pathogens. C4 is proteolytically activated by C1s or

MASP-2 to form C4a and C4b. C4b becomes one of the components of the classical/lectin pathway C3 convertase. C4 is an acute phase reactant and is upregulated by cytokines (Kulics, Colten et al. 1990; Andoh, Fujiyama et al. 1993). Deficiency in C4 has been implicated in SLE, immune complex disease of the kidneys and susceptibility to multiple infections (Hauptmann, Tappeiner et al. 1988). Deletion of C4A, which can be detected through long range PCR, is associated with SLE (Kristjansdottir and Steinsson 2004).

ADAM Family

ADAM19 is part of the metalloprotease ADAM (a disintegrin and metalloprotease) family of proteins. ADAM proteins are type I transmembrane glycoproteins that contain disintegrin and metalloprotease. There are about 33 different kinds of ADAM proteins. These proteases function in ectodomain shedding during signaling processes of several proteins including cytokines, growth factors, receptors, etc. The substrates include TNFα, TGFα, L-selectin and p75 TNF receptor and IL-1 receptor II (Black, Rauch et al. 1997; Moss, Jin et al. 1997; Powell, Fingleton et al. 1999; Schlondorff, Lum et al. 2001).

ADAM19, also known as meltrin β, has been shown to cleave epidermal growth factor, TNFα, TRANCE and KL-1 (Chesneau, Becherer et al. 2003). The ADAM19 gene is located in human chromosome 5q32-33 and consists of 23 exons. The ADAM19 protein is composed of 918 amino acids. It is highly expressed in the bone, lungs and heart but is also expressed in leukocytes and glomerulus of the kidneys (Wei, Zhao et al. 2001). Because of its metalloprotease activity, ADAM19 is a possible metalloprotease that can cleave membrane-bound complement protein like CR1, CR2 and MCP. All

these proteins are expressed in leukocytes and renal tissues. The three membrane-bound complement proteins are cleaved by metalloproteases to generate soluble form found in the serum. Metalloproteases cleaving CR1 and CR2 are still unknown. However, MCP is cleaved by ADAM10 *in vitro* (Gelderman, Hakulinen et al. 2003).

Case-Control Association Studies

In order to identify possible candidate genes that are involved in the pathogenesis of DDD, one approach that can be used is an association study. An association study is a statistical statement of the co-occurrence of alleles and phenotypes. It is a powerful tool to determine how particular alleles at a marker locus are co-inherited to predispose to a disease. In general, cases and appropriately matched controls are used for these comparisons.

Statistical analyses used for these studies include Chi square test and logistic regression. Since common human diseases have multiple genetic and environmental causes, association studies can be used to detect underlying risk factors and provide insight into the biologic effects of these variants in relation to a disease. This can lead to public health strategies for prevention and proper diagnosis and treatment for the disease. Case-control association studies are commonly utilized to understand the role of genes and gene-environment interactions in the identification of risk of complex diseases. Various statistical analyses have been employed for association studies.

Statistical Analyses for Genetic Associations

Chi Square Test of Independence

The Chi square test of independence is employed using two categorical variables in the same population. It determines significant association between the two variables (e.g. genotypes and disease status). The null hypothesis states that the two variables are independent from one another. In contrast, the alternative hypothesis states that one variable affects the other and hence both variables are associated. The analysis will test if the null hypothesis can be rejected based on a specified significance level (0.05). This will be determined by computing the p-value which is the probability of observing a test statistic (χ^2) at least as extreme as the observed sample statistic. The p-value and significance level are compared to assess if the null hypothesis is going to be rejected or accepted. If the p-value is less than or equal to significance level (0.05), then the null hypothesis is rejected and the result is said to be statistically significant.

Cochran-Armitage Trend Test

The Cochran-Armitage trend test is used for ordered categorical data analysis (Cochran 1954; Armitage 1955). It tests for association in a 2 x k contingency table. For genotypes, there are three levels: homozygous for major allele, homozygous for minor allele and heterozygous group. In association studies, the trend test is able to predict the linear trend between the presence of risk alleles and disease (two risk alleles, increase susceptibility to disease). The Chi square test of independence may not be able to detect a trend in the ordered data but the trend test can do so because the test statistic is able to reflect the anticipated trend. In association studies, the null hypothesis states that there is no linear trend in binomial proportions of genotypes. The alternative hypothesis states

that there is a linear trend in binomial proportion of genotypes. If the p-value is less than the significance level, then the null hypothesis is rejected which means that the linear trend between genotypes and disease is statistically significant.

Haplotype Analysis

A haplotype is a group of alleles in multiple loci that are inherited together at the same chromosome. In order to better understand the role of candidate genes in the development of a particular human disease, haplotype information is often used. One problem for doing haplotype analyses is that it is difficult to predict the exact haplotype based on the genotypes unless genotypes of family members are accessible. The most common algorithm to predict the haplotypes is the expectation-maximization (EM) algorithm. The EM algorithm utilizes maximum-likelihood estimates of molecular haplotype frequencies under the assumption of Hardy-Weinberg proportions (Excoffier and Slatkin 1995). It works well with large sample and control sizes. The association analysis of haplotypes is done by logistic regression shown as odds ratios, 95% confidence interval and p-value. The most frequent haplotype is automatically selected as the reference category.

Gene-Gene Interactions

In human genetics, the most important aim is to identify variations in the DNA that confer susceptibility or risk in developing a certain human disorder. A variation in the DNA that arises in at least once in every 100 copies in autosomal chromosomes or 50 copies in sex chromosomes is called a polymorphism. The most common type of polymorphism is single nucleotide polymorphism (SNP). SNPs can change an amino acid in the coding region. However, many SNPs are silent with no effect on gene

products. But these can still be valuable genetic markers in disease association studies. In general, functionally significant effects of genetic polymorphisms are most likely related to 1) an amino acid substitution in the gene product, 2) a frameshift mutation due to deletion or insertion in the coding region, 3) a gene is completely null or 4) the polymorphism directly affects gene transcription, RNA splicing, mRNA stability or mRNA translation.

SNPs are differentiated from rare variants if the minor allele has a frequency of 1% or more in the population. Since genetic and phenotypic variability exists among individuals, it is hypothesized that SNPs present in an individual can play a role in disease susceptibility and therapy response (Masood 1999). For association studies, a major challenge in identifying risk alleles is to recognize how and when certain genetic variants or combination of variants are associated with the disease. Genetic interaction among dozen or possibly hundreds of disease genes in most complex disease like DDD is poorly understood. The inheritance of combinations of functional and commonly occurring SNPs may additively or synergistically affect the AP of the complement system, leading to uncontrolled complement activity. Therefore, individuals who carry several interacting risk or susceptibility alleles could have a high risk of developing DDD.

Because of gene-gene interactions, the relationship between genetic causes and disease susceptibility is not always linear. Nonlinearities in this relationship can be due to locus heterogeneity, phenocopy, dependence on environmental factors and genotypes at other loci. In human and population genetics, epistasis is defined as interaction between different genes. It is recognized as deviations from Mendelian segregation ratios

or deviations from additivity in a linear statistical model. There are two types of epistasis: biological and statistical. Biological epistasis is due to physical interactions between biomolecules (DNA, RNA, proteins, enzymes, etc.). On the other hand, statistical epistasis occurs when there are interindividual DNA variations at the population level. It is important that the relationship between biological and statistical epistasis is clearly understood to generate biological inferences from statistical results.

Logistic Regression

In association studies, logistic regression (LR) is a standard parametric method in identifying effects and interactions with binary response data or genes. It is used to predict the probability of occurrence of an event by fitting the data to a logistic curve. It is considered a generalized linear model that is being used for binomial regression. It utilizes several predictor variables, numerical or categorical, similar to many forms of regression analysis. Unfortunately, LR models have some weakness when used in association studies. The three-level genotype factors and the interactions can produce many parameters and problems with overfitting. Also, multiple candidate genes and increased interaction order can give false positive results leading to the degradation of the fit model.

Focused Interaction Testing Framework

Another parametric approach in identifying genetic interactions is the Focused Interaction Testing Framework (FITF). It is a powerful testing framework based on likelihood ratio tests (LRTs) which simultaneously tests multilocus effects across various orders of genetic interaction while modifying the threshold for significance by adjusting false discovery rates (FDR) (Millstein, Conti et al. 2006). The screening

algorithm utilizes a goodness-of-fit χ^2 statistic which compares observed to expected genotype frequencies in cases and controls. Its output is the False Discovery Rate (FDR) which is the expected false positive rate. A cutoff is determined for each level of interaction (main effect, two-way and three-way). If the computed FDR for each set is lower than the cutoff, then the effect or interaction is significant.

Multifactor Dimensionality Reduction

Statistical epistasis is difficult to detect and characterize in human association studies because of its inherent nonlinearity especially in genome-wide association studies. Modeling nonlinear interactions need special statistical analyses because parametric approaches (e.g. logistic regression) can have less power for determining genetic interactions than independent main effects. Non-parametric methods, which use distribution free methods not relying on assumptions that the data are drawn form a given probability distribution, are useful in this case. One such statistical analysis is the Multifactor Dimensionality Reduction (MDR). MDR is a nonparametric data mining approach for detecting and characterizing interactions among attributes or independent variables to influence a dependent or class variable (Hahn, Ritchie et al. 2003). It is primarily designed for identification of genetic interactions among discrete variables that influence binary outcome. It can identify nonlinear interactions among discrete genetic and environmental attributes. The MDR analysis consists of attribute selection, attribute construction, classification, cross-validation, and visualization in order to give a comprehensive and powerful approach in identifying and analyzing nonlinear interactions among attributes or variables.

Purpose of the Study

The purpose of this study is to explore the role of the alternative pathway of the complement system in the pathogenesis of DDD. We hypothesize that the pathophysiologic basis of DDD is related to the uncontrolled systemic activation of the alternative pathway of the complement cascade and that damage to the GBM of the kidney reflects inadequate protection from complement-mediated injury. Although the inciting triggers that initially activate the alternative pathway are not known, we further hypothesize that activation of the alternative pathway is possible only on a permissive genetic background and that this background contributes to complement dysregulation. We therefore sought to identify complement genes that are associated with DDD and determine their relative contribution to the DDD phenotype through functional analyses and animal models.

Currently, there is no effective treatment for DDD. Renal transplantation in most patients with DDD is associated with disease recurrence and graft failure (Andresdottir, Assmann et al. 1999). Other treatment options used for DDD patients include corticosteroids, immunosuppressants (Kiyomasu, Shibata et al. 2002; Iitaka, Nakamura et al. 2003), anticoagulants (Kher, Makker et al. 1982), antithrombolytics, plasmapheresis (Kurtz and Schlueter 2002) and plasma exchange. Most of these therapies are empirical or desperate measures to attempt to prevent end stage renal failure (Cansick, Lennon et al. 2004). The better understanding of the pathophysiology and pathobiology of DDD may foster the development of an effective treatment to prevent the progression to end stage renal disease.

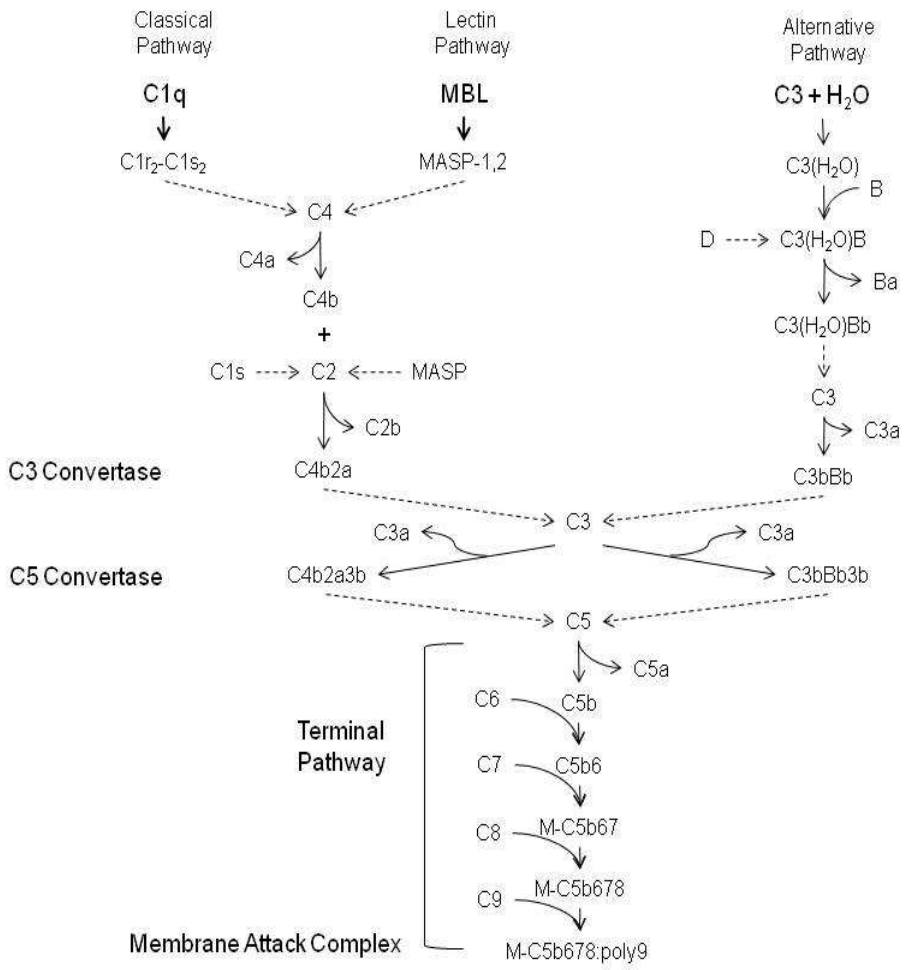

Figure 1-1.Overview of the three pathways of the Complement System. Dashed arrows represent enzymatic cleavage.

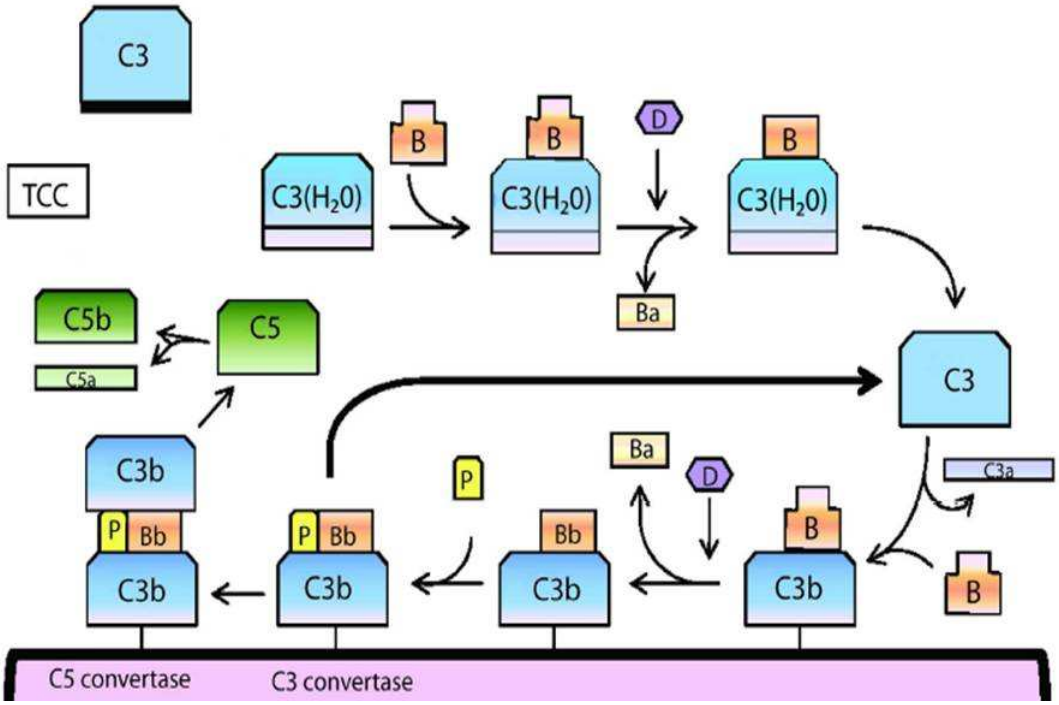

Figure 1-2. Activation of the Alternative Pathway of the Complement System. C3 is spontaneously hydrolyzed to C3(H2O) or C3i , which forms an intermediate convertase called C3iBb. This intermediate convertase cleaves C3 to generate C3a and C3b, the latter of which binds factor B (B). The action of factor D (D) on Factor B leads to the formation of C3bBb. Properdin (P) binds to the complex to stabilize the C3 convertase, which cleaves additional C3 to amplify C3b generation (bold arrow). The binding of additional C3b to C3bBb results in formation of C5 convertase, which leads to the terminal complement complex (TCC).

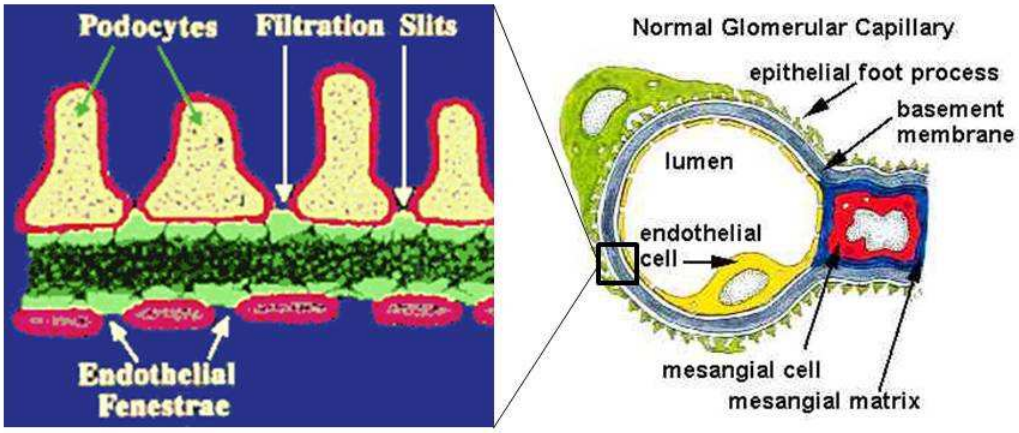

Figure 1-3. Structure and layers of the glomerulus. The glomerulus is composed of endothelial lining, glomerular basement membrane, podocytes and mesangial cells that help in the filtration process.

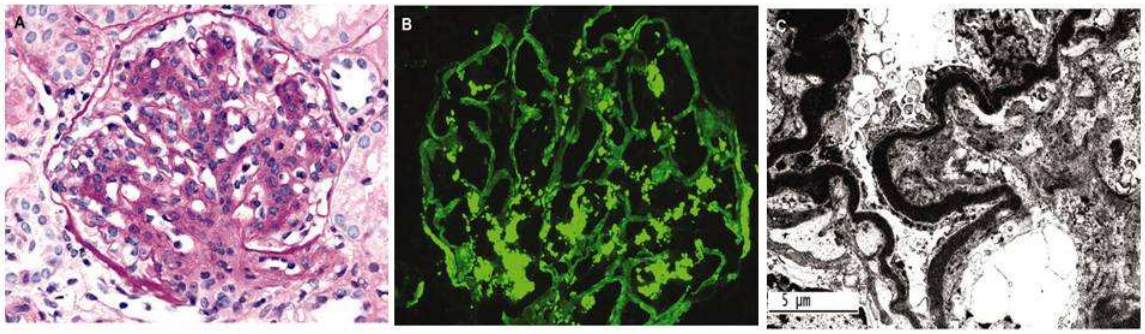

Figure 1-4. Renal Pathology of Dense Deposit Disease.Figure A) The light microscopic appearance shows capillary wall thickening and mesangial cell proliferation (periodic acid-Schiff stain). (B) C3 is present in loops and mesangial areas using fluorescein-conjugated anti-C3 antibody stain. (C) Presence of electron-dense deposits in the glomerular basement membranes (GBM) of the kidney is characteristic of DDD (unstained). Magnifications: x400 in A and B; x5000 in C.

Source: (Smith, Alexander et al. 2007)

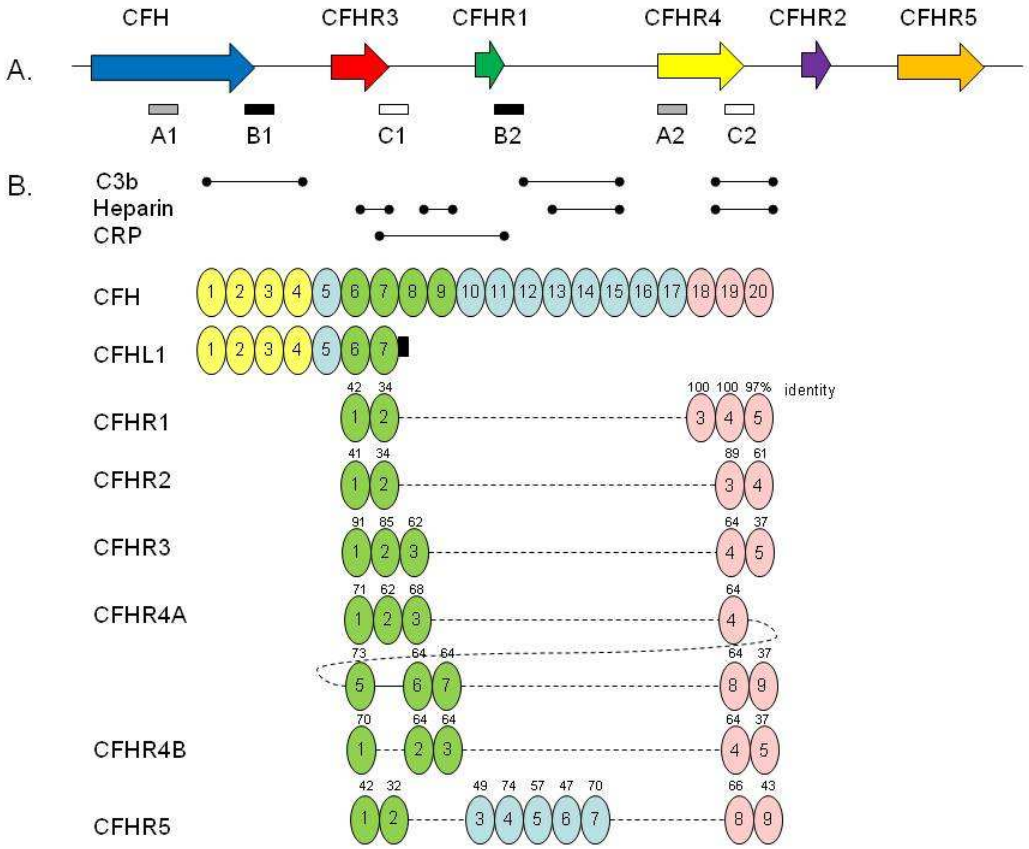

Figure 1-5. Factor H and Factor H-related gene cluster and protein structures. **(A)** This gene cluster consists of homologous repeat regions (labeled as A1–C2), which can result in deletion of large fragments due to non-homologous recombination and gene conversion. **(B)** The CFH family proteins are composed of short consensus repeat (SCR) domains. CFHR proteins contain SCRs homologous to CFH as indicated by the same color. %homology are numbers indicated on top of SCR domains. Homologous domains are indicated by vertical alignment. Various binding sites in CFH are also noted.

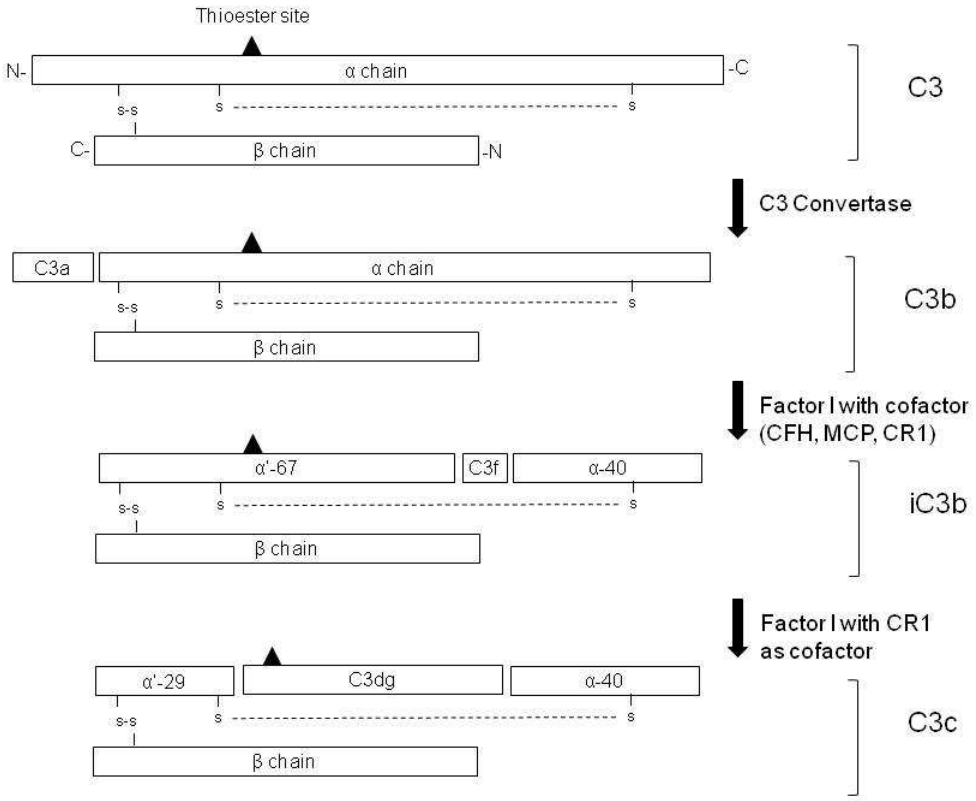

Figure 1-6. Activation and cleavage of C3. Complement C3 is activated and cleaved by the C3 convertase to produce C3a and C3b. Factor I and complement regulators as cofactor are responsible for the inactivation of C3b.

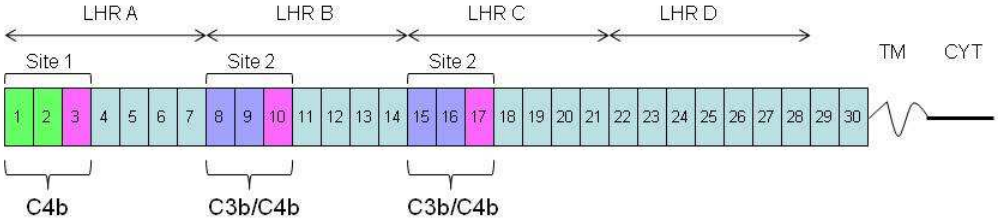

Figure 1-7. Schematic diagram of the most common structural variant of CR1 (F or A). CR1 is composed of 30 short consensus repeats (SCRs) arranged into 4 long homologous repeats (LHRs). The SCRs are followed by transmembrane domain (TM) and cytoplasmic tail (CYT).

CHAPTER II

ALLELE VARIANTS OF GENES OF THE ALTERNATIVE PATHWAY ASSOCIATED WITH DENSE DEPOSIT DISEASE PHENOTYPE

Introduction

The complement system is our innate immunity against infection. It has several functions which include lysis of pathogens, opsonization, immune clearance and binding to complement receptors (Walport 2001). There are three major pathways in the complement system: the classical, the lectin and the alternative pathways. The three pathways all generate homologous variants of the C3 convertase. The classical complement pathway typically requires antibodies for activation (specific immune response), whereas the alternative and mannose-binding lectin pathways can be activated by C3 hydrolysis or antigens without the presence of antibodies (non-specific immune response). In all pathways, a C3-convertase cleaves and activates component C3, creating C3a and C3b and causing a cascade of further cleavage and activation events. C3b binds to the surface of pathogens leading to greater internalization by phagocytic cells by opsonization (Pangburn and Muller-Eberhard 1980). C5a is an important chemotactic protein, helping recruit inflammatory cells. Both C3a and C5a have anaphylatoxin activity, directly triggering degranulation of mast cells as well as increasing vascular permeability and smooth muscle contraction. C5b initiates the membrane attack pathway, which results in the membrane attack complex (MAC), consisting of C5b, C6, C7, C8, and polymeric C9. MAC is the cytolytic end product of the complement cascade; it forms a transmembrane channel, which causes osmotic lysis of the target cell.

The alternative pathway (AP) is continuously activated at a low rate in human plasma (Pangburn and Muller-Eberhard 1983). Plasma C3, the key component of AP, undergoes slow and spontaneous hydrolysis to C3(H_2O) due to the breakdown of the thioester bond via condensation reaction. This provides a subunit for the initial C3 convertase of AP, C3(H_2O)Bb, which cleaves fluid phase C3, generating metastable C3b. Within a short period, this metastable C3b can attach covalently to any surface. The surface-bound C3b acts as a part of the C3 convertase, which cleaves and activates other C3 molecules. This leads to a positive feedback loop and progressively increased deposition of C3b on the target surface. Upon binding with a cellular membrane, C3b is bound by Factor B to form C3bB. This complex in presence of factor D is cleaved into Ba and Bb. Bb remains covalently bonded to C3b to form C3bBb together with properdin which is the alternative pathway C3-convertase. The C3bBb complex, which is "hooked" onto the surface of the pathogen, will catalyze the hydrolysis of C3 in the blood into C3a and C3b, which positively affects the number of C3bBb hooked onto a pathogen. After hydrolysis of C3, C3b complexes to become C3bBbC3b, which cleaves C5 into C5a and C5b. C5b binds with C6, C7, C8, and C9 to form the membrane attack complex (MAC) or terminal complement complex (TCC).

Because the AP cascade does not recognize any target-specific activator structures, it must be strictly regulated. As AP is part of innate immunity, it must discriminate between activator and nonactivator structures to destroy only the harmful targets. The main regulator of AP in plasma is Factor H (CFH). It acts as an accelerator of the decay of C3bBb and as a cofactor for Factor I in the proteolytic inactivation of C3b. When CFH has high affinity for a surface or for surface-bound C3b, AP activation

is stopped; when affinity is low, AP activation proceeds leading to efficient opsonization and lysis.

The alternative pathway has been implicated in the pathogenesis of Dense Deposit Disease (DDD), which is a primarily a disease of the kidneys. The kidneys receive 20% of the cardiac output and during the course of the day filter 180 liters of blood. The glomerulus is composed of capillary vessels actively involved in the filtration of blood in the kidneys. In DDD, dense deposits which are composed of C3 degradation products occur in the glomerular basement membrane for which the disease is named. DDD is a rare glomerular disease defined by histology as having capillary wall thickening and mesangial cell proliferation compared to a normal glomerulus. It preferentially affects a younger age group with an incidence of 2-3 in 1,000,000 individuals. It has varied signs and symptoms including hypocomplementemia, nephrotic syndrome, hypertension, hematuria, proteinuria and renal insufficiency and failure. It has been shown to be caused a continuous activation of the alternative complement pathway. Also, an autoantibody against C3 convertase called C3 Nephritic factor (C3NeF) is present in most DDD patients (Daha, Austen et al. 1978). Autoantibodies for C3 and CFH have also been reported in patients with DDD which can either prolong the half-life of the C3 convertase or interfere with regulatory function of CFH (Dragon-Durey, Loirat et al. 2005)

Major genetic contributions in the pathogenesis of DDD include mutations and polymorphisms in complement regulator, Factor H (CFH), which can either cause CFH deficiency or a non-functional CFH protein (Dragon-Durey, Fremeaux-Bacchi et al. 2004; Abrera-Abeleda, Nishimura et al. 2006; Licht, Heinen et al. 2006; Pickering, de Jorge et al. 2007). CFH is a soluble glycoprotein that is produced mainly in the liver and

circulates in blood. It destabilizes C3 convertase by binding to three sites of the C3 protein. This binding inactivates C3 convertase and inhibits complement activity. CFH can also bind to other molecules on cells and membranes to protect these surfaces from damage due to the activation of the complement system. .

Mutations in CFH gene are also associated with another renal disease, atypical hemolytic uremic syndrome (aHUS) (Rodriguez de Cordoba, Esparza-Gordillo et al. 2004), while missense mutations of CFH are associated with AMD (Hageman, Anderson et al. 2005). These changes in the Factor H protein may affect secretion of Factor H into the circulation or impair the binding of Factor H to C3 and other substrates of the alternative complement system. Mutations and polymorphisms in C3, Factor B, C2, C4, MCP/CD46 and Factor I have been implicated in aHUS or AMD (Hageman, Anderson et al. 2005; Klein, Zeiss et al. 2005; Saunders, Abarrategui-Garrido et al. 2007; Johnson and Taylor 2008). We hypothesize that specific allele variants of genes of the alternative pathway of the complement system are associated with the DDD phenotype.

Materials and Methods

DDD Cases and Controls

Seventy one (71) patients with biopsy-proven DDD were ascertained in nephrology divisions and enrolled in this study under IRB-approved guidelines. One control group consisted of 103 unrelated persons in whom age-related macular degeneration (AMD) had been excluded (AMD(-)). This group matched the DDD patient group by ethnicity and sex but did not match by age, a necessity in order to exclude persons with AMD. Another group of controls consisting of 165 age- and sex-matched individuals labeled as Iowa controls. The status of these individuals, if they would have

AMD or any complement-mediated disease in the future, was unknown. Demographics of cases and controls are shown in Table 2-1.

Mutation Screening and Analyses

Coding regions and intron-exon boundary junctions of C3 (NM_000064), Factor B (NM_001710), Factor D (NM_001928), Factor I (NM_000204), CFH (NM_000186), CFHR5 (NM_030787), Clusterin (NM_001831), C3aR1 (NM_004054), C5aR1 (NM_001736), Properdin (NM_001145252), CR1 (NM_000651), CRIg (NM_007268) and ADAM19 (NM_033274) were amplified and screened for mutations and polymorphisms using bi-directional sequencing. Genomic DNA was extracted from blood samples of patients and controls using standard DNA blood extraction kit (Qiagen; Valencia, CA). The PCR reaction included 20ng genomic DNA, 2x NH_4 buffer, 3mM $MgCl_2$, 400uM each dNTP, 50U/ml Taq polymerase and 10% DMSO. PCR amplification consisted of initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds; and final extension at 72°C for 10 minutes.

For C4A deletion, two sets of primers were used to amplify the non-deleted C4A (5.4kb band) and a deleted C4A (5.2kb) (Grant, Kristjansdottir et al. 2000). Long Range PCR for C4A deletion utilized LA Taq (TAKARA Bio, Inc., Japan) with PCR conditions consisting of initial denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 95°C for 1 minute and annealing/extension at 68°C for 1 minute/kb.

The quantitative alleles, H/L, for CR1 was determined using restriction digest with *HindIII* restriction enzyme. The H/L alleles were restriction length fragment polymorphisms due to a base change in intron 27 which was recognized by *HindIII*. H

allele showed 1.8kb band while L allele had 1.3kb and 0.5kb bands (Wilson, Murphy et al. 1986).

Predictions of possible functional effects of mutations in the protein structure were done using Conseq, PolyPhen, SIFT (Sunyaev, Ramensky et al. 2001; Ng and Henikoff 2003; Berezin, Glaser et al. 2004) and SNPs3D (Yue, Melamud et al. 2006). Primer sequences are listed in Appendix A.

Statistical Analyses

The chi-square test of independence was used to detect differences in allele frequencies between cases and controls and p-values$\leq$ 0.05 were considered significant (Preacher 2001, April). The Cochran-Armitage trend test was used to identify linear trends in genotypes and compare these trends between the cases and controls (Armitage 1955). Haplotype analyses for all genes were performed using an expectation-maximization algorithm in Haploview and SNPStats programs (Barrett, Fry et al. 2005; Sole, Guino et al. 2006). For each polymorphism, odds ratio (ORs), 95% confidence interval (CI) and Hardy-Weinberg equilibrium (HWE) were also computed using SNPStats (Sole, Guino et al. 2006).

Once associated variants were identified, the best genetic model for each polymorphism was established by determining the best inheritance model (codominant, dominant or additive) and interaction analysis with covariate sex using SNPStats (Sole, Guino et al. 2006). The best fit model for each polymorphism has the most significant p-value and the lowest Akaike's information criterion (AIC) value which is a tool for model selection by measuring the goodness of fit of an estimated statistical model.

Determination of possible gene-gene interactions was done using three statistical analysis methods. The first was conditional logistic regression (LR) using a full model (SAS 9.1.3). p-values less than 0.05 were considered statistically significant. Odds ratios (OR) and 95% confidence interval (CI) were also computed for each genotype or interaction. The second analysis was the Focused Interaction Testing Framework (FITF) which could perform likelihood-ratio tests in stages that increase in the order of interaction (Millstein, Conti et al. 2006). The output for this program analysis was the false discovery rate (FDR) or the expected false positive rate. A cutoff was determined for each level of interaction (main effect, two-way and three-way). If the computed FDR for each set was lower than the cutoff, then the effect or interaction was significant. The last analysis was done using the Multifactor Dimensionality Reduction (MDR) method, a nonparametric data mining and genetic model-free alternative to logistic regression for detecting and characterizing nonlinear interactions among discrete genetic and environmental attributes (www.epistasis.org). It merges attribute selection, attribute construction, classification, cross-validation and visualization to identify and interpret nonlinear interactions among polymorphisms (Hahn, Ritchie et al. 2003). Results were shown as testing balance accuracy (TBA) in which TBA >0.55 were significant with a cross validation consistency (CVC) of 5/10 or higher. This detects up to three-way interactions and creates a dendogram showing the degree of interaction among the genes in this model using entropy-based measures.

Results

Novel Mutations

Four novel mutations: C3 K1203R (MPGN2-15), C3aR1 L84S (MPGN2-44 and 46), CR1 V1222L (MPGN2-63-1) and ADAM19 G507S (MPGN2-24) were identified upon screening DDD patients (Table 2-2, Figure 2-1). These changes were not found in the 268 controls and in SNP databases. All mutations found changed an amino acid in the protein sequence. Each of these mutations was found in a DDD patient except C3aR1 L84F which was found in two DDD patients. All mutations were not highly conserved based on ConSeq. However, PolyPhen showed possibly damaging effects in C3aR1 L84F and SIFT showed effect in protein function with mutation C3 K1203R. CR1 V1222L and ADAM19 G507S were benign and not damaging based on PolyPhen and SIFT.

Polymorphisms

Several polymorphisms were identified in the genes that were screened. Allele frequencies for major and minor alleles were determined and compared between DDD cases and controls (Appendices B, C, D). Using a Chi square test of independence, polymorphisms with p-value <0.05 were considered significant and hence associated with DDD phenotype. These polymorphisms were CFH Y402H (rs1061170), C3 R102G (rs2330199), CR1 H1022Q (rs3738467), CR1 R1208H (rs2274567), CR1 R1827P (rs3811381), CFHR5 -249 T>C (rs9427661), CFHR5 -20 T>C (rs9427662), CFHR5 P84S (rs1209755), C5aR1 N2D (rs4467185), C5aR1 N279K (rs1100889) and ADAM19 S284G (rs1422795). Polymorphisms in CR1, CFHR5 (except P84S) and C5aR1 were in

linkage disequilibrium (LD). A copy number variant in C4A which rendered a null allele was also shown to be associated with DDD. The quantitative alleles, H/L, in CR1 were also associated with DDD. All variants satisfied the Hardy Weinberg equilibrium (HWE). In most variants, the minor allele was the risk allele except for CFHR5 -249 C>T and -20 C>T which were protective alleles (Table 2-3). Using the Cochran-Armitage trend test, we found that the same polymorphisms as in the Chi-square test had significant p-values (Table 2-4). Possible functional effects and results from Conseq, PolyPhen and SIFT for each associated SNP were also listed (Table 2-5). All SNPs were not highly conserved based on ConSeq. CR1 H1208R and P1827R were noted to be damaging in both PolyPhen and SIFT.

Haplotype analyses were performed using an EM algorithm in all the genes that were screened. For SNPs in the CFH gene, a similar haplotype analysis is done by Pickering et al. using five CFH SNPs: -331T>C (rs3753394), V62I (rs800292), Y402H (rs1061170), Q673 (rs3753396) and D936E (rs1065489) to determine association with the DDD phenotype (Pickering, de Jorge et al. 2007) (Table 2-6A). In this study, haplotype frequencies are computed by EM algorithm but ORs are computed manually (OR of most common haplotype in both cases and control is not 1). Performing the same haplotype analysis, our results showed the same risk haplotype H1 (Table 2-6B). Another risk haplotype H5 was also identified in our results. In contrast, different protective haplotypes were identified in both studies. The protective haplotype H2 was identified in the previous study while H4 haplotype was identified as the protective haplotype in our study. Performing another haplotype analysis using SNPStats to compute the haplotype frequencies and ORs, the H4 haplotype was verified to be the

protective haplotype in our study (Table 2-7). In SNPStats, OR of the most common haplotype is considered to be 1 (Sole, Guino et al. 2006).

A risk haplotype in C3 gene was also identified. Since several polymorphisms were found in this gene, an LD block analysis was performed to identify different blocks in this gene and pick out one interesting block for further analysis (Figure 2-2). Haplotype analysis was performed using the first block because this region contained the risk allele, R102G which was associated with DDD using Chi-square test and Cochran-Armitage trend test. The haplotype analysis showed an at-risk haplotype consisting of 4 SNPs in this block of C3: R102G (rs2230199), R304 G>A (rs2230201), P314L (rs1047286) and P518 C>A (rs1047286) (Table 2-8).

Best inheritance model and interaction with covariate sex

The best inheritance model was identified for each polymorphism using SNPStats (Appendix E). For most polymorphisms, the best inheritance pattern was the dominant model. SNPs in CFHR5 promoter and ADAM S284G had log-additive model as best inheritance pattern. This revealed that DDD was a complex disease but the predominant model of inheritance among the SNPs associated with this disorder was the dominant model (Table 2-9). We also looked at any possible interaction with covariate sex using the best inheritance model for each polymorphism. Our results showed no interaction between SNPs and covariate sex.

Gene-gene interaction

Possible gene-gene interactions among SNPs associated with DDD were determined using three different statistical analyses and algorithm namely MDR, FITF and LR. FITF and LR were not able to show any gene-gene interaction, but had verified

association of C3R102G, C3P314L and FHY402H with DDD phenotype (Table 2-9). The MDR program was able to show a synergistic interaction between CFH V62I and C3 P314L (Figures 2-3 and 2-4). C3R102G as a main effect was verified to be associated with DDD.

Four SNPs - CFHY402H, CHFV62I, C3R102G and C3P314L - were shown to be associated with DDD using different statistical analyses and algorithms. Main effects, two-variant and three-variant combination analysis were performed using these 4 SNPs by utilizing the EM algorithm in predicting allele combination frequencies (Table 2-11). We then computed the ORs, 95% confidence intervals and p-values to determine significant allele combinations. The most significant combination was the CFHY402H x CFHV62I x C3P314L which had the highest OR and significant p-value. Another interesting combination was CFHY402H x C3P314L which also gave the second highest OR and significant p-value. This showed that the presence of two or more risk alleles in the different genes increased the risk in developing DDD.

Discussion

We performed an association study using DDD cases and controls to identify risk allele variants in the genes of the complement system, particularly the alternative pathway, that were associated with DDD. We identified four novel missense mutations in different genes of the complement system in only DDD cases and not in controls. The effect of these mutations in the complement pathway has not been determined. Several possible explanation could elucidate how these mutations affect protein function or structure. The C3 K1203R is located in the TED domain (residues 963-1268) of C3 which is responsible for the binding of target surfaces. The mutation is also in a CFH

binding site (residues 1187-1249). Arginine, which is bulkier than lysine, can cause a conformational change in the CFH binding site which can hinder the binding of C3b to CFH. Because of this, Factor B will be able to bind more to C3b to produce the C3 convertase leading to complement activation. CR1 V1222L is located in SCR19 which is part of LHR-C. This mutation is near the C3b binding site of LHR-C which can possibly change the binding capacity of CR1 to C3b for ineffective regulation of the alternative pathway. C3aR1 L84F is found near the transmembrane domain of the C3aR1 protein. An amino acid change from leucine to phenylalanine can also cause a conformational change in the transmembrane domain which can disrupt the transmembrane domain structure. This change can lead to the inefficient binding of C3a, an anaphylatoxin, to C3aR1. Binding of C3a to its receptor creates signaling pathways that are important in the immune system. ADAM19 G507S mutation is located in the cysteine-rich domain which regulates proteolytic activity of the ADAM19 protein. Mutations in this region can cause the inefficient cleavage of target substrates like complement proteins by ADAM19.

To identify allele variants associated with DDD phenotype, allele frequencies between cases and controls were compared using Chi-square test of independence with significance of $p<0.05$; linear trends were identified among genotype proportions using Cochran-Armitage trend test based on an additive inheritance model. The Cochran-Armitage trend test does not look if the genotype proportions differ significantly between DDD cases and controls, but whether they show significant linear trend with the ordering of the groups. One advantage of using Cochran-Armitage trend test in association studies

instead of Chi-square test is that if HWE is not satisfied, then allele-based test becomes invalid but the Cochran-Armitage Trend test can still be used (Armitage 1955).

Interestingly, the same polymorphisms were associated with DDD in both Chi square test and Cochran-Armitage trend test in our study. For most of these SNPs, the best inheritance pattern was the dominant model, which meant that the presence of one risk allele could influence the phenotype. Possible functional effects of SNPs were assessed by PolyPhen and SIFT. Three polymorphisms were shown to be damaging to protein structure and function. CR1 H1208R, CR1 P1827R and CFHR5 P84S were shown to be damaging in both PolyPhen and SIFT. These mutations are located in a C3b binding site in CR1 and CFHR5 genes and thus can decrease the binding of CR1 and CFHR5 to C3b leading to diminished regulation of complement activation.

Most of the genes associated with DDD were part of the complement system except for ADAM19 which was a metalloprotease. Several complement proteins are membrane proteins but are cleaved by metalloproteases to form a soluble form. One example is CR1, which is an integral membrane glycoprotein expressed in erythrocytes, leukocytes and podocytes of the glomerulus. In vitro, soluble CR1 (sCR1) is released from leukocytes through proteolytic cleavage (Danielsson, Pascual et al. 1994) by elastase and a metalloproteinase not yet identified. Cleavage of CR1 is blocked by protease inhibitors like elafin against elastase (30% inhibition) and 1,10-phenanthroline against metalloprotease (70% inhibition)(Sadallah, Hess et al. 1999). ADAM19 is a membrane glycoprotein that has been shown to cleave cytokines, receptors and transmembrane proteins. It is expressed in the leukocytes and glomerulus similar to the expression pattern of CR1 (Dehmel, Janke et al. 2007). Also, it has been shown to be

inhibited by 1,10-phenanthroline. The role of ADAM19 in the complement system has not been proven but in our study, a SNP in the metalloprotease domain of ADAM19 is associated with Dense Deposit Disease. We hypothesize that ADAM19 can possibly cleave CR1 in leukocytes to produce sCR1.

Besides analyzing allele frequencies and genotype proportions, we determined whether there were risk or protective haplotypes in the different genes we screened. A risk haplotype in C3 which contained C3R102G and C3 P314L was identified in the haplotype analyses (Table 2-8). These polymorphisms are associated with other autoimmune diseases like AMD and aHUS (Finn, Li et al. 1994; Miyagawa, Yamai et al. 2008; Spencer, Olson et al. 2008; Francis, Hamon et al. 2009; Park, Fridley et al. 2009). We also identified the same risk haplotype in CFH as Pickering et al (Pickering, de Jorge et al. 2007) (Table 2-6B). However, we identified another protective haplotype different from the previous study (Tables 2-6B and 2-7). Interestingly, our protective haplotype for DDD has been shown to be a protective haplotype for AMD (Hageman, Anderson et al. 2005; Pickering, de Jorge et al. 2007). Discrepancy between the two studies may be due to the smaller number of cases (n=15) and controls (n=139) in Pickering et.al. compared to our cases (n=71) and controls (n=268). Estimating haplotype frequencies by EM algorithm can be affected if small number cases and controls are used (Excoffier and Slatkin 1995).

In the analyses of gene-gene interactions associated with DDD, we noted that four SNPs in CFH and C3 namely CFH Y402H, CFH V62I, C3 R102G and C3 P314L were consistently shown to be associated with DDD. In the MDR analysis, the two-way interaction between CFH V62I and C3 P314L was shown to be significant and

synergistic. Computing the ORs of risk alleles in CFH and C3 showed that the presence of two or more of these risk alleles increased the ORs in developing DDD (Table 2-11). This implied that in DDD the interplay of genes in the alternative complement pathway could contribute to the pathogenesis of the disorder.

Two SNPs in CFH, Y402H and V62I, have been shown to be associated with AMD and aHUS (Hageman, Anderson et al. 2005; Haines, Hauser et al. 2005; Tortajada, Hakobyan et al. 2007). The presence of H402 decreases the binding of CFH to C-reactive protein (CRP) and heparin, and also affects surface attachments of CFH to membrane surfaces in the eye (Herbert, Deakin et al. 2007; Laine, Jarva et al. 2007; Skerka, Lauer et al. 2007). The V62I is in SCR1 of CFH which is part of a C3b binding site. The I62 allele has been shown to increase the binding of CFH to C3b and thus increasing its cofactor activity (Tortajada, Montes et al. 2009). Since the pathogenesis of DDD involves both systemic and local complement activation, it is most likely that these two SNPs play a role in the development of the disease.

The C3 polymorphisms associated with DDD have been shown to be associated with other autoimmune disease like SLE and aHUS. These SNPs are located in the macroglobulin domains of C3. The C3 R102G, which is also known as C3 F/S allele, is located in MG1 which is near the TED domain. Based on the three dimensional structure of C3 using SNPs3D, the variant is located on the protein surface (Figure 2-5). With arginine at this position, an electronegative interface is created on MG1 that can interact with the strong electropositive region on the exposed surface of the TED domain to stabilize C3b. Glycine, in contrast, is not electronegative and in the absence of stabilizing electrostatic interactions, the binding of the TED domains to target surfaces

can be affected or there can be emergence of new binding sites for C3 autoantibodies (Figure 2-6). A previous study has shown an association between C3F or C3 G102 and the presence of C3 NeF in the serum (Finn and Mathieson 1993). The C3 P314L, also known as the HAV4-1+/- allele, is located in MG3 which is a binding site for Factor B (Torreira, Tortajada et al. 2009). Polyphen and SIFT showed that this SNP was probably damaging and could affect protein function (Table 2.4). This variant is positioned on the protein surface based on SNPs3D (Figure 2-7). The C3 protein with L314 allele reacts to the monoclonal antibody, HAV4-1. The amino acid change from proline to leucine in residue 314 creates a structural alteration that exposes an epitope recognized by the antibody. This structural change in MG3 may enhance the binding of Factor B to C3b via MG3 domain leading to the increased stability of the C3 convertase.

The synergistic interaction of these CFH V62I and C3 P314L is possibly due to fact that the CFH variant decreases binding to C3b which makes it harder to displace Factor B in the C3 convertase. And if the C3 P314L variant, which increases binding to Factor B, is added then CFH will have a doubly hard time to displace Factor B in the C3 convertase. Hence in this scenario, C3 convertase will not be degraded due to inability of CFH to regulate the AP and hence the AP is highly activated.

In summary, an association study using DDD cases and controls to identify mutations and risk alleles associated with DDD which are found mostly in the genes of the complement system. The ones that showed strong association using various statistical analyses are CFH Y402H, CFH V62I, C3 R102G and C3 P314L. A synergistic interaction is also noted between CFH V62I and C3 P314L. Based on our results from the association study, we predict that individuals carrying these mutations or risk alleles

have a higher chance of developing DDD. In order to determine if the novel mutations and associated polymorphisms are pathogenic in nature, functional studies and assays are necessary.

Acknowledgments

We would like to thank all the DDD patients and their families for their participation in this project. We would like to acknowledge all the staff and students in our lab who helped with the mutation screening and analyses. Also, we would like to express our gratitude to Dr. Deborah Dawson for her suggestions with the statistical analyses and to Dr. Joshua Millstein for his help with the FITF program.

Table 2-1. Demographics of DDD cases and controls.

	DDD Cases	AMD(-) Controls	Iowa Controls	AMD(-) + Iowa Controls
Number	71	103	165	268
Males (%)	26 (38%)	38 (37%)	60 (37%)	98 (37%)
Females (%)	45 (62%)	65 (63%)	102 (63%)	167 (63%)
Average Age $\pm$ SD	19.89 $\pm$ 9.86	75.38 $\pm$ 12.11	18.44 $\pm$ 1.37	40.32 $\pm$ 28.76

Note: SD (standard deviation)

Table 2-2. Novel mutations found in DDD patients and not in controls.

Gene	Mutation	Patients (#)	Conseq	Polyphen	SIFT
C3	K1203R	Heterozygote(1)	1	Benign	Affect protein function
C3aR1	L84F	Heterozygote (2)	4	Possibly damaging	Tolerated
CR1	V1222L	Heterozygote (1)	2	Benign	Tolerated
ADAM19	G507S	Homozygote (1)	1	Benign	Tolerated

Table 2-3. Risk and protective alleles associated with DDD.

Gene	Variant	Region	HWE	OR	95% CI
CFH	Y402H	Exon 9	0.18	1.90	1.22-2.95
CFHR5	-249 T>C	Promoter	0.69	0.356	0.15-0.84
	-20 T>C	Promoter	0.69	0.356	0.15-0.84
	P84S	Exon 2	1.00	7.28	0.86-64.74
CR1	Q1022H	Exon 20	0.64	1.86	1.02-3.37
	H1208R	Exon 22	0.64	1.86	1.02-3.37
	P1827R	Exon 33	0.64	1.86	1.02-3.37
	H/L	Intron 27	0.64	1.86	1.02-3.37
C3	R102G	Exon 3	0.54	1.80	1.10-2.93
ADAM19	S284G	Exon 10	0.18	1.61	1.04-2.51
C4A	deletion		0.59	1.88	1.00-3.51
C5aR1	N2D	Exon 2	1.00	4.50	0.89-22.62
	N279K	Exon 2	1.00	4.50	0.89-22.62

Note: HWE (Hardy-Weinberg equilibrium); OR (Odds Ratio); CI (confidence interval). Risk alleles highlighted in red. Protective alleles highlighted in green.

Table 2-4. Risk alleles for developing DDD determined by Chi-square test and Cochran-Armitage trend test.

Gene	Variant	DDD Cases # of minor allele	DDD Cases allele freq	AMD (-) +Iowa Controls # of minor allele	AMD(-) + Iowa Controls allele freq	Chi-square p-value	Cochran-Armitage p-value
CFH	Y402H	63	0.44	188	0.35	0.0051	0.0085
CFHR5	-249 T>C	7	0.049	65	0.12	0.0133	0.0122
	-20 T>C	7	0.045	65	0.12	0.0133	0.0122
	P84S	5	0.035	5	0.009	0.029	0.0219
CR1	Q1022H	32	0.22	81	0.15	0.0348	0.0321
	H1208R	32	0.22	81	0.15	0.0348	0.0321
	P1827R	32	0.22	81	0.15	0.0348	0.0321
	H/L	32	0.22	81	0.15	0.0348	0.0321
C3	R102G	44	0.30	112	0.21	0.0095	0.0136
ADAM19	S284G	63	0.44	150	0.28	0.00018	0.00031
C4A	deletion	25	0.18	40	0.07	0.0026	0.00013
C5aR1	N2D	6	0.04	6	0.011	0.012	0.0118
	N279K	6	0.04	6	0.011	0.012	0.0118

Note: Minor alleles are either highlighted in red (risk) or green (protective). p-values<0.05 are statistically significant.

Table 2-5. Nonsynonymous SNP effects in protein structure and function.

Gene	Variant	Conseq	PolyPhen	SIFT
CFH	Y402H	3	Benign	Tolerated
CFHR5	P84S	3	Damaging	Tolerated
CR1	Q1022H	5	Benign	Tolerated
	H1208R	5	Damaging	Damaging
	P1827R	5	Damaging	Damaging
C3	R102G	3	Benign	Tolerated
ADAM19	S284G	1	Benign	Tolerated
C5aR1	N2D	5	Benign	Tolerated
	N279K	1	Benign	Tolerated
CFH	V62I	6	Benign	Tolerated
C3	P314L	2	Benign	Damaging

Table 2-6. Haplotype analysis of CFH polymorphisms. Risk haplotype is highlighted in pink. Protective haplotype is highlighted in green. p-values<0.05 are statistically significant.

A.

Haplotype	-331T>C	V62I (G>A)	Y402H (T>C)	Q673 (A>G)	D936E (G>T)	Controls Frequency	DDD Cases Frequency	OR (95%CI)	p-value
H1	C	G	C	A	G	0.228	0.47	2.99 (1.38-6.45)	0.0068
H2	C	A	T	A	G	0.266	0.13	0.42 (0.14-1.24)	NS
H3	T	G	T	G	T	0.192	0.17	-	NS
H4	C	G	T	A	G	0.167	0.17	-	NS
H5	T	G	C	A	G	0.075	0.07	-	NS
H6	C	G	T	G	T	0.039	-	-	-

Source: (Pickering, de Jorge et al. 2007)

B.

Haplotype	-331T>C	V62I (G>A)	Y402H (T>C)	Q673 (A>G)	D936E (G>T)	Controls Frequency	DDD Cases Frequency	OR (95%CI)	p-value
H1	C	G	C	A	G	0.2935	0.3949	1.57 (1.07-2.31)	0.021
H2	C	A	T	A	G	0.2056	0.1798	0.87 (0.54-1.77)	NS
H3	T	G	T	G	T	0.0849	0.0804	0.89 (0.45-1.77)	NS
H4	C	G	T	A	G	0.1607	0.1014	0.57 (0.31-1.04)	0.06
H5	T	G	C	A	G	0.0333	0.0758	2.41 (1.11-5.24)	0.021
H6	C	G	T	G	T	0.0088	0.0229	2.29 (0.54-9.73)	NS

Table 2-7. Haplotype analysis of CFH SNPs using SNPStats.

Haplotype	-331T>C	V62I (G>A)	Y402H (T>C)	Q673 (A>G)	D936E (G>T)	Controls Frequency	DDD Cases Frequency	OR (95%CI)	p-value
H1	C	G	C	A	G	0.2935	0.3949	1.00	--
H2	C	A	T	A	G	0.2056	0.1798	0.69 (0.40-1.17)	0.17
H3	T	G	T	G	T	0.0849	0.0804	0.77 (0.38-1.55)	0.46
H4	C	G	T	A	G	0.1607	0.1014	0.46 (0.22-0.93)	0.033
H5	T	G	C	A	G	0.0333	0.0758	1.34 (0.59-3.03)	0.48
H6	C	G	T	G	T	0.0088	0.0229	1.61 (0.37-7.05)	NS

Note: Protective haplotype is highlighted in green. Reference haplotype which is the most common haplotype in both groups, has OR =1. p-values<0.05 are statistically significant.

Table 2-8. Haplotype analysis for C3 SNPs using SNPStats.

Haplotype	R102G (C>G)	R304 (A>G)	P314L (C>T)	P518 (C>A)	DDD Cases Freq.	Controls Freq.	OR (95% CI)	p-value
1	C	G	C	C	0.5276	0.6304	1.00	--
2	G	G	T	A	0.2225	0.1395	1.92 (1.06-3.50)	0.033
3	C	A	C	C	0.103	0.1179	1.24 (0.63-2.44)	0.54
4	C	G	T	A	0.0522	0.0463	0.67 (0.27-1.66)	0.38

Note: Risk haplotype is highlighted in pink. Reference haplotype which is the most common haplotype in both groups, has OR =1. p-values<0.05 are statistically significant.

Table 2-9. Summary of best inheritance model.

Polymorphism	Best Inheritance Model	Odds Ratio (95% CI)	p-value
CFH Y402H	Dominant	2.13 (1.20-3.77)	0.0075
C3 R102G	Dominant	2.17 (1.27-3.69)	0.0043
C3 P314L	Dominant	2.06 (1.21-3.50)	0.0074
C3 P518	Dominant	1.77 (1.04-3.02)	0.0360
CFHR5 -249C>T -20C>T	Log-additive	0.37 (0.16-0.83)	0.0069
CR1 H1022Q R1208H R1827P H/L	Dominant	1.81 (1.05-3.11)	0.0330
ADAM19 S284G	Log-additive	1.96 (1.35-2.85)	4E-04

Note: $p<0.05$ are statistically significant.

Table 2-10. Interaction of genes associated with DDD.

Multifactor Dimensionality Reduction (MDR)	Testing Balance Accuracy (TBA)	Cross validation consistency (CVC)
C3R102G	0.5506	5/10
CFH V62I x C3 P314L	0.5746	5/10
Focused Interaction Testing Framework (FITF)	**False Discovery Rate Cutoff (FDRc) p-value**	**False Discovery Rate (FDR) p-value**
CFH Y204H	0.00625013	0.0055939
C3 R102G	0.00625013	0.00618517
C3 P314L	0.00625013	0.0597087
Logistic Regression (LR)	**Odds Ratio (95% CI)**	**p-value**
CFH Y204H	YY: 1.00 (reference) HY: 1.99 (1.08-3.67) HH: 2.47 (1.18-5.15)	0.0277
C3 R102G	RR: 1.00 (reference) RG: 2.20 (1.26-3.84) GG: 2.00 (0.72-5.46)	0.0164
C3 P314L	PP: 1.00 (reference) PL: 2.02 (1.57-3.52) LL: 2.28 (0.87-5.97)	0.0264

Note: For MDR, TBA>0.55 and CVC at least 5/10 to be considered statistically significant. For FITF, FDRc>FDR to be statistically significant. For LR, $p<0.05$ to be statistically significant.

Table 2-11. Odds ratios of main effects and variant combinations between CFH and C3.

Main effects	**Risk alleles**	**OR**	**95% CI**	**p-value**
CFH Y402H	H	2.13	1.20-3.77	0.0073
CFH V62I	I	0.75	0.43-1.28	0.29
C3 R102G	G	2.17	1.28-3.68	0.0042
C3 P314L	L	2.06	1.22-3.50	0.0072
Two variant combinations				
CFH Y402H x CFH V62I	H x V	1.75	1.15-2.66	0.0096
CFH Y402H x C3R102G	H x G	2.53	1.34-4.79	0.0046
CFH Y402H x C3P314L	H x L	3.14	1.66-5.93	5.00E-04
CFH V62I x C3 R102G	V x G	1.6	0.96-2.68	0.072
CFH V62I x C3P314L	V x L	2.29	1.40-3.72	0.001
C3 R102G x C3 P314L	G x L	1.82	1.16-2.84	0.009
Three variant combinations				
CFH Y402H x CFH V62I x C3 R102G	H x V x G	2.7	1.32-5.51	0.0066
CFH Y402H x CFH V62I x C3 P314L	H x V x L	4.06	2.02-8.15	1.00E-04
CFH Y402H x C3 R102G x C3 P314L	H x G x L	2.23	1.01-4.90	0.048
CFH V62I x C3 R102G x C3 P314L	V x G x L	2.11	1.15-3.87	0.016

Note: $p<0.05$ are statistically significant.

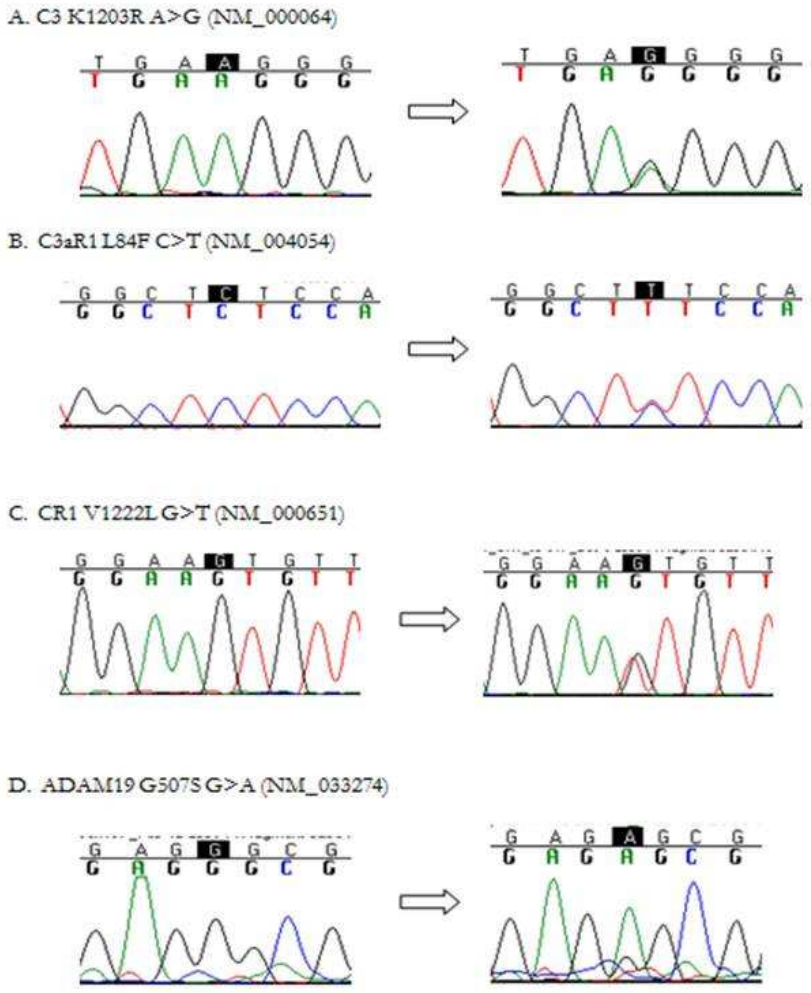

Figure 2-1. Novel mutations found in DDD patients. These mutations were not found in controls.

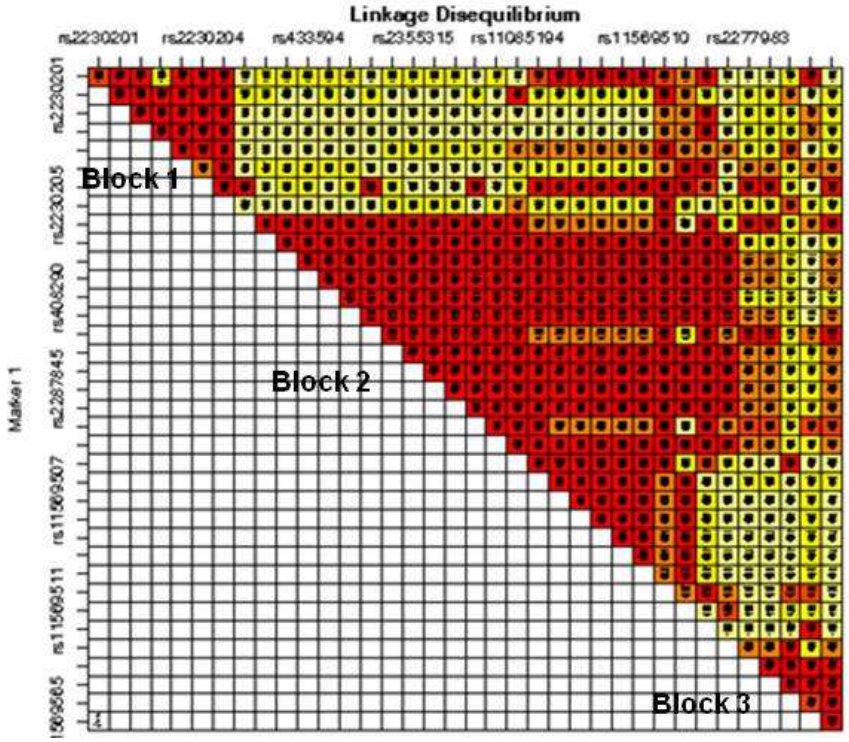

Figure 2-2. Linkage disequilibrium (LD) block analysis of C3 polymorphisms. Using SNPStats, three LD blocks were produced in our study. Red squares signified marker or SNP pairs were in LD. Yellow squares signified marker or SNP pairs were in linkage equilibrium.

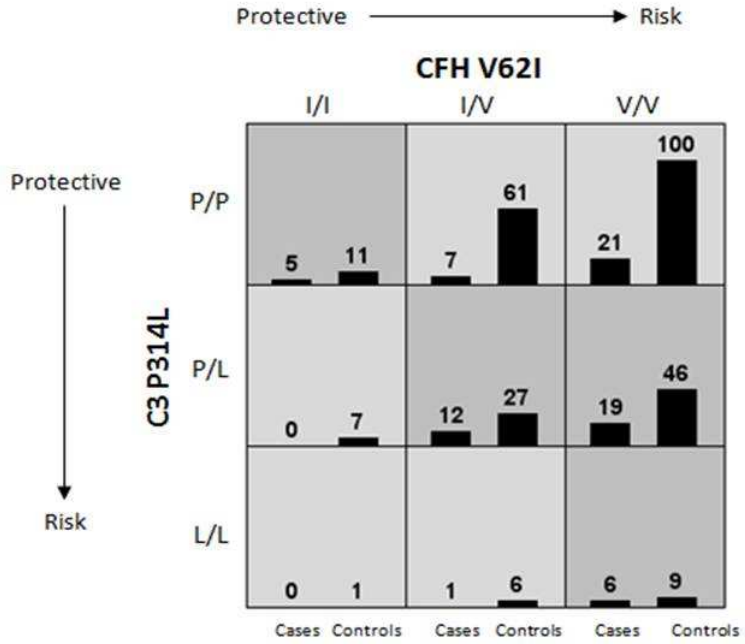

Figure 2-3. Summary of two-loci genotype combinations associated with DDD. For each cell, the cases are indicated by the left bar and controls the right bar. Each multilocus genotype combination is regarded as high risk (dark shaded box) when ratio of cases and controls are over a certain threshold equal to the ratio of cases and controls in each population. If the ratio does exceed the threshold then the combination is considered low risk (light shaded box). The presence of risk alleles, CFH V62 and C3 L314 in the genotype combinations were considered high risk.

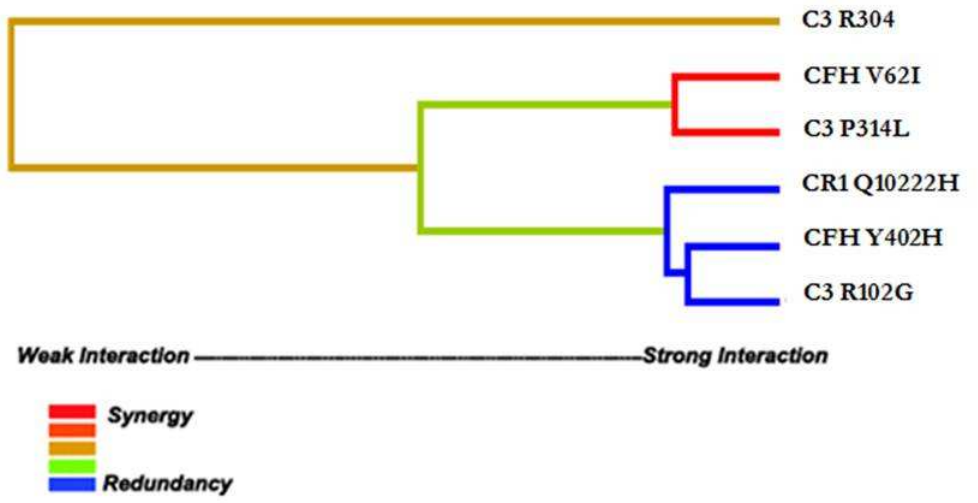

Figure 2-4. Dendogram illustrating the synergistic interaction between CFH and C3. Red lines indicate strong synergistic interaction. Blue lines indicate redundant interaction or no interaction.

Figure 2-5. Location of C3 R102G in the protein surface of C3. This variant is on the protein surface of MG1 domain based on the 3D structure of C3 (SNPs3D).

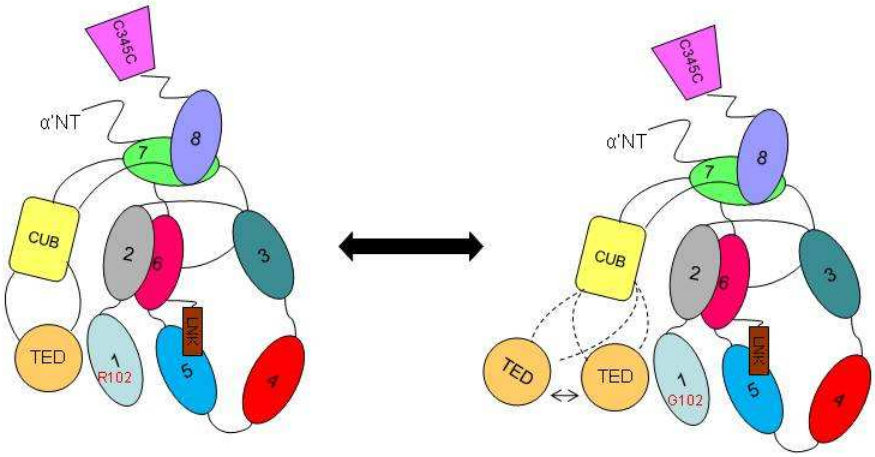

Figure 2-6. Structure of C3b. C3b is composed of several domains including eight macroglobulin domains (MG1-8) and a thioester domain (TED) which is important in the binding of C3b to target surfaces. MG1 (electronegative) and TED (electropositive) closely interact with one another and the interaction is stabilized by the differences of charges between the two domains. The presence of glycine in residue 102 can decrease that electronegativity of MG1 leading to instability of its interaction with TED. The instability of the interaction between MG1 and TED can expose epitopes for autoantibodies (e.g. C3NeF).

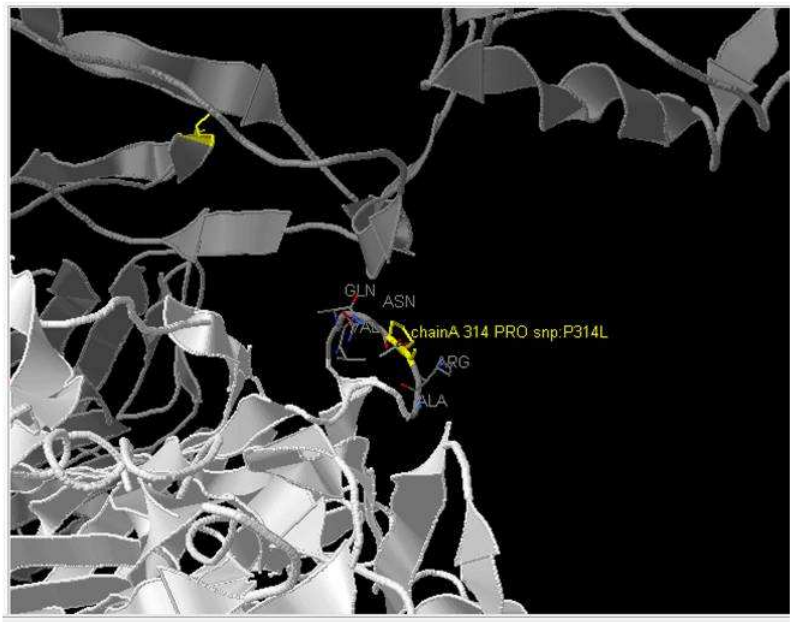

Figure 2-7. . Location of C3 P314L in the protein surface of C3 This variant is on the protein surface of MG3 based on the 3D structure of C3 (SNPs3D).

CHAPTER III

FUNCTIONAL ACTIVITY OF THE ALTERNATIVE PATHWAY OF THE COMPLEMENT SYSTEM

Introduction

Dense Deposit Disease (DDD) is a complex and rare disease primarily characterized by abnormal electron-dense deposits at the glomerular basement membrane of the capillary walls of the kidneys. It is usually diagnosed in children 5-15 years of age and affects both sexes equally. About half of affected persons develop end-stage renal disease with a comorbidity of visual impairment due to the development of drusen (ocular deposits in the Bruch's membrane) (Colville, Guymer et al. 2003). DDD can also be associated with acquired partial lipodystrophy (APL) (Mathieson and Peters 1997), a condition in which subcutaneous fat is lost in the upper half of the body resulting in a strikingly haggard facial appearance.

The pathogenesis of DDD is related to the dysregulation of the alternative pathway of the complement cascade in the fluid phase. Levels of serum C3, the most abundant complement protein in the serum, are decreased as a result of continuous autoactivation and C3 consumption (West and McAdams 1999). Regulatory mechanisms controlling activation of the alternative pathway include Factor H, Factor H-like 1, Factor I, C4 binding protein (C4BP), membrane co-factor protein (MCP, CD46), decay accelerating factor (DAF, CD55) and complement receptor 1 (CR1, CD35), all proteins encoded by genes in the Regulation of Complement Activation (RCA) locus on chromosome 1q32 except Factor I. The similar feature shared by proteins in the RCA

locus is the presence of a 60-amino acid short consensus repeat (SCR) of varying numbers in each protein (Appel, Cook et al. 2005).

Factor H (CFH), the most abundant complement regulator protein in the serum, is a soluble glycoprotein that is produced mainly in the liver and circulates in blood. It destabilizes C3 convertase by binding into three sites of the C3 protein. This binding inactivates C3 convertase and inhibiting complement activity. CFH can also bind to other molecules on cells and membranes to protect these surfaces from damage due to the activation of the complement system. Of note, CFH binds to heparin (Rodriguez de Cordoba, Esparza-Gordillo et al. 2004). The discriminatory activity of CFH depends on the specific SCRs it contains, which recognize sialic acid and other negatively charged glycoaminoglycans (Meri and Pangburn 1990). The function of CFH has not only been studied in humans but also in pigs and mice by generating CFH deficient animal models. Both the CFH-deficient pig and mouse models develop a renal phenotype similar to human DDD (Hogasen, Jansen et al. 1995; Hegasy, Manuelian et al. 2002; Pickering, Cook et al. 2002), providing evidence that DDD is caused by the uncontrolled activation of the complement cascade.

In the previous chapter, we showed that allelic variants of C3, CFH and other complement proteins were associated with DDD. These variants affect the binding capacity of C3 and CFH for complement activators, complement regulators, pathogens and other substrates. Since the alternative pathway is continuously activated at a low rate in human plasma, we hypothesize that the activity of the alternative complement pathway differs among individuals depending on the allele variants one carries. We further hypothesize that individuals who carry risk alleles for DDD have intrinsically higher

complement activity. In contrast, individuals with protective alleles have lower complement activity.

Materials and Methods

DNA and Serum Samples

One hundred two (102) blood samples were obtained from the Mississippi Valley Regional Blood Center in Davenport, Iowa and spun down to separate serum from blood cells. Serum was immediately frozen and kept at -70C until use. DNA was extracted from white blood cells using standard DNA blood extraction kit (Qiagen, Valencia, CA). Each sample was genotyped and screened for CFH and C3 SNPs associated with DDD (C3 R102G, R304, P314L, P518; CFH -331T>C, V62I, Y402H, Q673, D936E) (Appendix F).

C3 and CFH Serum Level Determination

Serum levels of C3 and CFH were determined using commercially available kits (Genway Biotech, Inc., San Diego, CA and Hycult Biotechnology, The Netherlands respectively). Both kits are solid-phase enzyme-linked immunosorbent assays based on the sandwich principle. Serum concentrations were calculated from a standard plot using either a linear or a four-parameter fit (Appendix G).

Alternative Pathway Hemolytic Assay

The AP hemolytic assay was based on a standard hemolytic assay protocol (Whaley 1985) in which rabbit red blood cells are washed and resuspended in buffer to a concentration of $1x10^8$/ml. Serum is diluted 1:10 and increasing volumes of diluted serum (30, 50, 60, 70, 80, 90, 100, 120 ul) are placed in a tube, adding buffer to a total

volume of 150ul (negative control, 150ul buffer; positive control, 150ul distilled water). 20ul of rabbit RBCs are added to each tube and tubes are incubated at 37°C for 1 hour. Tubes are then centrifuged and 100ul of the supernatant is transferred to a microtiter plate. Absorbance (Abs) is measured at 415nm using a microplate reader. Fractional hemolysis for each reading is computed as

% hemolysis (y) = (Abs sample - Abs blank)/(Abs positive control - Abs blank) x 100.

Each value is plotted against serum dilution (x) for each individual to create an S-shaped curve, which is converted to a linear function of serum dilution (x) vs y/(1-y). The volume at which y/(1-y) = 1 is defined as the APH50, i.e. that volume of serum in which 50% of RBCs hemolyze. This definition means that the lower the APH50, the more active the AP complement activity (Appendix G).

Alternative Pathway Functional Immunoassay

In brief, the assay utilized microtitre strips coated with specific activators of the AP. Test serum was diluted with diluents containing specific blockers to ensure only AP was activated. Upon addition of test serum into the wells, complement was activated by the specific coating. The C5b-9 formed during the complement cascade was detected with a specific alkaline phosphatase-labeled antibody. Detection of specific antibodies was done upon addition of alkaline phosphatase substrate solution. The amount of AP complement activity was correlated to the intensity of color produced in the reaction and absorbance was measured at 405nm. %AP was calculated as $(Abs_{sample}-Abs_{negative\ control})/(Abs_{positive\ control}-Abs_{negative\ control})x100$. The interpretation of data from this assay is that a lower %AP signifies an activated AP complement activity (Wieslab® COMPL AP330 kit; ALPCO Immunoassays, Salem, NH) (Appendix G).

Statistical Analyses

For testing association of complement protein levels (C3 and CFH) or complement activity (APH50 and %AP) with CFH and C3 risk alleles, two groups were considered: one group consisted of all individuals homozygous for the major allele; the second group consisted of all individuals homozygous or heterozygous for the minor allele. Association with complement activity was also determined using two variant combinations so that one group consisted of major alleles in all variants and another group consisted of at least a minor allele in all variants. Conditional logistic regression analysis (LR) using a full model was performed in which p-values <0.05 were considered significant. P-values for Pearson goodness of fit test was obtained to determine if the predicted model was able to describe the data well (p-value>0.05 rejected null hypothesis that data did not fit model or simply that goodness-of-fit model was a good model for the observed data). Odds ratio and 95% confidence interval were computed to determine if alleles are risk or protective. Association of predicted probabilities and observed responses was analyzed by computing %concordant, %disconcordant and concordance index (c). Association was also analyzed by performing a Mann-Whitney U-test (two variables) or Kruskal-Wallis Test (three or more variables) by comparing mean and standard deviation (SD) values between groups of major and minor alleles. All statistical analyses were done using SAS 9.1.3.

Results

Significant association was found between APH50 and CFH V62I, C3 R102G and C3 P314L polymorphisms using conditional logistic regression ($p= 0.0193, 0.0166, 0.0054$ respectively). Pearson goodness-of-fit test p-value for the models were $p>0.05$,

which indicated that the predicted model was a good model for the observed data. Odds ratio indicated that presence of risk alleles CFH V62, C3 G102 or C3 L314 increased the risk to 2x of developing low APH50 or high complement activity. Concordance index for the models was approximately about 0.56-0.57 which implied about 56-57% of the time that this model could discriminate the response. The association between APH50 and C3 R102G and C3 P314L were verified using Mann-Whitney U-test by comparing the means between homozygote for R102 or P314 and homozygote and heterozygote for G102 or L314. The group with either G102 or L314 had significantly lower APH50 compared to the group with R102 or P314 (p=0.045, 0.0176) (Table 3-1 and Figures 3-1 and 3-2). Using two variant combinations, the group with G102 and L314 together demonstrated lower APH50 thus higher complement activity compared to the group with R102 and P314 (Figure 3-4)

There was no association with any CFH or C3 polymorphism with %AP using LR analyses. However, comparing the two groups with major and minor alleles showed that the group having the CFH H402 had lower %AP or high complement activity than the group homozygote for the CFH Y402 (p=0.0345) (Figure 3.5).

A significant association was found in LR analyses between CFH serum level and CFH Y402H (p=0.0145). Interestingly, association between CFH serum level and C3 R102G and C3 P314L was also noted in LR analyses (p=0.056, 0.043 respectively). Pearson goodness-of-fit test for CFH Y402H, C3 R102G and C3 P314L indicated a good model fit for the observed data (p>0.05). Odds ratio showed that presence of CFH H402, C3 G102 or C3 L314 doubled the risk of having low CFH serum level. Concordance index ranged from 0.55-0.568 indicating that about 55-57% of the time that the model

could discriminate the response (Table 3-1). No association was found with genotypes and C3 serum levels.

Discussion

Uncontrolled alternative pathway complement activation can be caused by several factors including low levels of CFH, the presence of C3 Nephritic Factor (C3NeF) which stabilizes C3 convertase, and increased binding between C3 and Factor B. Complement activity can be measured in the serum through functional assays like hemolytic assays, immunoassays and determination of complement protein serum levels. These functional assays are able to gauge deficiencies in complement protein or in any specific complement pathway. C3 and CFH serum levels can be used to assess complement activity. Low C3 serum levels or hypocomplementemia indicate high complement activity since C3 is being used up to produce more C3 convertase during the amplification loop in the complement cascade. Genetic and environmental factors can influence CFH serum levels in humans (Esparza-Gordillo, Soria et al. 2004). Interestingly, the CFH H402 allele has been shown to be a low-expressing allele compared to Y402 in heterozygotes indicating that genetic variations can affect CFH serum levels (Tortajada, Hakobyan et al. 2007).

Two different types of assays were used to measure complement activity. First is the alternative pathway hemolytic assay (APHA) which utilizes rabbit red blood cells since these cells do not have membrane-bound complement regulators, making it an activated surface for AP activation. During AP activation, the C3 convertase forms on the cell surface which leads to the formation of the complement cascade and this lyses the RBCs. Complement regulators present in the serum are able to regulate the activity of

AP in these cells and prevent the cell lysis. The AP hemolytic assay is able to monitor complement initiation to the terminal stage and thus, can directly measure functional complement activity or regulation based on hemolysis of RBCs. An advantage in using hemolytic assays is that it can detect consumption-related complement activity and complement protein deficiencies. The disadvantage of hemolytic assays is the problem in standardization of the assay which makes it difficult to adapt it as a routine laboratory work (Jaskowski, Martins et al. 1999)..

The second assay we performed using ELISA is an alternative assay to determine complement activity by measuring the formation of C5b-9 complex in the presence of the neoantigen in the wells of the assay plates. Thus, this assay indirectly measures complement activity or regulation. The immunoassay is more sensitive than the hemolytic assays, which are not sensitive enough to identify small changes in complement activity due to difficulty in standardization and technique errors Also, results of hemolytic assays can be influenced in the presence of deficiencies of certain complement proteins like Factor B, Factor D. On the other hand, the ELISA assay quantitates the level of C5b-9 formed in the test samples but it does not show if the complex is functional to produce cell lysis which can be shown in the hemolytic assays. Despite the differences, the risk alleles identified in our association study for DDD were the same risk alleles identified that affect complement activity.

In our study, we determined that genetic variants in C3 and CFH could influence complement activity and levels of CFH in the serum. We predicted that risk alleles in C3 and CFH could lead to high complement activity and low CFH serum levels. These risk alleles which included CFH V62I, CFH Y402H, C3 R102G and C3 P314L were also the

same variants associated with DDD in our previous study. However, possible interaction between CFH V62I and C3 P314L had not been determined in this study. One possible reason was that the minor allele CFH I62 had a low frequency in the sample population which could affect results from the statistical analyses. This was the first time that risk alleles in CFH and C3 had been shown to affect complement activity. CFH H402 has been shown to be a low-expressing allele in serum of aHUS patients (Tortajada, Hakobyan et al. 2007). We had looked at possible effects of CFH Y402H in the protein expression and determined that there was a removal of exon splice enhancer (ESE) site for splicing factorbbbb SRp55 in H402 alleles using ESEfinder program. SR proteins play a role in the constitutive and regulated splicing of pre-mRNA (Lin and Fu 2007). Removal of ESE sites for SR proteins like SRp55 can lead to the incorrect splicing of mRNA which can generate alternative protein forms that can be nonfunctional or dysfunctional (Manley and Tacke 1996; Long and Caceres 2009). Based on the concordance index, presence of any of these risk alleles can predict complement activity about 57% of the time. Environmental factors including age and history of smoking have been considered to affect CFH serum levels (Esparza-Gordillo, Soria et al. 2004). It is still possible that other unknown genetic and environmental factors can also influence complement activity and serum levels of complement protein.

Since DDD patients have highly active AP complement system which could be due to increased activation or decreased regulation of AP, we wanted to determine if we would be able to predict the functional activity of the AP complement system based on an individual's genetic makeup. Since CFH and C3 are the most abundant complement proteins in the serum, we have determined the presence of risk alleles in these genes in

the serum of normal individuals. Next, we have measured complement activity through hemolytic assay and immunoassay. Our results showed that risk alleles in CFH and C3 increased complement activity which could in fact lead to the development of DDD especially if the complement cascade had been activated or could not be regulated. Thus, the presence of the risk alleles in normal individuals could be used as a predictor if DDD or other complement-mediated disease would occur in these individuals.

Future Directions

Serum and blood samples used in this study are from individuals randomly obtained from a blood bank. The same experiment can be performed using serum samples from DDD patients and age- and sex-matched controls which will be able to predict more genetic factors that can influence complement activity leading to the development of DDD. We have only analyzed C3 and CFH variants in this study but we suspect that other complement activators and regulators can also influence complement activity. For future experiments, serum levels of the different complement components and regulators (e.g. Factor B, Factor I, etc.) can be measured and analyzed for possible association with the different risk alleles in these genes. Identification of the different genetic factors that affect complement activity can lead to determine the susceptibility of an individual carrying risk alleles for developing DDD. Also, this can provide new knowledge that will aid in developing appropriate diagnosis and treatment for DDD or other complement-mediated disorders.

Acknowledgments

We would like to thank Dr. Yuzhou Zhang for performing most of the AP ELISA assays. We are also grateful to Dr. Louis Katz and the staff of the Mississippi Valley Regional Blood Center in Davenport, Iowa for providing the blood and serum samples.

Table 3-1. Association of complement activity with risk alleles.

Main Effect	Pearson Goodness of Fit	Analysis of Effect p-value	Odds Ratio	95% Confidence Interval	% Concordant	% Disconcordant	Concordance Index (c)
APH50							
C3R102G	0.2871	0.0166	2.387	1.172-4.865	29.9	17.4	0.562
C3 P314L	0.3472	0.0054	2.773	1.353-5.686	30.9	16.3	0.573
CFH V62I	0.3591	0.0193	2.337	1.148-4.758	29.9	17.4	0.562
CFH Serum Level							
CFH Y402H	0.3392	0.0145	2.388	1.188-4.796	31.4	17.8	0.568
C3 R102G	0.3817	0.0566	1.986	0.981-4.022	29.2	18.1	0.555
C3 P314L	0.5005	0.0433	2.073	1.022-4.203	29.4	17.8	0.558

Note: Analysis of effects p-value<0.05 are statistically significant. Pearson of Goodness of Fit p-value>0.05 have a good fit of the logistic curve.

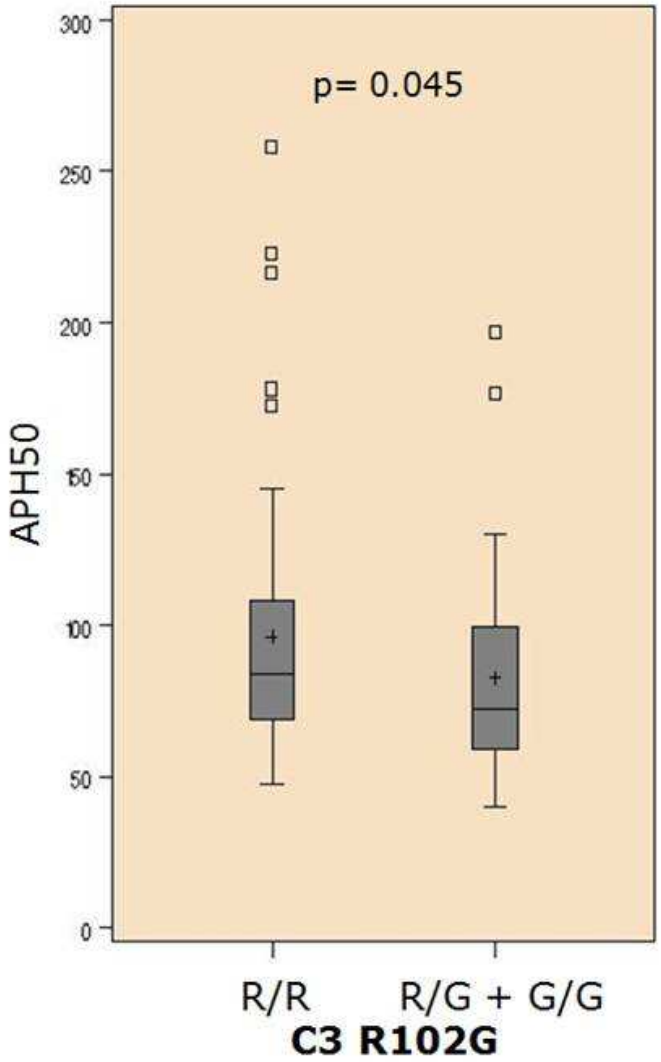

Figure 3-1..Association between C3 R102G and APH50 using Mann-Whitney U-test. Box plot shows that individuals with risk allele G102 have lower APH50 than those who are homozygous of protective allele R102 (p-value = 0.0450). Legends: (+) – mean; line in box – median or 50th percentile ; lower side of box – 25th percentile; upper side of box – 75th percentile; lower side of linear bar – minimum value; upper side of linear bar – maximum value.

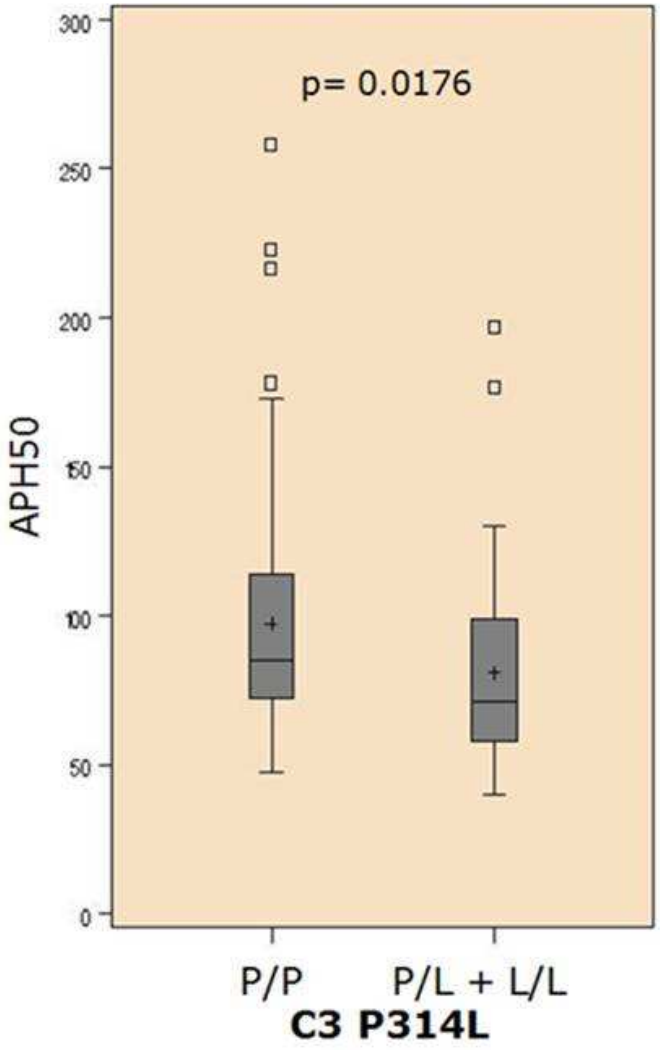

Figure 3-2. Association between C3P314L and APH50 using Mann-Whitney U-test. Individuals with risk allele L314 have lower APH50 than those who are homozygous of protective allele P314 (p-value = 0.0176). Legends: (+) – mean; line in box – median or 50^{th} percentile ; lower side of box – 25^{th} percentile; upper side of box – 75^{th} percentile; lower side of linear bar – minimum value; upper side of linear bar – maximum value.

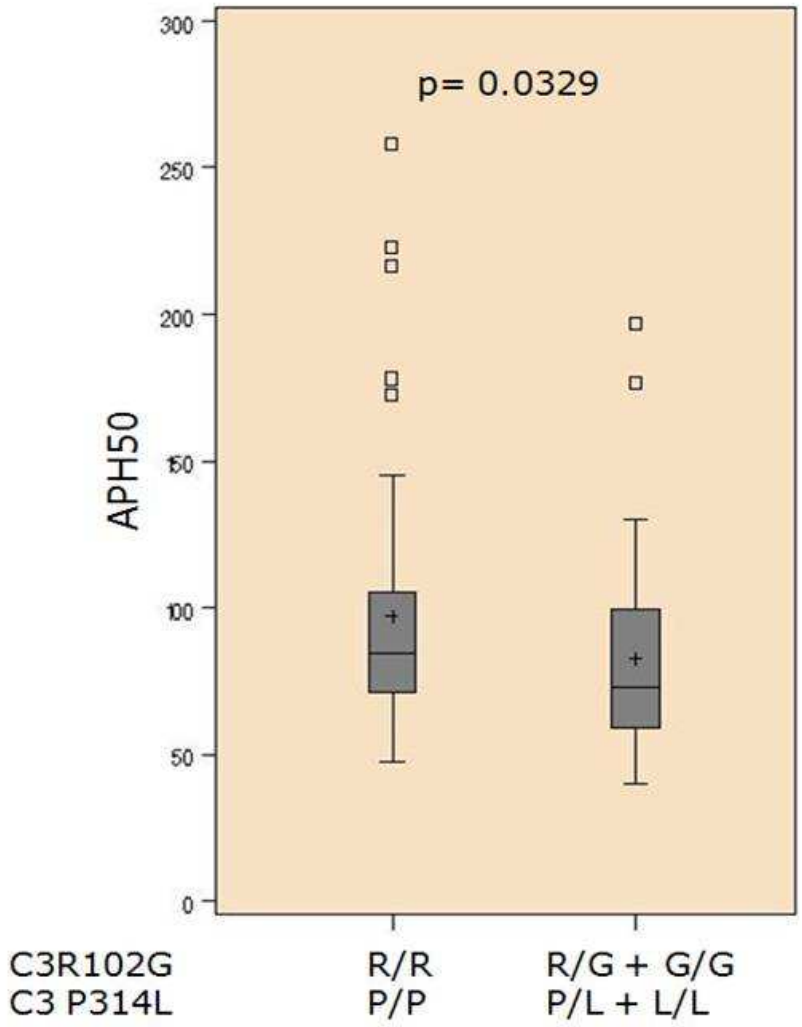

Figure 3-3. Association between two-variant combination of C3 R102G and C3 P314L with APH50 using Mann-Whitney U-test. Individuals with G102 and L314 have lower APH50 compared to individuals with R102 and P314. Legends: (+) – mean; line in box – median or 50th percentile ; lower side of box – 25th percentile; upper side of box – 75th percentile; lower side of linear bar – minimum value; upper side of linear bar – maximum value.

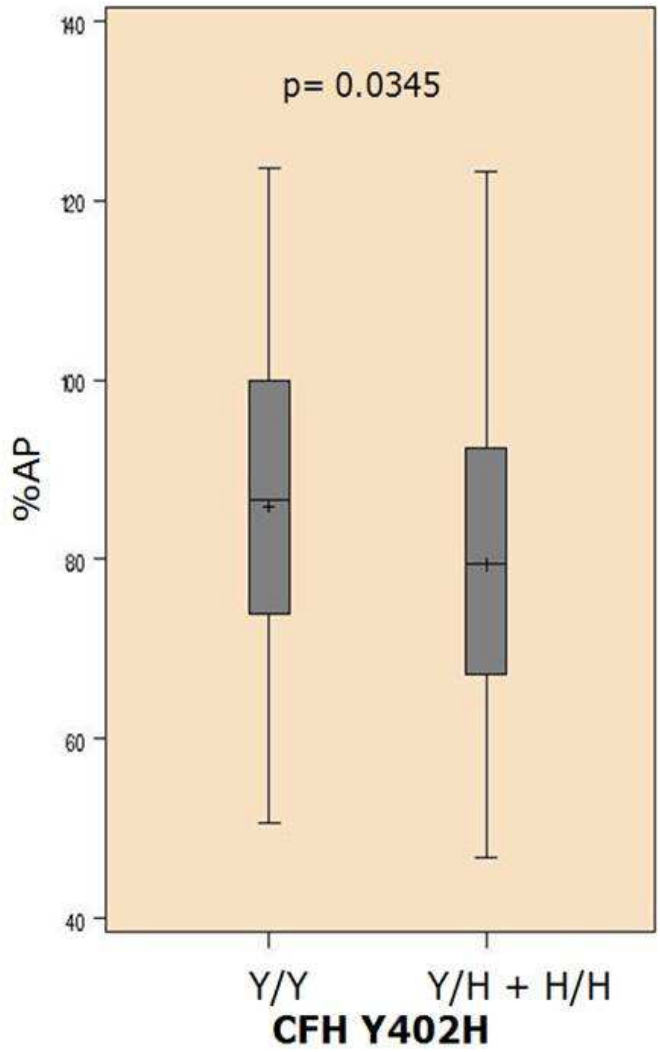

Figure 3-4 Association between CFH Y402H with %AP using Mann-Whitney U-test. Individuals with risk allele H402 have lower %AP than those who are homozygous of protective allele Y402 (p-value=0.0345). Legends: (+) – mean; line in box – median or 50^{th} percentile ; lower side of box – 25^{th} percentile; upper side of box – 75^{th} percentile; lower side of linear bar – minimum value; upper side of linear bar – maximum value.

CHAPTER IV

DEFICIENCY OF COMPLEMENT FACTOR D ABOLISHES DENSE DEPOSIT DISEASE IN FACTOR H-DEFICIENT MICE

The following paper by Sethi et. al. is recently submitted to Journal of American Society of Nephrology (JASN) for publication. A paper we have previously published (Abrera-Abeleda, Xu et al. 2007) have demonstrated that *Cfh*$^{-/-}$ mice have abnormal renal function, active complement activity and abnormal renal pathology similar to DDD in humans. On the other hand, *Cfd*$^{-/-}$ mice demonstrated mesangial deposits containing C3 and IgM in the kidneys. Our results suggest the Factor D or AP activity is needed to prevent spontaneous accumulation of C3 and IgM deposits in the mesangium. In this study, we have generated a *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ double knockout and assessed the renal function, complement activity and renal phenotype in comparison to wildtype, *Cfh*$^{-/-}$ and *Cfd*$^{-/-}$ mice.

I was involved with breeding of the double knockout mice, harvesting renal and hepatic tissues, performing renal function tests, determining expression levels and performing statistical analyses. I was also involved in writing, editing and revising the paper and creating figures and tables for the paper.

Deficiency of Complement Factor D abolishes Dense Deposit Disease in Factor-H deficient mice

Sanjeev Sethi [1#], Maria Asuncion Abrera-Abeleda [2#], Yuanyuan Xu [3], Richard J.H.Smith [2,4*]

[1]Department of Laboratory Medicine and Pathology, The Mayo Clinic, Rochester, MN; [2]The Interdepartmental PhD Program in Genetics and [4]Departments of Otolaryngology and Internal Medicine, University of Iowa, Iowa City, IA; [3]Department of Medicine, University of Alabama, Birmingham, AL.

# These persons contributed equally to this work

* Corresponding author:

Richard J.H. Smith

Carver College of Medicine

Department of Internal Medicine – Division of Nephrology

University of Iowa

5270 CBRB

Iowa City, IA 52242, USA

Tel: +319 335 6501

Fax: +613 353 5869

E-mail: richard-smith@uiowa.edu

Abstract

Factor H and Factor D are regulatory proteins of the complement alternative pathway. Glomeruli of mutant mice with a targeted deletion of Factor H (*Cfh*-/- mice) show features of Dense Deposit Disease (DDD, also known as membranoproliferative glomerulonephritis type II) with mesangial expansion, endocapillary proliferation, intense C3 deposition along glomerular capillary walls and subendothelial electron-dense deposits. Mutant mice with a targeted deletion of Factor D ($Cfd^{-/-}$ mice), in contrast, develop mesangial immune-complex glomerulonephritis with an increase in mesangial matrix and cellularity accompanied by para-mesangial deposition of C3 and IgM. To determine whether Factor D plays an essential role in the development of DDD in $Cfh^{-/-}$ mice, we generated $Cfh^{-/-}.Cfd^{-/-}$ mice. These animals do not develop membranoproliferative or mesangial immune-complex glomerulonephritis and have normal renal function. These findings suggest that therapy targeting Factor D or abrogating its function in the complement cascade could play role in treatment of DDD.

Introduction

Activation of the alternative pathway (AP) of the complement cascade leads to the generation of two convertases that are integral to AP amplification and the development of membrane attack complex (MAC), the C3 and C5 convertases, respectively. Because low levels of C3 convertase are continually produced in a process known as 'tick-over', exquisite AP control is essential if untoward complement-mediated damage is to be prevented (Appel, Cook et al. 2005; Turnberg and Cook 2005). In the absence of AP control, a spectrum of renal diseases can develop that includes Dense Deposit Dense (DDD, also known as membranoproliferative glomerulonephritis type II) and atypical Hemolytic Uremic Syndrome (aHUS), a type of thrombotic microangiopathy (Rodriguez de Cordoba, Esparza-Gordillo et al. 2004; Smith, Alexander et al. 2007; Jozsi and Zipfel 2008; Skerka, Licht et al. 2009).

Factor D is a highly specific serine protease with only one known function – to catalyze the enzymatic reaction that leads to the formation of C3bBb (C3 convertase) from C3bB. In this step factor B (93kDa) is cleaved to release a 30kDa activation fragment called Ba while the larger portion called Bb (63kDa) remains bound to C3b. Factor D is indispensable for AP amplification and in its absence cleavage of factor B cannot occur and the C3 convertase cannot form (Volanakis and Narayana 1996). Mice deficient in Factor D (*Cfd-/-* mice) develop mesangial immune complex glomerulonephritis characterized by mesangial immune deposits that stain for C3 and IgM and are associated with both mild mesangial expansion and mild mesangial proliferative features in the absence of infiltration by extrinsic cells (Abrera-Abeleda, Xu et al. 2007).

Factor H is the major fluid-phase regulator of AP activation. Encoded by the Complement Factor H (*CFH*) gene on chromosome 1, this protein belongs to the regulators-of-complement-activation (RCA) family and controls AP activity in the fluid phase in three ways. It has: 1) decay accelerating activity (DAA), which facilitates the decay of C3bBb to C3b and Bb; 2) cofactor activity for factor I-mediated inactivation of C3b to iC3b (inactive C3b); and, 3) competitive activity with factor B for Cb3 binding. Factor H also provides surface regulation of AP activity through its cofactor activity and its ability to bind to biological membranes (Rodriguez de Cordoba, Esparza-Gordillo et al. 2004). The importance of factor H in AP control is illustrated by the observation that individuals with genetic deficiency of factor H develop DDD in the face of a markedly reduced plasma C3 level, which arises as a consequence of uncontrolled spontaneous AP activation and C3 consumption (Pickering, Cook et al. 2002; Appel, Cook et al. 2005; Smith, Alexander et al. 2007). Mice deficient in factor H (*Cfh*-/- mice) reflect the human phenotype – they have extremely low plasma C3 levels and increased mortality secondary to kidney failure. Renal histology shows marked mesangial matrix expansion, mesangial hypercellularity, peripheral capillary loop thickening with deposition of periodic acid–Schiff (PAS)-positive material, double-contouring of the glomerular basement membranes (GBMs) and the presence of subendothelial electron-dense deposits (Pickering, Cook et al. 2002).

Since deficiency of factor H results in uncontrolled C3 convertase activity and factor D is required for C3 convertase formation, we hypothesized that deficiency of factor D might protect *Cfh-/-* mice from developing renal disease. To test this hypothesis

we studied 8-month-old *Cfh-/-.Cfd-/-* mice and compared renal function and pathology of these animals with that of *Cfh*-/- and *Cfd*-/- mice and wild-type controls.

Materials and Methods

Animals

Study animals included factor H deficient ($Cfh^{-/-}$), factor D deficient ($Cfd^{-/-}$) and factors H and D deficient ($Cfh^{-/-}.Cfd^{-/-}$) mice congenic on a C57BL/6 background; 8-month-old C57BL/6 mice were used as controls. Each study group consisted of five mice - two males and three females. All procedures were approved by the University Animal Care and Use Committee (UACUC) of the University of Iowa.

Genotyping

Mouse genotypes were determined by a PCR-based assay using DNA extracted from mouse tail snips (Wizard® Genomic DNA Purification Kit; Promega Corp, Madison, WI). Primers used were CFH forward (5'-GTAAAGGTCCTCCTCCAAGAG-3'), CFH reverse (5'-GGTATAAACAACAACCTTTGCACC-3'), CFH insert (5'-GGGGATCGGCAATAAAAAGAC-3'), CFD forward (5'-ATGACGACTCTGTGCAGGTG-3'), CFD reverse (5'GGTTGCTCTCTGCACACAT-3') and CFD insert (5'GGAGAACCTGCGTGCAAT-3'). PCR products were amplified using the following conditions: initial denaturation at 95°C for 5 minutes; 35 cycles of denaturation at 95°C for 30 seconds, annealing at 63°C for 30 seconds and extension at 72°C for 30 seconds; and a final extension at 72°C for 10 minutes. In $Cfh^{-/-}$ mice, PCR amplification resulted in either 600bp (wild-type) or 500bp products ($Cfh^{-/-}$ mice); in $Cfd^{-/-}$ mice, PCR products were either 580bp (wild-type) or 480bp ($Cfd^{-/-}$ mice). To verify

loss of factor H and factor D protein expression, immunoblotting was done using protein lysates from mouse liver or kidney (PARIS™; Ambion, Austin TX). Cell lysates were run in 10% SDS-PAGE and protein expression was visualized using antibodies against Cfh (ab8842; Abcam Inc, Cambridge, MA) and Cfd (Adipsin, sc-12402; Santa Cruz Biotechnology, Santa Cruz, CA). Beta-tubulin (ab6046; Abcam Inc, Cambridge, MA) expression was used as a control.

Renal Function

All mice were housed in individual metabolic cages to collect urine for 24 hours prior to being euthanized. At the time of euthanasia, blood was taken to assess renal function by measuring plasma creatinine and blood urea nitrogen (BUN) using the Vitros® 350 (Ortho Clinical Diagnostics; Langhorne, PA) at the Animal Fluid Analysis Core in the University of Iowa. Urine creatinine and albumin were measured using colorimetric methods (QuantiChrom™ Creatinine and BCP Albumin Assay Kits, BioAssays Systems; Hayward, CA). Creatinine clearance (ul/min) was used as an index of glomerular filtration rate and was calculated as the product of the urinary creatinine and 24-hour urinary flow rate divided by the serum creatinine. The presence of microalbuminuria was assessed by computing the ratio of urine albumin to creatinine (ug/ug). All data were expressed as the mean $\pm$ SD. Statistical significance was determined using one-way analysis of variance (ANOVA) (GraphPad Prism, San Diego, CA).

C3 Plasma and Expression Levels in Liver and Kidney

C3 plasma levels were determined using a commercially available kit utilizing a two-site enzyme-linked immunoassay (Kamiya Biomedical Co., Seattle, WA), which detects full-length C3. Plasma concentrations were calculated from a standard plot using third-order polynomial regression. C3 plasma levels for each group were compared using one-way analysis of variance (ANOVA; GraphPad Prism, San Diego, CA). Total RNA was extracted from mouse kidney and liver using RNeasy Mini Kit (Qiagen; Valencia, CA). Reverse transcription was done using SuperScript® III Reverse Transcritase (Invitrogen, Carlsbad, CA). To measure C3 mRNA relative quantificantion in the mouse liver and kidneys, a real-time PCR was performed using 7500 Fast Real-Time PCR System (Applied Biosystems; Foster City, CA). Pre-designed commercially available probes for C3 (Mm437875_g1) and Gapdh (FAM-MGB) were used for the PCR reaction (Applied Biosystems, Foster City, CA). Fold changes were obtained relative to the expression of control/wildtype samples.

Histology

After mice were euthanized using carbon dioxide inhalation followed by cervical dislocation, kidneys were harvested and preserved in 10% formaldehyde for light microscopy, with the exception of the poles of the kidneys, which were preserved in 2.5% glutraldehyde for ultrastructural analyses by electron microscopy. A portion of each kidney was also immediately frozen for immunofluorescence studies.

Light Microscopy: Formalin-fixed tissue was embedded in paraffin and 4 μM sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS) and silver stain for histological analysis. The total number cells per glomerulus were counted

(a minimum of least 20 glomeruli were counted per section). Mesangial proliferative and endocapillary proliferation was graded as 1+ to 3+ depending on severity (1+ mild, 2+ moderate, 3+ severe).

Immunofluorescence Microscopy: Renal tissue was snap frozen and embedded in Tissue Tek OCT (Miles Inc, Elkhart, IN). 4 μM sections were cut and direct immunofluorescence studies were done using fluorescein isothacyante (FITC)-conjugated sheep antibody against IgM, IgG, IgA and C3c (Sigma, St Louis, MO, USA and Binding Site, CA, USA). Immunofluorescence staining was graded by intensity as 1+ (mild), 2+ (moderate) or 3+ (severe).

Electron Microscopy: Semithin (1 μM) sections were stained with toluidine blue and ultra-thin sections were stained with uranyl acetate and lead citrate for examination using a Jeol 100S electron microscope. The electron-dense deposits were graded based on the extent of mesangial or capillary wall involvement (1+ mild, 2+ moderate, 3+ severe).

Results

Genotyping and Immunoblotting

Genomic DNA from tail snips was used for PCR-based genotyping of *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice. PCR amplification generated 480bp and 500bp products reflecting disruption of the *Cfd* and *Cfh* genes, respectively. Immunoblotting of cell lysates using antibodies against Cfh and Cfd confirmed the absence of these proteins in *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice and was consistent with genotypic data (Figure 4-1).

Renal Function

When compared to wild-type controls, renal function in *Cfh-/-* mice was impaired as evidenced by an increase in plasma creatinine (p=0.0013) and blood urea nitrogen (BUN) (p=0.0054), microalbuminuria (p=0.0476) and reduced creatinine clearance (p=0.0001). In contrast, *Cfd-/-* and *Cfh-/-.Cfd-/-* mice had normal renal function (Table 4-1, Figure 4-2).

C3 Plasma and Hepatic and Renal Expression

C3 plasma levels in $Cfh^{-/-}.Cfd^{-/-}$ (610±410mg/l) were similar to levels in the wildtype (554±154mg/l). On the other hand, low C3 plasma levels were noted in the $Cfh^{-/-}$ (24±18mg/l) and high levels in $Cfd^{-/-}$ (1041±636mg/l). The $Cfh^{-/-}.Cfd^{-/-}$ had similar tissue expression levels of C3 in the liver and kidney with the wildtype. $Cfh^{-/-}$ had 2-fold increase in expression level of C3 in both tissues relative to the levels in the wildtype. $Cfd^{-/-}$ had similar expression level of C3 in the liver but had a 1.3 fold increase in the expression of C3 in the kidneys (Figure 4-3).

Glomerular Lesions

Wild-type mice: Wild-type (control) mice had normal appearing glomeruli on light microscopy with no significant mesangial or endocapillary proliferation. Immunofluorescence studies showed no or mild mesangial staining for C3, and electron microscopy typically did not show capillary wall electron dense deposits, although occasional mesangial deposits were noted (Figure 4-4, top panel).

$Cfh^{-/-}$ mice: $Cfh^{-/-}$ mice had a membranoproliferative pattern of glomerular injury on light microscopy with mesangial hypercellularity due to mononuclear cells. The GBMs were thickened and other features of membranoproliferative glomerulonephritis

including segmental endocapillary proliferation and lobular accentuation of glomerular tufts were noted in many glomeruli. Global glomerulosclerosis was not present and neither was there significant tubulointerstitial disease. An important and significant diagnostic finding was the intense C3 deposition along glomerular capillary walls on immunofluorescence microscopy. On electron microscopy, dense deposits (arrows) were noted in a subendothelial location along the capillary walls and in the mesangium (Fig 4-4, 2nd panel).

Cfd$^{-/-}$ *mice*: Light microscopy showed mild-to-moderate mesangial proliferative glomerulonephritis with an increase in mesangial matrix and mild-to-moderate mesangial hypercellularity. Mesangial C3 staining was seen on immunofluorescence microscopy and mesangial electron dense deposits were noted by electron microscopy. *Cfd*$^{-/-}$ mice did not have endocapillary proliferation and neither C3 deposition (by immunofluorescence microscopy) nor dense deposits (by electron microscopy) were observed along glomerular capillary walls (Figure 4-4, 3rd panel).

Cfh$^{-/-}$.*Cfd*$^{-/-}$ *mice*: Glomeruli of *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice had mild mesangial proliferative changes with an average cellularity of 29.4 (±2.7), midway between wild-type mice (25 ± 1.5 cells) and *Cfd*$^{-/-}$ mice (31± 3 cells). Immunofluorescence microscopy showed no-to-minimal (1+) staining for C3 with no capillary wall C3 staining in any *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice. Electron microscopy confirmed these findings – there were no capillary wall dense deposits and no or only occasional mesangial dense deposits. In all respects, glomeruli from *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ and wild-type mice appeared very similar (Figure 4-4, bottom panel) (Tables 4-2 and 4-3).

Discussion

In this study, we generated *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice to determine whether absence of factor D prevents the DDD renal phenotype that develops in *Cfh*$^{-/-}$ mice secondary to absence of factor H. Our findings are striking for the glomerular normalcy we observed. The proliferative features of DDD in *Cfh*$^{-/-}$ mice were absent, as were capillary C3 staining and electron dense deposits. In the mesangium, C3 staining was minimal and electron dense deposits were insignificant, features remarkably similar to those observed in wildtype mice. Consistent with this histological picture, the renal function in *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice was similar to that in wild-type mice. In an analogous study that prompted this work, Pickering and colleagues showed that mutant mice deficient for both factors H and B (*Cfh*$^{-/-}$.*Cfb*$^{-/-}$ mice) also have a normal renal phenotype. This outcome would be predicted from the structure of the AP C3 convertase (C3bBb), which requires factor B for its formation. Absence of factor B in *Cfh*$^{-/-}$.*Cfb*$^{-/-}$ mice prevents generation of C3 convertase making factor H-mediated control of its activity unnecessary and therefore the absence of factor H inconsequential (Pickering, Cook et al. 2002). In *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice, although C3bB can form, cleavage of factor B cannot occur and C3 convertase does not form (Volanakis and Narayana 1996). The absence of rampant fluid-phase C3 convertase activity is therefore prevented and DDD does not develop. It is germane to note that the absence of factor H also prevents mesangial immune-complex glomerulonephritis in *Cfd*$^{-/-}$ mice. *Cfd*$^{-/-}$ mice develop mild mesangial cell proliferation with para-mesangial deposition of C3 and IgM, which may reflect accumulation of C3 at sites of synthesis (Abrera-Abeleda, Xu et al. 2007). Consistent with this possibility, we observed a slight increase of C3 mRNA expression levels in the kidney of *Cfd*$^{-/-}$ mice (Figure 4-3). In the

absence of factor D, most of C3 will be in its hydrolyzed form (C3(H_2O)). Like C3b, C3(H_2O) can bind to target surfaces, complement regulators and factor B. We therefore assayed factor B mRNA hepatic and renal expression and found it to be significantly increased in both *Cfh*$^{-/-}$ and *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice as compared to *Cfd*$^{-/-}$ and wild-type controls (Figure 4-5). This finding raises the possibility that in the absence of factor D, C3(H_2O) bind preferentially to factor B to form an inactive proenzyme in the fluid phase reduces its deposition on mesangial cells.

There are currently no disease-specific treatments for persons with DDD, although studies implicating fluid-phase dysregulation of both C3 and C5 convertases make Eculizumab, a humanized anti-C5 monoclonal antibody, an attractive consideration (Hogasen, Jansen et al. 1995; Sethi, Gamez et al. 2009) .Data from this study suggest that therapies targeting factor D or abrogating its function by preventing factor B cleavage should also be explored. Noteworthy in this regard is CRIT (C2 receptor inhibitor trispanning), a three transmembrane receptor first discovered on the *Shistosoma* parasite surface that acts as a decoy receptor for C2 to protect cells from assembly of C4bC2 and C2 cleavage. The 11 amino acids at the Cterminal portion of the first extracellular domain of CRIT (CRIT-H17) bind the Bb fragment of factor B to inhibit factor D-mediated cleavage of factor B and limit AP C3 convertase activity. Binding prevents neither DAA nor C3b-factor B coassembly, implying that C3b and CRIT-H17 bind to different regions of factor B. Other important functional relationships, such as C3b-CR1 binding, are also preserved (Hui, Magnadottir et al. 2006).

The development of a monoclonal antibody to the epitope on factor B recognized by CRIT-H17 would therefore be predicted to prevent formation of a functional

C3convertase, which could be therapeutically relevant to the treatment of DDD. Possible untoward effects of C3 convertase inhibition would be predicted to include increased risk of meningococcal infection and an abnormal inflammatory reaction.

In summary, our studies show that deficiency of factor D prevents the development of DDD in *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice. These findings suggest that therapies to prevent factor D-mediated cleavage of factor B to produce the C3 convertase should be explored as a treatment option for DDD.

Acknowledgments

We would like to acknowledge the staff in our lab who helped with this project and to the undergraduate students in our lab who obtained the tail snips and performed the DNA extraction from the mouse tails. We are also thankful to the Animal Care Facilty and Animal Fluid Analysis Core for helping us with our mouse experiments and renal function tests respectively. We are grateful to Dr. Matthew Pickering for providing us the CFH-deficient mouse and to Dr. Yuanyuan Xu for providing the CFD-deficient mouse.

Table 4-1 Renal function in *Cfh*$^{-/-}$, *Cfd*$^{-/-}$, *Cfh*$^{-/-}$.*Cfd-/-* and *WT* mice.

Group	Average 24hour urine volume (ml)	Plasma creatinine (mg/dl)	Plasma BUN (mg/dl)	Urine Creatinine (mg/dl)	Urine Albumin (mg/dl)	Urine albumin: creatinine ratio (ug/ug)	Creatinine Clearance (ul/min)
Cfh$^{-/-}$	1.00	0.34 ± 0.03	26.84 ±1.79	43.44 ± 4.67	11.86 ± 5.95	0.29 ± 0.11	89.66 ± 4.16
Cfd$^{-/-}$	1.00	0.21 ± 0.04	20.00 ±3.70	46.68 ± 5.88	8.20 ± 4.25	0.14 ± 0.06	153.60 ± 25.41
Cfh$^{-/-}$.*Cfd*$^{-/-}$	1.08	0.19 ±0.06	17.10 ±4.54	38.27 ± 4.81	7.35 ± 4.21	0.15 ± 0.07	149.70 ± 20.14
WT	1.00	0.19 ±0.72	16.78 ±6.59	42.74 ± 4.81	7.49 ± 6.24	0.12 ± 0.03	156.20 ± 31.63
p-value (ANOVA)		0.0111	0.0142	0.1975	0.5259	0.0985	0.008

Note: Values are in mean ± standard deviation. Normal values for plasma creatinine is 0.05-0.2 mg/dl. Normal values for plasma BUN is 12-28 mg/dl. Normal values for urine albumin:creatinine ratio is 0.1-0.2 ug/ug. Normal values for creatinine clearance is 120-200 ul/min.

Table 4-2. Glomerular lesions in *Cfh*$^{-/-}$, *Cfd*$^{-/-}$, *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ and *WT* mice.

	Light Microscopy	**Immunofluorescence Microscopy**	**Electron Microscopy**
Wild-type mice	No proliferative changes	Minimal-to-no mesangial C3; no capillary wall C3	Rare mesangial deposits
Cfh$^{-/-}$ mice	Membranoproliferative glomerulonephritis	Mesangial and capillary wall C3	Mesangial and capillary wall electron dense deposits
Cfd$^{-/-}$ mice	Mild-to-moderate mesangial proliferative glomerulonephritis	Mesangial C3; no capillary wall C3	Mesangial electron dense deposits
Cfh$^{-/-}$.*Cfd*$^{-/-}$ mice	Minimal-to-no proliferative changes	Minimal-to-no mesangial C3; no capillary wall C3	Rare mesangial deposits

Table 4-3. Cellularity per glomerulus.

	Total Cellularity	**Mesangial Cellularity**	**Endocapillary Cellularity**
Wild-type mice	25 (±1.5)	20.25 (± 4.8)	0
Cfh$^{-/-}$ mice	41.65 (±4.2)	29.6 (±4.3)	12.05 (±3.3)
Cfd$^{-/-}$ mice	31 (± 3.0)	23.9 (±4.5)	0
Cfh$^{-/-}$.*Cfd*$^{-/-}$ mice	29.4 (±2.7)	22.45 (±3.8)	0

* At least 20 glomeruli were counted per mice. Total cellularity includes all cell types including endothelial cells (except podocytes); mesangial cellularity includes mesangial cells and other infiltrating cells in the mesangium; and endocapillary cellularity includes cells within the glomerular capillaries and includes endothelial cells.

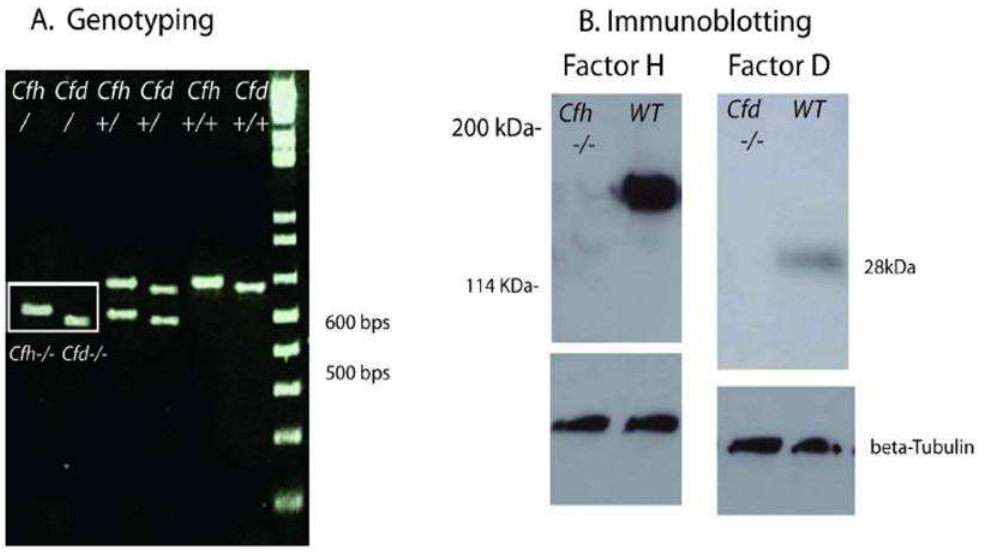

Figure 4-1. . Identification of knockout mice. A. The *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mutant mouse was identified by PCR-based genotyping, which generated 480bp and 500bp products associated with disruption of the *Cfd* and *Cfh* genes, respectively. B. Disruption of these genes was confirmed by immunoblotting.

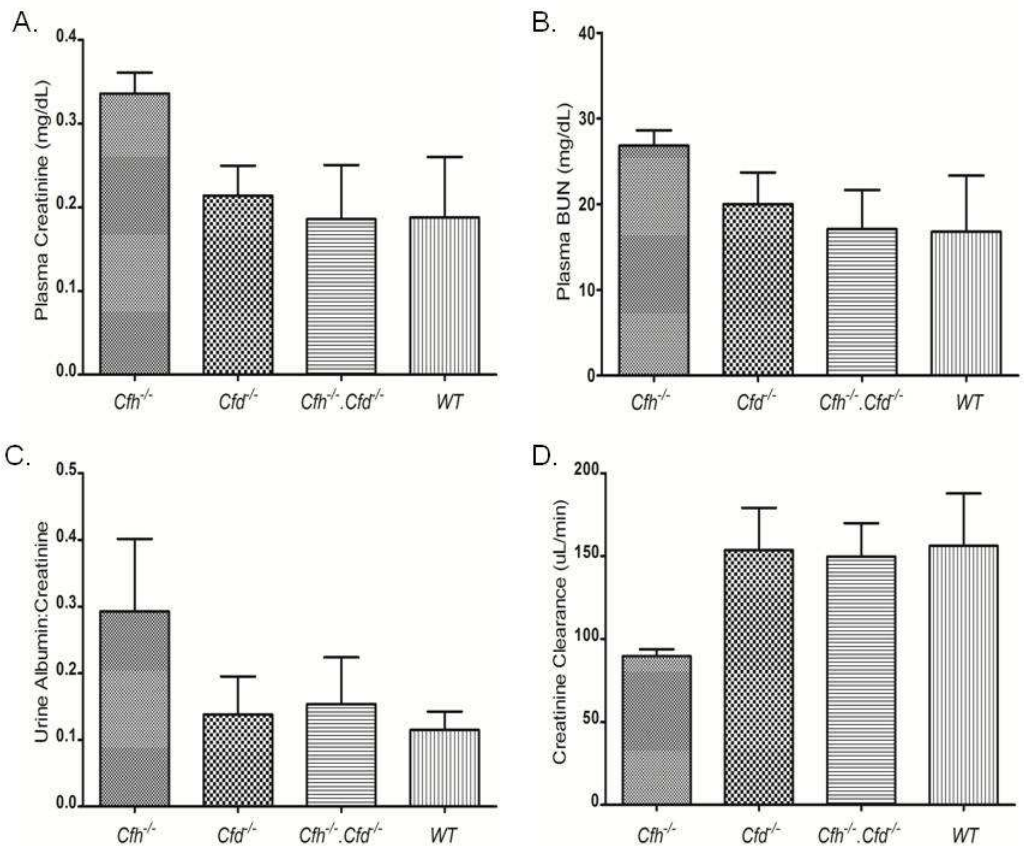

Figure 4-2. Renal function tests in mice. Renal function in *Cfh*$^{-/-}$ mice was significantly abnormal as compared to wild-type controls as exemplified by increased plasma creatinine (A), increased plasma BUN (B), microalbuminuria (C) and low creatinine clearance (D). Renal function in *Cfd*$^{-/-}$ and *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice was not different from renal function in wild-type controls (*Cfd*$^{-/-}$ mice vs wild-type controls: p=0.2457 (A); p=0.2065 (B); p=0.4206 (C); p=0.1216 (D); *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice vs wild-type controls: p=0.4822 (A); p=0.4654(B); p=0.2738 (C); p=0.1634 (D)).

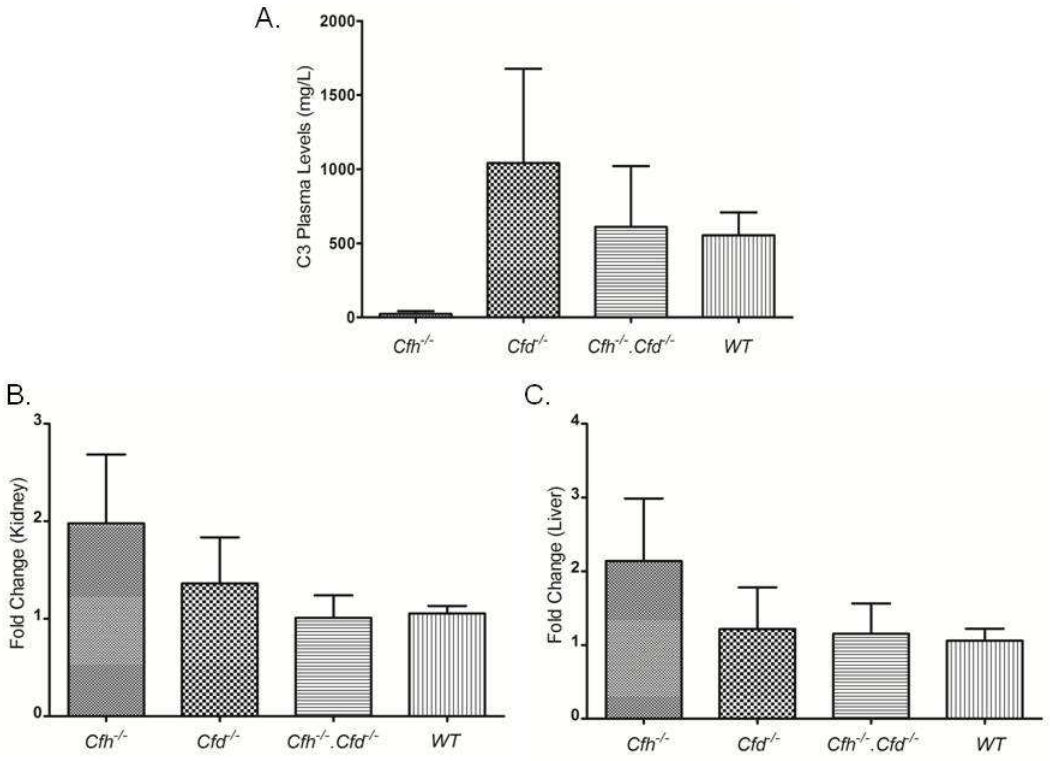

Figure 4-3. C3 plasma and tissue levels. C3 plasma levels in *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice were not significantly different than levels measured in wild-type controls (p=0.4025). *Cfh*$^{-/-}$ mice had lower C3 plasma levels while *Cfd*$^{-/-}$ mice had higher C3 plasma levels as compared to wild-type controls (p<0.0001 and p=0.0973, respectively)(A). *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice had similar C3 mRNA expression levels in both hepatic and renal tissues as compared to wild-type controls; *Cfh*$^{-/-}$ mice had higher C3 mRNA expression levels in both tissues while *Cfd*$^{-/-}$ mice had similar hepatic but slightly increased renal expression (B, C) (ANOVA p=0.05).

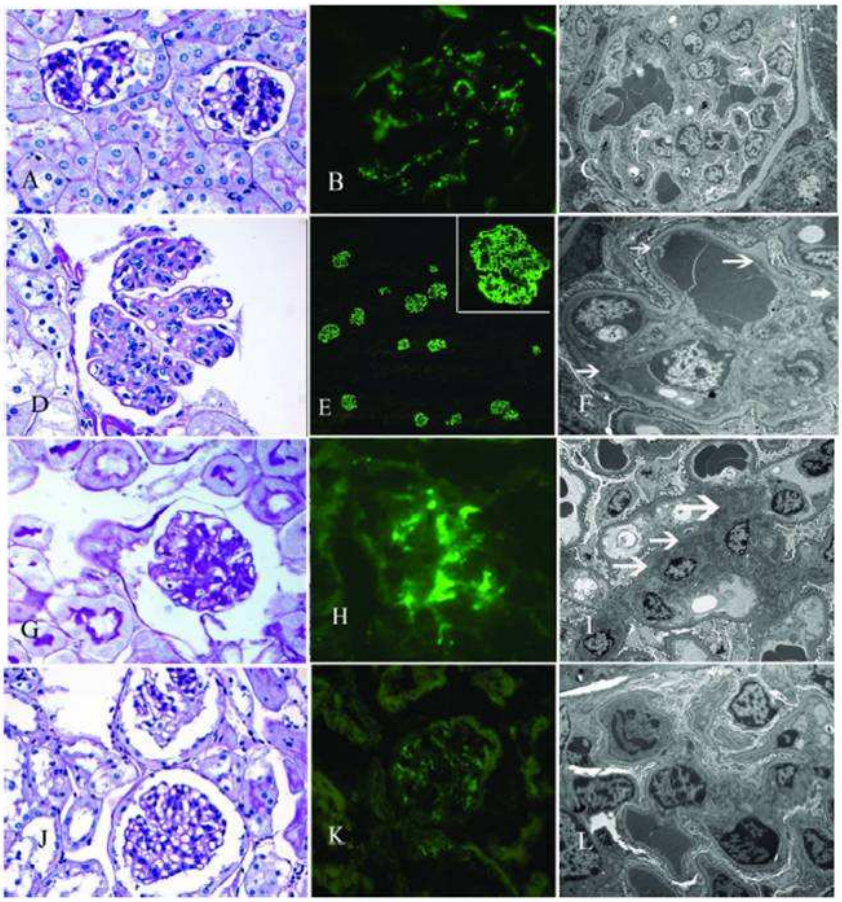

Figure 4-4 Glomerular histology in *WT*, *Cfh*$^{-/-}$, *Cfd*$^{-/-}$ and *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice.

A-C: Top panel (wild-type mice): Light microscopy shows normal appearing glomeruli (A);immunofluorescence microscopy for C3 shows mild mesangial deposition of C3 (B); electronmicroscopy shows no significant mesangial deposits (C).

D-F: 2nd panel (*Cfh*$^{-/-}$ mice): Light microscopy shows membranoproliferative glomerulonephritis (D); immunofluorescence microscopy shows prominent C3 deposition along the capillary walls (E); electron microscopy shows capillary wall deposits (arrows) (F).

G-I: 3rd panel (*Cfd*$^{-/-}$ mice): Glomeruli from Cfd-/- mice show mild mesangial expansion with mild increase in mesangial cellularity (G); immunofluorescence microscopy shows prominent mesangial C3 staining (H); electron microscopy shows numerous mesangial electron dense deposits (I).

J-L: bottom panel (*Cfh*-/-.*Cfd*-/- mice): Light microscopy shows normal appearing glomeruli (J); immunofluorescence microscopy shows mild mesangial deposition of C3 (K); electron microscopy show no significant mesangial deposits (L).

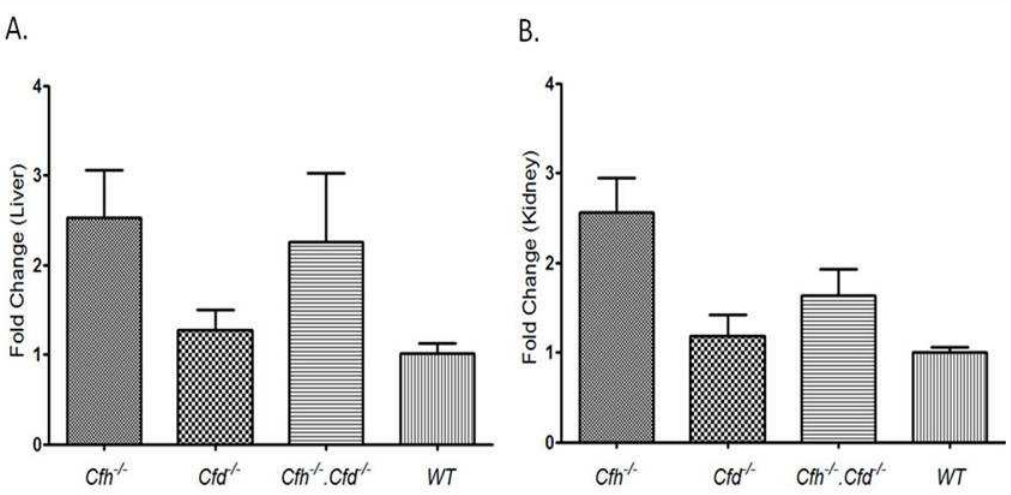

Figure 4-5. Factor B expression levels in mice. Factor B mRNA expression levels in hepatic and renal tissues were significantly elevated in *Cfh*$^{-/-}$ and *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice relative to expression levels in wild-type controls (*Cfh*$^{-/-}$ mice vs wildtype controls: p=0.004 (A), p=0.0064 (B); *Cfh*$^{-/-}$.*Cfd*$^{-/-}$ mice vs wild-type controls: p=0.0253 (A), p=0.032 (B)). Factor B mRNA expression levels in *Cfd*$^{-/-}$ mice and wild-type controls were similar (p=0.0719 (A), p=0.2027 (B)).

CHAPTER V

CONCLUSIONS AND FUTURE DIRECTIONS

My thesis work aims to understand the role of the alternative complement pathway (AP) in the development of Dense Deposit Disease. The first part of my work was an association study using DDD cases and controls in order to determine specific allele variants in genes of the AP that are associated with the DDD phenotype. The genes screened include complement components and regulators. Four novel missense mutations in the genes of AP had been identified in DDD cases but not in all the controls. Further functional analyses are required to determine if these mutations are pathogenic and can contribute to DDD phenotype. We also identified polymorphisms in the genes of AP that were associated with DDD. Some of these polymorphisms have been associated with other complement-mediated disease like SLE, aHUS and AMD. Out of all the SNPs, we determined four SNPs: CFH V62I, CFH Y402H, C3 R102G and C3 P314L, that were identified to be associated with DDD in several statistical analyses. We predict that these SNPs may contribute to the DDD phenotype. Interaction between CFH V62I and C3 P314L had also been ascertained by statistical analyses.

The second part of my thesis work involved the functional analyses of CFH and C3 polymorphisms. The alternative pathway is continuously activated at a low rate in human plasma. Our hypothesis is that the activity of the alternative complement pathway is different among individuals depending on the genotypes one carries. Individuals with risk alleles have more active AP of complement system. On the other hand, individuals with the protective alleles have normal activity of AP. In our study, complement activity was determined using several assays including hemolytic assay and

immunoassay. We also measured CFH and C3 serum levels since individuals with high complement activity could have low CFH and/or C3 serum levels. Statistical analyses had demonstrated that individuals with risk alleles in CFH V62I, CFH Y402H, C3 R102G and C3 P314L had elevated AP activity compared to individuals with the protective alleles. The risk allele in CFH Y402H had been determined to decrease expression of CFH in the serum (Tortajada, Hakobyan et al. 2007).

The last part of my thesis involved the characterization of CFH and CFD deficient mice in comparison to CFH-deficient and CFD-deficient mice. Mice that are deficient in CFH develop similar renal pathology seen in DDD patients. Surprisingly, CFD deficient mice have developed mesangial deposits in the kidneys which can be attributed to C3 accumulation in the local area. Since Factor D is needed to produce the C3 convertase during the AP complement cascade, we predict that abolishing the expression of Factor D in CFH-deficient mice will prevent the progression of renal disease in these mice. We had generated a mouse that was deficient in both CFH and CFD. Renal function tests and renal histology had shown that the double knockout mice did not have any renal pathology. Complement activity were also noted to be normal in the double knockout but there was an observed slight increase in Factor B expression which could explain the disappearance of mesangial deposits found in the kidneys of our CFD-deficient mice. Thus, the absence of Factor D in CFH-deficient mice could hinder the progression of renal diseases in these mice.

DDD is a complex disorder that is not inherited in a simple Mendelian order. It is predicted that several genes are involved in the pathogenesis of DDD. There are about 60 genes involved in the AP and not all of them have been screened for risk alleles

associated with DDD. Good candidates for screening include complement components in the classical, lectin and terminal complement pathway. Complement C5-C9 produce the membrane attack complex (MAC). Complement regulators, clusterin and vitronectin binds to MAC to prevent its formation. Mutations and polymorphisms in C5-C9 can possibly hinder the binding of the complement regulators to MAC leading to lysis of target cells. Other possible candidates are membrane-bound complement regulators, MCP and DAF. MCP is expressed in the kidneys and is a co-factor for Factor-I mediated cleavage of C3b and C4b which protects the cell where it is anchored (Seya and Atkinson 1989; Johnstone, Russell et al. 1993). On the other hand, DAF is expressed in most blood elements and protects host cells from autologous complement attack by accelerating the decay of the C3 and C5 convertases (Nicholson-Weller, Burge et al. 1982; Medof, Kinoshita et al. 1984). It is interesting to note that a soluble variant of DAF is present in body fluids which can inhibit complement activation in the fluid-phase (Medof, Walter et al. 1987; Lass, Walter et al. 1990). The pathogenesis of DDD involves both systemic and local activation of the complement cascade which makes these two complement regulators good candidates for possible association with DDD.

There are 5 Factor H-related genes in the RCA locus. We have screened CFHR5 for our association studies. The rest of the Factor H-related genes are good candidates for screening because they also have complement regulatory function by binding to C3b and heparin (Hellwage, Jokiranta et al. 1999; McRae, Cowan et al. 2001). A polymorphism in CFHR1 has been shown to be associated with aHUS. The risk allotype which is most similar to the sequence of CFH gene can possibly compete with CFH which leads to decrease protection of cellular surfaces against complement activity (Abarrategui-

Garrido, Martinez-Barricarte et al. 2009). One difficulty in screening Factor H-related genes is the similarity of the sequences with the sequence of Factor H gene. The CFH-CFHR1-5 region in the RCA locus contains several large genomic duplications involving various exons of the CFH and CFHR1-5 genes caused by gene conversion and non-homologous recombination. Other mutation screening techniques can be utilized to identify allele or copy number variants like Southern blotting, Multiplex Ligation-dependent Probe Amplification (MLPA), etc.

The complement system is part of the innate immunity. It has been shown to interact or "cross-talk" with other pathways in the immune system or defense mechanism of the body. One pathway is the coagulation system. The coagulation system is involved in hemostasis and is activated early after damage or injury. Pathogenesis of DDD may involve renal damage which can lead to activation of the coagulation system. Some DDD patients develop hematuria or blood in the urine. Interaction between these two systems has been proposed often. The coagulation factors, FXa, FXIa and plasmin are able to cleave C5 and C3 to generate the anaphylatoxins, C5a and C3a which are important in the inflammatory response (Amara, Rittirsch et al. 2008). It will be interesting if mutations and variants in the coagulation factors are associated with DDD.

Other possible candidate genes associated with DDD are interleukins and tumor necrosis factor superfamily. These are cytokines that are involved in the inflammatory processes. Cytokines are present in body fluids and tissues at low concentrations. However, an increase in mean concentrations of most cytokine is noted in chronic kidney disease and end-stage renal failure. Because of this, cytokines are used as prognostic biomarkers for chronic kidney diseases (Stenvinkel, Carrero et al. 2008). Mutations and

polymorphisms in interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) have been implicated in several chronic kidney disease including IgA nephropathy (Bantis, Heering et al. 2008), diabetic nephropathy (Navarro, Milena et al. 2005; Vionnet, Tregouet et al. 2006) and mesangial proliferative glomerulonephritis (Horii, Iwano et al. 1993). Expression of complement proteins like C3 and Factor B are influenced by levels of interleukins and tumor necrosis factor (Ramadori, Sipe et al. 1985; Perlmutter, Dinarello et al. 1986; Beuscher, Fallon et al. 1987; Ramadori, Van Damme et al. 1988; Circolo, Pierce et al. 1990).

Our animal studies have shown that absence of Factor D can stop the progression of renal diseases due to low or deficient CFH expression. This can be a possible therapy for DDD or other complement-mediated diseases caused by deficiency and non-functional complement regulators. Antibody against Factor D has been ascertained to inhibit AP in human serum (Tanhehco, Kilgore et al. 1999). Another study have shown that the Factor D antibody can inhibit complement and leukocyte activity in the baboon model of cardiopulmonary bypass (Undar, Eichstaedt et al. 2002). We can test the Factor D antibody in our *Cfh*$^{-/-}$ mice to determine if it will stop the progression of renal disease in these mice.

The long term goal of my study is to develop an effective treatment for DDD. We found risk allele variants in CFH and C3 that are associated with DDD and high complement activity. Possible effective treatment for DDD or other complement-mediated diseases is to target or eliminate the mutant CFH or C3 proteins from the system. Recently, concentrated plasma of CFH is being currently obtained for patients with aHUS and DDD associated with CFH mutations (Mazurier 2006). Replacement of

mutant proteins can be done through plasma exchange. However, immunologic problems can arise in replacement therapy. An alternative route is to use gene therapy that will enhance expression of the wildtype protein in the system. Recent advances regarding vector safety and transfection efficiency can make this type of therapy viable to DDD patients. Another way of targeting the mutant proteins for therapeutic purposes is the use of antibodies. Antibody therapy utilizes antibodies that target proteins or cells which can stimulate the immune system to attack and eliminate these targets. Antibodies against CFH or C3 proteins with specific risk alleles can be developed as therapeutic agents. Examples are CFH Y402H and C3 P314L variants in which certain antibodies can detect the risk alleles CFH H402 nad C3 L314. Identification of risk alleles associated with DDD can lead to the development of a more effective treatment for DDD or other complement-mediated diseases.

APPENDIX A

PRIMER SEQUENCES

Table A-1. Sequence of primers for PCR genotyping.

Exon	Forward	Reverse	MW
C3			
1	GGAAAGGCAGGAGCCAG	ATCCACAAACACCCAAATGC	291
2	ATCCTCTCCAGGACCAGAGG	AGGGAGAAGACAGAAGGGG	400
3-4	GACCAAGAATAATGGGCAGG	CCCTTCCGGTGTGTCTTTC	521
5-7	AGACACAGGTCGGGGAGAG	CCCACCTGGTCTTCACCTG	604
8-9	GGCTCCAGTCTTCAGCACA	CAGGCTGGAATCCATCTTCA	593
10-11	GCACAGGCCCAGTATGAAAG	TGCGCAGGAGAGAACCTC	630
12	CACCAATTCCCAGGTCTCA	AGTGGCAGGGAACGCAG	394
13	GGGACTCCTTCCCCCATC	GAGAGAAAAGGAGAAAGGGAGA	398
14	CCCCCAACCTTTCTGTCTTT	GCACTGCCCTCTCCAGTC	352
15-16	GGGGATCCCAATTGTCAG	TCCCCTCCTCCCTCTCTG	613
17	GAAGTCCTCCCTGGGGTC	TCCCTCCTCAGACAGGAGTC	337
18	GTGACCGCTGAGGAAGTAGG	TTTTCTAGGTTGGGCTGTG	725
19	GACCTACATGGAGGCACCC	TCCTGGTAACAACACCTCTGTG	353
20-21	AGCCTGGCCTTGTCCAC	TCAGAGCCTGGATCTCCAAC	600
22-23	AGTGCCCTGCTGACCATC	GAAGGGGAAGAGAAAGGTGC	464
24	CTCGCCTGTCCCTAACCC	GGAGGGCGTGGTCTTGAG	413
25	TTTTCCACCTCCTCGTTCTG	AGCGCTTGCCTGGACTCT	399
26	GAAGGCAAACCAGGCAAAC	ACATGGAGGTGGGGCTC	364
27	AGCGGGGTAACACCTAGAAG	GAGCTGCAAATTCCCTGAAG	324
28	ATGCTCAGGAAGTGCTGCTC	CTAAAAGCCATGCATCCCAG	328
29	AGGTAAGGCAGGAGCCAAGT	TTCTAGAACCTATAGATGGGTTTGC	498
30-31	GATGTCCCAGCTCTGATTTG	CTAGGACTGCTGGGGACAAG	553
32-33	GACCATCTCCTCTTGTCCCC	ACTTGGAAAGTACTGAATATCATGG	431
34-35	TGCTGCTATGTGGGAATCAG	CCCTACAACTCAGCAGCACA	531
36	TCATCTTTGCCACTTCCTCC	GAATGCTCAAATCCCTGGAC	342
37-38	GGGGTTGAAGACCTAGCATC	CCACACCCACAGCCTGAA	600
39-40	TTCTGCTTGGGAGAGAGAGC	GGGGCGTGACAATGGTG	487
41	AGTGAGTGCTTTCCCTGCG	CTGGGGATTTCAGCCTCTC	363
CFH			
1	TGGGAGTGCAGTGAGAATTG	TCCTGTGAAAAGCATCATTAGC	351
2	CCTGTGACTGTCTAGGCATTTT	GGCAATAGTGATATAATTCAGGCATA	86

Table A-1. Continued

3	GCTTTGCTATGTTTAATTTTCCTT	CCAGATTTAATTATTTCCATCATAGTT	400
4	TGCATATGCTGTTCATTTTC	GAGACTTTAAGATATTTTAATGTAAGAC	279
5	TTTCCTCCAATCTTATCCTGAG	TGCTGATATTCCTTAGAATGAACG	380
6	CCTGATGGAAACAACATTTCTG	TGAACTTTTCTGGCCCTGTT	491
7	GGACAAATAAATAACACCCACTTTT	TGCTTCCAACAGCCTTACTTT	399
8	CCTAGAAACCCTAATGGAATGTG	TTTGGTCACTTTGCTTGAACA	464
9	TAGTAACTTTAGTTCGTCTTCAG	TTAGAAAGACATGAACATGCTAGG	372
10	TGAATGCTTATGGTTATCCAGGT	GCTTTGTTCCTGCAGGTTTT	372
11	TCTTAGAATGGGAAATACTCAGATTG	TGAAAATGAATTTCTGAAAAACCA	283
12	ATGTAAAATTAACTTTGGCAATGA	CAAAGTTCTAATTCTTATTTCAGCAA	287
13	CCATTCTTGATTGTTTAGGATGC	CAGCCATGTTCAAGTTCAGG	353
14	AAAACACATACATCATGTTTTCACAA	GACTGGAAATGTTGAGGCATATC	346
15	GTTGGTTTGATTCCTATCATTTG	GACACACATACCTATTACTTTTCCAA	299
16	CTATGAGAATACAAGCCAAAAGTTC	TTGTTTACACGAAGCACAAGAGA	495
17	AACCCTTTGATTTTCATTCTTCA	TCAATTATTCCCCTCACTTTGA	300
18	AATTTATGAGTTAGTGAAACCTGAAT	GGTACCACTTACACTTTGAATGAAGA	283
19	ACAAAATGGCTAATATATTTTCTCAAG	GGCTGGGCCCACACATTA	380
20	CTGTTATCAGTTGATTTGCTACTC	GGAAAATAATATACCCTATTACTTGTG	426
21	CTGTGTTTGCGTTTGCCTTA	GATTTTTCCAGCCACGTGAA	299
22	TTTCTACATAGTTGGTTTGGAT	CCAAAATTTTACGTATGAATAAAAAGG	299
5’UTR	GTACACATTGTCTGGGTGCTG	GATGCTCCGCCTATCAGAAAC	370
Factor B			
1-2	CAAGCAGACAAGCAAAGCAA	CTGCCACCCTAAAACTGCTC	593
3	GGAGGGATACACCTAAGGCAG	GAGTGCCCACTCCTCGG	318
4	TGCTCTCTACCTTGCTCACG	ACGGGGGATCATGGGTTT	399
5	GGTCAGATCCTGGTCTTCCA	GTCTGATCCATCTAGCACCAG	477
6	GGAAAATGGAGAAGGGACAG	CAGGGAGCTAGTCCTGGAAG	266

Table A-1. Continued

7	GTCGCTGAAATCTCCCAATC	CACCCTGAACCTCCTGACC	253
8	TGGAGGTTAGTGGGAACCTG	TGATCACAGGGCTTAGGAAGA	487
9-10	GTGTATCCCTGCCTTTAGCC	GGAAGAATGAATTACTTCAGGGG	450
11-12	TACCCGTGGTCTTTCCCTTT	GCAGCTTCCTGCCTCTGG	668
13-14	TGAAGTTAGGAATGACACGGG	TCTCATGCCCCACTTGTCTC	460
15-17	CTGGCCCAGAACCTAGCTC	AGGCTCTGCCTACCTCGAAT	678
18	GCTGGGTCCCTAGTCTGATTC	CTGTTCCCCACTCCCTTG	365
Factor I			
1	CAAAAGTACAAAGCTCTTTAGGAGG	TTGCTGACTATAGAGTGGCATTG	329
2	TTGAAGCCACCAGACAACAC	GGCAACCCCTGATTTGTTTAG	573
3	CGTAAAATGATTGCTTACTATTACTTG	TGATGCACATAGTTAATTTTCTTAGG	431
4	CTTGCCCAAGCTGTAACTCC	AACGAGGCATCAATCATTTG	396
5-6	TCCATAAGCCAGGTTTGACC	CCATAGTTCTGCAAATGCCC	698
7	GCCTTTGCATTTTAGTAATGCTT	GAAAGTTTATCACACTGAGAAAAGG	274
8	GGGAGGATAAGTTTTAAGGCAG	CCAAAACTACTTGTTGCTTGAATC	252
9-10	TCCAGCCTGTCTTGTACTGG	CTTTGCAATTAATATAGTGGAGTTTG	630
11	AAGCATTTACAAAATTCTGGGG	GGCTGGATGTTTACTTTTCTGG	539
12	CACCCTTTCATAATCCCAATG	CCAAATGGAGGGAATTAGGG	312
13	AAGGAGAGCCCATGCTATTG	GGCATAAACTCTGTGGAGACC	496
Factor D			
1	GAGTCTGGCAGGAGGTAACCCAGTC	GCGTTCAGAGCCTTCCATTAGTGAG	365
2	GAGAGCTGGGATCCCGTCAGGCAGC	GAGTCCGCGGTCGGTGCCAGCCGACTC	283
3	GAGTCGGCTGGCACCGACCGCGGACTC	CATGCAGCAGGAGAGGTCGAGGCTGG	297
4	CTCCCCGAGCCTAGCGGCATTCTCC	TCATGCTCCGCCCATCTTCCAGTTC	383
5	ACTAGTGAAGACCAAATTAACACGG	GCAGGAGTGGATGACTTCATTGCTCG	352
CFHR5			
1	CAGTCCATTTCTGATTGTTCCA	CCCCTTCAAATTATCCTCAGC	493
2	GTGATTCATCGATGTAGCTCTTT	TTCCAGCTCCTCTGGTCATT	366
3	TGATGTCAGTTTTCAAAGTTTTCC	ATAATTAGCAAAACTGAGAGAGTGGT	314
4	CACATTAAATTTGTTTCTGCAATGA	TCAAATTCTTGTTTCATCACTTCT	275

Table A-1. Continued

5	CCATTTAAGCATTATTTATGGTTTC	TGCAAAGTAATAGTAACTGTCCTGTT	265
6	AAATATTTTCAGAGTAAGCACTCATTT	ACAATCCCAATCAAAATGATAAA	280
7	TGCAGATATTTTATTGACATAATTGTT	TCTTGTAAAGAAGCAACAAGATCAAC	282
8	CCATTTTCCTGAAACACTACCC	TTGGGGTACAGTGCAACAGA	377
9	AATTATTTGAATTTCCAGACACCTT	GGGTTATTCTATGAAATTAGTCCAAAA	375
10	CTTAAATGCAATTTCACTAATCTATGA	CAGAATTGGCTACATAATGGCTA	354
CLU			
1	GTTCAGGCTCTTCCCTACTG	GTTGTGACTGCGAGCTGTGTC	420
2	CATGGCAAGTCTGGTCATTTC	GAGGCACTGAGCAGAATTAG	379
3	GAACTGAACGGGCCTTTCTG	CTTAATTGCAGCCTCAGCATC	389
4	CTTGAACTGGAGCAAGGGTAG	GCCAGACATTACCAATGGAG	469
5	CTGTTGGTCACTTGCTGTGTG	CACCCTTGTGATATTGCTG	654
6	GCTGCAGTGAGCTATGATTG	CTGCAGAGCTGGAATCCAAAC	387
7	GTCGTGGACAATTCCAGTTTG	CTGCCGTGTGATAAATGCTC	478
8-9	GCAGGAGGACTTCCTTATCAG	GTTACTTGGTGACGTGCAGAG	592
Properdin			
1	GAGCACCGCACACTCACTTC	CCTCGCGCTCCTCCCAGCAAC	237
2-3	CTCTTTCCCTGCTGATTCCAG	GTATGGTCACGATCAGGGCCTC	533
4	CACTCTGGAGTCCCACATGC	CTCTGTACCGATCGTTCCCAC	315
5	GAAGCAAAGAGCCCTGAATG	CTGGCAGTTCCCTGCTGTAAC	307
6	GGAACTGCCAGGCTGAGAGGAC	CATGGTCACATGGCCTAGGAAC	362
7	GTCTGCTTCAAGGGTCTAGGG	CAGCAGCAGGAGCTGGGCAGC	324
8-9	CATTCCTTCCTCTGAACCCC	GCAGATACCTTAGATTCAGC	638
10	CCTCGGCATATAGCAGGCACTC	GTGTTAGAGTTCCTCTTCCTC	277
ADAM19			
1	AGTGGGCAGGTATGGCTGAG	GCAGAACGTGGGAACAAAG	318
2	CTGCCGCCAGTATTTGAGTTC	CTTCCGGGTTCATGCCATTC	190
3	GTGCATGTGTGGCTTAGCTG	AATGGTGATGACTGGGATTC	230
4	CAATGTTGGAGCGCTGTG	CTTCCTTCCAGAACAGCGAC	248
5	ATCACTCGTTGTTGCTAGGAG	GAGAGACCAACTCCATCAG	244
6	GGAAAGCTCTTTGCAGGTTG	CTCAATGGACTCCTTCCCAAG	325

Table A-1. Continued

7	GTCTCACGAAGGGGTACATG	CAAAGTAAGCAACCTCAGAG G	420
8	GCTTATCCAGCAGCTCTGTTTC	GCAACATTTCCCCAAAGC	240
9	CTGTGGTGGGGAAGACAATTG	CACTGCTCATTCCCTTTCAG	319
10	CAGGTGGTATTGACAGATAC	GCAGCCAAGGACAACACAAC	252
11	GAATGACCTGGGAAAGTCAG	GATGACCTCTGGTCTTGAAA GG	270
12	CTAAGGATAAGGCAGGGATG	GCACATTTGCCTTGGTCTTC	386
13	CTAAGGGGCCAGAATGTATG	CTCCAACTGGAAGGCTTCTT C	497
14	GAGATAGTTGCTAGAGAGCTG	GAAACTCTGTTGCCATCACA G	323
15	CTTATGAGCCCTTGGGCAAC	GAGGTAGTATCTAGAGGTGC	388
16	GAAGAGGCTGAGTATTCCCTC	CTGATTGACGTAAAGGTGCA G	350
17	GAAAGACTGAAGCTCTCTGGT G	CTCAATCACTCTGCTCTGCAG	338
18-19	CCATGGGTCCTCATATGTTC	CAATGTGGATGCTCTGCAAC	485
20	CCTGAAGAAGAAGCACGAATA C	GAGAGGGCAGCATGTATCTT G	370
21	CCAGAGCAGACTTGGGAAAG	GAAAAATACAGCATGGCCCT G	274
22	GTGTGTCTGTTCTCCCTTTG	CTTCCCCACCTTTGCCATTAC	408
23	CAGCTTGCTCTCCTGACTTC	GTCCAGACCACAAGAAGCAT G	440
24	CCTTCCTTCTAAAATCGGGAG	CATGCTTCTTCAGGGTTCCAT G	231
C3aR1			
1_1	CGTGAGGACCTTCTGGTTTC	TTCAAGAGACCTGTTTTCTAG TGG	780
1_2	TATCTGGGTGGTGGCTTTTG	GTGATCGTTATTGCCACGAG	580
1_3	TGTTCCCTAGCGCTTCTAGC	CGCTGGATTGATTCTTTGAC	683
	C5aR1		
1	TTTCCTCCCTGCATCTTCC	AGGACCCACTGGAGCAAAG	168
2_1	ATGCCTGAGCCAGGATG	TTAAACACCAGCAGAAAGCG	492
2_2	ATCACCACTGGCCCTTTG	AAAGAAACTGGCCACCACTG	460
2_3	CACGCTCACGATTTGTTACAC	CCATCCCACGAAAAGTGAAG	507
CR1			
1	GTCAAAAGCATTTTGTCCCG	GTCTCCAGTGCGCAGAAGG	455
3	GCAAAATCTGTGGAACCATC	GAGCAGATGGTAATAGTTTA GTTGTCC	338
4	AATTGGGTAGTTGACCTGTGT	TCTTCAGCCGTGTGAAGGAC	238
5	CATCATATTATGCCCATTGAA G	GAAAATTTGCTCCTCGACCC	687
8	TGCTGTTCCAGGGTCAGAG	ATTAGGGGAGGTCATGGGTC	546
9	ACACCAATCATAGCACCCTG	GAGGGCCTGGGTTAGATATT C	358

Table A-1. Continued

10	TGGCATCAAATCTACCAAGG	TTCAGAAAAGGTTTCTCATATCCC	356
11	TCAAGGTGCCAAAATCTGTG	TCTTTGTTCATTTCAATCAGGAG	362
12	TGCTGTCAGGAAGTTGATGAG	TCCATTCTTTGTAATTCTAGGAGG	222
13	AGCAAGACTCTGTCCCAAGG	CTCCTGATCCAACAGCAACG	496
14-15	CCTTGGCCAGTTTAACAGTGAG	TTAGACTGATTCCCAACCCC	500
16	TCAAAATCCTGAAATTGGGG	ATTGAGGGAGGCCATAGGAC	257
17	TGCAGAATACCTCTGGTGAAAC	ATATGGAGGGGCTGGGTTAG	400
18	TGGCATCAAATCTACCAAGG	TTCAGAAAAGGTTTCTCATATCCC	356
19	TCAAGGTGCCAAAATCTGTG	TCTTGTTCATTTCAATCAGGAG	362
20	TTGCAGTCCACAGTGAAGCC	CTCAACCAACAGAAGAGGAC	340
21	TGGGTGACAGCAAGACTCTG	TCCTGATCCAACAGCAACAC	522
22-23	TTGTGCTAGGGAGAATTGGG	CTCCCTGGATTCTTCTTCTCC	600
24	CGAAGGGAAGAGAGAAATGG	ATAATCCCATGGGGCAAAC	214
25	GCTTAATTGGCAACACAGTCA	CAGAAACATGAGGGATTGGG	394
26	AAGAGGAGGTAGGGTGGAAGTC	CGGTCCCACCTATGCTTTAAC	297
27	TGTTTAGTATGCCACACTCCAG	AAGTTGGCTCTGCTGTTCTG	240
28	ACCTGCTATTATTTTCTAAATGGTG	GTACTCCAGGGGCAAGTCAG	215
29	AATATGAGCAGAACCCATATCATAC	TGCCAATTTCATAGTCCTTATACAC	679
30-31	TTAATAGCTGCACTCTGCAATG	TCCTTAATTGGATTAACCCCTG	555
32	CTGGGCCTGAACCTAAGACC	CAGTGAGCTGTGATTGCACC	617
33	AGTTGCCTCTCAACAAAAGC	GCAAAGCCATTTTCACAAGC	475
37	GCATTGGCAATCTGTAGTGG	CTTTTGACTTCCAAGAGGGG	236
38	GCAAAGATATCAGGAACTTAAGGC	TGGTATCAATGACCTACCTAGTGC	332
39	TCAGCCTGTAAATTCTGGGTAG	AATCAATTGGCTCCTTTCCC	150
Intron27	CAGCAGAGCCAACTTCTGACCCAGT	GAGCCCTTGTAAGGCAAGTCTGGTG	1.3KB
CRIg			
1	GGGAAGGAAGGGAAGTAAGC	GCTGAGAAGACTCACCTGCC	280
2	GCAAAAGGGCACAGGTACAC	TGCCTGTCCACTTCCTCTG	650
3	CTCCAAAGTCAGAAGCCCC	GGATCCTCCTCTCCCAATTC	520
4	CAAGCAACACAGGACTTACCA	TCTCTTTCCTTTTGCCTCAGA	430
5	AGTGGTTGATAACATGGTCAGG	TGCTTGGCACATAGCAGGTA	344

Table A-1. Continued

6	TGGCTAGCTAAGAAGGTTAGGAG	CTGCACCCCAACCTTAACTC	380
7	CCTCCTCTGTACCCTTCCC	CTTAGGATTGGGTCTGTGCC	235
8	GCAAGAATTGAGGCAGAAGG	GTGTTGGTAGGCGGACACTT	440
C4A			
non	TCTAGCTTCAGTACTTCCAGCCTGT	TGGTCCCCAACATGTCTGTGCATGCT	5.2kb
del	TCTAGCTTCAGTACTTCCAGCCTGT	GATGACACAAAATACCAGGATGTGA	5.4kb

APPENDIX B

DDD CASES

Table B-1. Age, sex and genotypes of DDD cases.

DDD cases id	Sex (M/F)	Age (years)	CFH -331C>T (rs3753394)	CFH V62I G>A (rs800292)	CFH Y402H T>C (rs1061170)	CFH Q673 A>G (rs3753396)	CFH E936D G>T (rs1065489)	CFHR5 -249 T>C (rs9427661)	CFHR5 -20 T>C (rs9427662)
MPGN2-1	F	22	T/T	G/G	T/T	G/A	G/T	T/T	T/T
MPGN2-2	F	29	C/T	G/G	C/T	G/A	G/T	T/T	T/T
MPGN2-7	F	-	C/T	G/G	C/T	G/A	G/T	T/T	T/T
MPGN2-9	F	23	C/T	G/G	C/T	G/A	G/T	T/T	T/T
MPGN2-10	M	16	C/T	G/G	C/T	A/A	G/T	T/T	T/T
MPGN2-11	F	-	C/C	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-12	M	-	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-13	F	-	C/T	G/G	C/T	G/A	G/T	T/T	T/T
MPGN2-14	F	-	C/T	G/G	C/T	G/A	G/T	T/T	T/T
MPGN2-15	F	-	T/T	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-16	F	-	C/T	G/G	T/T	G/A	G/T	C/T	C/T
MPGN2-17	F	-	T/T	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-18	M	-	C/T	G/G	C/T	G/A	G/G	T/T	T/T
MPGN2-19	F	-	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-20	F	-	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-21	F	-	C/C	G/A	T/T	G/A	G/T	T/T	T/T
MPGN2-22	F	-	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-23	M	-	T/T	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-24	F	19	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-27-2	F	10	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-29	F	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-30	M	49	C/T	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-31	F	11	C/T	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-32	M	16	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-33-1	M	19	C/C	G/A	C/T	A/A	G/G	T/T	T/T

Table B-1. Continued

MPGN2-34-1	M	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-35	F	25	C/T	G/G	T/T	G/A	G/G	C/T	C/T
MPGN2-36	F	23	C/T	G/A	T/T	G/A	G/T	T/T	T/T
MPGN2-37	F	10	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-382	M	21	C/C	A/A	T/T	G/A	G/G	T/T	T/T
MPGN2-39	M	44	C/C	G/G	T/T	A/A	G/T	T/T	T/T
MPGN2-40A	F	17	C/T	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-42	M	21	C/T	G/A	T/T	A/A	G/G	T/T	T/T
MPGN2-43	M	14	C/C	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-44	M	34	C/T	G/A	T/T	G/A	G/T	T/T	T/T
MPGN2-46	F	17	C/T	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-47-1	F	14	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-48-1	M	17	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-49	F	7	C/C	G/G	T/T	G/G	T/T	T/T	T/T
MPGN2-50-1	F	22	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-52-1	M	11	C/T	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-53	F	30	C/C	A/A	C/T	A/A	G/G	T/T	T/T
MPGN2-54	F	9	C/T	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-55	F	14	C/C	G/G	C/T	A/A	G/G	C/T	C/T
MPGN2-56	F	31	C/C	A/A	T/T	G/A	G/T	T/T	T/T
MPGN2-57	F	25	C/C	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-58-1	M	-	C/C	G/A	C/T	A/A	G/G	C/T	C/T
MPGN2-59	M	15	C/T	G/A	T/T	A/A	G/G	T/T	T/T
MPGN2-60	F	47	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-61	F	21	C/T	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-62	F	13	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-63-1	F	4	C/T	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-64	F	17	T/T	G/G	T/T	G/G	T/T	T/T	T/T
MPGN2-65	F	29	C/C	A/A	T/T	A/A	G/G	T/T	T/T
MPGN2-66	F	14	C/C	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-67	M	43	C/C	G/G	C/T	A/A	G/G	C/T	C/T
MPGN2-68	M	9	C/C	G/G	C/T	A/A	G/G	T/T	T/T
MPGN2-26	M	14	C/T	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-69	M	15	C/C	G/A	T/T	A/A	G/G	C/T	C/T
MPGN2-71	F	-	C/T	G/A	T/T	A/A	G/G	C/T	C/T

Table B-1. Continued

MPGN2-72	M	20	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-73	M	-	C/T	G/G	T/T	A/A	G/G	T/T	T/T
MPGN2-74	M	16	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-82	F	26	C/C	G/G	C/C	A/A	G/G	T/T	T/T
MPGN2-85	F	15	C/C	A/A	T/T	A/A	G/G	T/T	T/T
MPGN2-87	M	19	C/C	G/A	T/T	A/A	G/G	T/T	T/T
MPGN2-92	M	12	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-93	F	30	T/T	G/G	C/T	G/A	G/T	T/T	T/T
MPGN2-94	F	13	C/C	G/A	C/T	A/A	G/G	T/T	T/T
MPGN2-96	F	8	T/T	G/A	T/T	G/A	G/G	T/T	T/T
MPGN2-98	M	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
DDD cases id	Sex (M/F)	Age (years)	CFHR5 P84S C>T (rs12097550)	CR1 Q1022H G>T (rs3738467)	CR1 H1208R A>G (rs2274567)	CR1 P1827R C>G (rs3811381)	CR1 H/L alleles	ADAM19 S284G A>G (rs1422795)	C4A deletion (nd, del)
MPGN2-1	F	22	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-2	F	29	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-7	F	-	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-9	F	23	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-10	M	16	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-11	F	-	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
MPGN2-12	M	-	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-13	F	-	C/T	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-14	F	-	C/T	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-15	F	-	C/T	G/T	A/G	C/G	H/L	G/G	nd/del
MPGN2-16	F	-	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
MPGN2-17	F	-	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-18	M	-	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-19	F	-	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
MPGN2-20	F	-	C/T	G/T	A/G	C/G	H/L	G/G	nd/nd
MPGN2-21	F	-	C/C	G/G	A/A	C/C	H/H	G/G	nd/del

Table B-1. Continued

MPGN2-22	F	-	C/C	T/T	G/G	G/G	L/L	A/G	nd/nd
MPGN2-23	M	-	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
MPGN2-24	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
MPGN2-27-2	F	10	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-29	F	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
MPGN2-30	M	49	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
MPGN2-31	F	11	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
MPGN2-32	M	16	C/C	G/T	A/G	C/G	H/L	G/G	nd/nd
MPGN2-33-1	M	19	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
MPGN2-34-1	M	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-35	F	25	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
MPGN2-36	F	23	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-37	F	10	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
MPGN2-382	M	21	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-39	M	44	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-40A	F	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-42	M	21	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
MPGN2-43	M	14	C/C	G/T	A/G	C/G	H/L	G/G	nd/del
MPGN2-44	M	34	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
MPGN2-46	F	17	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-47-1	F	14	C/C	T/T	G/G	G/G	L/L	A/A	nd/del
MPGN2-48-1	M	17	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
MPGN2-49	F	7	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-50-1	F	22	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-52-1	M	11	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-53	F	30	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-54	F	9	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-55	F	14	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-56	F	31	C/C	G/T	A/G	C/G	H/L	A/A	nd/del
MPGN2-57	F	25	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-58-1	M	-	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
MPGN2-59	M	15	C/C	G/T	A/G	C/G	H/L	A/A	nd/del
MPGN2-60	F	47	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-61	F	21	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
MPGN2-62	F	13	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd

Table B-1. Continued

MPGN2-63-1	F	4	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-64	F	17	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
MPGN2-65	F	29	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-66	F	14	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
MPGN2-67	M	43	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-68	M	9	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-26	M	14	C/T	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-69	M	15	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-71	F	-	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-72	M	20	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-73	M	-	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
MPGN2-74	M	16	C/C	G/T	A/G	C/G	H/L	G/G	nd/nd
MPGN2-82	F	26	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
MPGN2-85	F	15	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-87	M	19	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
MPGN2-92	M	12	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
MPGN2-93	F	30	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-94	F	13	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
MPGN2-96	F	8	C/C	G/T	A/G	C/G	H/L	G/G	nd/del
MPGN2-98	M	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd

DDD cases id	Sex (M/F)	Age (years)	C3 R102G C>G (rs2330199)	C3 R304 G>A (rs12230201)	C3 P314L C>T (rs1047286)	C3 P518 C>A (rs2230203)	C5aR1 D2N A>G (rs4467185)	C5aR1 N279K T>G (rs11880097)
MPGN2-1	F	22	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-2	F	29	G/G	G/G	T/T	A/A	A/A	T/T
MPGN2-7	F	-	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-9	F	23	C/G	G/A	C/T	C/C	A/A	T/T
MPGN2-10	M	16	G/G	G/G	C/T	C/A	A/A	T/T
MPGN2-11	F	-	C/C	G/G	C/T	C/A	A/A	T/T
MPGN2-12	M	-	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-13	F	-	C/G	G/G	C/C	C/C	A/A	T/T

Table B-1. Continued

MPGN2-14	F	-	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-15	F	-	C/G	G/G	T/T	A/A	A/A	T/T
MPGN2-16	F	-	G/G	G/G	T/T	A/A	A/A	T/T
MPGN2-17	F	-	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-18	M	-	C/C	A/A	C/C	C/C	A/A	T/T
MPGN2-19	F	-	C/C	A/A	C/C	C/C	A/A	T/T
MPGN2-20	F	-	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-21	F	-	C/C	G/A	C/C	C/C	A/A	T/T
MPGN2-22	F	-	G/G	G/G	T/T	C/C	A/A	T/T
MPGN2-23	M	-	G/G	A/A	C/C	C/C	A/A	T/T
MPGN2-24	F	19	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-27-2	F	10	C/G	G/G	C/T	C/C	A/A	T/T
MPGN2-29	F	18	C/G	G/A	C/T	C/C	A/G	T/G
MPGN2-30	M	49	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-31	F	11	C/C	G/G	C/T	C/C	A/A	T/T
MPGN2-32	M	16	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-33-1	M	19	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-34-1	M	18	C/G	G/G	C/C	C/C	A/A	T/T
MPGN2-35	F	25	C/C	G/A	C/C	C/C	A/A	T/T
MPGN2-36	F	23	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-37	F	10	C/G	G/A	C/T	C/A	A/A	T/T
MPGN2-382	M	21	C/G	G/G	C/C	C/C	A/A	T/T
MPGN2-39	M	44	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-40A	F	17	C/C	G/G	C/T	C/A	A/A	T/T
MPGN2-42	M	21	G/G	G/G	T/T	A/A	A/A	T/T
MPGN2-43	M	14	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-44	M	34	C/G	G/G	C/T	C/A	A/G	T/G
MPGN2-46	F	17	C/C	G/G	C/C	C/C	A/G	T/G
MPGN2-47-1	F	14	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-48-1	M	17	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-49	F	7	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-50-1	F	22	C/C	A/A	C/C	C/C	A/A	T/T
MPGN2-52-1	M	11	C/C	G/G	C/T	C/A	A/A	T/T
MPGN2-53	F	30	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-54	F	9	C/C	G/G	C/C	C/C	A/A	T/T

Table B-1. Continued

MPGN2-55	F	14	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-56	F	31	C/C	G/G	C/C	C/C	A/G	T/G
MPGN2-57	F	25	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-58-1	M	-	C/G	G/A	C/T	C/A	A/A	T/T
MPGN2-59	M	15	C/C	A/A	C/C	C/C	A/A	T/T
MPGN2-60	F	47	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-61	F	21	C/G	G/G	T/T	A/A	A/A	T/T
MPGN2-62	F	13	C/C	G/G	C/C	C/C	A/G	T/G
MPGN2-63-1	F	4	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-64	F	17	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-65	F	29	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-66	F	14	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-67	M	43	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-68	M	9	C/G	G/G	T/T	A/A	A/A	T/T
MPGN2-26	M	14	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-69	M	15	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-71	F	-	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-72	M	20	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-73	M	-	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-74	M	16	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-82	F	26	C/C	G/A	C/T	C/A	A/A	T/T
MPGN2-85	F	15	C/G	G/G	C/C	C/C	A/G	T/G
MPGN2-87	M	19	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-92	M	12	C/G	G/A	C/T	C/A	A/A	T/T
MPGN2-93	F	30	C/G	G/G	C/T	C/A	A/A	T/T
MPGN2-94	F	13	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-96	F	8	C/C	G/G	C/C	C/C	A/A	T/T
MPGN2-98	M	18	C/G	G/G	C/T	C/A	A/A	T/T

Note: (-) – unknown; nd – nondeletion; del-deletion

APPENDIX C

AMD(-) CONTROLS

Table C-1. Age, sex and genotypes of AMD(-) controls.

DDD cases id	Sex (M/F)	Age (years)	CFH -331C>T (rs3753394)	CFH V62I G>A (rs800292)	CFH Y402H T>C (rs1061170)	CFH Q673 A>G (rs3753396)	CFH D936E G>T (rs1065489)	CFHR5 -249 T>C (rs9427661)	CFHR5 -20 T>C (rs9427662)
269-00	F	91	C/C	G/G	C/C	A/A	G/G	T/T	T/T
287-00	F	87	C/C	G/G	C/T	A/A	G/G	C/T	C/T
365-00	F	85	C/C	A/A	T/T	G/A	G/T	T/T	T/T
366-00	M	88	C/T	G/G	T/T	A/A	G/G	C/T	C/T
370-00	F	88	C/T	G/G	T/T	A/A	G/G	C/T	C/T
374-00	M	85	C/C	G/A	C/T	A/A	G/G	T/T	T/T
388-00	F	87	C/T	G/A	T/T	A/A	G/G	T/T	T/T
45-01	M	85	C/T	G/G	C/T	A/A	G/G	C/T	C/T
101-01	F	89	C/C	G/G	C/T	A/A	G/G	T/T	T/T
106-01	F	86	C/T	G/G	T/T	A/A	G/G	C/T	C/T
107-01	F	87	C/C	G/G	C/T	A/A	G/G	C/T	C/T
411-01	M	87	C/C	G/G	C/T	G/A	G/T	T/T	T/T
139-02	F	90	C/C	G/A	T/T	A/A	G/G	T/T	T/T
190-02	F	90	T/T	G/G	T/T	G/A	G/T	T/T	T/T
191-02	M	93	C/C	A/A	T/T	A/A	G/G	T/T	T/T
192-02	F	88	T/T	G/G	C/C	A/A	G/G	T/T	T/T
207-02	F	93	C/T	G/G	C/T	A/A	G/G	T/T	T/T
209-02	F	88	T/T	G/G	T/T	G/G	T/T	T/T	T/T
215-02	M	89	C/C	G/G	C/T	A/A	G/G	T/T	T/T
266-00	F	73	C/C	G/A	C/T	A/A	G/G	T/T	T/T
274-00	F	77	C/C	G/A	T/T	A/A	G/G	T/T	T/T
276-00	F	76	C/T	G/A	T/T	G/A	G/T	T/T	T/T
293-00	F	83	C/C	G/G	C/T	A/A	G/G	C/T	C/T
367-00	F	80	C/C	G/G	T/T	A/A	G/G	C/T	C/T
369-00	F	75	C/T	G/G	T/T	G/A	G/T	C/T	C/T

Table C-1. Continued

371-00	M	83	C/C	G/A	C/T	A/A	G/G	T/T	T/T
380-00	F	77	C/C	G/A	C/T	A/A	G/G	T/T	T/T
382-00	F	77	C/C	G/A	T/T	G/A	G/T	T/T	T/T
385-00	F	82	C/C	G/G	C/T	A/A	G/G	C/T	C/T
386-00	F	81	C/C	G/G	C/T	A/A	G/G	C/T	C/T
390-00	F	84	C/T	G/G	C/T	A/A	G/G	T/T	T/T
397-00	F	83	C/T	G/G	C/T	A/A	G/G	T/T	T/T
103-01	F	82	T/T	G/G	T/T	G/A	G/T	T/T	T/T
104-01	F	83	C/T	G/A	T/T	A/A	G/G	T/T	T/T
105-01	F	83	C/C	A/A	T/T	G/A	G/T	T/T	T/T
111-01	F	75	C/C	G/A	C/T	A/A	G/G	C/T	C/T
113-01	F	79	C/C	G/G	C/T	A/A	G/G	T/T	T/T
359-01	M	77	C/T	G/G	C/T	A/A	G/G	T/T	T/T
403-01	F	71	C/C	G/A	C/T	A/A	G/G	T/T	T/T
405-01	F	83	C/T	G/A	C/T	A/A	G/G	T/T	T/T
406-01	F	68	C/T	G/A	C/T	A/A	G/G	T/T	T/T
115-02	F	78	C/C	G/A	C/T	A/A	G/G	T/T	T/T
116-02	M	77	C/T	G/G	C/C	A/A	G/G	T/T	T/T
117-02	F	70	C/C	G/A	T/T	A/A	G/G	T/T	T/T
120-02	M	72	C/C	G/G	T/T	A/A	G/G	C/C	C/C
140-02	F	82	C/T	G/G	C/T	A/A	G/G	T/T	T/T
143-02	F	75	C/T	G/G	C/C	A/A	G/G	T/T	T/T
145-02	F	84	C/T	G/G	C/C	A/A	G/G	T/T	T/T
161-02	F	84	C/C	G/A	C/T	A/A	G/G	T/T	T/T
162-02	F	80	C/T	G/A	T/T	G/G	G/G	T/T	T/T
164-02	M	79	C/T	G/G	C/T	G/A	G/T	T/T	T/T
167-02	M	71	C/T	G/G	T/T	A/A	G/G	T/T	T/T
168-02	F	72	C/T	G/A	T/T	G/A	G/G	T/T	T/T
169-02	M	70	C/C	G/G	C/T	A/A	G/G	C/T	C/T
170-02	M	74	C/C	G/A	C/T	A/A	G/G	T/T	T/T
172-02	F	72	C/C	G/G	C/C	A/A	G/G	T/T	T/T
173-02	F	76	C/C	G/G	C/C	A/A	G/G	T/T	T/T
174-02	M	78	C/T	G/A	T/T	G/A	G/T	T/T	T/T
177-02	M	72	C/C	G/A	T/T	G/A	G/T	C/T	C/T
178-02	F	77	C/C	G/G	C/C	A/A	G/G	T/T	T/T

Table C-1. Continued

179-02	M	72	C/C	G/G	C/T	A/A	G/G	T/T	T/T
180-02	F	76	C/C	G/A	T/T	G/A	G/T	C/T	C/T
181-02	M	74	C/C	G/A	C/T	A/A	G/G	T/T	T/T
186-02	F	77	T/T	G/G	T/T	A/A	G/G	T/T	T/T
188-02	F	76	C/C	G/A	T/T	A/A	G/G	C/T	C/T
194-02	F	77	C/C	G/G	C/T	A/A	G/G	C/T	C/T
195-02	F	81	C/C	G/G	C/T	A/A	G/T	C/T	C/T
198-02	F	79	C/C	G/G	C/T	A/A	G/G	T/T	T/T
199-02	F	80	T/T	G/G	T/T	G/A	G/G	T/T	T/T
200-02	F	82	C/T	G/A	T/T	A/A	G/G	T/T	T/T
201-02	F	74	C/T	G/A	T/T	G/A	G/G	T/T	T/T
202-02	F	78	C/C	A/A	T/T	A/A	G/G	T/T	T/T
206-02	M	83	C/C	G/A	T/T	A/A	G/G	C/T	C/T
208-02	M	74	C/C	A/A	T/T	A/A	G/G	T/T	T/T
211-02	M	81	C/C	G/G	C/C	A/A	G/G	C/T	C/T
212-02	F	78	T/T	G/G	T/T	G/A	G/G	T/T	T/T
213-02	F	75	T/T	G/G	T/T	G/A	G/G	T/T	T/T
283-00	F	50	C/T	G/G	C/C	A/A	G/G	T/T	T/T
109-01	M	52	C/T	G/G	T/T	G/A	G/G	C/T	C/T
356-01	F	31	C/T	G/G	T/T	G/A	G/T	C/T	C/T
358-01	F	50	C/C	G/G	C/C	A/A	G/G	T/T	T/T
379-01	M	46	C/T	G/G	C/T	G/A	G/G	T/T	T/T
382-01	M	45	T/T	G/G	T/T	G/G	G/G	T/T	T/T
384-01	M	41	T/T	G/G	C/T	G/A	G/G	T/T	T/T
15-02	F	48	T/T	G/G	T/T	G/A	G/G	T/T	T/T
16-02	F	42	C/T	G/A	T/T	A/A	G/G	T/T	T/T
359-02	F	88	C/T	G/G	C/T	A/A	G/G	T/T	T/T
430-99	F	69	C/T	G/A	T/T	A/A	G/G	T/T	T/T
431-99	M	68	C/C	G/A	T/T	A/A	G/G	T/T	T/T
435-99	M	64	C/T	G/G	C/C	A/A	G/G	T/T	T/T
308-00	M	74	C/C	G/A	T/T	A/A	G/G	C/T	C/T
345-00	F	67	C/T	G/G	C/T	A/A	G/G	T/T	T/T
444-00	M	52	C/T	G/A	T/T	A/A	G/G	C/T	C/T
21-01	M	60	C/C	G/G	T/T	A/A	G/G	C/T	C/T
50-01	M	72	C/T	G/A	T/T	A/A	G/G	T/T	T/T

Table C-1. Continued

148-01	M	64	C/C	G/G	C/T	A/A	G/G	T/T	T/T
149-01	M	69	T/T	G/G	T/T	A/A	G/G	T/T	T/T
292-01	M	75	C/C	G/G	C/C	A/A	G/G	T/T	T/T
236-03	M	83	C/T	G/G	T/T	G/A	G/T	C/T	C/T
286-03	F	59	C/T	G/A	T/T	G/G	T/T	T/T	T/T
325-03	M	83	C/T	G/G	T/T	A/A	G/G	T/T	T/T
340-03	M	75	C/C	G/G	C/T	A/A	G/G	T/T	T/T
361-02	M	70	C/C	G/G	C/C	A/A	G/G	T/T	T/T
DDD cases id	Sex (M/F)	Age (years)	CFHR5 P84S C>T (rs12097550)	CR1 Q1022H G>T (rs3738467)	CR1 H1208R A>G (rs2274567)	CR1 P1827R C>G (rs3811381)	CR1 H/L alleles	ADAM19 S284G A>G (rs1422795)	C4A deletion (nd, del)
269-00	F	91	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
287-00	F	87	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
365-00	F	85	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
366-00	M	88	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
370-00	F	88	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
374-00	M	85	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
388-00	F	87	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
45-01	M	85	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
101-01	F	89	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
106-01	F	86	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
107-01	F	87	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
411-01	M	87	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
139-02	F	90	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
190-02	F	90	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
191-02	M	93	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
192-02	F	88	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
207-02	F	93	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
209-02	F	88	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
215-02	M	89	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
266-00	F	73	C/C	T/T	G/G	G/G	L/L	A/G	nd/nd

Table C-1. Continued

274-00	F	77	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
276-00	F	76	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
293-00	F	83	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
367-00	F	80	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
369-00	F	75	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
371-00	M	83	C/C	G/T	A/G	C/G	H/L	A/A	nd/del
380-00	F	77	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
382-00	F	77	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
385-00	F	82	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
386-00	F	81	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
390-00	F	84	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
397-00	F	83	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
103-01	F	82	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
104-01	F	83	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
105-01	F	83	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
111-01	F	75	C/C	G/T	A/G	C/G	H/L	G/G	nd/del
113-01	F	79	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
359-01	M	77	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
403-01	F	71	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
405-01	F	83	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
406-01	F	68	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
115-02	F	78	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
116-02	M	77	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
117-02	F	70	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
120-02	M	72	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
140-02	F	82	C/T	T/T	G/G	G/G	L/L	A/G	nd/nd
143-02	F	75	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
145-02	F	84	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
161-02	F	84	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
162-02	F	80	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
164-02	M	79	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
167-02	M	71	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
168-02	F	72	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
169-02	M	70	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
170-02	M	74	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd

Table C-1. Continued

172-02	F	72	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
173-02	F	76	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
174-02	M	78	C/C	G/T	A/G	C/G	H/L	G/G	nd/del
177-02	M	72	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
178-02	F	77	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
179-02	M	72	C/C	G/T	A/G	C/G	H/L	G/G	nd/del
180-02	F	76	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
181-02	M	74	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
186-02	F	77	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
188-02	F	76	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
194-02	F	77	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
195-02	F	81	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
198-02	F	79	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
199-02	F	80	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
200-02	F	82	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
201-02	F	74	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
202-02	F	78	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
206-02	M	83	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
208-02	M	74	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
211-02	M	81	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
212-02	F	78	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
213-02	F	75	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
283-00	F	50	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
109-01	M	52	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
356-01	F	31	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
358-01	F	50	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
379-01	M	46	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
382-01	M	45	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
384-01	M	41	C/C	G/T	A/G	C/G	H/L	A/A	nd/del
15-02	F	48	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
16-02	F	42	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
359-02	F	88	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
430-99	F	69	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
431-99	M	68	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
435-99	M	64	C/C	G/T	A/G	C/G	H/L	G/G	nd/del

Table C-1. Continued

308-00	M	74	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
345-00	F	67	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
444-00	M	52	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
21-01	M	60	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
50-01	M	72	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
148-01	M	64	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
149-01	M	69	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
292-01	M	75	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
236-03	M	83	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
286-03	F	59	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
325-03	M	83	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
340-03	M	75	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
361-02	M	70	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
DDD cases id	Sex (M/F)	Age (years)	C3 R102G C>G (rs2330199)	C3 R304 G>A (rs12230201)	C3 P314L C>T (rs1047286)	C3 P518 C>A (rs2230203)	C5aR1 D2N A>G (rs4467185)	C5aR1 K279N T>G (rs11880097)	
269-00	F	91	C/C	G/G	C/C	C/C	A/A	T/T	
287-00	F	87	C/G	G/G	T/T	A/A	A/A	T/T	
365-00	F	85	C/C	G/G	C/C	C/C	A/A	T/T	
366-00	M	88	C/C	G/G	C/C	C/C	A/A	T/T	
370-00	F	88	C/C	G/G	C/C	C/C	A/A	T/T	
374-00	M	85	C/G	G/G	C/T	C/A	A/A	T/T	
388-00	F	87	C/C	G/A	C/C	C/C	A/A	T/T	
45-01	M	85	C/C	G/G	C/C	C/C	A/A	T/T	
101-01	F	89	C/C	G/A	C/C	C/C	A/A	T/T	
106-01	F	86	C/G	G/G	C/T	C/A	A/A	T/T	
107-01	F	87	C/C	G/A	C/C	C/C	A/A	T/T	
411-01	M	87	C/C	G/G	C/C	C/C	A/A	T/T	
139-02	F	90	C/C	G/A	C/C	C/C	A/A	T/T	
190-02	F	90	C/C	G/A	C/C	C/C	A/A	T/T	
191-02	M	93	C/C	G/G	C/T	C/A	A/A	T/T	
192-02	F	88	C/C	G/G	C/C	C/C	A/A	T/T	

Table C-1. Continued

207-02	F	93	C/C	G/G	C/C	C/C	A/A	T/T
209-02	F	88	C/C	G/G	C/C	C/C	A/A	T/T
215-02	M	89	C/C	G/A	C/C	C/C	A/A	T/T
266-00	F	73	C/C	G/A	C/C	C/C	A/A	T/T
274-00	F	77	C/C	G/G	C/C	C/C	A/A	T/T
276-00	F	76	C/C	G/G	C/T	C/A	A/A	T/T
293-00	F	83	C/C	G/G	C/C	C/C	A/A	T/T
367-00	F	80	C/C	G/G	C/C	C/C	A/A	T/T
369-00	F	75	C/G	G/G	C/T	C/A	A/A	T/T
371-00	M	83	C/G	G/G	C/T	C/A	A/A	T/T
380-00	F	77	G/G	G/G	T/T	A/A	A/A	T/T
382-00	F	77	C/C	G/G	C/C	C/C	A/A	T/T
385-00	F	82	C/C	G/A	C/C	C/C	A/A	T/T
386-00	F	81	C/G	G/G	C/T	C/A	A/A	T/T
390-00	F	84	C/C	G/G	C/T	C/A	A/A	T/T
397-00	F	83	C/G	G/G	C/T	C/A	A/A	T/T
103-01	F	82	C/G	G/A	C/T	C/A	A/A	T/T
104-01	F	83	C/C	G/G	C/T	C/A	A/A	T/T
105-01	F	83	C/G	G/G	C/T	C/A	A/A	T/T
111-01	F	75	C/C	G/G	C/C	C/C	A/A	T/T
113-01	F	79	C/G	G/G	C/T	C/A	A/A	T/T
359-01	M	77	C/C	G/G	C/C	C/C	A/A	T/T
403-01	F	71	C/G	G/G	C/T	C/A	A/A	T/T
405-01	F	83	C/C	G/G	C/C	C/C	A/A	T/T
406-01	F	68	C/C	G/G	C/C	C/C	A/G	T/G
115-02	F	78	G/G	G/G	T/T	A/A	A/A	T/T
116-02	M	77	C/G	G/G	C/T	C/A	A/A	T/T
117-02	F	70	C/C	G/G	C/C	C/C	A/A	T/T
120-02	M	72	C/C	G/A	C/C	C/C	A/A	T/T
140-02	F	82	C/G	G/A	C/C	C/C	A/G	T/G
143-02	F	75	C/C	G/G	C/C	C/C	A/A	T/T
145-02	F	84	C/G	G/G	C/T	C/A	A/A	T/T
161-02	F	84	C/C	G/G	C/C	C/C	A/A	T/T
162-02	F	80	C/G	G/G	C/T	C/A	A/A	T/T
164-02	M	79	C/G	G/G	C/T	C/A	A/A	T/T

Table C-1. Continued

167-02	M	71	G/G	G/G	T/T	A/A	A/A	T/T
168-02	F	72	C/C	A/A	C/C	C/C	A/A	T/T
169-02	M	70	C/G	G/G	C/T	C/A	A/A	T/T
170-02	M	74	C/G	G/G	C/T	C/A	A/A	T/T
172-02	F	72	C/C	G/G	C/C	C/C	A/A	T/T
173-02	F	76	C/C	G/G	C/C	C/C	A/A	T/T
174-02	M	78	C/C	G/G	C/C	C/C	A/A	T/T
177-02	M	72	C/C	G/A	C/T	C/A	A/A	T/T
178-02	F	77	C/C	G/A	C/C	C/C	A/A	T/T
179-02	M	72	C/C	G/A	C/C	C/C	A/A	T/T
180-02	F	76	C/G	G/G	C/C	C/C	A/A	T/T
181-02	M	74	C/C	G/G	C/C	C/C	A/A	T/T
186-02	F	77	C/C	G/G	C/C	C/C	A/A	T/T
188-02	F	76	G/G	G/G	C/T	C/A	A/A	T/T
194-02	F	77	C/G	G/G	C/C	C/C	A/A	T/T
195-02	F	81	C/C	G/G	C/C	C/C	A/A	T/T
198-02	F	79	C/G	G/G	C/T	C/A	A/A	T/T
199-02	F	80	C/C	G/G	C/C	C/C	A/A	T/T
200-02	F	82	C/C	G/G	C/C	C/C	A/A	T/T
201-02	F	74	C/C	G/G	C/C	C/C	A/A	T/T
202-02	F	78	C/C	G/G	C/C	C/C	A/A	T/T
206-02	M	83	C/C	G/G	T/T	A/A	A/A	T/T
208-02	M	74	C/C	G/G	C/C	C/C	A/A	T/T
211-02	M	81	C/C	G/G	C/C	C/C	A/A	T/T
212-02	F	78	C/G	G/G	C/C	C/C	A/A	T/T
213-02	F	75	C/C	G/A	C/C	C/C	A/A	T/T
283-00	F	50	C/G	G/G	C/T	C/A	A/A	T/T
109-01	M	52	C/C	G/A	C/C	C/C	A/A	T/T
356-01	F	31	C/C	G/G	C/T	C/A	A/A	T/T
358-01	F	50	C/C	G/G	C/C	C/C	A/A	T/T
379-01	M	46	C/C	G/G	C/C	C/C	A/A	T/T
382-01	M	45	C/C	G/G	C/C	C/C	A/A	T/T
384-01	M	41	C/G	G/G	C/C	C/C	A/A	T/T
15-02	F	48	C/C	G/G	C/C	C/C	A/A	T/T
16-02	F	42	C/G	G/G	C/T	C/A	A/A	T/T

Table C-1. Continued

359-02	F	88	C/C	G/G	C/C	C/A	A/A	T/T
430-99	F	69	C/C	G/G	C/C	C/C	A/A	T/T
431-99	M	68	C/C	G/G	C/C	C/C	A/A	T/T
435-99	M	64	C/G	G/G	T/T	A/A	A/A	T/T
308-00	M	74	C/C	G/G	T/T	A/A	A/A	T/T
345-00	F	67	G/G	G/G	C/C	C/C	A/A	T/T
444-00	M	52	C/C	G/G	C/C	C/C	A/A	T/T
21-01	M	60	C/C	G/G	C/C	C/C	A/A	T/T
50-01	M	72	C/G	G/G	T/T	A/A	A/A	T/T
148-01	M	64	C/G	G/G	T/T	A/A	A/A	T/T
149-01	M	69	C/C	G/G	C/T	C/A	A/A	T/T
292-01	M	75	C/C	G/G	C/T	C/A	A/A	T/T
236-03	M	83	C/G	G/G	C/C	C/C	A/A	T/T
286-03	F	59	C/C	A/A	C/C	C/C	A/A	T/T
325-03	M	83	C/C	G/G	C/T	C/A	A/A	T/T
340-03	M	75	C/G	G/G	C/C	C/C	A/A	T/T
361-02	M	70	C/G	G/G	C/C	C/C	A/A	T/T

Note: (-) – unknown; nd – nondeletion; del-deletion

APPENDIX D

IOWA CONTROLS

Table D-1. Age, sex and genotypes of Iowa Controls.

DDD cases id	Sex (M/F)	Age (years)	CFH -331C>T (rs3753394)	CFH V62I G>A (rs800292)	CFH Y402H T>C (rs1061170)	CFH Q673 A>G (rs3753396)	CFH D936E G>T (rs1065489)	CFHR5 -249 T>C (rs9427661)	CFHR5 -20 T>C (rs9427662)
IA0004	M	17	C/C	A/A	C/T	A/A	G/G	C/T	C/T
IA0005	M	18	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA0006	M	16	T/T	G/G	T/T	G/G	T/T	T/T	T/T
IA0010	F	16	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA0023	F	17	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA0024	M	17	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA0025	M	18	C/T	G/G	T/T	G/A	G/T	T/T	T/T
IA0052	M	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0053	M	17	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0055	F	17	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA0056	M	16	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0057	M	17	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA0058	M	17	C/C	G/G	C/T	A/A	G/G	T/T	T/T
IA0060	M	16	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0061	F	17	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA0062	F	17	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0065	M	17	C/T	G/G	C/T	A/A	G/G	C/C	C/C
IA0067	M	16	C/C	G/A	C/C	A/A	G/G	T/T	T/T
IA0068	M	16	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA0069	F	17	C/C	A/A	C/C	A/A	G/G	T/T	T/T
IA0071	F	17	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA0078	M	17	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0079	F	16	C/T	G/G	C/T	A/A	G/G	C/T	C/T
IA0081	F	18	T/T	G/G	T/T	G/G	T/T	T/T	T/T
IA0082	F	16	C/T	G/G	T/T	A/A	G/G	C/T	C/T

Table D-1. Continued

IA0084	M	13	T/T	G/G	T/T	G/A	G/T	T/T	T/T
IA0091	M	20	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0094	F	18	C/C	G/A	T/T	A/A	G/G	C/T	C/T
IA0095	M	19	C/T	G/G	T/T	A/A	G/G	T/T	T/T
IA0097	F	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0099	F	18	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA0100	M	19	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA0101	M	18	C/T	G/G	T/T	A/A	G/G	T/T	T/T
IA0102	F	19	C/C	G/G	C/T	A/A	G/G	T/T	T/T
IA0103	F	18	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0106	F	18	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0108	F	20	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA0109	F	20	C/T	G/A	C/C	A/A	G/G	T/T	T/T
IA0110	F	19	C/C	G/G	C/T	A/A	G/G	T/T	T/T
IA0112	F	20	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA0113	F	18	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA0114	F	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0172	M	20	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA0195	M	17	T/T	G/G	T/T	A/A	G/G	T/T	T/T
IA0233	F	13	T/T	G/G	C/T	G/A	G/T	T/T	T/T
IA0336	F	18	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA0337	F	19	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0416	M	17	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA0469	M	18	T/T	G/G	T/T	G/A	G/T	T/T	T/T
IA0473	F	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0475	F	20	C/T	G/A	T/T	A/A	G/G	C/T	C/T
IA0482	F	19	C/C	G/G	T/T	G/A	G/T	C/T	C/T
IA0483	F	18	C/C	G/A	T/T	G/A	G/T	C/T	C/T
IA0484	F	19	C/C	A/A	T/T	G/A	G/T	T/T	T/T
IA0542	F	16	C/C	G/A	C/T	A/A	G/G	C/T	C/T
IA0597	M	20	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0599	F	18	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA0611	F	17	C/T	G/A	T/T	G/G	T/T	T/T	T/T
IA0632	F	19	C/C	G/G	T/T	A/A	G/G	C/C	C/C
IA0671	F	20	C/T	G/A	T/T	A/A	G/G	T/T	T/T

Table D-1. Continued

IA0673	M	17	C/C	G/A	T/T	G/A	G/T	T/T	T/T
IA0756	M	18	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0760	F	20	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA0775	F	17	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA0790	M	20	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA0807	F	19	T/T	G/G	T/T	A/A	G/G	T/T	T/T
IA0824	F	19	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA0828	M	19	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA0850	M	19	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA0863	M	20	C/C	G/A	T/T	A/A	G/G	C/T	C/T
IA1026	F	17	C/C	G/A	T/T	A/A	G/G	T/T	T/T
IA1059	F	17	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA1071	M	17	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1095	F	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1096	F	20	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA1098	F	18	C/T	G/G	T/T	G/A	G/T	C/T	C/T
IA1100	F	19	C/C	A/A	C/T	A/A	G/G	T/T	T/T
IA1104	F	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1109	F	19	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1116	M	19	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA1164	F	20	C/C	G/G	C/T	A/A	G/G	T/T	T/T
IA1185	F	18	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA1187	M	19	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1190	F	18	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA1191	F	19	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA1199	F	18	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1200	F	20	C/T	G/G	T/T	G/A	G/T	C/T	C/T
IA1201	F	19	C/T	G/G	T/T	G/A	G/T	C/T	C/T
IA1202	F	18	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA1204	F	20	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA1211	F	19	C/T	G/A	T/T	A/A	G/G	C/T	C/T
IA1251	F	19	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA1277	M	18	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA1279	F	19	C/T	G/A	T/T	G/A	G/T	T/T	T/T
IA1299	M	20	C/C	G/G	C/T	A/A	G/G	T/T	T/T

Table D-1. Continued

IA1304	F	18	T/T	G/G	T/T	G/A	G/T	T/T	T/T
IA1335	F	20	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA1344	M	18	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA1345	F	19	C/T	G/A	T/T	G/A	G/T	C/T	C/T
IA1347	M	19	T/T	G/G	T/T	A/A	G/G	T/T	T/T
IA1348	M	20	C/C	G/A	T/T	A/A	G/G	T/T	T/T
IA1349	F	20	C/T	G/G	C/C	A/A	G/G	T/T	T/T
IA1353	F	20	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA1354	F	18	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA1356	M	18	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA1368	M	19	T/T	G/G	T/T	G/A	G/T	T/T	T/T
IA1370	F	19	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA1371	F	19	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1374	F	18	C/C	G/A	C/T	A/A	G/T	T/T	T/T
IA1375	F	19	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA1376	F	19	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA1377	F	19	C/T	G/A	T/T	A/A	G/G	C/T	C/T
IA1379	M	20	C/C	G/G	C/T	A/A	G/G	T/T	T/T
IA1383	F	20	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA1384	F	20	C/T	G/G	T/T	G/A	G/T	C/T	C/T
IA1390	F	19	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA1399	F	19	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA1401	M	20	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA1402	M	18	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA1407	M	19	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1409	F	19	C/C	G/A	T/T	A/A	G/G	C/T	C/T
IA1416	F	20	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA1433	M	16	C/T	G/G	C/T	A/A	G/T	T/T	T/T
IA1465	F	20	C/C	G/A	C/T	G/A	G/G	T/T	T/T
IA1524	M	18	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA1525	F	19	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA1526	F	18	C/T	G/A	T/T	A/A	G/G	C/T	C/T
IA1527	F	19	C/T	G/G	T/T	A/A	G/G	C/T	C/T
IA1528	F	19	C/T	G/G	T/T	G/A	G/T	T/T	T/T
IA1530	F	18	C/C	G/A	C/T	A/A	G/G	T/T	T/T

Table D-1. Continued

IA1532	F	18	C/T	G/G	C/C	G/A	G/T	T/T	T/T
IA1533	F	18	C/T	G/A	C/T	G/A	G/T	T/T	T/T
IA1534	F	20	C/C	G/A	T/T	A/A	G/G	T/T	T/T
IA1535	F	18	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA1536	F	20	C/T	G/G	T/T	A/A	G/G	C/T	C/T
IA1537	F	20	C/C	G/G	C/T	A/A	G/G	T/T	T/T
IA1539	F	20	C/T	G/G	T/T	A/A	G/G	T/T	T/T
IA1540	F	18	C/C	G/A	T/T	A/A	G/G	C/T	C/T
IA1541	F	20	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA1544	F	18	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA1550	F	19	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA1551	M	18	C/T	G/G	C/C	A/A	G/G	T/T	T/T
IA1552	M	20	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA2074	F	23	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA2075	F	18	C/T	G/G	T/T	G/A	G/T	C/T	C/T
IA2076	F	21	C/C	A/A	T/T	A/A	G/G	T/T	T/T
IA2077	M	20	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA2078	M	17	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA2079	F	16	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA2083	M	20	C/T	G/G	C/C	A/A	G/G	T/T	T/T
IA2084	F	19	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA2085	F	18	C/T	G/A	C/T	A/A	G/G	T/T	T/T
IA2086	M	18	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA2087	F	20	C/C	G/G	T/T	A/A	G/G	C/T	C/T
IA2093	M	19	C/C	G/A	C/T	A/A	G/G	T/T	T/T
IA2103	M	19	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA2123	M	19	C/C	G/G	C/C	A/A	G/G	T/T	T/T
IA2127	M	20	C/C	G/A	T/T	A/A	G/G	T/T	T/T
IA2128	F	19	C/T	G/A	T/T	A/A	G/G	T/T	T/T
IA2129	F	19	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA2136	F	20	C/C	G/G	C/T	A/A	G/G	C/T	C/T
IA2139	F	19	C/T	G/G	C/T	A/A	G/G	T/T	T/T
IA2159	M	18	C/T	G/G	C/T	G/A	G/T	T/T	T/T
IA2169	M	19	C/C	G/A	C/T	G/A	G/T	T/T	T/T
IA2173	M	18	T/T	G/G	T/T	G/G	T/T	T/T	T/T

Table D-1. Continued

DDD cases id	Sex (M/F)	Age (years)	CFHR5 P84S C>T (rs12097550)	CR1 Q1022H G>T (rs3738467)	CR1 H1208R A>G (rs2274567)	CR1 P1827R C>G (rs3811381)	CR1 H/L alleles	ADAM19 S284G A>G (rs1422795)	C4A deletion (nd, del)
IA0004	M	17	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
IA0005	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0006	M	16	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0010	F	16	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0023	F	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0024	M	17	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
IA0025	M	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA0052	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0053	M	17	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0055	F	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0056	M	16	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0057	M	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0058	M	17	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0060	M	16	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0061	F	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0062	F	17	C/T	G/T	A/G	C/G	H/L	A/A	nd/nd
IA0065	M	17	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0067	M	16	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0068	M	16	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0069	F	17	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
IA0071	F	17	C/C	G/T	A/G	C/G	H/L	A/A	nd/del
IA0078	M	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0079	F	16	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0081	F	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA0082	F	16	C/C	G/G	A/A	C/C	H/H	G/G	nd/del
IA0084	M	13	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0091	M	20	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0094	F	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/del

Table D-1. Continued

IA0095	M	19	C/C	G/T	A/G	C/G	H/L	G/G	nd/nd
IA0097	F	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0099	F	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0100	M	19	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0101	M	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0102	F	19	C/C	G/T	A/G	C/G	H/L	A/G	nd/del
IA0103	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0106	F	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0108	F	20	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0109	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0110	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0112	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0113	F	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0114	F	18	C/C	T/T	G/G	G/G	L/L	A/A	nd/nd
IA0172	M	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0195	M	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0233	F	13	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA0336	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA0337	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0416	M	17	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0469	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0473	F	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0475	F	20	C/C	T/T	G/G	G/G	L/L	A/A	nd/nd
IA0482	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA0483	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0484	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0542	F	16	C/C	G/T	A/G	C/G	H/L	G/G	nd/nd
IA0597	M	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA0599	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0611	F	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA0632	F	19	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0671	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0673	M	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0756	M	18	C/C	T/T	G/G	G/G	L/L	A/A	nd/nd
IA0760	F	20	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd

Table D-1. Continued

IA0775	F	17	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
IA0790	M	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0807	F	19	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA0824	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0828	M	19	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA0850	M	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA0863	M	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/del
IA1026	F	17	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1059	F	17	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1071	M	17	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1095	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1096	F	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1098	F	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1100	F	19	C/C	T/T	G/G	G/G	L/L	A/A	nd/nd
IA1104	F	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/del
IA1109	F	19	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1116	M	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1164	F	20	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1185	F	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1187	M	19	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1190	F	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1191	F	19	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA1199	F	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1200	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1201	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1202	F	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA1204	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1211	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1251	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1277	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1279	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA1299	M	20	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA1304	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1335	F	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1344	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd

Table D-1. Continued

IA1345	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1347	M	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA1348	M	20	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1349	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA1353	F	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1354	F	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1356	M	18	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
IA1368	M	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1370	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1371	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1374	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA1375	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1376	F	19	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1377	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1379	M	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1383	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA1384	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1390	F	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1399	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1401	M	20	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
IA1402	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1407	M	19	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1409	F	19	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA1416	F	20	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1433	M	16	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1465	F	20	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1524	M	18	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1525	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1526	F	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA1527	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1528	F	19	C/C	G/G	A/A	C/C	H/H	G/G	nd/del
IA1530	F	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1532	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1533	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1534	F	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd

Table D-1. Continued

IA1535	F	18	C/T	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1536	F	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1537	F	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1539	F	20	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA1540	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1541	F	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA1544	F	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/del
IA1550	F	19	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA1551	M	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA1552	M	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA2074	F	23	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA2075	F	18	C/C	G/T	A/G	C/G	H/L	A/G	nd/nd
IA2076	F	21	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA2077	M	20	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA2078	M	17	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
IA2079	F	16	C/T	G/G	A/A	C/C	H/H	A/A	nd/nd
IA2083	M	20	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA2084	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA2085	F	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA2086	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA2087	F	20	C/C	G/T	A/G	C/G	H/L	A/A	nd/nd
IA2093	M	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA2103	M	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA2123	M	19	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
IA2127	M	20	C/C	G/T	A/G	C/G	H/L	G/G	nd/nd
IA2128	F	19	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA2129	F	19	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
IA2136	F	20	C/T	G/G	A/A	C/C	H/H	A/A	nd/nd
IA2139	F	19	C/C	G/G	A/A	C/C	H/H	G/G	nd/nd
IA2159	M	18	C/C	G/G	A/A	C/C	H/H	A/G	nd/nd
IA2169	M	19	C/C	G/G	A/A	C/C	H/H	A/A	nd/nd
IA2173	M	18	C/C	G/G	A/A	C/C	H/H	A/A	nd/del

Table D-1. Continued

DDD cases id	Sex (M/F)	Age (years)	C3 R102G C>G (rs2330199)	C3 R304 G>A (rs12230201)	C3 P314L C>T (rs1047286)	C3 P518 C>A (rs2230203)	C5aR1 D2N A>G (rs4467185)	C5aR1 K279N T>G (rs11880097)
IA0004	M	17	C/C	G/G	C/C	C/C	A/G	T/G
IA0005	M	18	C/G	G/A	C/T	C/A	A/A	T/T
IA0006	M	16	C/C	G/G	C/C	C/C	A/G	T/G
IA0010	F	16	C/G	G/G	C/T	C/C	A/A	T/T
IA0023	F	17	C/G	G/G	C/T	C/C	A/A	T/T
IA0024	M	17	C/G	G/G	C/T	C/C	A/G	T/G
IA0025	M	18	C/G	G/G	C/T	C/A	A/A	T/T
IA0052	M	18	C/G	G/G	C/C	C/C	A/A	T/T
IA0053	M	17	C/G	G/G	C/T	C/C	A/A	T/T
IA0055	F	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0056	M	16	C/G	G/G	C/T	C/C	A/A	T/T
IA0057	M	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0058	M	17	G/G	G/A	C/T	C/A	A/A	T/T
IA0060	M	16	C/G	G/A	C/T	C/A	A/A	T/T
IA0061	F	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0062	F	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0065	M	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0067	M	16	C/C	A/A	C/C	C/C	A/A	T/T
IA0068	M	16	C/C	G/G	C/C	C/C	A/A	T/T
IA0069	F	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0071	F	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0078	M	17	C/C	G/A	C/C	C/C	A/A	T/T
IA0079	F	16	C/C	G/G	C/C	C/C	A/A	T/T
IA0081	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0082	F	16	C/C	G/G	C/C	C/C	A/A	T/T
IA0084	M	13	C/C	G/A	C/C	C/C	A/A	T/T
IA0091	M	20	C/C	G/G	C/C	C/C	A/A	T/T

Table D-1. Continued

IA0094	F	18	C/G	G/G	C/T	C/C	A/A	T/T
IA0095	M	19	G/G	G/G	T/T	A/A	A/G	T/G
IA0097	F	18	G/G	G/A	C/T	C/C	A/A	T/T
IA0099	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0100	M	19	C/G	G/A	C/T	C/A	A/A	T/T
IA0101	M	18	G/G	G/G	C/T	C/A	A/A	T/T
IA0102	F	19	C/C	G/A	C/C	C/C	A/A	T/T
IA0103	F	18	C/C	G/G	C/T	C/A	A/A	T/T
IA0106	F	18	C/C	G/A	C/C	C/C	A/A	T/T
IA0108	F	20	C/G	G/G	C/T	C/A	A/A	T/T
IA0109	F	20	C/C	G/A	C/C	C/C	A/A	T/T
IA0110	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA0112	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA0113	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0114	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0172	M	20	C/G	G/G	C/T	C/C	A/A	T/T
IA0195	M	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0233	F	13	C/C	G/A	C/C	C/C	A/A	T/T
IA0336	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0337	F	19	C/G	G/A	C/T	C/A	A/A	T/T
IA0416	M	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0469	M	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0473	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0475	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA0482	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA0483	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0484	F	19	C/G	G/G	C/T	C/A	A/A	T/T
IA0542	F	16	C/G	A/A	C/C	C/C	A/A	T/T
IA0597	M	20	C/C	G/G	C/T	C/A	A/A	T/T
IA0599	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA0611	F	17	C/C	G/A	C/C	C/C	A/A	T/T
IA0632	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA0671	F	20	C/C	G/A	C/C	C/C	A/A	T/T
IA0673	M	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0756	M	18	C/C	A/A	C/C	C/C	A/A	T/T

Table D-1. Continued

IA0760	F	20	C/G	G/A	C/T	C/A	A/A	T/T
IA0775	F	17	C/C	G/G	C/C	C/C	A/A	T/T
IA0790	M	20	C/C	G/G	C/C	C/C	A/A	T/T
IA0807	F	19	C/C	G/A	C/C	C/C	A/A	T/T
IA0824	F	19	C/C	G/A	C/C	C/C	A/A	T/T
IA0828	M	19	C/C	G/G	C/C	C/C	A/A	T/T
IA0850	M	19	G/G	G/G	T/T	A/A	A/A	T/T
IA0863	M	20	C/G	G/G	C/T	C/A	A/A	T/T
IA1026	F	17	C/C	G/G	C/C	C/C	A/A	T/T
IA1059	F	17	G/G	G/G	T/T	A/A	A/A	T/T
IA1071	M	17	C/C	G/G	C/C	C/C	A/A	T/T
IA1095	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1096	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1098	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1100	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1104	F	18	C/G	G/G	C/T	C/A	A/A	T/T
IA1109	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1116	M	19	C/C	A/A	C/C	C/C	A/A	T/T
IA1164	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1185	F	18	C/G	G/G	C/C	C/A	A/A	T/T
IA1187	M	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1190	F	18	C/C	G/A	C/C	C/C	A/A	T/T
IA1191	F	19	C/G	G/G	C/T	C/A	A/A	T/T
IA1199	F	18	C/G	G/G	C/T	C/A	A/A	T/T
IA1200	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1201	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1202	F	18	C/G	G/G	C/T	C/A	A/A	T/T
IA1204	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1211	F	19	C/G	G/A	C/T	C/A	A/A	T/T
IA1251	F	19	C/G	G/G	C/T	C/A	A/A	T/T
IA1277	M	18	G/G	G/G	T/T	A/A	A/A	T/T
IA1279	F	19	C/C	A/A	C/C	C/C	A/A	T/T
IA1299	M	20	C/C	A/A	C/C	C/C	A/A	T/T
IA1304	F	18	C/G	G/G	C/T	C/A	A/A	T/T
IA1335	F	20	C/C	G/G	C/C	C/C	A/A	T/T

Table D-1. Continued

IA1344	M	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1345	F	19	C/G	G/A	C/T	C/A	A/A	T/T
IA1347	M	19	C/C	A/A	C/C	C/C	A/A	T/T
IA1348	M	20	C/C	G/A	C/C	C/C	A/A	T/T
IA1349	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1353	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1354	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1356	M	18	C/G	G/A	C/T	C/A	A/A	T/T
IA1368	M	19	C/G	G/G	C/T	C/A	A/A	T/T
IA1370	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1371	F	19	C/G	G/G	C/T	C/A	A/A	T/T
IA1374	F	18	C/G	G/G	C/T	C/A	A/A	T/T
IA1375	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1376	F	19	C/C	G/G	C/T	C/A	A/A	T/T
IA1377	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1379	M	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1383	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1384	F	20	G/G	A/A	C/T	C/A	A/A	T/T
IA1390	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1399	F	19	C/G	G/G	C/T	C/C	A/A	T/T
IA1401	M	20	C/G	G/G	C/T	C/A	A/A	T/T
IA1402	M	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1407	M	19	C/C	G/A	C/C	C/C	A/A	T/T
IA1409	F	19	C/C	G/A	C/C	C/C	A/A	T/T
IA1416	F	20	C/G	G/G	C/T	C/C	A/A	T/T
IA1433	M	16	C/C	G/G	C/C	C/C	A/A	T/T
IA1465	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1524	M	18	C/G	G/G	C/T	C/A	A/A	T/T
IA1525	F	19	C/C	G/A	C/C	C/C	A/A	T/T
IA1526	F	18	C/C	A/A	C/C	C/C	A/A	T/T
IA1527	F	19	C/C	G/A	C/C	C/C	A/A	T/T
IA1528	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1530	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1532	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1533	F	18	C/C	G/G	C/C	C/C	A/A	T/T

Table D-1. Continued

IA1534	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA1535	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1536	F	20	C/C	G/G	C/T	C/A	A/A	T/T
IA1537	F	20	C/G	G/G	C/C	C/C	A/A	T/T
IA1539	F	20	C/C	G/G	C/T	C/A	A/A	T/T
IA1540	F	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1541	F	20	G/G	G/G	T/T	A/A	A/A	T/T
IA1544	F	18	C/G	G/G	C/T	C/A	A/A	T/T
IA1550	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA1551	M	18	C/C	G/G	C/C	C/C	A/A	T/T
IA1552	M	20	C/C	G/A	C/C	C/C	A/A	T/T
IA2074	F	23	C/C	G/G	C/T	C/A	A/A	T/T
IA2075	F	18	C/C	G/A	C/C	C/C	A/A	T/T
IA2076	F	21	C/C	G/A	C/C	C/C	A/A	T/T
IA2077	M	20	C/C	G/G	C/C	C/C	A/A	T/T
IA2078	M	17	C/C	G/A	C/C	C/C	A/A	T/T
IA2079	F	16	C/G	G/G	C/T	C/A	A/A	T/T
IA2083	M	20	C/C	G/G	C/C	C/C	A/A	T/T
IA2084	F	19	C/G	G/G	C/T	C/A	A/A	T/T
IA2085	F	18	G/G	G/G	T/T	A/A	A/A	T/T
IA2086	M	18	C/G	G/G	C/T	C/A	A/A	T/T
IA2087	F	20	C/G	G/G	C/T	C/A	A/A	T/T
IA2093	M	19	C/G	G/G	C/T	C/A	A/A	T/T
IA2103	M	19	C/G	G/A	C/C	C/C	A/A	T/T
IA2123	M	19	C/C	G/A	C/C	C/C	A/A	T/T
IA2127	M	20	C/C	G/G	C/C	C/C	A/A	T/T
IA2128	F	19	C/C	G/G	C/C	C/C	A/A	T/T
IA2129	F	19	C/C	G/A	C/C	C/C	A/A	T/T
IA2136	F	20	C/C	G/G	C/C	C/C	A/A	T/T
IA2139	F	19	C/G	G/G	C/T	C/A	A/A	T/T
IA2159	M	18	C/G	G/G	C/T	C/A	A/A	T/T
IA2169	M	19	G/G	G/G	C/T	C/A	A/A	T/T
IA2173	M	18	C/G	G/G	T/T	A/A	A/A	T/T

Note: (-) – unknown; nd – nondeletion; del-deletion

APPENDIX E

BEST INHERITANCE MODELS

Table E-1. Best inheritance model by SNPStats.

CFHY402H association with response status (n=339, crude analysis)						
Model	**Genotype**	**status=Control**	**status=Case**	**OR (95% CI)**	**P-value**	**AIC**
Codominant	T/T	122 (45.5%)	20 (28.2%)	1.00		
	C/T	104 (38.8%)	34 (47.9%)	**1.99 (1.08-3.67)**	0.023	346.4
	C/C	42 (15.7%)	17 (23.9%)	**2.47 (1.18-5.15)**		
Dominant	T/T	122 (45.5%)	20 (28.2%)	1.00	0.0073	344.8
	C/T-C/C	146 (54.5%)	51 (71.8%)	**2.13 (1.20-3.77)**		
Recessive	T/T-C/T	226 (84.3%)	54 (76.1%)	1.00	0.11	349.4
	C/C	42 (15.7%)	17 (23.9%)	1.69 (0.90-3.20)		
Overdominant	T/T-C/C	164 (61.2%)	37 (52.1%)	1.00	0.17	350.1
	C/T	104 (38.8%)	34 (47.9%)	1.45 (0.86-2.45)		
Log-additive	---	---	---	**1.60 (1.12-2.28)**	0.0091	345.2

C3R102G association with response status (n=339, crude analysis)						
Model	**Genotype**	**status=Control**	**status=Case**	**OR (95% CI)**	**P-value**	**AIC**
Codominant	C/C	175 (65.3%)	33 (46.5%)	1.00		
	C/G	77 (28.7%)	32 (45.1%)	**2.20 (1.26-3.84)**	0.016	345.7
	G/G	16 (6%)	6 (8.4%)	1.99 (0.72-5.46)		
Dominant	C/C	175 (65.3%)	33 (46.5%)	1.00	0.0042	343.8
	C/G-G/G	93 (34.7%)	38 (53.5%)	**2.17 (1.28-3.68)**		
Recessive	C/C-C/G	252 (94%)	65 (91.5%)	1.00	0.46	351.4
	G/G	16 (6%)	6 (8.4%)	1.45 (0.55-3.86)		
Overdominant	C/C-G/G	191 (71.3%)	39 (54.9%)	1.00	0.01	345.4
	C/G	77 (28.7%)	32 (45.1%)	**2.04 (1.19-3.48)**		
Log-additive	---	---	---	**1.69 (1.13-2.53)**	0.011	345.6

C3P314L association with response status (n=339, crude analysis)						
Model	**Genotype**	**status=Control**	**status=Case**	**OR (95% CI)**	**P-value**	**AIC**
Codominant	C/C	172 (64.2%)	33 (46.5%)	1.00		
	C/T	80 (29.9%)	31 (43.7%)	**2.02 (1.16-3.53)**	0.026	346.7
	T/T	16 (6%)	7 (9.9%)	2.28 (0.87-5.97)		
Dominant	C/C	172 (64.2%)	33 (46.5%)	1.00	0.0072	344.7
	C/T-T/T	96 (35.8%)	38 (53.5%)	**2.06 (1.22-3.50)**		
Recessive	C/C-C/T	252 (94%)	64 (90.1%)	1.00	0.27	350.7
	T/T	16 (6%)	7 (9.9%)	1.72 (0.68-4.36)		
Overdominant	C/C-T/T	188 (70.2%)	40 (56.3%)	1.00	0.03	347.3
	C/T	80 (29.9%)	31 (43.7%)	**1.82 (1.06-3.12)**		
Log-additive	---	---	---	**1.69 (1.13-2.52)**	0.011	345.5

Table E-1. Continued

CR1Q1022H association with response status (n=339, crude analysis)						
Model	**Genotype**	**status=Control**	**status=Case**	**OR (95% CI)**	**P-value**	**AIC**
Codominant	G/G	191 (71.3%)	41 (57.8%)	1.00		
	G/T	71 (26.5%)	28 (39.4%)	**1.84 (1.06-3.19)**	0.099	349.3
	T/T	6 (2.2%)	2 (2.8%)	1.55 (0.30-7.97)		
Dominant	G/G	191 (71.3%)	41 (57.8%)	1.00	0.032	347.4
	G/T-T/T	77 (28.7%)	30 (42.2%)	**1.82 (1.06-3.12)**		
Recessive	G/G-G/T	262 (97.8%)	69 (97.2%)	1.00	0.78	351.9
	T/T	6 (2.2%)	2 (2.8%)	1.27 (0.25-6.41)		
Overdominant	G/G-T/T	197 (73.5%)	43 (60.6%)	1.00	0.037	347.6
	G/T	71 (26.5%)	28 (39.4%)	**1.81 (1.04-3.12)**		
Log-additive	---	---	---	**1.62 (1.01-2.61)**	0.048	348

CFHR55UTR association with response status (n=339, crude analysis)						
Model	**Genotype**	**status=Control**	**status=Case**	**OR (95% CI)**	**P-value**	**AIC**
Codominant	T/T	206 (76.9%)	64 (90.1%)	1.00		
	C/T	59 (22%)	7 (9.9%)	**0.38 (0.17-0.88)**	0.022	346.4
	C/C	3 (1.1%)	0 (0%)	0.00 (0.00-NA)		
Dominant	T/T	206 (76.9%)	64 (90.1%)	1.00	0.0085	345
	C/T-C/C	62 (23.1%)	7 (9.9%)	**0.36 (0.16-0.83)**		
Recessive	T/T-C/T	265 (98.9%)	71 (100%)	1.00	0.23	350.5
	C/C	3 (1.1%)	0 (0%)	0.00 (0.00-NA)		
Overdominant	T/T-C/C	209 (78%)	64 (90.1%)	1.00	0.014	346
	C/T	59 (22%)	7 (9.9%)	**0.39 (0.17-0.89)**		
Log-additive	---	---	---	**0.37 (0.16-0.83)**	0.0068	344.6

ADAM19S284G association with response status (n=339, crude analysis)						
Model	**Genotype**	**status=Control**	**status=Case**	**OR (95% CI)**	**P-value**	**AIC**
Codominant	A/A	145 (54.1%)	22 (31%)	1.00		
	A/G	97 (36.2%)	35 (49.3%)	**2.38 (1.32-4.30)**	0.0013	340.6
	G/G	26 (9.7%)	14 (19.7%)	**3.55 (1.61-7.82)**		
Dominant	A/A	145 (54.1%)	22 (31%)	1.00	5e-04	339.7
	A/G-G/G	123 (45.9%)	49 (69%)	**2.63 (1.50-4.58)**		
Recessive	A/A-A/G	242 (90.3%)	57 (80.3%)	1.00	0.028	347.1
	G/G	26 (9.7%)	14 (19.7%)	**2.29 (1.12-4.65)**		
Overdominant	A/A-G/G	171 (63.8%)	36 (50.7%)	1.00	0.046	348
	A/G	97 (36.2%)	35 (49.3%)	**1.71 (1.01-2.91)**		
Log-additive	---	---	---	**1.96 (1.35-2.85)**	4e-04	339.3

APPENDIX F

BLOOD DONOR SAMPLES

Table F-1. Genotypes of blood donor samples.

Sample id	CFH -331C>T (rs3753394)	CFH V62I G>A (rs800292)	CFH Y402H T>C (rs1061170)	CFH Q673 A>G (rs3753396)	CFH D936E G>T (rs1065489)	C3 R102G C>G (rs2230199)	C3 R304 G>A (rs12230201)	C3 P314L C>T (rs1047286)	C3 P518 C>A (rs2230203)
P50569	C/C	G/A	T/T	A/A	G/G	C/C	G/A	C/C	C/C
P50570	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
P50571	C/T	G/A	T/T	A/A	G/G	C/G	G/G	C/T	C/A
P50574	T/T	G/G	T/T	A/G	G/T	C/C	G/G	C/C	C/C
P53334	C/C	G/G	C/C	A/A	G/G	C/G	G/G	C/T	C/A
P53335	C/C	G/G	T/C	A/A	G/G	C/C	G/A	C/C	C/C
P53339	C/C	G/A	T/C	A/A	G/G	C/G	G/G	C/T	C/A
P53340	C/C	G/A	T/T	A/A	G/G	C/G	G/A	C/T	C/A
P53341	C/C	G/G	C/C	A/A	G/G	G/G	G/G	T/T	A/A
P53342	C/C	A/A	T/T	A/A	G/G	C/G	G/G	C/T	C/A
P53343	C/T	G/G	T/C	A/A	G/G	C/G	G/G	C/C	C/C
P53344	T/T	G/G	T/T	A/G	G/T	C/C	G/A	C/C	C/C
P53345	C/C	G/G	C/C	A/A	G/G	G/G	G/G	T/T	A/A
P53346	C/C	A/A	T/T	A/A	G/T	C/C	G/A	C/C	C/C
P53347	C/C	G/A	T/C	A/A	G/G	C/G	G/G	C/T	C/A
P53348	T/T	G/G	T/T	G/G	T/T	C/C	G/A	C/C	C/C
P53349	C/C	G/A	T/T	A/A	G/G	C/C	A/A	C/C	C/C
P53350	C/T	G/G	T/T	A/G	G/T	C/G	G/G	C/T	C/A
P53351	C/T	G/G	C/C	A/A	G/G	C/G	G/G	C/T	C/A
P53352	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
P53353	C/C	G/A	T/T	A/A	G/G	C/C	G/G	C/C	C/C
P53354	C/C	G/G	C/C	A/A	G/G	C/C	G/A	C/C	C/C
P53355	C/C	G/A	T/C	A/A	G/G	C/C	G/G	C/C	C/C
P53356	C/T	G/G	T/C	A/A	G/G	C/C	G/G	C/C	C/C

Table F-1. Continued

P53357	C/T	G/A	T/T	A/G	G/T	C/C	G/G	C/C	C/C
P53358	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
P53359	C/C	G/G	T/C	A/G	G/G	C/C	G/G	C/C	C/C
P53360	C/C	G/A	T/T	A/A	G/G	C/G	G/G	C/T	C/A
P53361	C/C	G/G	C/C	A/A	G/G	C/G	G/G	C/T	C/A
P53363	C/T	G/A	T/T	G/G	T/T	C/C	G/G	C/C	C/C
P53364	C/T	G/G	T/C	A/A	G/G	C/C	G/A	C/C	C/C
P53365	C/T	G/G	T/T	A/G	G/T	C/G	G/G	C/T	C/A
P53366	C/T	G/A	T/T	G/G	T/T	C/C	G/G	C/C	C/C
P53367	C/T	G/G	T/C	A/A	G/G	G/G	G/G	T/T	A/A
P53368	C/T	G/G	T/T	A/G	G/T	C/G	G/G	C/T	C/A
P53369	C/C	G/A	T/C	A/A	G/G	C/G	G/G	C/T	C/A
P53370	C/T	G/G	T/T	A/G	G/T	C/C	G/G	C/C	C/C
P53371	C/C	G/G	T/C	A/A	G/G	G/G	G/G	T/T	A/A
P53372	C/T	G/A	T/T	A/G	G/T	C/C	G/G	C/C	C/C
P53373	C/T	G/A	T/T	A/G	G/T	C/C	G/A	C/C	C/C
P53374	C/C	G/A	T/C	A/A	G/G	C/G	G/G	C/T	C/A
P53375	C/C	A/A	T/T	A/A	G/G	C/C	G/G	C/C	C/C
P53376	C/C	G/A	C/C	A/A	G/G	G/G	G/G	C/T	C/A
P53377	C/C	G/A	T/C	A/A	G/G	C/C	G/G	C/C	C/C
P53378	C/C	G/G	T/T	A/A	G/G	C/C	G/A	C/T	C/A
P53379	T/T	G/G	T/T	G/G	T/T	C/G	G/G	C/T	C/A
P53380	C/C	G/A	T/T	A/A	G/G	C/G	G/G	C/T	C/C
P53382	C/T	G/A	T/T	A/G	G/T	C/G	G/G	C/T	C/A
P53383	C/C	G/A	T/C	A/A	G/G	C/C	A/A	C/C	C/C
P53384	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
P53385	C/T	G/G	T/C	A/A	G/T	G/G	G/G	T/T	A/A
14778	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
14779	C/C	G/G	T/C	A/A	G/G	C/C	G/G	C/C	C/C
14873	T/T	G/G	T/T	A/G	T/T	C/G	G/G	C/T	C/A
14874	T/T	G/G	T/C	A/G	G/T	C/C	G/G	C/T	C/A
14875	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
14876	C/C	A/A	T/T	A/G	G/T	C/C	G/G	C/C	C/C
14878	C/C	A/A	T/T	A/A	G/G	C/C	A/A	C/C	C/C
14880	C/C	G/A	T/C	A/A	G/G	C/C	G/G	C/C	C/C

Table F-1. Continued

14935	C/C	G/G	C/C	A/A	G/G	C/C	G/A	C/C	C/C
14936	C/C	G/G	T/C	A/A	G/G	C/G	G/G	C/T	C/A
14937	C/T	G/A	T/T	A/G	G/T	C/C	G/G	C/C	C/C
14938	C/T	G/A	T/T	A/G	G/T	C/G	G/G	C/T	C/A
17410	C/T	G/A	T/C	A/A	G/G	C/C	G/G	C/C	C/C
17500	C/C	G/G	T/C	A/A	G/G	C/G	G/G	C/T	C/A
17501	C/C	G/G	T/C	A/G	G/T	C/C	G/A	C/C	C/C
17502	T/T	G/G	T/T	A/G	G/T	C/C	G/G	C/C	C/C
17503	C/T	G/A	T/T	A/A	G/T	C/C	G/G	C/C	C/C
17504	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
17505	C/C	G/G	T/C	A/G	G/T	C/G	G/G	C/T	C/A
17506	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
17507	C/T	G/G	T/C	A/G	G/T	C/C	G/G	C/C	C/C
17508	C/C	G/A	T/T	A/G	G/T	C/C	G/G	C/C	C/C
17509	C/T	G/G	T/C	A/G	G/T	C/G	G/G	C/T	C/A
17510	C/C	G/A	T/T	A/A	G/G	C/C	G/G	C/C	C/C
17511	C/T	G/A	T/T	A/A	G/G	C/C	G/G	C/C	C/C
17512	C/C	G/G	C/C	A/A	G/G	C/C	G/A	C/C	C/C
17513	C/C	G/A	T/T	A/A	G/G	C/C	G/A	C/C	C/C
17514	C/C	G/G	C/C	A/A	G/G	C/G	G/G	C/T	C/A
17515	C/T	G/G	T/T	A/A	G/G	C/C	G/G	C/C	C/C
17516	T/T	G/G	T/T	A/G	G/T	C/C	G/G	C/C	C/C
17517	C/T	G/G	T/C	A/A	G/G	C/C	G/G	C/C	C/C
17518	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
17519	C/C	G/G	T/T	A/G	G/T	C/C	G/A	C/C	C/C
17520	T/T	G/G	T/T	A/G	G/T	C/G	G/G	C/T	C/A
17521	C/C	G/G	C/C	A/A	G/G	C/G	G/G	C/C	C/C
17522	C/C	G/G	T/C	A/A	G/G	C/G	G/G	C/T	C/A
17523	C/T	G/A	T/C	A/G	G/T	C/G	G/G	C/T	C/A
17524	C/C	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
17525	C/C	G/G	T/C	A/G	G/T	C/C	G/G	C/C	C/C
17526	C/C	G/G	C/C	A/A	G/G	C/G	G/G	C/T	C/A
17527	C/C	G/A	T/C	A/A	G/G	C/C	G/G	C/C	C/C
17528	C/T	G/G	C/C	A/A	G/G	C/C	G/G	C/C	C/C
17529	C/C	G/G	T/C	A/G	G/T	C/C	G/G	C/C	C/C

Table F-1. Continued

17530	C/T	G/G	T/T	A/A	G/G	C/G	G/G	C/T	C/A
17531	C/C	G/G	T/C	A/A	G/G	C/C	G/G	C/C	C/C
17532	T/T	G/G	T/T	A/G	G/T	C/C	G/G	C/C	C/C
17535	C/T	G/G	C/C	A/A	G/G	C/C	G/G	C/T	C/A
17536	C/T	G/A	T/C	A/A	G/G	C/C	G/A	C/C	C/C
17537	C/C	G/G	C/C	A/A	G/G	C/C	A/A	C/C	C/C
17538	C/C	G/G	T/C	A/A	G/G	C/G	G/G	C/C	C/C
17539	C/T	G/G	T/T	A/A	G/G	C/C	G/G	C/C	C/C

APPENDIX G

COMPLEMENT SERUM LEVELS

Table G-1. CFH and C3 serum levels and complement activity of blood donor samples.

Sample id	CFH serum level (mg/ml)	C3 serum level (mg/ml)	APH50 (U)	%AP
P50569	1.05	0.94	54.54	103.8
P50570	2.45	4.09	81.17	88.8
P50571	0.96	1.68	71.73	87.7
P50574	3.58	2.3	63.14	123.7
P53334	1.47	4.25	87.91	73.9
P53335	1.18	5.74	62.41	86.2
P53339	2.53	2.79	60.5	95.3
P53340	1.4	1.31	58.88	111.5
P53341	2.36	0.63	57.73	98.5
P53342	2.08	3.14	48.9	112.8
P53343	2.36	0.59	76.39	88.8
P53344	1.68	0.91	74.45	92.9
P53345	2.19	2.49	70.9	115.3
P53346	1.31	1.19	77.3	63.7
P53347	2.7	1.89	46.56	123.3
P53348	2.23	2.33	62.26	111.4
P53349	1.47	0.12	59.31	74
P53350	2.29	2.12	58.88	100.8
P53351	1.76	0.51	67.22	77.6
P53352	2.51	1.96	122.86	92.6
P53353	1.45	2.14	76.44	83.2
P53354	2.77	1.34	52.12	102.9
P53355	1.55	1.63	89.26	78.3
P53356	1.05	1.4	57.38	94.9
P53357	2.54	0.34	85.25	104.42
P53358	2.11	1.61	62.21	111.8
P53359	2.14	1.31	73.92	87.2
P53360	1.85	2.06	97.8	84.6
P53361	2.01	1.22	63.56	90.3

Table G-1. Continued

P53363	2.01	1.08	84.44	101.4
P53364	2.48	0.98	63.86	108.28
P53365	1.16	0.87	68.7	94.11
P53366	1.86	0.33	74.31	78.23
P53367	1.46	1.46	65.75	83.01
P53368	0.94	0.75	73.08	107.2
P53369	2.19	1.61	74.86	92.41
P53370	1.50	1.01	71.03	99.63
P53371	0.82	0.29	59.11	106.88
P53372	2.05	1.44	83.4	79.14
P53373	2.87	1.54	73.59	67.01
P53374	1.73	0.97	39.9	86.88
P53375	1.90	2.50	49.32	69.53
P53376	2.37	2.51	48.9	85.13
P53377	1.67	1.99	47.21	68.22
P53378	1.07	2.11	54.22	69.43
P53379	1.59	1.54	48.53	79.07
P53380	1.49	2.69	41.63	104.17
P53382	1.66	2.05	44.55	78.45
P53383	1.64	1.95	48.73	81.09
P53384	1.05	2.87	53.13	79.66
P53385	1.48	1.56	47.96	50
14778	3.11	0.84	105.114	70.41
14779	3.47	1.11	99.937	62.9
14873	3.06	1.22	98.932	65.02
14874	3.08	0.91	110.874	72.19
14875	2.18	0.71	134.812	94.32
14876	1.57	1.07	66.142	99.92
14878	3.01	0.35	95.992	86.54
14880	4.11	1.00	89.668	94.05
14935	3.32	1.14	73.209	71.89
14936	1.96	0.59	176.671	51.37
14937	1.75	0.5	98.846	75.88
14938	2.76	0.65	93.222	73.96
17410	2.77	0.91	145.041	53.1

Table G-1. Continued

17500	1.73	0.55	113.551	75.58
17501	2.31	0.61	83.558	51.48
17502	2.26	0.86	113.015	92
17503	1.73	0.24	144.913	68.71
17504	2.55	0.72	130.567	90.87
17505	2.14	0.29	196.854	84.28
17506	2.78	0.59	143.77	79.56
17507	4.63	0.78	101.252	88.51
17508	2.88	0.87	79.772	90.41
17509	2.73	0.53	79.571	68.69
17510	3.1	0.87	172.902	92.39
17511	2.53	0.57	103.644	60.06
17512	4.66	1.16	97.717	95.24
17513	2.74	0.53	98.203	96.47
17514	2.27	0.34	99.64	63.38
17515	4.84	1.43	87.29	50.62
17516	1.22	0.07	119.119	52.08
17517	3.97	1.19	178.223	71.91
17518	2.55	0.75	91.634	54.11
17519	3.56	0.67	66.836	94.78
17520	2.24	0.45	103.273	94.49
17521	2.37	0.27	114.459	91.29
17522	2.52	0.64	114.459	46.73
17523	2.19	0.42	128.185	67.05
17524	2.49	0.46	258.46	67.1
17525	3.19	0.32	83.601	77.81
17526	2.23	0.41	130.321	47.66
17527	2.68	0.73	131.382	69.05
17528	2.13	0.41	79.061	73.1
17529	2.39	0.31	222.834	52.52
17530	3.13	0.79	86.506	63.36
17531	2.81	0.98	119.699	74.38
17532	2.81	1.17	93.59	75.26
17535	4.88	0.72	75.496	62.55

Table G-1. Continued

17536	3.75	0.92	83.555	62.79
17537	4.5	1.33	216.653	54.11
17538	3.89	0.79	125.753	99.69
17539	3.00	0.93	100.98	82.12

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