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The complement system: a gateway to gene-environment interactions in schizophrenia pathogenesis

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Abstract

The pathogenesis of schizophrenia is considered to be multi-factorial, with likely gene-environment interactions (GEI). Genetic and environmental risk factors are being identified with increasing frequency, yet their very number vastly increases the scope of possible GEI, making it difficult to identify them with certainty. Accumulating evidence suggests a dysregulated complement pathway among the pathogenic processes of schizophrenia. The complement pathway mediates innate and acquired immunity, and its activation drives the removal of damaged cells, autoantigens and environmentally-derived antigens. Abnormalities in complement functions occur in many infectious and auto-immune disorders that have been linked to schizophrenia. Many older reports indicate altered serum complement activity in schizophrenia, though the data are inconclusive. Compellingly, recent genome-wide association studies suggest repeat polymorphisms incorporating the complement 4A (*C4A*) and 4B (*C4B)* genes as risk factors for schizophrenia. The *C4A/C4B* genetic associations have re-ignited interest not only in inflammation-related models for schizophrenia pathogenesis, but also in neurodevelopmental theories, because rodent models indicate a role for complement proteins in synaptic pruning and neurodevelopment. Thus, the complement system could be used as one of the 'staging posts' for a variety of focused studies of schizophrenia pathogenesis. They include GEI studies of the *C4A/C4B* repeat polymorphisms in relation to inflammation-related or infectious processes, animal model studies and tests of hypotheses linked to auto-immune diseases that can co-segregate with schizophrenia. If they can be replicated, such studies would vastly improve our understanding of pathogenic processes in schizophrenia through GEI analyses and open new avenues for therapy.

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CONFLICT OF INTEREST

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INTRODUCTION

The multi-factorial polygenic threshold model (MFPT) of schizophrenia posits a large number of genetic risk factors with discrete, cumulative, small effects and environmental factors that can act discretely or interact with the genetic risk factors.[1] The MFPT model has been supported by recent genome wide association studies (GWAS).[2] In parallel, many environmental risk factors have also been identified, including maternal infection, season of birth (winter excess), urban birth and residence, obstetric complications, maternal malnutrition, substance abuse (particularly cannabis use) and childhood trauma.[3–11] Though the MFPT model provides a sound foundation for etiological research in schizophrenia, it challenges simplistic notions of causality. In particular, risk could increase through interactions between genetic and environmental risk factors.[12] Initial GEI studies relied on familiality as a proxy for genetic risk.[13, 14] As more DNA variant data were generated, the amount of data and the complexity of GEI analyses has increased.[15–17] With the availability of GWAS data, the complexity has mushroomed.[18–20] Using SNP-based GEI analyses, even if one analyzes the phenotype of schizophrenia as a syndrome, ignoring secondary features, simple ‘two hit’ models involving one genetic and one environmental risk factor can invoke several models of interactions, increasing the number of analyses and the likelihood of false positive results.[21–23] For example, Avramopoulos et al[19] evaluated multiple infections agents, as well as indices of inflammation in conjunction with genome-wide DNA variant data; interestingly, they found suggestive associations with cytomegalovirus infections, reminiscent of an earlier study by Borglum and colleagues.[18, 19] Furthermore, typical case-control designs can be confounded if a correlation exists between the genetic and environmental risk factors, or the risk variables confer risk through more than one pathway.[22, 24–28] These complexities explain the difficulties in identifying GEI consistently.[29, 30]

What can be done in the face of the analytic challenges? One practical solution is a step-wise progression, starting with well-accepted genetic risk factors that are paired with established or plausible environmental risk factors or pathogenic processes. We illustrate this approach with respect to the complement pathway. Recent GWAS analyses implicate complement gene variation in schizophrenia pathogenesis. The complement system is also dysfunctional in many other disorders linked to schizophrenia; thus it provides a nexus for numerous lines of enquiry, including GEI analyses. In the following sections, we initially provide an overview of the complement system and its roles in the immune system, as well as its recently discovered effects on the brain. We next review the putative links between the complement system and schizophrenia: through a possible role in aberrant neurodevelopment, through links to infectious risk factors and through auto-immune disorders that can segregate with schizophrenia. We conclude by suggesting avenues for future research.

The complement system in innate immunity

The complement system encompasses a dynamic, orchestrated array of soluble plasma factors, proteases, cleavage products, cell surface receptors and regulatory protein complexes, all of which serve immune protection of the host.[31] This system is best known

for its role in halting and destroying invading pathogens by augmenting the effects of antibodies and phagocytes on target antigens and microorganisms.[32] The complement system can be activated via three pathways, namely the classical, lectin and alternative pathways, all of which converge on complement C3 (Figure 1). C3 and its activated products covalently bind to cell surface residues to localize the related innate immune inflammatory cascade to specific cellular and tissue sites. The classical pathway is primarily initiated after complement C1q binds to immune complexes composed of immunoglobulin antibodies bound to antigen. Activation of the classical pathway leads to the cleavage and activation products of C4 and C2, which fuse and drive amplification and cleavage of C3. C3 amplification through C4 and C2 cleavage also follows activation of the lectin pathway that occurs when pattern recognition receptors such as mannose-binding lectin (MBL) and the ficolins recognize carbohydrate patterns on damaged cell surfaces or invading microbes. The third pathway, called the alternative pathway, is activated by spontaneous hydrolysis of C3 which prompts a perpetual cycle of amplification that in turn also activates downstream components C5 through C8 and eventually the membrane attack complex. These pathways are intricately controlled, enabling amplification and suppression via complement inhibitors, binding proteins and factors, control genes and cell surface receptors. Thus the complement system maintains a critical role in immune surveillance with important ramifications for protection against infectious agents. Genetically encoded disruption/s could alter responses to environmentally-derived or endogenous antigens perceived as foreign (Figure 1).[33–35] As such, susceptibility to infection and autoimmune disorders is increased when there are defects in the complement pathway.[36]

The complement system AND the brain

The role of complement proteins in synapse formation and elimination has been one of the most fascinating recent discoveries in neuroscience research.[37] In the healthy brain, complement proteins are expressed at relatively low levels that vary with stages of maturation.[37, 38] The complement proteins C1q, C3 and C4 are detectable on cell bodies, neuronal processes and synapses of discrete neuronal groups. Although neurons express complement proteins, microglia and astrocytes are the major sources of these proteins, suggesting diverse roles.[37, 39, 40] In rodent models, the complement system is recruited for removing dysfunctional neuronal cells and dendritic processes.[41–43] Through elegant experiments, Stevens and colleagues have suggested that the complement system could also be involved in sculpting neurons even during normal neurodevelopment. They reported that C1q and C3 proteins mediate activity-dependent synaptic elimination in the developing rodent brain, preferentially tagging less active synapses for later elimination by microglia.[39, 44, 45] In support, C1q and C3 knockout mice have deficits in synapse elimination.[37] As these landmark findings in rodents have invigorated the study of the complement system in neurodevelopment, they merit replications by independent laboratories. In particular, it is important to investigate whether similar processes also occur in other brain regions implicated in the pathogenesis of schizophrenia, e.g., the prefrontal and temporal regions.

Aging, as well as several human brain diseases associated with abnormalities in complement systems, usually stem from infectious or inflammatory pathology. They include Alzheimer’s

disease, Down syndrome, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease and Rett's Syndrome.[38, 46–48] In Alzheimer's disease, for example, specific protein motifs found in amyloid plaques can trigger the activation of the classical complement pathway.[38, 42, 49] A recent translational study in mice and humans documented the accumulation of C1q at synapses in aging animals, suggesting that age-associated cognitive decline may be the result of synapse level vulnerability to extra-CNS and environmental insults. Triggers such as ischemia, trauma and infection could activate the complement cascade and result in inappropriate synapse loss at locations where C1q is over-represented.[50] Findings from this study as well as from Hong et al's study of Alzheimer's disease are important because of the links they suggest between synaptic C1q and cognitive decline - an indication that disorders like schizophrenia may also be impacted by similar processes.[42] Indeed, as elaborated in the following section, Sekar et al suggested a similar synaptic role for C4 in schizophrenia, but with an exquisite genetic twist.[40] In summary, components of the complement system could not only help to mold the brain during neurodevelopment[39, 51] but also could contribute to dysfunction in the adult brain.

The complement system and the pathogenesis of schizophrenia

Early studies of the complement pathway in schizophrenia utilized the complement hemolytic activity assay to quantify activity of total complement and of specific component proteins in serum samples, based on the percentage of antibody-coated erythrocytes that were lysed *in vitro* following exposure to the serum (Spivak et al[52], and reviewed by Mayilyan et al[53]). Results from these studies were varied, though altered complement activity was noted in schizophrenia by several investigators.[54] Studies that specifically included C4 targets indicated significant elevations of hemolysis by serum from patients with schizophrenia[55–58] and similar examinations of C1, C2, and C3 also demonstrated significant schizophrenia-associated complement abnormalities.[56, 57] Using immunoassays to quantify serum or plasma concentrations of specific components, Maes and colleagues[59] reported higher levels of complement components C3C and C4 and Mayilyan et al[58] reported increased lectin-activated complement activation capacity. On the other hand, non-significant case-control differences or even reduced complement activity were also reported.[52, 60, 61] The enzymatic nature and varied half-lives of activated complement components likely contributed to difficulties in replicating associations.

A role for complement in schizophrenia could arguably be detected more reliably in the presence of infection or other environmentally-derived antigenic stimuli. For example, complement C1q-bound immune complexes containing food antigens were found at increased rates in individuals with schizophrenia compared with controls.[62] Furthermore, elevated levels of complement C1q have been found in the mothers of infants who later developed schizophrenia[63], suggesting a role for the complement pathway in early neurodevelopmental events associated with schizophrenia. Notably in this study of unselected mothers, levels of C1q-associated antibodies were significantly correlated with a number of viral antigens, including herpes simplex virus, type 2 (HSV-2) and adenovirus. Thus, the activation of maternal complement by external and intrinsic antigens during a critical period of synaptic pruning may represent an important risk factor for the future

development of schizophrenia. In a later section, we discuss more fully the complement system in relation to neurodevelopmental hypotheses of schizophrenia.

Several genetic association studies of complement gene polymorphisms have also been reported. The early studies utilized relatively small samples; unsurprisingly, they yielded inconsistent results.[60, 64] Greater clarity has emerged from a recent GWAS. Through a collaborative effort, the Psychiatric Genomics Consortium (PGC) analyzed DNA from 28,799 patients with schizophrenia and 35,986 controls to identify 108 uncorrelated single nucleotide polymorphisms (SNPs) that confer risk for schizophrenia.[65] Among the most statistically significant risk variants were those in the human leukocyte antigen (HLA) region; like other schizophrenia-associated SNPs, the risk conferred by individual alleles was modest (odds ratios <2.0). Sekar and colleagues subsequently determined that variation at three uncorrelated loci could explain the observed associations in the HLA region (rs13194504, *C4A* and rs210133; localized to the distal extended HLA region, the HLA class III and the HLA class II regions, respectively).[40] Further, they demonstrated that variation at a polymorphic copy number variant (CNV) spanning the *C4A–C4B* complement genes accounted for the risk in the HLA Class III region. The primary risk variants represented by the two other SNPs in the HLA region have not been identified yet.

To understand the genetic associations of *C4* with schizophrenia, it is necessary to understand the functional impact of the CNV. The CNV cassette (denoted RCCX) comprises *STK19* (*RP1*), *C4* (*C4A* or *C4B*), *CYP21A2*, and *TNXB*.[66] A recombination site at *CYP21A2* leads to mono-, bi-, and trimodular cassettes with 1–3 functional copies of C4A or C4B, respectively, while retaining just one functional copy of the remaining genes (Figure 2). The *C4A* and *C4B* genes, which have over 95% sequence homology, nevertheless encode proteins with different substrate affinities.[67] Earlier studies indicated positive correlations between the gene copy number and serum protein concentrations for C4A and C4B.[68] To add further complexity, the *C4A* and the *C4B* genes can be present in long or short forms, based on the insertion of a human endogenous retroviral (HERV) element. The HERV sequence insertions are associated with increased gene expression, but the mechanism is uncertain. The HERV sequence is present more frequently in the *C4A* genomic sequences and likely accounts for the observation that *C4A* expression levels are two to three times greater than expression levels of *C4B*, even after controlling for relative copy numbers in each genome.[69] There is substantial ethnic variation in the distribution of alleles comprising the CNV.[70, 71]

Using an innovative droplet digital PCR (ddPCR) assay, Sekar and colleagues assayed the CNVs in a family-based sample and used this information in conjunction with SNP-based data to impute the number of copies of the *C4A* and the *C4B* genes in the PGC dataset. In post-mortem brain samples from the Stanley Medical Research Institute (SMRI) and the Genome Tissue Expression (GTEx) consortia, they showed that the levels of C4A and C4B mRNA increased proportionally with the number of copies of *C4A* and *C4B*, respectively. Separately, *C4A* was also expressed at significantly higher levels in five brain regions in post-mortem samples from patients with schizophrenia, a consistent result was reported with the larger CommonMind Consortium post-mortem dataset.[72]

In sum, three lines of evidence support the involvement of complement C4A in the pathogenesis of schizophrenia: the early serological studies, the genetic association studies and the post-mortem brain expression analyses. Even though the magnitude of the risk conferred by C4A is similar to, or less than other well established risk factors, the independent lines of evidence lend credence in altering risk for schizophrenia.

Links between the complement system and the neurodevelopmental hypothesis of schizophrenia

Many risk factors for schizophrenia, such as obstetric complications, season of birth effects or nutritional deficiencies could be traced to prenatal maternal influences *in utero*. The maternal influences, in turn, suggest pathology during the early neurodevelopmental period (spanning prenatal to early postnatal life, when much neuronal proliferation and differentiation occurs). Other lines of evidence implicate the late neurodevelopmental period; i.e., until late adolescence to young adulthood, when synaptic pruning predominantly occurs.

Schizophrenia was proposed as a disorder of faulty programmed synaptic elimination by Feinberg (1982)[73] based on convergent evidence from studies of sleep EEG, evoked response potential, brain metabolic rate, dendritic spine variations from new born through age 90 years, and patterns of onset of schizophrenia. These abnormalities were synthesized with evidence that indicated similar temporal patterns for onset of schizophrenia and known age related changes in synaptic density and dendritic spine density. Huttenlocher (1979)[74] showed that synaptic density in the middle frontal gyrus increases to a peak in early childhood, and subsequently decreases in late childhood and reaches a plateau in early adolescence, although the pruning continues during the third decade of life before stabilizing at adult level. In some regions that are critical to schizophrenia pathogenesis, e.g. dorsolateral prefrontal cortex, a protracted pruning is observed starting at age 9 years to 22 years. However, different dendritic segments prune dendritic spines with different chronology, e.g. basal and proximal dendrites started to prune at 7–9 years but the distal dendrites do not begin until 17 years of age.[75] In primates, substantial reduction in the dendritic spine density occurred in adolescence.[76] Overall, the number of synapses decrease in an age-related manner in monkeys and humans[77, 78] and these changes could underlie age-related gray matter reductions observed in neuroimaging studies of schizophrenia.[79] The factors determining the type or timing of synaptic pruning are uncertain, though much research suggests that immature synapses or those showing lower levels of activity are more likely to be eliminated.[80]

Thus, the work of Stevens and colleagues[37] regarding complement proteins C1q and C3 as mediators of synaptic sculpting in the developing visual system, has important implications for Feinberg's hypotheses. From a neurodevelopmental perspective, the inappropriate activation of complement or the failure of complement to function correctly in the developing CNS could conceivably disrupt neuronal networks. Faulty complement activity could be generated through environmental factors (such as maternal infection). For example, in a rodent study, adult offspring of dams exposed to prenatal poly(I:C) had significantly

elevated expression of prefrontal cortical C1q compared with adult offspring of vehicle treated mothers.[81] As discussed earlier, Sekar and colleagues have suggested that risk variants of the *C4A* CNV could also mediate accelerated synaptic pruning in schizophrenia, consistent with Feinberg's hypothesis.[40] However, there are important caveats: human *C4A* and *C4B* sequences do not occur in the mouse genome. Instead, there are two other forms of *C4*, namely *C4* and *Slp* (sex limited protein).[82] Insertion of a retroviral long-terminal repeat in the promoter region of *Slp* leads to restricted expression of *C4* in the mouse.[83] Future studies will also need to assay point mutations in *C4A* that can abrogate function.[84, 85] In summary, Sekar's hypothesis needs to be tested in humans, keeping genomic variations and variations related to brain region and chronological age in mind.

Links between the complement system and the possible role of infection and inflammation in the etiopathogenesis of schizophrenia

Can the *C4A* genetic associations inform infection as an environmental risk factor for the pathogenesis of schizophrenia or for some aspect of schizophrenia? The answer depends on the strength of evidence linking not only complement system dysfunction and infectious agents, but also the evidence linking infectious agents with schizophrenia.

Complement deficiencies are associated not only with increased levels of bacterial infections,[86] but also with viral infections.[87–98] Separately, a role for complement in the immune response to *Toxoplasma gondii*, a protozoan organism was first suggested based on an increased susceptibility of *C5*-deficient mice to *Toxoplasma* infection.[99] It was subsequently reported that virulent strains of *Toxoplasma* have a diminished ability to activate the classical complement pathway though interactions with C3.[100] In a rodent model, chronic *Toxoplasma* infection could lead to complement-induced changes in cell connectivity and synaptic pruning,[101] as well as the generation of antibodies to the NMDA receptor.[102] Thus, complement dysfunction is demonstrable in the pathogenesis of several types of infectious diseases.

With regard to the second question, it is challenging to determine etiological links between infectious agents and schizophrenia due to many technical limitations. Most infections in immune competent individuals result in viral replication for 3–14 days. Thus, evidence for active infection are not expected even in the premorbid period among individuals at high risk for schizophrenia. Hence, most studies of viral infections rely on the immune response to viral proteins, such as circulating antibody molecules or immunoglobulins, but they do not indicate the precise timing of the initial exposure. The difficulty in measuring antibodies within the central nervous system without the performance of cerebrospinal puncture is another substantial barrier. Still, there are several possible mechanisms linking infectious agents such as *Toxoplasma gondii* infection with schizophrenia.[103–106] Conceptually, infectious agents could also elevate risk for certain features of a disorder. For example, several studies have linked the neurotropic herpes simplex virus, type 1 (HSV-1) with cognitive impairment, particularly among patients with schizophrenia[107]. In sum, proving a link between infections and schizophrenia is challenging; the bulk of evidence suggests several indirect effects.

Several lines of investigation are needed to gain a better understanding of these mechanisms. It would be instructive to evaluate correlations between peripheral and central indices of complement function; e.g., through post-mortem or animal model studies. As it can be difficult to prove causality based on epidemiological studies alone, animal models could be invoked to test causal effects in the association between infection and schizophrenia-relevant brain dysfunctions, as reviewed by Kannan et al.[108] The links between infections and complement activation also indicate an intriguing paradox. The genetic association studies of Sekar et al, as well as other studies suggest increased complement activity among patients with schizophrenia. On the other hand, reduced complement system activity facilitates infection and/or increases bacterial/viral loads, and infection or the infectious disease process is a putative risk factor for schizophrenia.[109] Thus, it would be of interest to investigate whether individuals with deficiencies in complement system proteins have elevated risk for schizophrenia.

Links between the complement system, auto-immune diseases and schizophrenia

Dysfunction in the complement system can also predispose to well-recognized auto-immune diseases, such as systemic lupus erythematosus (SLE), systemic sclerosis, and rheumatoid arthritis (RA).[110] Complete or partial C4 deficiency leads to increased risk of infection and autoimmune diseases, such as SLE.[70] It is well-established that reduced concentrations of complement C4 protein or reduced serum complement activity occur with active disease in SLE.[111] Though infrequent, absence of complement components C4A and C4B are strongly associated with risk for SLE or lupus-like disease, after controlling for HLA background and ethnicity. A review of 35 studies indicated that heterozygous and homozygous deficiencies of C4A were present in 40–60% of SLE patients from almost all ancestral groups investigated.[111] Complement dysfunction has also been linked to other non-infectious diseases, including age-related macular degeneration.[112, 113] The prevalence of several auto-immune diseases, including SLE is increased among patients with schizophrenia and their relatives, whereas RA prevalence is reduced among schizophrenia patients and their relatives.[114, 115] Systematic studies of complement levels among schizophrenia patients in relation to these auto-immune diseases are, therefore, needed.

The complement system as a PLATFORM for investigating schizophrenia pathogenesis

The application of current knowledge about the complement proteins to schizophrenia research could be fruitful in several directions. Examples include GEI analyses of *C4A* polymorphisms alongside infection exposure data. Similarly, neurodevelopmental processes in brain imaging studies could be combined with *C4A* polymorphism data. On a different plane, studies of *Toxoplasma gondii* infections could be paired with C1q, C3 and C5 levels in the serum. It would also be instructive to investigate whether abnormalities in the complement system, including alterations in levels of complement 4, explain the co-morbidity of schizophrenia and auto-immune diseases.

Most components of the human complement system have a characteristic domain structure; it is likely that the current complexity, exemplified by over 30 proteins, arose partly through multiple gene duplication events.[116, 117] In a similar vein, the CNV bearing C4A and the HERV insertion provides a rich source of information about human population history.[70, 71] Those data, combined with haplotype analyses may enable future dissection of the origins and geographical variations of schizophrenia.

Such focused analyses, followed by replicative studies could identity pathologic processes for some aspects or sub-groups of schizophrenia, motivating focused therapeutics in the future. More broadly, this scheme could be extended to other genetic risk factors. Indeed, several SNPs identified through schizophrenia GWAS have been linked to immune regulation[65] and other studies indicate that genetic factors play an important role in the control of infectious agents and the generation of the immune response to infection.[118] The step-wise progression would begin with a single reproducible genetic risk factor - a choice that reflects the difficulty in establishing causal links conclusively for some non-genetic risk factors. Based on its known biological functions, plausible environmental risk factors for schizophrenia could be picked and analyzed next in relation to the selected genetic risk factor. The design and the samples for the joint analyses would be dictated by the biological question. For example, if genetic and environmental risk factors are independent, case only analyses are suitable.[28, 119] In other contexts, such as tests of neurodevelopmental hypotheses, premorbid analyses in population-based cohorts may be needed. If plausible GEIs are detected, independent replications would be sought.

CONCLUSIONS

Consistent with the MFPT model of pathogenesis, recent genetic association studies indicated that a portion of the risk for schizophrenia is conferred by copy number variation in the *C4A* gene; it was also proposed that the pathogenic effects of *C4A* may be mediated through dysfunction in synaptic pruning. The complement pathway also mediates innate and acquired immunity, suggesting additional plausible mechanisms of pathogenesis and future opportunities for testing novel therapies for schizophrenia – a concept being considered for other diseases.[120] We also advocate additional studies of complement function in complement deficient individuals, those with auto-immune disorders and carefully selected animal models studies, as well as post-mortem human samples.

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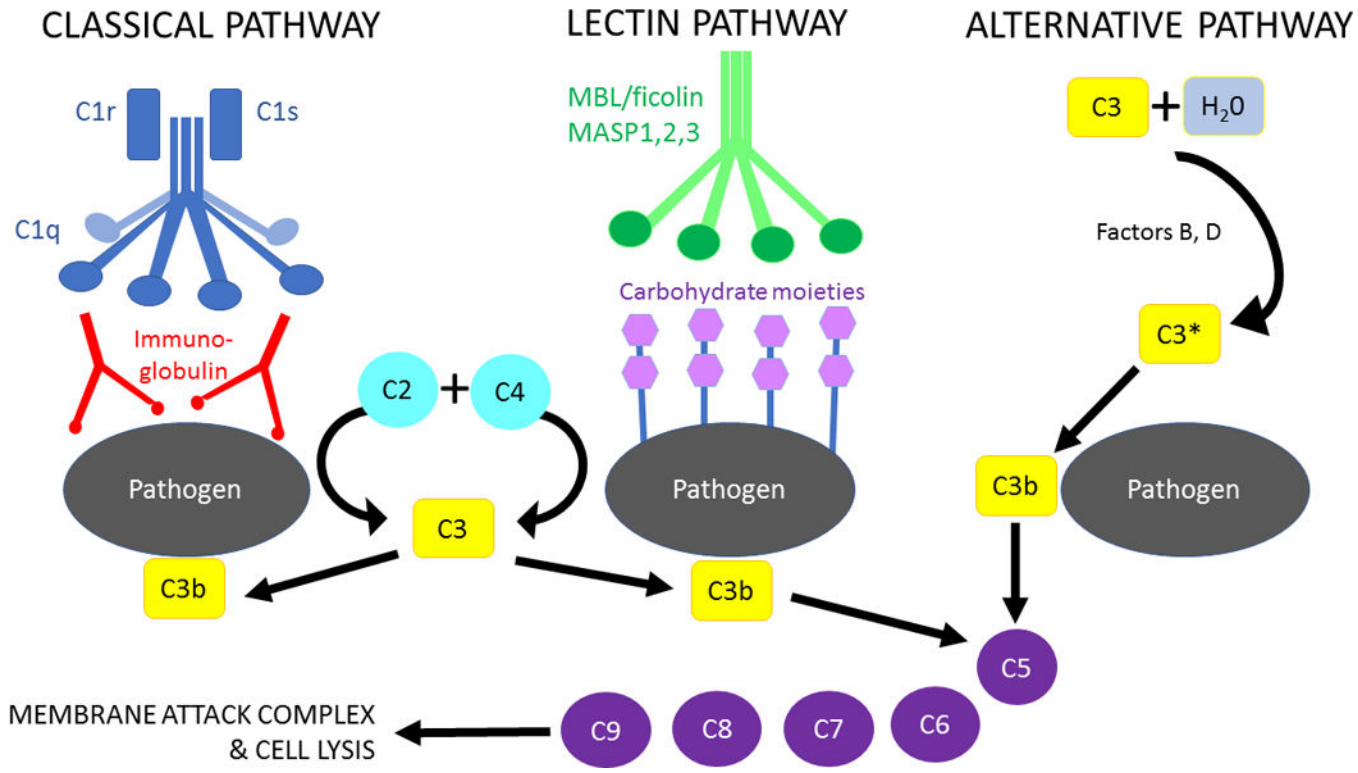

Figure 1. The complement pathway

The complement system can be activated along three major pathways. The classical pathway is initiated after C1q interacts with IgM and IgG class antibodies bound to antigen. The lectin pathway is activated by carbohydrate pattern recognition receptors such as mannose-binding lectin (MBL) and the ficolins which are complexed with enzymes known as MBL-associated serine proteases (MASPs). Both the classical and lectin pathways cleave C4 and C2, with subsequent activation of C3. Cleavage of C3 causes C3b to bind to the surface of pathogens and accelerate phagocytic activity. The alternative pathway is activated by spontaneous hydrolysis of C3 and functions as an amplification loop for the cleavage of C3; the generation of C3b involves interactions with the protease factors B and D. In addition to the covalent attachment of C3b to target surfaces, C3b can change substrate specificity of C3 convertases to C5, which leads to assembly of the C5b-C9 membrane attack complex that can lyse targeted cells.

C3*: C3 in its hydrolyzed state.

(Adapted from[96, 121]).

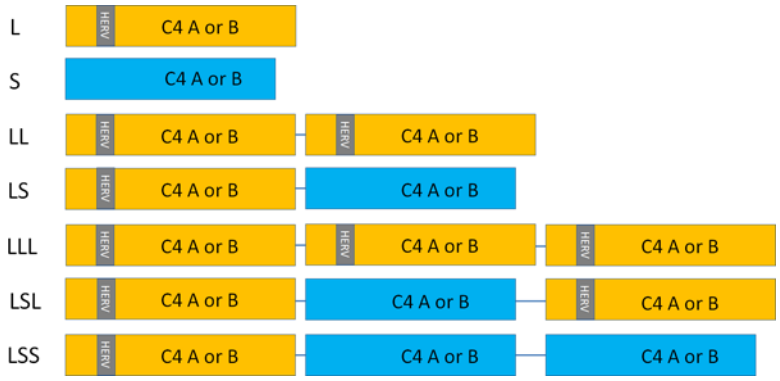

Figure 2. Copy number variation at the *C4A* and *C4B* loci

The figure illustrates the range of structural variation at the *C4A* and *C4B* loci, indicated as boxes. For clarity, flanking genes (*RP1*, *RP2*, *CYP21A*, *CYP21B*, *TNXA*, and *TNXB*) are not shown; nor are some variants that are less frequent in Caucasian ancestry samples. The gray bar labeled 'HERV' indicates a retroviral insertion that produces longer variants (C4A-L or C4B-L, shown in ochre); its absence indicates shorter variants (C4A-S or C4B-S, shown in blue). Each individual can have 0–6 copies of *C4A* and 0–5 copies of *C4B*. L: long variant; S: short variant. Additional mutations that can yield non-functional 'null alleles' are not shown.

Table 1

Genetic associations between copy number variants incorporating Complement 4A and Complement 4B genes and selected disorders/diseases.

Disease / disorder	Genetic Associations	Genotype assays	References
Schizophrenia (SZ)	Increased *C4A* copy number associated with risk for SZ	Droplet digital PCR	Sekar et al[40]
Behcet's disease (BD)	Increased *C4A* expression and IL-6 levels with 2 or >2 C4A copy number.	qPCR	Hou S et al[122]
Systemic lupus erythematosus (SLE)	Deficiency - high risk for SLE; 0 or 1 copy of C4A - elevated risk for SLE; 3 or more copies of C4A - protective against SLE	PFGE of *Pme*I-Digested DNA qPCR qPCR	Yang et al[123] Wu et al[124] Yih et al[125]
Grave's disease (GD)	<2 copies of *C4A* associated with risk for vitiligo in patients with GD	qPCR	Liu et al[126]
Crohn's disease (CD)	CD patients have overall lower C4L and higher C4S copies compared to controls	qPCR	Cleynen et al[127]
Type1 Diabetes Mellitus	>2 copies of HERV-C4 in patients	qPCR	Mason et al[66]
Alzheimer's disorder	Overall increased copies of *C4A* or *C4B* in patients	qPCR	Zorzetto et al[128]

PCR – polymerase chain reaction; PFGE - Pulsed-field gel electrophoresis; qPCR – quantitative PCR.