THE GENETICS OF THE SIXTH AND SEVENTH COMPLEMENT COMPONENTS IN MAN

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by

© Laura Jean York, B.Sc.

A Thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Faculty of Medicine
Memorial University of Newfoundland
September 1981

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ABSTRACT

The purpose of this thesis was to study the human complement components, C6 and C7, in Newfoundland and to pursue the genetics of these proteins within the limits of the material obtained and the time allowed.

Approximately 2400 sera from three Newfoundland populations were allotyped by the method of isoelectric focusing in polyacrylamide gel followed by specific hemolytic overlay. The frequencies of the common alleles were calculated for two of these populations. The West Coast community had frequencies of C6*a (0.6321) and C6*B (0.3670) and the Newfoundland families plus random individuals in a group had C6*A (0.6947) and C6*B (0.2980). These frequencies are similar to those found in other populations. The C6 alleles observed in the three populations were inherited in a manner consistant with a single autosomal locus and there was no evidence to suggest selection against the inheritance of the rare alleles. Examination for linkage of C6 with HLA reiterated the negative results of previous studies. An attempt to link the C6 and SOD (superoxide dismutase) loci in a West Coast family having a rare SOD variant was made but the data were uninformative.

The homozygous C7*1 pattern was by far the most commonly observed, occurring in more than 99% of the samples allotyped. The novel observation and investigation of an artifact associated with prolonged serum storage of the C7*1 protein such that it is modified and mimics the migration of the C7*3 protein during isoelectric focusing is described. Genuine C7*3 alleles were also observed, two in heterozygote form, C7 3-1, and one which was due to either C7*3 homozygosity or C7 3-0. Data suggesting the C7*2 allele is of Chinese origin are presented.

ADDENDUM

Since the final writing of this thesis, nine unrelated local Chinese people have been bled and their serum typed for C7. Of the nine, three were heterozygous for C7*2. This supports the suggestion that this allele is of Chinese origin and appears with a frequency of approximately 17%. Further studies are currently in progress to accumulate family data for linkage efforts.

SECOND ADDENDUM

Following evaluation of this thesis it has been pointed out that the term "allele" has been used here, as it sometimes is in published literature on C6 & C7, in an imprecise manner. The DNA sequence coding for a polymorphic polypeptide is, strictly speaking the "allele". whilst the polymorphic polypeptide or protein should be referred to as the "gene product". The thesis should be read with this reservation in mind.

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CHAPTER I INTRODUCTION

In this chapter, the review of the pertinent literature is divided into three parts, each becoming more detailed than the last. An overview of the structure and function of the complement system is followed by a review of the genetic discoveries and ideas about the system. Finally, a detailed review of the literature on the genetics of the sixth and seventh complement components, the subject of the present thesis, is presented.

OVERVIEW OF THE COMPLEMENT SYSTEM, STRUCTURE AND FUNCTION

For this overview, the following review articles have been used to provide much of the material for the classical pathway:

Müller-Eberhard, H.J., 1968
Müller-Eberhard, H.J., 1970-71
Müller-Eberhard, H.J., 1974
Frank, M.M., 1975

and the following for the alternative pathway:

Osler, A.G. and A.L. Sandberg, 1973
Götze, O. and H.J. Müller-Eberhard, 1976
Müller-Eberhard, H.J. and R.D. Schreiber, 1980
Feardon, D.T. and K.F. Austen, 1980.

When first identified, complement was though to be a single serum factor that acted in conjunction with antibody to cause cell lysis. Its first investigator, Pfeiffer, in 1894, studied the bacteriolytic property of serum from immunized guinea pigs. Heating of the serum at 56°C for one half hour resulted in loss of the bacteriolytic activity. However, upon addition of fresh serum from non-immunized guinea pigs, the sensitized cells could be lyzed. Four years later, Bordet identified the requirement for this same thermolabile serum factor for immune hemolysis. Since that time, complement has been shown to be a multifactor system made up of at least twenty discrete proteins that have been isolated and each assigned various activities.

With properties such as chemotaxis, opsonization, immune adherence, anaphylatoxin activation, promotion of blood coagulation and virus neutralization, as well as immune hemolysis, the complement system is complex and intricate. It can, however, be looked at as a simple cascade of the components acting upon each other in a precise sequence. The multistep system limits the amount of host cell destruction in the vicinity of the recognized antigen by two means. Firstly, the components exist in serum and the body fluids as inactive proteins and secondly, when activated by specific biochemical steps, the enzymatic half-life of the proteins is very short.

When describing the mode of action by which the complement system works, a three unit model has been adopted (Götze, O. and H.J. Müller-Eberhard, 1970): a recognition unit comprised of subcomponents Clq, Clr and Cls; an activation unit of C4, C2 and C3; and finally, the membrane attack unit of C5, C6, C7, C8 and C9. Not one of the proteins is dispensible and the cascade must proceed in a precise sequence for efficient cytolysis. Slow lysis will occur without the addition of C9 (Stolfi, 1968) but upon its addition the rate is greatly increased (Haxby and Kolb, 1972).

The Recognition Unit

Complement is activated by antibodies of the IgM and most IgG classes (strongly by γ_1 and γ_2, weakly by γ_3) and not activated by the immunoglobulins IgA, IgD, IgE or γ_4 (Ishizaka et al. 1966; Ishizaka et al. 1967; Augener et al. 1971).

The first component, Cl, exists in serum as a complex of subunits Clq, Clr and Cls in the ratio of 1:2:4 respectively (Naff et al. 1964). The macromolecule is stabilized by calcium ions (Lepow et al. 1963).

Recognition of the antibody-antigen complexes is mediated by the globular tulip-like head structures of C1q. C1q has six of these heads, each on its own thin tendril attached to the central unit. C1q binds to the Fc portion of the immunoglobulin (Kehoe and Fougereau, 1969) and it is thought that the action of the binding causes a change in the conformation of the central unit which results in a modification of C1r, revealing the C1r enzymatic site which activates C1s.

Esterolytic activity resides in the C1s subunit and will enzymatically cleave the unactivated precusors of C3-convertase, C4 and C2 (Naff et al. 1964).

The Activation Unit

C4 is enzymatically fragmented into a small molecular weight unit, C4a, which is released into free solution. The remaining activated larger fragment, C4b, attaches itself to the membrane around the site of the immunoglobulin-C$\overline{1}$ complex. Activated C1 (C$\overline{1}$) fragments hundreds of C4 molecules. A protecting inhibitor, C1-INH, binds to the $\overline{C1s}$ site and thus limits the consumption of C4 (Levy and Lepow, 1959). Also, the binding of the C4b fragment to the antigen membrane is inefficient, only approximately 10% of all the fragmented C4b doing so. The free C4b has a very short half-life, soon becoming $C4b_i$, and this again decreases the amount of host cell damage.

C4b bound to the membrane exhibits a binding site for C2 which is also activated by the esterolytic action of $\overline{C1s}$. C2 is fragmented as was C4, into C2a and C2b. The larger, C2a, binds to C4b to form $\overline{C4b2a}$, known as C3-convertase (Mayer and Miller, 1965).

The C3-convertase activity resides in the C2a subunit and enzymatically activates C3 (Cooper, 1971). As previously, activation requires

the fragmentation of the component into small and large molecular weight subunits, C3a and C3b respectively. This step is of great importance in that the bound $\overline{C4b2a}$ converts thousands of C3 molecules into its two fragments. There is a focal distribution about the $\overline{C4b2a}$ site as C3b binds to the membrane and C3a is released into the fluid phase (Müller-Eberhard et al. 1966). Only the C3b closest to the site of the bound $\overline{C4b2a}$ will form $\overline{C4b2a3b}$, necessary for the formation of the membrane attack unit.

The Membrane Attack Unit

Activated, membrane bound $\overline{C4b2a3b}$ cleaves the next complement protein, C5, into C5a and C5b. The larger molecular weight fragment, C5b, binds to the membrane and upon doing so, reveals a binding site for C6. There is thought to be no fragmentation of the C6 molecule, nor is there of C7 which binds to the activated $\overline{C5b6}$ complex to form $\overline{C5b67}$. It may be that binding of C7 to $\overline{C5b6}$ reveals a hydrophobic site which allows association between the complex and the membrane lipids (Polley et al. 1971). The discovery of tributyrinase activity attributable to C7 may include this component as an active participant in membrane interaction, possibly in the capacity of a lipase (Delâge et al. 1973).

Binding of C8 to $\overline{C5b67}$ results in slow hemolysis which is rapidly increased upon addition of C9. There is disagreement on the number of C9 molecules required for effective lysis, it being either a single molecule (Rommel and Mayer, 1973; Kitamura and Inai, 1974) or up to six (Kolb and Haxby, 1972a; Kolb et al. 1972b).

Recently, the hypothesis of the five late components (C5, C6, C7, C8 and C9) binding to form a donut-shaped unit which, when inserted into the bilipid membrane layer, would allow diffusion and therefore, ly-

sis, has been challenged. Nascent C5b-9 complexes have been shown to aggregate spontaneously (Podack et al. 1978; Podack et al. 1980) and analysis of solute diffusion across the C5b-9 membrane lesion now suggests that the pore size is heterogenous and must result from aggregation of the bound C5b-9 complexes into multimeric units (Sims and Lauf, 1980).

The Alternative Complement Pathway

The complement system can also be activated by an alternative pathway or Properdin pathway. The early components C1, C4 and C2 are bypassed by Properdin, Factor D and Factor B.

Credit for the discovery of the Properdin system goes to Pillemer and associates, who first recognized that complement activities, specifically bactericidal, virus-neutralizing and hemolytic, could be initiated without the presence of immune aggregates, in conditions that inhibit C1 complex formation or in serum deficient in any one of the initial components (Pillemer et al. 1954).

The Properdin pathway is initiated by polysaccharides, endotoxins, cell walls and zymosan (Pillemer, 1956; Pillemer et al. 1955), requires magnesium ions (Pillemer et al. 1954) and consumes what was once known as C'3 (Pillemer et al. 1953; Pillemer et al. 1956), now known as C3, C5, C6, C7, C8, and C9 (Klein and Wellensiek, 1965; Wellensiek and Klein, 1965; Linscott and Nishioka, 1963; Nilsson and Müller-Eberhard, 1965). Early investigation isolated a Factor A necessary in the Properdin system but this was later identified as C3b (Müller-Eberhard and Götze, 1972). The Properdin pathway is thus non-specific in that specific antibody is not required for its activation. Presence of immune aggregates nevertheless may enhance the activation.

Small amounts of C3b are continuously produced by the slow interaction of C3, Factor B, Factor D and Properdin. The C3b molecules attach to cell surfaces (Law and Levine, 1977) and in the presence of magnesium ions, Factor B binds to the C3b. Factor D then cleaves the bound B into two fragments, Ba and Bb, the fragment which cleaves C3. The C3 cleaving ability of Bb is stabilized by the binding of Properdin to the C3b subunit.

The formation of BbC3b is controlled by β1H which inhibits by actively blocking the binding of B, by dissociating the B or Bb bound to the C3b and by increasing C3b susceptibility to cleavage by C3-inactivator. The outcome of the competition between β1H and Factor B is dependent upon whether the C3b subunit has attached to an activator. C3b on the surface of a non-activator has a 100 fold greater affinity for β1H than for Factor B. However, when bound to an activator surface, the uptake of B is favored (Feardon and Austen, 1977a; Feardon and Austen, 1977b). The C3-convertase activity provides, by cleavage of C3, more C3b for the formation of $\overline{BbC3b}$, (C3-convertase), and forms a positive feedback loop. It is at this stage that C3 inactivator exhibits its property of binding and cleaving C3b into two fragments, C3c and C3d, thus stopping the overconsumption of C3 (Ruddy and Austen, 1971). C3 inactivator also inactivates C4b, cleaving it into its inactive forms, C4c and C4d (Cooper, 1975).

The C3b generated by the alternative C3-convertase $\overline{BbC3b}$ then activates C5 and the late components are activated and function in the same fashion as in the classical cascade. The convergence of the two pathways is shown in Figure I which expresses clearly the importance of C3 in both.

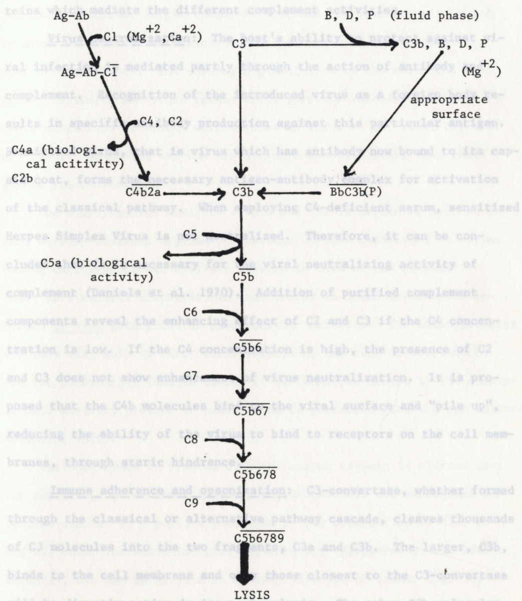


Figure I. Convergence of the classical and alternative pathways.

Mediation of Complement Activities

Development of techniques that allow separation and purification of the specific components allowed identification of the specific proteins which mediate the different complement activities.

Virus neutralization: The host's ability to protect against viral infection is mediated partly through the action of antibody and complement. Recognition of the introduced virus as a foreign body results in specific antibody production against this particular antigen. Sensitized virus, that is virus which has antibody now bound to its capsid coat, forms the necessary antigen-antibody complex for activation of the classical pathway. When employing C4-deficient serum, sensitized Herpes Simplex Virus is not neutralized. Therefore, it can be concluded that C4 is necessary for the viral neutralizing activity of complement (Daniels et al. 1970). Addition of purified complement components reveal the enhancing effect of C2 and C3 if the C4 concentration is low. If the C4 concentration is high, the presence of C2 and C3 does not show enhancement of virus neutralization. It is proposed that the C4b molecules bind to the viral surface and "pile up", reducing the ability of the virus to bind to receptors on the cell membranes, through steric hindrance.

Immune adherence and opsonization: C3-convertase, whether formed through the classical or alternative pathway cascade, cleaves thousands of C3 molecules into the two fragments, C3a and C3b. The larger, C3b, binds to the cell membrane and only those closest to the C3-convertase will be directly active in immune hemolysis. The other C3b molecules deposited on the cell membrane increase immune adherence and act as opsonizing factors, greatly increasing the cell's susceptibility to

phagocytosis (Lepow et al. 1970). Opsonization is also thought due to the C4 of the C14 complex (Cooper, 1969).

Anaphylatoxin activity: Cleavage of components C3 and C5 yields two small molecular weight fragments, C3a and C5a respectively, which by means of immunofluorescence can be shown to bind to receptors on mast cell membranes. The ensuing degranulation of the mast cell releases histamine and other agents which increase vascular permeability and constriction of smooth muscle (Dias da Silva et al. 1967; Lepow et al. 1970). Human C3a and C5a are stronger anaphylatoxins than their guinea pig counterparts.

The anaphylatoxin activity of C5a and C3a is dependent upon an intact carboxy-terminal arginine. Cleavage of this residue by an enzyme having carboxypeptidase activity results in loss of the activity. There is a natural anaphylatoxin inactivator in human serum which inhibits in vivo generation of anaphylatoxic activity. The anaphylatoxins are thus only able to exert their effects at the site of activation; and the potentially immense amount of imflammation caused by the anaphylatoxic activity present in human serum is prevented (Bokisch and Müller-Eberhard, 1970).

Purified human C2 treated with the enzyme trypsin is cleaved into two peptides, one of which exhibits physicochemical and biological properties similar to a peptide generated by the interaction of C$\overline{1s}$, C4 and C2, which increases vascular permeability (Klemperer et al. 1969). Recently, C4a was identified as an anaphylatoxin of lesser activity than C5a or C3a and similarly is inactivated upon removal of its carboxy-terminal arginine (Gorski et al. 1979).

Chemotaxis: Initial studies on the chemotactic activities of se-

rum indicated the role of complement components because heated serum or serum with the addition of EDTA were found to have no chemotactic activity. Both these serum states render the initial components inactive. Heating inactivates by disturbing the molecular configuration of C2, C4 and Factor B, and addition of EDTA chelates the divalent ions necessary in the initial stages of the two pathways; calcium for stabilization of the C1 complex and magnesium which allows binding of Factor B to C3b.

Chemotactic activity for neutrophil and eosinophil leukocytes is present in purified $\overline{C5b67}$ but not in $\overline{C5b6}$, suggesting C7 is an essential part of the complex. However, upon binding of $\overline{C5b67}$ to erythrocyte membranes or other hydrophilic surfaces, its chemotactic effect is lost. Therefore, it is not a primary factor in the chemotactic property of complement (Lachmann et al. 1970).

Further experimentation determined the majority of chemotactic activity in guinea pig serum was a product of C5 cleavage (Snyderman et al. 1969). Sucrose-density gradient centrifugation and Sephadex chromatography made possible isolation of two peaks having chemotactic activity for eosinophils and neutrophils (Kay, 1970). One peak was identified as C5a (Kay et al. 1971).

C5a is also a chemotactin for mononuclear leukocytes, important in wound healing in their function of phagocytosing degenerating polymorphonuclear leukocytes and tissue debris (Snyderman et al. 1971). Use of anti-C5 selectively inhibits chemotactic activity, while cleavage of C5 by trypsin artificially generates this activity (Snyderman et al. 1972).

Although cleavage of C3 by trypsin does not result in chemotactic

activity (Snyderman et al. 1971), use of the enzyme plasmin generates a peptide thought to be C3a having the activity (Ward, 1967).

Promotion of blood coagulation: Rabbits, identified as being deficient in component C6 (Rother et al. 1969), are noted as having prolonged clotting time and abnormal clot retraction. Both condition are corrected by the addition of purified C6 (Zimmerman et al. 1971). Although similar studies in humans deficient in C6 reveal no such abnormalities (Lim et al. 1976; Heusinkveld et al. 1974), it has been suggested by Zimmerman et al. (1971) that C6 and therefore complement acts in some unknown manner in normal blood coagulation.

COMPLEMENT GENETICS

Similarities Between Complement Components

A number of the complement components exhibit similarities both physicochemically and biologically and it is thought that the common proteins may have arisen from successive gene duplication. These similarities are shown in Table I, where the number of peptide chains and molecular weight for each component is listed.

Subcomponents C1r and C1s: Both subcomponents are single polypeptide chains and having similar amino acid composition, share similar molecular weights (Sim and Porter, 1976).

Components C2 and Factor B: Similar in molecular weight; C2, 102,000 and Factor B, 100,000, share an analogous function in that each forms C3-convertase and possess the enzymatic site for C3 conversion. Single, heat-labile polypeptide chains with similar amino acid composition (Kerr and Porter, 1978), they also show similarities in their susceptibility to proteolysis and there is a limited degree of sequence homology at the sites of cleavage (Kerr, 1979). Also, being

Table I. Proteins of the complement system.

Components Classical Components	Number of Peptide Chains	Molecular Weight	Approximate Serum Concentra- tion (μg/ml)
C1q	18	410,000	180
C1r	1	83,000	50
C1s	1	83,000	50
C4	3	206,000	400
C2	1	102,000	15
C3	2	180,000	1200
C5	2	180,000	80
C6	1	124,801	75
C7	1	120,806	55
C8	3	163,000	80
C9	1	79,000	230
Alternative Pathway Components			
Properdin	4	223,000	25
Factor B	1	100,000	250
Factor D	1	25,000	Trace
Control Proteins			
C1-inhibitor	1	90,000	18
C3b-inactivator	-	100,000	40
C6-inactivator		---	---
β1H globulin	1	150,000	600
C4-binding protein	Several	540-590,000	---
Anaphylatoxin inhibitor	-	310,000	---

(Hauptmann, 1979)

coded by genes within the HLA region suggests that they may have evolved from a common ancestral gene.

Components C3 and C5: Both C3 and C5 consist of two polypeptide chains with the sum molecular weight of 180,000. Both components are cleaved into two fragments, the small molecular weight C3a and C5a responsible for anaphylatoxic and chemotactic activities and the larger weight C3b and C5b playing actively in immune cytolysis.

Parital amino acid sequencing of the small molecular weight fragments C3a and C5a show marked similarities, eight of the first twenty-five amino acid residues are identical with only minor manipulation in positioning of the two peptides (Fernandez et al. 1977). Anaphylatoxic activity of both lies in an intact arginine at the C-terminus of the peptide chain. The amino acid sequence similarities and the intact arginine dependent anaphylatoxic activity are also evident in C4a. The anaphylatoxic activity of C4a is of a lesser magnitude than C3a or C5a.

Components C6 and C7: Please see detailed section, page 23.

Deficiencies of Complement Components, Disease Association and Linkage

More information pertaining to complement genetics is gained from three sources: (i) from the study of polymorphism of the individual components,

(ii) from the study of complement deficiencies,

(iii) and from the study of linkage of the components to other known genetic loci.

The first complement component to demonstrate electrophoretic polymorphism was the slow β protein (Weime, 1965), later identified as C3 (Alper and Propp, 1968). Being a major serum protein, it is the

only complement protein which can be analyzed by electrophoresis in agarose gel followed by direct protein staining.

In 1972, polymorphism of Factor B was detected by electrophoresis in agarose gel and immunofixation with anti-Factor B (Alper et al. 19-72a). This method is applicable to other complement components wher-ever there is sufficient specific antibody available and the concentra-tion of the component is high enough.

A means of detecting both polymorphism and functional activity was pioneered by Hobart et al. (1975), in which isoelectric focusing in polyacrylamide gel is followed by a hemolytic overlay of erythro-cytes and hemolytic reagents necessary for detecting the particular component in question. First used for C6 allotyping, this method is extremely adaptable for the detection of other lytic proteins, as well as the other complement proteins.

Since the discovery of C3 polymorphism, deficiency or polymorphism has been detected in all classical components and in Factors B and D, and both states have been used to study the possibility of linkage to known genetic markers.

With the advance of diagnostic techniques, deficiencies of iso-lated complement components have been detected and these deficiencies show association with specific disease states (see Table II).

Cl-inhibitor: The first disease shown to be associated with a ge-netic abnormality (specifically a deficiency) of a protein in the com-plement system was hereditary angioedema. This disease is due to the deficiency of the Cl inhibitor and therefore, complement activity is unrestrained. The defect is transmitted genetically as an autosomal dominant (Donaldson et al. 1963; Donaldson et al. 1977). Defective

Table II. Complement deficiencies in man.

Component	Number of cases (pedigrees)	Clinical manifestations
C1q	3(2)	SLE-like syndrome, renal disease, healthy
C1r	2(2)	Infection, SLE-like syndrome
C1s	1(1)	SLE
C4	4(4)	SLE-like, glomerulonephritis (abortions)
C2	>40	About 50% healthy, SLE, immune complex disease including Schönlein-Heaoch purpura, glomerulonephritis
C3	4(4)	Severe immune deficiencies, fever, arthralgia
C5	2(2)	SLE, gonococcal sepsis, healthy
C6	4(4)	Gonococcal sepsis, recurrent meningococcal meningitis, healthy
C6 + C7	1(1)	Healthy
C7	6	Glomerulonephritis, gonococcal sepsis, meningococcal infection, Raynaud's disease, rheumatoid arthrits, SLE, healthy
C8	5(2)	Gonococcal sepsis, healthy, SLE-like
C9	2(2)	Healthy
Factor B (heterozygous and/or functionally deficient	14(4)	In association with C2 deficiency, recurrent bacterial infections, healthy
C1 inhibitor	many	HAE with immune complex disease, nephritis
C3b inactivator	2(2)	Severe immune deficiency, recurrent meningitis

SLE = systemic lupus erythematosus; HAE = hereditary angioedema
(Rittner and Bertrams, 1981)

suppression of the normal gene was shown by the de-repressive effects apparent with the use of androgen therapy. With this treatment, C1 protein levels rose toward normal in those individuals exhibiting either markedly reduced levels of C1-INH or high to normal levels of dysfunctional C1-INH protein (Colten et al. 1981; Gelfand et al. 1976).

Component C1 - subunit C1q: Deficiencies of subcomponent C1q have for the most part been the product of hypercatabolism of C1q in patients exhibiting hypogammaglobulinemia (C1q is decreased due to an increased catabolism and higher extravascular distribution), with multiple myeloma and common variable immunodeficiency (Kohler and Müller-Eberhard, 1972). However, recently two families have been identified as antigenically deficient due to a familial defect which results in a nonfunctional molecule (Thompson et al. 1980; Leyva-Cobián et al. 1981). Homozygosity of the defective gene has been associated with systemic lupus erythematosus (SLE)-like disease and subsequent renal disease but may also be associated with health. Detection of the heterozygous state by quantitation was difficult, presumably because the normal gene contributes more to the serum level of C1q than the abnormal gene. There has been no indication of linkage of the C1q deficiency gene and HLA (Arnáiz-Villena et al. in press).

Component C1 - subunit C1r: Complement studies revealing no C1r, decreased C1s and elevated C4 levels were first described by Pickering et al. (1970). If the C1r molecule is depleted due to activation, C4 levels are expected to be low and therefore, the defect described was an inability to synthesis C1r.

Deficiency of the C1r molecule has been associated with multiple infections, SLE-like symptoms, renal disease and arthritis (Pickering

et al. 1970; Day et al. 1975; Mittal et al. 1976). An inherited defect, the autosomal gene controlling C1r deficiency segregated independently of the genes of the HLA and ABO systems (Mittal et al. 1976).

Component C1 - subunit C1s: C1s deficiency was first described by Pondman and associates (1968) showing an association with SLE. SLE-like disease was also evident in the individual described by Blum et al. (1976). C1s deficiency was due to a null gene at the site that codes for the synthesis of C1s. No linkage to HLA has been demonstrated.

Component C4: Complete C4 deficiency, described in only four individuals (Hauptmann et al. 1974; Ochs et al. 1977; Ballow et al. 1979; Hauptmann, 1979), was associated with clinical symptoms of SLE and glomerulonephritis. The deficiency was inherited as an autosomal co-dominant trait (Hauptmann et al. 1974) and family studies revealed its linkage to the major histocompatibility complex (Rittner et al. 1975; Ochs et al. 1977).

Component C2: The most common deficiency within the complement system is that of the second component. Although about half of the deficient individuals were healthy, the deficiency state has been associated with Hodgkin's disease, chronic lymphocytic leukemia, infectious fatal dermatomyositis, membranoproliferative glomerulonephritis and anaphylactoid purpura (Day et al. 1978; Gewurz et al. 1978).

The C2 deficiency gene was closely linked to HLA (Fu et al. 1975; Fu et al. 1977) and allelic association was apparent between Aw25, B18, Dw2 and the deficiency gene (Day et al. 1975; Gibson et al. 1976; Hauptmann et al. 1977a). Within the human population, the Aw25, B18, Dw2 haplotype occurs rarely and approximately 50% exhibit the C2 deficiency gene (Mortensen et al. 1980).

Component C3: Deficiency of the third component, complete (Alper et al. 1969) or partial (Hoppe et al. 1978), is associated with recurrent infections since the individual lacks the biological activities mediated by this component and those following in the cascade (Alper et al. 1972b; Alper et al. 1976). Only four cases of homozygous C3 deficiency have been described (Alper et al. 1972b; Davis et al. 1977; Ballow et al. 1975; Lachmann and Rosen, 1978).

C3 was also found deficient when the C3 inactivator protein was absent. In an inherited deficiency of C3-INA, the C3 molecules are overconsumed during pathway activation. Clinical maifestations were identical to C3 deficiency in that there was increased susceptibility to infection. Two such cases have been described (Alper et al. 1970; Thompson and Lachmann, 1977).

Component C5: First identified immunochemically (Rosenfeld et al. 1976), hereditary C5 deficiency was not linked to HLA (Rosenfeld et al. 1977; Snyderman et al. 1978; Snyderman et al. 1979), nor was it linked to complement component C6 (Rosenfeld et al. 1978). The deficiency state was compatible with good health but has been associated with SLE (Rosenfeld et al. 1976) and predisposition to disseminated gonococcal infections (Snyderman et al. 1978; Snyderman et al. 1979).

Component C6: Please see detailed section, page 25.

Component C7: Please see detailed section, page 26.

Component C8: Deficiency of C8 seemingly predisposed to gonococcal infection (Merritt et al. 1976; Petersen et al. 1976; Snyderman et al. 1978; Petersen et al. 1979) and the immune-complex disease, SLE (Jasin, 1977).

HLA linkage was first suggested for C8 (Merritt et al. 1976; Peter-

sen et al. 1976) but was disputed with later family studies (Jasin, 1977; Giraldo et al. 1977; Tedesco et al. 1980). The deficiency gene was not found to be linked to the C6 locus (Tedesco et al. 1980).

Component C9: To date, only three individuals have been described as having C9 deficiency (Lint et al. 1980; Inai et al. 1979). The small number was thought due to the fact that lysis will proceed, though slowly, without addition of C9 (Stolfi, 1968). Though bacterial killing was 35 times slower than the normal killing time of 3 minutes, C9 deficient serum was still capable of killing bacteria (Lint et al. 1980). Individuals deficient in the ninth complement component would therefore be easily hidden. Linkage studies have not yet been described.

Polymorphism of Components and Linkage

C4 polymophism: Population heterogeneity of the fourth complement component was first suggested by two-dimensional immunoelectrophoresis of EDTA-plasma against anti-C4 serum (Rosenfeld et al. 1969; Bach et al. 1971). Subsequent work using the technique of high voltage electrophoresis in agarose gel followed by immunofixation was used to reveal a true C4 polymorphism (Teisberg et al. 1976; Mauff et al. 1978). The current method in which better separation of the C4 protein bands was found, entails pre-treatment of serum samples with neuraminadase before high voltage electrophoresis and immunofixation (Awdeh and Alper, 1980).

C4 allotyping of family groups has since the beginning given data which are not wholly compatible with the concept of a single co-dominant autosomal locus. Immunochemical and genetic data led O'Neill et al. (1978a; 1978b) to suggest that two loci may code for the C4 protein on each HLA haplotype and that the antigens of the Chido and Rodgers blood groups are components of C4. One locus, A, having 7 alleles which

have faster electrophoretic mobility than the 3 at the B locus, appears to code for molecules that are Rodgers positive and Chido negative. The B locus alleles are Rodgers negative and Chido positive. One allele at the A locus may be hemolytically inactive and shows allelic association with HLA-B17 (O'Neill et al. 1980). Null alleles presumably exist for both loci as evidenced by complete C4 deficiency and are postulated by O'Neill to occur frequently. Other workers are more conservative and simply conclude that duplicated loci exist on some haplotypes but cannot be demonstrated yet on others. Homozygosity for null genes at both loci may perhaps be selected against since there have been so few complete C4 deficiencies observed (see C4 deficiency, page 17). The most common haplotype is A3B1 and accounts for approximately 80% of the population (Skanes, personal communication).

Strong alleleic association has been shown between HLA-B types and C4 (Teisberg et al. 1977) and between C4 and Bf but not between C4 and GLO-I (Mauff et al. 1978). Therefore, the two loci for C4 are considered to be within the HLA complex and close to Bf and HLA-B, a conclusion which is supported by limited data on recombinants.

C2 polymorphism: First described by Hobart and Lachmann (1976a), the C2 polymorphism revealed close linkage between the C2 locus and HLA. Isoelectric focusing in thin-layer polyacrylamide gel, followed by a hemolytic overlay deficient in C2, revealed three alleles for the second component. C2*1, the most common, had a frequency of approximately 0.96 (Meo et al. 1976; Meo et al. 1977; Alper, 1976; Olaisen et al. 1978a; Marshall et al. 1980a). Family studies suggested close linkage between C2 and HLA/Bf (Allen, 1974; Alper, 1976).

C3 polymorphism: The high concentration of C3 in serum aids in

its ease of allotyping. The protein can be visualized after high voltage electrophoresis in agarose or starch gels by simple staining.

C3 polymorphism is controlled by a single autosomal locus and there are two common co-dominant alleles and at least 22 rare ones. The more common allele, C3S, has a frequency of 0.77 to 0.81 in Caucasians. The faster migrating common allele, C3F, occurs at a frequency of 0.18 to 0.21 in Caucasian populations (Hauptmann, 1979). Population studies have shown a higher frequency of C3S in Negroid and Mongoloid populations as compared to Europeans (Seth et al. 1976) and a very high frequency of C3S in Lapps (Teisberg, 1971). The total frequency of rare variants varies between 0.004 and 0.1, except in the Lapps and Finns where no rare variants have been identified (Seth et al. 1976).

Although the C3 structural locus was found close to the histocompatibility complex H-2 in the mouse (Natsuume-Sakai et al. 1978; Penalva da Silva et al. 1978), extensive studies had not linked human C3 to any firmly established linkage group (Gedde-Dahl et al. 1974; Lamm et al. 1975; Weitkamp et al. 1975), other than a suggestion of loose linkage between the C3 locus and those of the Lewis blood group (Weitkamp et al. 1974) and familial hypercholesterolemia (Ott et al. 1974).

C5 polymorphism: Isoelectric focusing followed by hemolytic overlay revealed no polymorphism in any population studied (Rosenfeld et al. 1977; Vas-Guedez et al. 1978), except that of the Melanesians (Hobart et al. 1981). Two co-dominant alleles exist; C5*1, the most common with a frequency of 0.93 and C5*2, with a frequency of 0.07. Linkage data had not linked the C5 locus to HLA or C6/C7.

C6 polymorphism: Please see detailed section, page 28.

C7 polymorphism: Please see detailed section, page 35.

C8 polymorphism: Genetic polymorphism of C8 had only recently been reported by Alper et al. (1979). Isoelectric focusing and hemolytic overlay revealed two alleles, A and B, and a third allele, A1, detected only in Blacks.

Factor B polymorphism: A functionally deficient variant was found of Factor B (Mauff et al. 1980) and polymorphism of Factor B has been studied since 1972 (Alper et al. 1972a), showing the locus to be closely linked to HLA (Allen, 1974; Olaisen et al. 1975; Rittner et al. 1975; Rittner et al. 1976a; Albert et al. 1977; Larsen et al. 1977; Bender et al. 1977; Arnason et al. 1977). Polymorphism was detected by agarose gel electrophoresis and immunofixation. Functional activity of the variants was realized by employing hemolytic overlay. There are two common alleles, F and S, and two less common, F1 and S0.7 described (Alper et al. 1972a) and there are at least seven very rare alleles (Hauptmann et al. 1976; Naff et al. 1976; Hauptmann et al. 1977b; Larsen et al. 1981).

The Bf locus was thought to be close to that of HLA-B since significant associations had been found between BfS and alleles B7 and B8; between BfF and B12 and Bw35; between BfS0.7 and Bw50 and between BfF1 and B18 (Olaisen et al. 1975; Rittner et al. 1975; Bender et al. 1977; Larsen et al. 1977; Arnason et al. 1977).

Factor D polymorphism: Isoelectric focusing and functional overlay deficient in Factor D revealed a single banded pattern. Variants have been observed only in African samples (Hobart and Lachamnn, 1976a; Marshall, personal communication).

Summary: Information on the genetics of complement, obtained from studies of deficiency and polymorphism of the individual components,

suggests that the components actively forming the C3-convertases (C4, C2 and Factor B) are linked to the major histocompatibility complex. and deficiencies of these components predispose particularly to SLE-like syndromes. The exact position of the loci for C4, C2 and Factor B is still unknown. Most evidence suggests a tentative map with the loci between HLA-B and HLA-D/DR, closer to B, as shown in Figure II. Studies of the late acting components do not suggest linkage to HLA or to any other known marker and are thus far not assigned to any chromosome.

THE GENETICS OF COMPLEMENT COMPONENTS C6 AND C7

A genetic relationship has been proposed on the basis of similarities in physical and chemical properties and functional activities for Clr and Cls; C3, C4 and C5; and C2 and Factor B. C2 and Factor B have been found to be linked with each other and with HLA (Fu et al. 1977; Allen, 1974). Genetic linkage has also been proposed between C6 and C7, the suggestion substantiated by physicochemical similarities (Podack et al. 1976; Podack et al. 1979), combined deficiency (Hobart and Lachmann, 1978; Glass et al. 1978) and polymorphism studies of the two proteins (Hobart et al. 1978).

Similarities Between C6 and C7

C6 and C7, isolated and purified from human serum with immunoabsorbent columns of Sepharose coupled with anti-C6 IgG and anti-C7 IgG, are almost indistinguishable by amino acid composition. Only four amino acids; aspartic acid, isoleusine, lysine and proline, differ by more than 1 mole percent. Although no sequence data are available, a degree of homology is expected (Podack et al. 1979). Determined to be single polypeptide chians (Podack et al. 1976), both are glycoproteins (Podack

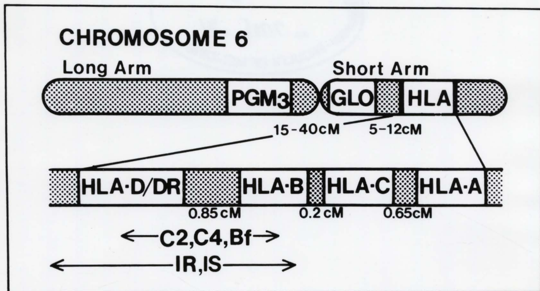


Figure II. A tentative map of Chromosome 6. IR = immune response genes. IS = immune suppressor genes. GLO = glyoxylase I. PGM3 = phosphoglucomutase 3. (Rittner and Bertrams, 1981)

et al. 1979). The molecular weights are calculated from amino acid composition; 124,801 for C6 and 120,806 for C7, and are similar to those found earlier (Arroyave and Muller-Eberhard, 1971; Podack et al. 1976). Upon isoelectric focusing, the major bands of C6 focus at pH 6.4; those of C7 at pH 6.25. Circular dichroism spectra show similar secondary structure in that both molecules contain 20 to 30% α-helical and β-structure (Podack et al. 1979).

These similarities in the physicochemical properties of C6 and C7 support the theory that the two proteins are the product of gene duplication (Hobart and Lachmann, 1976a; Lachmann and Hobart, 1978a).

Deficiency of C6 and C7, Disease Association and Linkage

Component C6: First described by Leddy et al. (1974), C6 deficiency was repeatedly shown to increase susceptibility to recurrent infections due to a lack of bacteriolytic activity against organisms such as Neisseria gonococcus and Neisseria meningitidis (Lim et al. 1976; Vogler et al. 1977; Glass et al. 1978; Lee et al. 1979; Petersen et al. 1979; Vogler et al. 1979; Clough et al. 1980; Haeney et al. 1980). To date, only one study had implied that C6 deficiency predisposed to connective tissue disease such as discoid lupus and Sjögren's syndrome (Trapp et al. 1980).

Chemotactic and opsonic activities were normal in these individuals and unlike C6 deficient rabbits, there was no visible clotting abnormality, the thrombin consumption time lying in the upper range of normal (Heusinkveld et al. 1974; Delâge et al. 1977). The hemolytic activity of C6 deficient serum was restored by addition of purified C6.

Genetic analyses suggested a co-dominant Mendelian inheritance of the trait. Originally thought to be HLA linked with a strong associa-

tion with HLA-Aw24 (Raum et al. 1976), more recent studies have repeatedly shown no segregation of the HLA complex genes with that of C6 deficiency (Mittal et al. 1976; Clough et al. 1978; Vogler et al. 1979; Delâge et al. 1979; Haeney et al. 1980; Trapp et al. 1980). Also, with the identification of a family having a combined deficiency of C6 and C2, it was determined that the deficiencies were two distinct hereditary defects, independent of one another (Delâge et al. 1979).

Component C7: As with C6 deficiency, the absence of the seventh component of complement was generally associated with recurrent infections caused by N. gonococcus and N. meningitidis (Lint et al. 1976; Lee et al. 1977; Lee et al. 1978; Lambert et al. 1979; Loirat et al. 1980) but was also associated with SLE (Zeitz et al. 1978), nephritis (Nemerow et al. 1978) and arthritis (Alcalay et al. 1980). C7 deficient sera showed no lack of opsonic or chemotactic activity nor of immune adherence, but bacteriolytic activity was absent. Early basic blood coagulation studies reported no abnormality (Boyer et al. 1975; Delâge et al. 1977) but recently C7 deficient serum was shown to determine a disorder in platelet aggregation in the presence of thrombin. The abnormality was correctable by the addition of purified C7 (Alcalay et al. 1980).

Wellek and Opferkuch (1975) reported a C7-inactivating principle in the serum of a healthy patient functionally and antigenically deficient in C7.

A single individual has been identified as deficient for both C2 and C7. Family studies revealed that both parents had transmitted their own singular deficiency and therefore the deficiencies are independent of one another.

Combined deficiency of C6 and C7: Two families have been shown to be simultaneously deficient in both C6 and C7 (see below), giving support to the theory that the genetic loci of these structurally similar proteins are closely linked.

There are a number of possible mechanisms by which a genetic defect could result in a combined deficiency. A large deletion within the region of the two structural genes could result in transcription of neither protein. Conversely, a smaller deletion could overlap the two genes such that various outcomes could reasonably occur. Conceivably, both proteins could be abnormal, either or both showing some function; or one protein could be abnormal while the other not made; or a protein comprising the N-terminal end of one protein and the C-terminal end of the other protein could be made. This "Lepore" molecule might show some function of either or both parent proteins. Alternatively, a defect in the structural gene of one protein could have a secondary effect on the structurally normal second protein, in that its synthesis could be affected or it could be hypercatabolized by a reaction with the abnormal protein. Deficiency could also be due to an effect of a control gene such that the synthesis of structurally normal proteins is affected, or due to the presence of an inactivating protein.

A family examined by Lachmann and co-workers (1978b) centers upon one gentleman who was in general good health but had low but detectable amounts of C6 and C7. The C6 molecule was antigenically deficient and was smaller than normal C6. The C7 molecule was normal both antigenically and structurally. Thus, the combined deficiency being due to a mutation at the level of a control gene is unlikely. A three gener-

ation study of the gentleman's family showed the deficiency to be inherited together as a single Mendelian trait, as is seen in Figure III. Heterozygotes had half levels for both the C6 and C7 proteins. Hemizygosity at the C6 locus was confirmed in the grandchildren in that those children who carried only the paternal C6B allele had half levels of both C6 and C7 while those who were phenotypically C6AB had normal levels.

Rapid in vivo catabolism of the normal C7 by the abnormal C6 cannot be excluded as a possible explanation for the combined deficiency but the more probable mechanism by which such a combined deficiency would occur is that a structural mutation at the C6 locus affects the rate of synthesis at the closely linked C7 locus. If the C6-C7 region is transcribed as a single message, a mutational event at one locus could conceivably interfere with transcription or post-transcriptional events at one or both loci. This explanation is applicable to the co-existent partial deficiency of C6 and C7 evident in the family presented by Glass and co-workers (1978), Figure IV. Functional and immunochemical assays did not detect synthesis of a dysfunctional protein. All the family members with low levels of C6 also had low levels of C7. Conversely, all those with normal C6 had normal C7 levels.

C6 Polymorphism

Electrophoretic variation of C6 has been demonstrated in rabbits (Goldman et al. 1981), dogs (Hobart, personal communication)[1], and primates (rhesus monkey, Hall et al. 1977; chimpanzee, Raum et al. 1980), including man (Alper et al. 1975; Hobart et al. 1975; Hobart and Lachmann, 1976a; Olving et al. 1977; Rittner et al. 1979; Olving et al. 1979).

1. Postal strike prevented receiving written unpublished data.

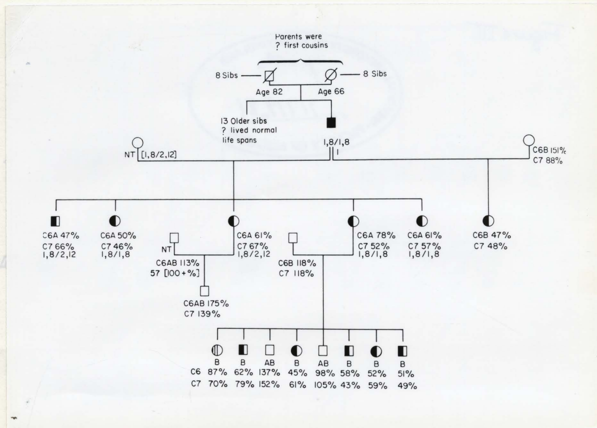


Figure III. Family having combined C6-C7 deficiency. Levels of allotypes of C6 are shown. Levels of C7 are shown. No allotype other than C7 1-1 was found. HLA types are shown where they were obtained. They are not informative for this family. (Lachmann et al. 1978)

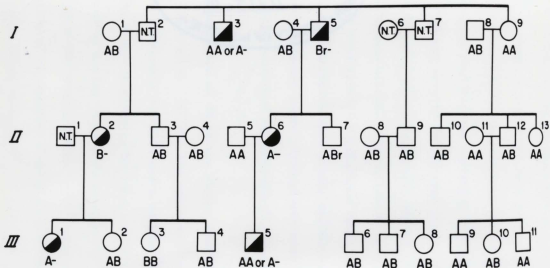


Figure IV. Family having co-existent partial deficiency of C6 and C7. (Glass et al. 1978). The half filled symbols are members with reduced C6 and C7 concentrations. NT. = Not tested.

C6 polymorphism in rabbits: The control of the structural variants of C6 in rabbits was studied by quantitation of functional and antigenic C6 levels and isoelectric focusing (Goldman et al. 1981). Three alleles were found, C6*A, the more acidic protein, C6*B, the more basic and C6*Q0, having no functional or antigenic activity. Heterozygotes of C6AQ0 or C6BQ0 had half levels of C6 protein and matings of heterozygotes produced the expected phenotypes and C6 activity levels, demonstrating that the two variants, C6*A and C6*B and the deficiency gene, C6*Q0 are allelic at the same genetic locus.

C6 polymorphism in primates: In the chimpanzee (Raum et al. 1980), the most common allele, C6*C, migrates to a slightly more basic isoelectric point than that of a reference human C6*A. Other alleles, C6*A and C6*B, respectively more acidic and more basic than the common allele, and different basic alleles were also demonstrated. Also, a blank allele confirmed immunochemically was not uncommon in the chimpanzee.

Polymorphism of C6 in the rhesus monkey was much like that found in man (Hall et al. 1977). Two major alleles, C6*A (frequency = 0.592) and C6*B (frequency = 0.354) and a single rare variant, C6*R (frequency = 0.053), were demonstrated by isoelectric focusing and hemolytic overlay. Natural mating between monkeys having the various alleles demonstrated that the three alleles were co-dominant and of a single locus. Interestingly, the allelic frequencies of the rhesus monkey were not unlike those found in man.

C6 polymorphism in man: Of the human populations studied, all have exhibited two major alleles, C6*A and C6*B, which occur with only slight differences in frequencies between the ethnic groups. Rare alleles have

been observed in all populations except those of India (Hobart and Lachmann, 1976a) and the Norwegian Lapps (Olving et al. 1979). The first study of Norwegians (non-Lapps) demonstrated no rare variants but further investigation yielded a combined frequency of 0.004 (Olving et al. 1979). Since only thirteen individuals were allotyped in the Indian population and because the frequency of rare alleles is small, the Indian study may not be indicative of the true frequency of rare variants within the population. Comparisons of C6 allele frequencies in various populations are given in Table III.

The common alleles code for proteins whose patterns differ by a single step in the banding pattern; C6*A being the more acidic pattern, C6*B, the basic. There have been 6 rare variants identified so far, coding for proteins which are not shifted a total pattern step. These patterns are shown schematically in Figure V.

The single step difference in the banding pattern of C6*A and C6*B can easily be produced by high voltage electrophoresis in agarose gel (Olving et al. 1977). Rare alleles are still more easily identified by using the principle method of isoelectric focusing in thin-layer polyacrylamide gel (Hobart et al. 1975). Regardless of the method of protein separation, visualization of the banding is usually through the use of a functional overlay of antibody sensitized sheep red blood cells and C6 deficient rabbit serum in agarose.

It is possible, through studying the inheritance of the C6 alleles within a family in which some members have low C6 serum levels, to determine the relationship between the structural genes and the deficiency. In four informative families (Glass et al. 1978), the genes determining electrophoretic variants and the low serum levels of C6

Table III. C6 allele frequencies in various populations

Population		n	Allele frequencies C6*A	C6*B	C6*R	References
Caucasians	British and N. American	189	0.621	0.360	0.018	Hobart and Lachmann, 1976a
	British	99	0.61	0.37	0.015	Hobart et al. 1975
	British	264	0.646	0.333	0.021	Alper et al. 1975
	Norway	81	0.61	0.39	0.0	Olving et al. 1977
	Norway	1623	0.59	0.379	0.004	Olving et al. 1979
	Germany	194	0.613	0.379	0.008	Rittner et al. 1979
	India	13	0.5	0.5	0.0	Hobart and Lachmann, 1976a
Lapps	Norway	167	0.53	0.47	0.0	Olving et al. 1979
Mongoloid	N. America	51	0.59	0.35	0.05	Hobart and Lachmann, 1976a
Negro	N. America, W. Indies, and Nigeria	165	0.551	0.403	0.045	Hobart and Lachmann, 1976a

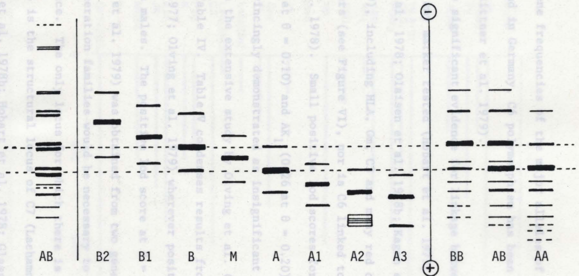


Figure V. Schematic representation of presently identified C6 allotypes. Complete pattern of common phenotypes at right, double banded pattern at left. In the central part only major bands and most pronounced minor bands are shown (indistinct acidic minor band in A2).
(Mauff et al. 1979)

were considered to be heterozygous for a normal gene and a silent or "null" gene which was the result of a mutation at the structural locus. These findings echoed similar suggestions made by Hobart and Lachmann (1976b).

The high gene frequencies of the major alleles of C6 make it a useful marker and in Germany, C6 polymorphism has been applied to paternity cases (Rittner et al. 1979).

There is no significant evidence for linkage betwen the C6 structural locus and any marker tested (Hobart et al. 1977; Olving et al. 1977; Olving et al. 1978; Olaisen et al. 1978b; Kagan et al. 1979; Olving et al. 1979), including HLA, Gm, C3 and many red cell antigens and enzyme markers (see Figure VI), nor is C6 linked to C5 deficiency (Rosenfeld et al. 1978). Small positive lod scores for Gm (0.23 at θ = 0.20), Gc (0.24 at θ = 0.10) and AK_1 (0.26 at θ = 0.20) (Hobart et al. 1977), were convincingly demonstrated as insignificant when taken in conjunction with the extensive study by Olving et al. (1979), as is illustrated in Table IV. Table V condenses results from the two studies (Hobart et al. 1977; Olving et al. 1979) wherever positive lod scores were obtained in males. The positive lod score at θ = 0.20 for P-C6 linkage (Olving et al. 1979) was obtained from two generation families only. Three generation families would be necessary to verify linkage at such a distance. The only locus for which there is good evidence of linkage to C6 is the structural locus of C7 (Lachamnn and Hobart, 1978a; Lachmann et al. 1978b; Hobart et al. 1978; Glass et al. 1978).

C7 Polymorphism

Screening of random samples by isoelectric focusing in thin-layer polyacrylamide gel enabled the identification of three structural forms

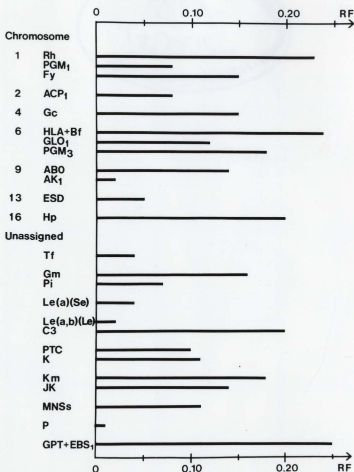


Figure VI. Diagram showing the parts of the male genome from which C6 has been excluded (lod score <-2.00). (Olving et al. 1979)

Table IV. Combined lod scores for Gm, Gc and AK_1.

Locus	Reference	Sex of Hetero-zygote	Two and three generation data; lod scores for recombination fractions of: 0.05	0.10	0.20	0.30	0.40
Gm	Olving et al. 1979	M	-6.20	-3.62	-1.47	-0.58	-0.17
	Hobart et al. 1977	M	-6.29	-3.32	-1.00	-0.21	-0.01
	Combined	M	-12.49	-6.94	-2.47	-0.79	-0.18
	Olving et al. 1979	F	-10.86	-6.15	-2.34	-0.67	-0.12
	Hobart et al. 1977	F	-5.27	-2.00	+0.23	+0.16	+0.12
	Combined	F	-16.13	-8.15	-2.11	-0.51	0.00
	Combined M + F		-28.62	-15.09	-4.58	-1.30	-0.18
Gc	Olving et al. 1979	M	-5.70	-3.31	-1.30	-0.47	-0.15
	Hobart et al. 1977	M	-6.55	-3.54	-1.15	-0.31	-0.05
	Combined	M	-12.25	-6.85	-2.45	-0.78	-0.20
	Olving et al. 1979	F	-8.07	-4.66	-1.80	-0.62	-0.13
	Hobart et al. 1977	F	-0.31	+0.25	+0.50	+0.38	+0.19
	Combined	F	-8.38	-4.41	-1.30	-0.24	+0.06
	Combined M + F		-20.63	-11.26	-3.75	-1.02	-0.14
AK_1	Olving et al. 1979	M	-1.44	-0.89	-0.38	-0.15	-0.04
	Hobart et al. 1977	M	-3.26	-1.15	+0.26	+0.46	+0.22
	Combined	M	-4.70	-2.04	-0.12	+0.31	+0.18
	Olving et al. 1979	F	-4.53	-2.68	-1.09	-0.40	-0.09
	Hobart et al. 1977	F	-3.72	-1.96	-0.57	-0.11	-0.02
	Combined	F	-8.25	-4.64	-1.66	-0.51	-0.11
	Combined M + F		-12.95	-6.68	-1.78	-0.20	+0.07

Table V. Joint lod scores for C6 linkage relations with positive lod scores in one of the two published materials, males only

Source of data	Male C6 lod scores to marker loci									
	AK_1 (θ=0.3)	ABO (θ=0.3)	HP (θ=0.4)	K (θ=0.3)	Km (θ=0.3)	ACP_1 (θ=0.3)	ESD (θ=0.4)	ADA (θ=0.05)	MNSs (θ=0.3)	P (θ=0.2)
Hobart et al. 1977	+0.46	+0.14	+0.21	+0.26	+0.16	-0.53	-0.21	-8.02	-0.18	-0.18
Olving et al. 1979	-0.15	-0.40	-0.18	-0.24	-0.90	+0.67	+0.10	+0.84	+0.47	+0.19
Both	+0.31	-0.26	-0.03	+0.02	-0.74	+0.14	-0.11	-7.18	+0.29	+0.01

The comparable recombination fractions with highest positive lod scores are used; θ_1 values are given in brackets.

(Olving et al. 1979)

of C7 proteins (Hobart et al. 1978). C7*1, C7*2 and C7*3 are products of three codominantly expressed alleles at a single autosomal locus. The banding patterns of the homozygote and heterozygote states are shown schematically in Figure VII.

The homozygote pattern C7 1-1 is by far the most common pattern. The less common alleles of C7*2 and C7*3 have only nominal charge differences compared to the common allele, resulting in very small changes in the pI of the proteins. The structural changes may be a substitution of one neutral amino acid with another having a different side chain which slightly changes the charge on a neighbouring residue.

The bands of C7*1 and C7*2 were of equal intensity but interestingly, those of C7*3 were found to be relatively weak and therefore posed a problem in that they may have been missed on a cursory inspection of the gel. Because of this difficulty in detection of the third allele, the gene frequencies were not calculated but it was believed that the frequency for both C7*2 and C7*3 was less than 0.01 (Hobart et al. 1978).

The largest contribution of evidence linking the loci of C6 and C7 was a single study of the polymorphic proteins of C7 and their mode of inheritance in reference to that of the C6 alleles (Hobart et al. 1978). The family tree as given, is shown in Figure VIII. The C6 and C7 genotypes for the first generation were reconstructed from their offspring. It is highly improbable that both were heterozygous for C7. Assuming the first generation has been correctly genotyped, there are ten informative children: II-1, 3, 7, 9; III-4, 12, 13, 14; and individuals II-5, 10 (who are inferred informative through their offspring), and there are no recombinations. The C6*A/C7*2 genotype has been inherited as a single unit. The odds in favour of linkage between C6 and C7 are

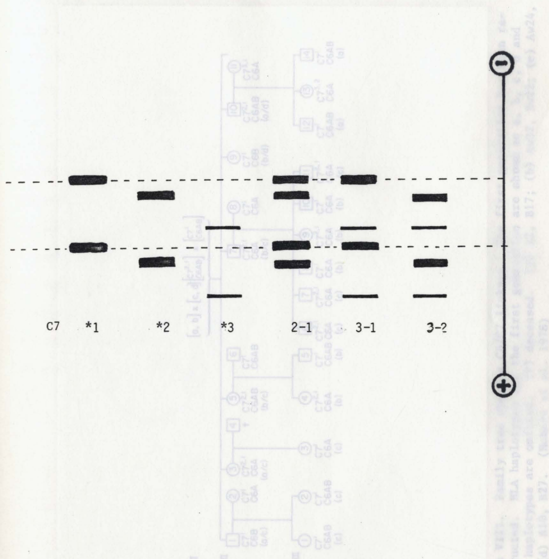


Figure VII. Schematic representation of homozygous and heterozygous patterns of the major bands of C7.

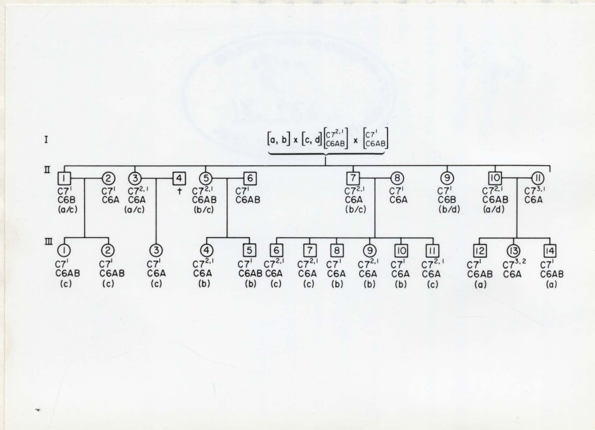


Figure VIII. Family tree showing C6/C7 linkage. The first generation has been reconstructed. HLA haplotypes for the first generation are shown as a, b, c, d and other haplotypes are omitted. (†) deceased. (a) A2, B17; (b) Aw32, Bw22; (c) Aw24, B40; (d) A10, B27. (Hobart et al. 1978)

suggested by the authors as greater than 1000:1.

The phase of the first generation is unknown but assuming linkage of HLA and C7, there are five recombinants and nine non-recombinants. Therefore, the C7 locus cannot be situated close to the HLA region. This is certainly expected since C6 has been shown to be not linked to HLA (Hobart et al. 1977; Olving et al. 1977; Olving et al. 1978; Olaisen et al. 1978b; Olving et al. 1979).

C6/C7 Linkage Summary

C6 and C7, as precusors of the membrane attack unit, act sequentially in the complement cascade. They share close physicochemical properties. Polymorphism has been demonstrated for both proteins and it has been further shown that the loci of the two components are closely linked. Two families exhibiting combined deficiency of C6 and C7 suggest that a single primary transcript is made for both loci. Post-transcriptional modification results in translation of two separate molecules. Based on all these findings, it is plausible to regard C6 and C7 as products of tandem gene duplication.

CHAPTER II OBJECTIVES

The general objective of this thesis is to contribute to the presently limited pool of information pertaining to the genetics of human C6 and C7. This is accomplished through typing various collections of sera available in this laboratory for C6 and C7 in order to:

(1) see which alleles are to be found in the sera.

(2) establish allele frequencies for Newfoundland.

(3) examine Mendelian ratios, looking particularly for irregularities which might suggest a more complicated genetics than one locus.

(4) check that rare alleles are inherited and not acquired.

(5) examine the data for its possible use in genetic linkage studies, with particular reference to families informative for both C6 and C7 so that the link between these loci can be examined further and to families in which a new marker exists which has not yet been examined for linkage to C6 or C7. As an example of a linkage investigation, the linkage of C6 with HLA will be re-examined.

CHAPTER III MATERIALS AND METHODS

Samples

The samples for this study were derived from three sources. The first, the West Coast community, was under study due to an increased frequency of common variable immunodeficiencies, Hodgkin's disease and other malignancies, in hopes to define a genetic basis for the development of such disorders (Buehler et al. 1975; Marshall et al. 1980b; Salmon et al. 1980). This community was visited for blood collection on four separate occasions. The sera collected in 1974 and 1975 were separated from the clot within 24 hours and have been kept at -20°C since then. The sera collected during the visits of 1976 and 1979 were separated within 4 hours, frozen in liquid nitrogen and stored frozen thereafter at -70°C. Sera were available for 1075 individuals from the community. The second collection included Newfoundland families plus random individuals; the family units usually under genetic investigation due to the necessity of kidney or bone marrow transplants, or for diseases such as ankylosing spondylitis, polycystic kidney disease, urolithiasis or diabetes. Samples of serum or heparin-plasma were separated and stored frozen at -70°C within approximately 3 hours of collection. The third collection consisted of an extended Labrador family of 110 members, 33 of whom have myotonic dystrophy (Larsen et al. 1979; Larsen et al. 1980). Heparin-plasma samples were separated and frozen in liquid nitrogen within 2 hours of collection and stored thereafter at -70°C.

A small number of other samples were available from South Africa (through the kindness of Dr. M. Hammond) and from mainland China (through the kindness of Dr. S.N. Huang).

Protein Separation

The polymorphic C6 and C7 proteins of human complement are separated by isoelectric focusing in thin-layer polyacrylamide gel and detection of the resulting banding is through the use of an appropriate overlay (see below). Because these two complement components have similar isoelectric focusing points, gels of identical chemical composition (see below) may be used. The slab gels used in this laboratory are ultra-thin; of obvious economic benefit but use of such gels also results in crisper bands of better separated proteins (Massi et al. 1979).

Preparation of Ultra-thin-layer Polyacrylamide Slab Gels

To prepare four, approximately 15 cm. X 20.5 cm. X 0.05 cm. slab gels, the following materials are needed:

60.0 ml. stock acrylamide solution (see below)
1.0 ml. ampholine pH 5-7 (LKB, 40% w/v, Fisher Scientific Ltd., Whitby, ON)
0.8 ml. ampholine pH 6-8 (LKB, 40% w/v, Fisher Scientific Ltd., Whitby, ON)
0.2 ml. ampholine pH 3.5-10 (LKB, 40% w/v, Fisher Scientific Ltd., Whitby, ON)
30.0 µl. TEMED (Eastman Kodack Co., Rochester, NY)
16.0 ml. 1 mg.% riboflavin (Sigma Chemical Co., St. Louis, MO)

The above chemicals are combined, in the order above, in a vacuum flask and de-gassed with swirling for five minutes or until no bubbles form on the flask bottom. The de-gassed solution is poured between two glass plates with the aid of a pipette and any bubbles are removed by gentle tapping. One glass plate (16.5 cm. X 21 cm.) is covered with self-adhesive transparent vinyl and gasket (four layers of 0.5 cm. wide vinyl), permanently bound to the plate. The second plate (16.4 cm. X 21.5 cm., glass prepared for coating, treated both sides, Ilford Nuclear Research, Markham, ON) is clamped to the covered plate and will

form the support for the gel.

Polymerization of the acrylamide mixture is initiated by direct light and is complete in approximately 2½ hours. The plates are pried apart and the polymerized gel and its support plate are wrapped with plastic wrap and cured at $4^{o}C$ overnight.

Preparation of Stock Acrylamide Solution

To prepare 500.0 ml. stock acrylamide solution, the following materials are needed:

16.68 g. taurine (BDH Chemicals, Toronto, ON)
33.3 g. acrylamide, enzyme grade (Eastman Kodack Co., Rochester, NY)
0.9 g. N-N'-methylene-bisacrylamide (Eastman Kodack Co., Rochester, NY)

The taurine is dissolved with gentle heating and stirring in approximately 300 ml. distilled water. The acrylamides are added and dissolved and the solution made up to 500.0 ml. in distilled water. This stock solution is refrigerated at $4^{o}C$ in a clean glass bottle covered with foil wrap. (The taurine is incorporated in the stock solution so that the slab gels are isotonic for the detecting cells used in the hemolytic overlay.)

Preparation of Gel for Isoelectric Focusing and Application of Samples

Materials needed:

cured polyacrylamide slab gel
sample papers, 6 mm. X 12 mm. (Whatman 40)
samples, quick-thawed at $37^{o}C$, on ice
electrofocusing strips (LKB, Fisher Scientific Co., Whitby, ON)
anode solution, 1 M H_3PO_4 (Fisher Scientific Co., Whitby, ON)
cathode solution, 1 N NaOH (BDH Chemicals, Toronto, ON)
Gilson pipetman, p20 (Mandel Scientific Co. Ltd., Rockland, ON)
pipette tips

For each slab gel, one electrofocusing strip is wet with anode solution, another with cathode solution and are then placed on the gel, approximately 1.5 cm. in on the lengthwise edges of the support plate. Sample papers are placed approximately 5 cm. from the anode strip and 3-6 µl. of sample is applied per paper. The smaller sample of 3 µl. is best as protein overload causes streaking and the homozygous C6BB may be mistyped as the heterozygous C6AB.

Because the homozygous C7*1 pattern is most common, samples may be screened for C7. This entails using sample papers of 2 mm. X 18 mm., placing the papers very close together on the gel and using 2 µl. of sample. The C7*2 and C7*3 patterns are easily distinguished from the common bands and this method allows more samples to be isoelectrically separated at one time.

Electrical Details of Isoelectric Focusing

Isoelectric focusing is carried out at 20 watts constant power, maximum voltage of 1500 volts (LKB 2103 Power Supply, Fisher Scientific Ltd., Whitby, ON) with cooling (Pharmacia Flatbed Apparatus FBE-3000, Dorval, PQ). The outport flow temperature is approximately 8°C throughout the focusing time, higher temperatures causing waving of the banding patterns and lower temperatures increasing protein viscosity.

The pH gradient is formed within the gel during the first half hour and is monitered by the increasing voltage and decreasing amps. Focusing of the C6 protein requires approximately 3½ hours after the pH gradient has been established, the C7 protein about 1 hour less due to its lower pI.

C6 Detection

Materials needed:

0.75% (w/v) agarose (Indubiose A37, Reactifs IBF, Gennevilliers) in CFD
10% (v/v) EA
1.5% (w/v) joining agar (Seakem, Marine Colloids Div., FMC Corp., Rockland, ME)
C6 deficient rabbit serum
four-sided plexiglass box, 19.5 cm. X 8 cm.
leveling table

CFD (complement fixation test diluent, Oxoid, England): The tablet form is solubilized in distilled water, 100.0 ml. distilled water per tablet.

EA (antibody sensitized sheep red blood cells): Sheep red blood cells in Alsever's solution are washed (X3) in CFD and made up to 20% in CFD. Rabbit anti-sheep red cell anti-serum (Gibco Grand Island Biological Co., Grand Island, NY or Wellcome Reagents Ltd., Beckenham, England), 0.1 ml. anti-serum per 0.5 ml. packed sheep cells, is added and the cells are stirred at 4°C for 20-30 minutes to allow attachment of the antibody to the cell membranes. The sensitized cells are then washed with CFD (X3), made up to 10% with CFD and stored at 4°C. (With the anti-serum used in this laboratory, 0.1 ml. anti-serum per 0.5 ml. packed cells always gives a minimal hemolytic dose of 6-10, as recommended by Lachmann and Hobart (1978c).

C6 deficient rabbit serum: C6 deficient rabbits are bled from the ear into glass tubes. The blood is allowed to clot for 45-60 minutes at room temperature. After separation from the clot, the serum is divided into 1 ml. aliquots and stored at -70°C.

Principle of the C6 overlay: After focusing, the C6 protein has assumed its characteristic banding pattern according to the protein's specific pI. The overlay contains all necessary components for lysis of the sensitized cells except C6. During an incubation period, the

C6 protein will diffuse up into the agarose overlay and completing the cascade, will cause bands of lysis.

Pouring of the overlay: The joining agar (1.5% w/v) and agarose (0.75% w/v) is completely melted and kept at 56°C. The detecting cells (10% in CFD) are kept at 37°C. Moments before the gel is finished focusing, the deficient serum (0.5 ml./gel) is quick-thawed at 37°C. The focused gel is removed from the electrofocusing apparatus and the four-sided plexiglass box is positioned approximately midway between the electrode strips. Using the 1.5% joining agar, the box is joined to the gel. 5.2 ml. agarose (0.75% w/v) is cooled to 45°C, 0.35 ml. EA (10% in CFD) added and the mixture is swirled to mix thoroughly. C6 deficient rabbit serum (0.5 ml./gel) is added. The mixture is again swirled and then poured into the area formed by the box. The overlay is spread by shaking the plate and placing it on the leveling table to ensure even distribution. (All these steps must be completed within a limited amount of time to ensure a clear overlay. If the agarose is cooled too long, it will solidify. Also, addition of detecting cells into agarose that is too hot will result in cell clumping.) The plate is then incubated in a moist chamber at 4°C for 30-45 minutes. Banding is visualized within 15 minutes at room temperature.

Allotyping of "older" serum: Older serum has a tendency towards thicker banding than fresh samples but with experience, the homozygous C6AA can be easily distinguished from the heterozygous C6AB. Plates should be read as soon as the bands are developed. If left too long, overdeveloped homozygous patterns may be misread as heterozygous C6AB.

C7 Detection

Materials needed:

0.75% (w/v) agarose (Indubiose A37, Reactifs IBF, Gennevilliers) in EDTA-PBS
10% (v/v) guinea pig cells in PBS
1.5% (w/v) joining agar (Seakem, Marine Colloids Div., FMC Corp., Rockland, ME)
$\overline{C56}$ serum
four-sided plexiglass box, 19.5 cm. X 8 cm.
leveling table

PBS (phosphate buffered saline, Oxoid, England): The tablet form is solubilized in distilled water, 100.0 ml. per tablet.

EDTA-PBS stock solution: A solution of 0.01 M EDTA (Sigma Chemical Co., St. Louis, MO), 0.2 M Na_2·EDTA (Fisher Scientific Ltd., Whitby, ON) and 0.2 M Na_4·EDTA (BDH Chemicals, Toronto, ON) is made in PBS. The pH is brought to 7.2 with 1 M NaOH (BDH Chemicals, Toronto, ON) if necessary. The stock solution is stored at 4°C and used as 5% in PBS.

Guinea pig cells: Cells obtained through heart puncture of healthy, normal guinea pig are washed (X3) in PBS and stored at 10% (volume packed cells/volume in PBS) at 4°C.

$\overline{C56}$ serum - preparation from acute phase serum: Three to five days post-trauma, whether it be childbirth, surgery or infection (bacterial or viral), specific serum proteins are known to increase in concentration, moreso in some individuals than in others (Thompson and Rose, 1968; Ganrot, 1974; Füst et al. 1976; Mak, 1978). Complement components C5 and C6 are two of these proteins. Blood from such subjects is obtained and serum collected off the clot is treated with yeast suspension, as prepared by Lachmann and Hobart (1978c), (a kind gift from Dr. M.J. Hobart), 50 µl. yeast suspension per 1.0 ml. serum, overnight at room temperature (see principle below). Centrifugation removes the yeast and the serum is aliquoted in 1 ml. volumes and is stored thereafter at -70°C.

The principle of the test for $\overline{C56}$ activity: Yeast activation of the alternative complement pathway results in consumption of the complement components. If there are elevated levels of C5 and C6, there will be great amounts of $\overline{C56}$ formed as the other components are depleted; C7 is usually at a limiting concentration and is exhausted by this treatment.

Serum deposited into the test plate well diffuses into the agarose and any $\overline{C56}$ in the test serum will attach to the guinea pig cell membranes. Normal human serum incorporated in the agarose will supply the required C7, C8 and C9 for lysis of the cells. Activation of the earlier components in the normal human serum is inhibited by the incorporation of EDTA in the agarose which chelates the magnesium and calcium ions required for activation of either pathway. Therefore, lysis occurs only in the areas where $\overline{C56}$ is present. The diameter of the ring is indicative of the concentration of $\overline{C56}$ in the test serum (see Figure IX). Serum giving no or minimal rings of lysis is discarded. No further purification is necessary.

Testing of the treated serum for $\overline{C56}$ activity: Materials needed are as follows:

2.0% agarose (w/v) (Seakem, Marine Colloids Div., FMC Corp., Rockland, ME)
10% (v/v) guinea pig cells in PBS
NHS (normal human serum)
5 cm. X 5 cm. glass plate, clean

Completely melted agarose which has been kept at 56°C is cooled to 37°C. 0.20 ml. NHS and 0.20 ml. guinea pig cells (10% v/v) are added and after thorough mixing, the mixture is poured onto the clean glass plate and spread evenly to all edges. The agarose is allowed to harden and wells are punched. 7 µl. of test serum is deposited per

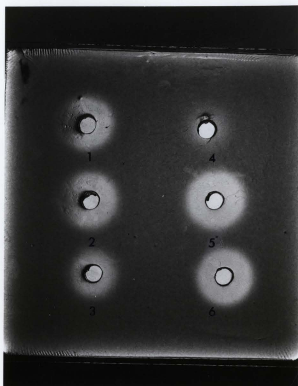


Figure IX. Test plate for C56 activity in yeast treated acute phase sera. Test sera deposited into wells, diffuse into agar containing guinea pig cells, EDTA and normal human serum (NHS). EDTA prevents <u>de novo</u> activation of complement. C56 present in the test sera attaches to the cell membranes. C7, C8 and C9 from the NHS complete the cascade and lysis occurs. Wells 5 and 6 contain sera having high C56 activity, wells 1, 2 and 3, adequate activity and well 4, inadequate activity.

well. The test plate is incubated at 4°C overnight and then placed in a 37°C incubator and warmed for 1-2 hours.

Principle of the C7 overlay: Once focused, the C7 protein is in a banded pattern according to the proteins's pI. C7 rapidly diffuses up into the overlay containing guinea pig cells and C56. The presence of the EDTA in the overlay inhibits any activation of the classical or alternative pathways. C7 complexes with the C56 which has attached to the cell membranes and C8 and C9 supplied in the C56 serum complete the cascade. Bands of lysis appear in the areas of high C7 concentration.

Pouring of the overlay: The reagents needed for C7 detection are made ready as in the C6 procedure, that is, the agarose for joining and overlay are completely melted and kept at 56°C, the detecting cells are kept at 37°C and the C56 serum is quick-thawed at 37°C just moments before the gel is finished focusing. The gel is removed from the apparatus and the box positioned about midway between the electrode strips and joined to the gel with the joining agar. 5.2 ml. agarose (0.75% w/v in EDTA-PBS) is cooled to 37°C. 0.5 ml. C56 serum and 0.35 ml. 10% (v/v in PBS) guinea pig cells are added, mixed thoroughly and poured, distributing evenly within the area formed by the box by shaking the plate and then placing it on the leveling table. As with the C6 overlay, these steps must be done in a limited amount of time. The plate is incubated at 4°C for 15 minutes.

CHAPTER IV RESULTS

C6 Results

Approximately 2400 sera were typed for C6 by the hemolytic overlay technique (see Methods), these being derived mainly from three sources: (i) West Coast community study, (ii) Newfoundland families plus random individuals and (iii) Labrador myotonic dystrophy family. Details of each collection are given in the Materials and Methods section.

Phenotypes and alleles: The typical pattern observed consisted of a ladder-like pattern of 3 to 4 bands of hemolysis (Figure X-A,B,C) spreading approximately between pH 6 and pH 8. By comparison with published reports, it was obvious that the majority of patterns were derived from two alleles in homozygous or heterozygous forms, a fact which was confirmed by family studies (see below). The two alleles have been called C6*A and C6*B.

Several rare phenotypes were observed. Serum H.D., a Newfoundland person, used from repeated bleeds as a reference serum, always showed a pattern shown in Figure X-D. This is interpreted as an A allele together with a more acidic variant allele whose lines interdigitate with those of C6*A. From descriptions of variants (Mauff et al. 1979), this is considered to be a C6*A1 but comparisons with a reference C6*A1 have not been possible. Fifteen other sera with this same allele were found, eleven were together with C6*A and four were C6*A1 with C6*B (see Figure X-E). Another rare pattern, shown in Figure X-F, is interpreted as an A allele with a variant B allele; clearly, the bands of the variant allele are shifted to a slightly more basic pH and interdigitate with those of the common C6*B allele (Figure X-G). It is because of this minor shift that this is thought probably to be a rare C6*B1 al-

lele, as described by Mauff et al. (1979). Unfortunately, no reference serum was available to confirm this. This C6*B1 allele was observed in six sera, only in combination with C6*A. A study of a single South African family fortuitously revealed the rare pattern shown in Figure X-H. This is considered to be a B allele together with a variant of neither the A or B allele since the lines interdigitate with both those of C6*A and C6*B. This is compatible with the allele described by Mauff et al. (1979) which was designated C6*M. This rare allele was observed in a nephew of the man just described but was in combination with C6*A (see Figure X-I).

The phenotype and actual numbers observed in each collection studied are given in Table VI.

Allele frequencies and inheritance: Although the West Coast community is known to contain many related individuals, all results were pooled as random samples for the calculation of the allele frequencies. In the Newfoundland families plus random individuals collection any known related persons were collected into family units and only the alleles from the parents or founders were used in the counting, all other samples were treated as random. The South African family was not included in the calculations. The Labrador collection of 110 individuals is an extended family and therefore allele frequencies were not calculated. The allele frequencies for the West Coast community and the Newfoundland families plus random individuals are given in Table VII. It can be seen that there is no significant difference between the allele frequencies of these two populations and those previously studied.

Single parent-offspring combinations, as well as mating pair-off-

Table VI. Observed phenotypes in three Newfoundland populations.

Collection	n		AA	AB	BB	AB1	A1B	A1A
West Coast community	1075	Observed	418	522	133	1	1	0
		Expected	429.5	498.8	144.8	0.3	0.2	
		χ^2	0.3079	1.0791	0.9616	1.6333	3.2	
Newfoundland families plus random individuals	689.5	Observed	351	249	79.5	2	3	5
		Expected	332.8	285.5	61.2	0.7	1.2	2.8
		χ^2	0.9953	4.6664	5.4721	2.4143	2.7	1.7286
Labrador myotonic dystrophy family	110	Observed	73	36	1			

Table VII. C6 allele frequencies in two Newfoundland populations.

C6 Allele	West Coast community	Newfoundland families plus random individuals
A	0.6321	0.6947
B	0.3670	0.2980
A1	0.0005	0.0058
B1	0.0005	0.0015

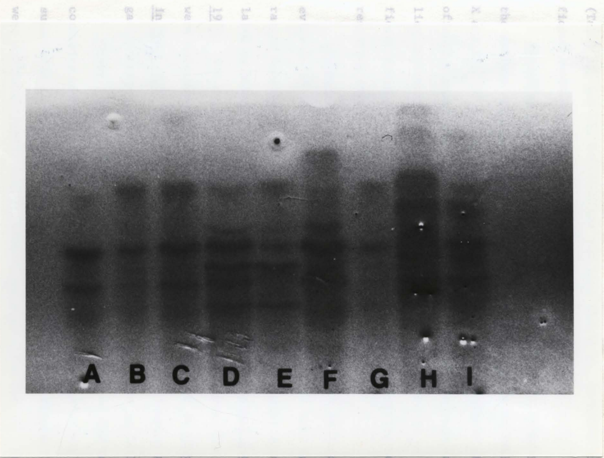


Figure X. C6 phenotypes observed in Newfoundland. Track A = C6AA, track B = C6BB, track C = C6AB, track D = C6A1A, track E = C6A1B, track F = C6AB1, track G = C6BB, track H = C6BM, track I = C6AM.

pring combinations, were observed in all three collections (see Tables VIII, IX, X, XI). No unusual combinations were observed. There was no evidence of selection against the inheritance of rare alleles. Mating pair frequencies, calculated only for the West Coast community (Table IX) and the Newfoundland families (Table X), revealed no significant deviation from the expected mating frequencies.

Mendelian ratios: Actual and predicted numbers of progeny from the matings observed in the three collections are shown in Tables IX, X and XI. Of significance is the excess of C6AA and deficit of C6AB offspring resulting from C6AB X C6AB matings in the Newfoundland families collection. However, in all other instances, there is no significant deviation between the expected and observed offspring number resulting from the various mating pairs.

Linkage studies with C6: Twenty-six families were studied for evidence of C6 linkage with the HLA complex. Since all were two generation families, z_1 scores (and e_1 scores where necessary) were calculated as shown by Alan E.H. Emery in Methodology in Medical Genetics, 1976. For those families with sibships larger than seven, lod scores were calculated as suggested by R.R. Race and R. Sanger in Blood Groups in Man, 1975. Combined lod scores for 19 males and 7 females gave negative values for all recombination fractions, as shown in Table XII.

Other linkage studies with C6: A previous study of the West Coast community revealed the increased incidence of a variant of red cell superoxide dismutase (SOD). The pedigree is given in Figure XI. Sera were available for 23 family members and were typed for C6. Unfortunately, the C6 results were uninformative and therefore an attempt to statistically link this SOD locus with that of C6 was not possible.

Table VIII. Single parent-offspring combinations.

Parental Genotype	Collection	n	Offspring Genotype AA	AB	BB	AB1	n
AA	West Coast community	44	77	35	0	0	112
	Newfoundland families	17	32	7	0	0	39
	Labrador M.D. family	3	1	5	0	0	6
AB	West Coast community	61	39	71	31	1	142
	Newfoundland families	32	49	50	15	0	114
	Labrador M.D. family	1	2	1	0	0	3
BB	West Coast community	16	0	18	9	0	27
	Newfoundland families	4	0	7	1	0	8
Total single parents		178		Total offspring			451

Table IX. Observed mating pairs and inheritance of C6 alleles in the West Coast community.

Mating Pair	n OBS(EXP)	χ^2	Offspring Genotype AA	AB	BB	χ^2	n
AA x AA	16(20.8)	1.1077	61(61)	0(0)	0(0)	-	61
AA x AB	55(48.4)	0.9	75(85.5)	96(85.5)	0(0)	2.578	171
AA x BB	5(14.0)	4.5714	0(0)	20(20.0)	0(0)	-	20
AB x AB	33(28.1)	0.8544	23(24.0)	53(48.0)	20(24.0)	1.230	96
AB x BB	16(16.3)	0.0055	0(0)	30(27.5)	25(27.5)	1.684	55
BB x BB	4(2.4)	1.0667	0(0)	0(0)	7(7.0)	-	7
A1B x AA	1		0	1	0		1
Total mating pairs	130		Total offspring				411

$\sum \chi^2_{mating} = 8.5057$, insignificant at $P > 0.05$, df = 5.

Table X. Observed mating pairs and inheritance of C6 alleles in Newfoundland families.

Mating pair	n OBS(EXP)	χ^2	Offspring Genotype AA	AB	BB	χ^2	n
AA x AA	13(18.2)	1.4857	36(36)	0(0)	0(0)	-	36
AA x AB	25(31.2)	1.2321	61(54.5)	48(54.5)	0(0)	1.5504	109
AA x BB	4(6.7)	1.0081	0(0)	8(8.0)	0(0)	-	8
AB x AB	20(13.4)	3.2507	33(18.5)	21(37.0)	20(18.5)	18.4095	74
AB x BB	9(5.7)	1.9106	0(0)	15(15.0)	15(15.0)	0.0	30
BB x BB	1(0.6)	0.2667	0(0)	0(0)	5(5.0)	-	5

Mating pair	n	Offspring Genotype AA	AB	A1A	A1B	AB1	χ^2	n
AA x A1A	1	0(0.5)	0(0)	1(0.5)	0(0)	0(0)	0.5	1
AA x A1B	1	0(0)	1(1.5)	2(1.5)	0(0)	0(0)	0.3334	3
AA x AB1	1	1(2)	0(0)	0(0)	0(0)	3(2)	1.0	4
AB x A1A	3	6(2.5)	1(2.5)	3(2.5)	0(2.5)	0(0)	5.9	10
Total mating pairs	78				Total offspring			280

$\sum \chi^2_{mating}$ = 9.2339, insignificant at P > 0.05, df = 5.

Table XI. Observed mating pairs and inheritance of C6 alleles in a Labrador myotonic dystrophy family.

Mating Pair	n	Offspring Genotype			χ^2	n
		AA	AB	BB		
AA x AA	4	26(26.0)	0(0)	0(0)	-	26
AA x AB	5	17(16.0)	15(16.0)	0(0)	0.135	32
AB x AB	2	2(1.0)	2(2.0)	0(1.0)	1.0	4
Total mating pairs	11		Total offspring			62

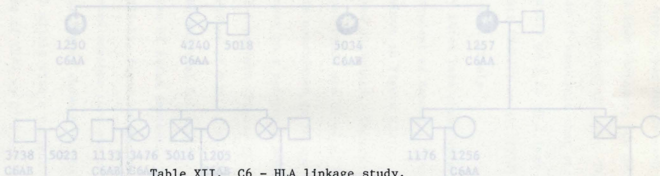
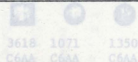


Table XII. C6 - HLA linkage study.

Sex of Heterozygote	n	Two generation families; lod score at recombination fraction θ= 0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
Male	19	-39.98	-18.65	-10.37	-6.13	-3.60	-2.01	-1.02	-0.45	-0.15	-0.03
Female	7	-23.30	-11.78	-7.13	-5.17	-3.04	-1.94	-1.16	-0.54	-0.21	-0.07
Combined	26	-63.28	-30.43	-17.50	-11.30	-6.64	-3.95	-2.18	-0.99	-0.36	-0.10



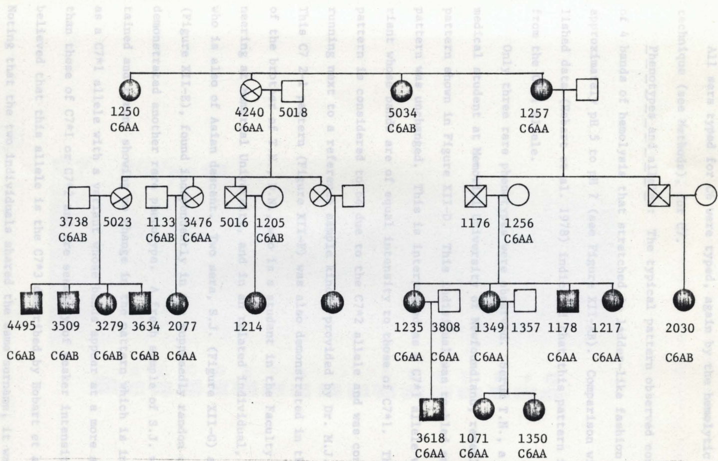


Figure XI. Pedigree for the C6 - SOD linkage attempt. Filled in figures represent individuals typed as heterozygous for the SOD variant, crossed figures are inferred heterozygous. C6 results are given. (used with permission of Ms. S.K. Buehler)

C7 Results

All sera typed for C6 were typed, again by the hemolytic overlay technique (see Methods), for C7.

Phenotypes and alleles: The typical pattern observed consisted of 4 bands of hemolysis that stretched in ladder-like fashion from approximately pH 5 to pH 7 (see Figure XII-AB). Comparison with published data (Hobart et al. 1978) indicated that this pattern is derived from the C7*1 allele.

Only three rare phenotypes were observed. Serum T.N., a Chinese medical student at Memorial University of Newfoundland, revealed the pattern shown in Figure XII-D. This individual was re-bled and the pattern was unchanged. This is interpreted as a C7*1 allele with a variant whose bands are of equal intensity to those of C7*1. This variant pattern is considered to be due to the C7*2 allele and was confirmed by running next to a reference sample kindly provided by Dr. M.J. Hobart. This C7 2-1 pattern (Figure XII-F) was also demonstrated in the serum of the brother of T.N., D.N., who is a student in the Faculty of Engineering at Memorial University, and in an related individual, L.H., who is also of Asian descent. Two sera, S.J. (Figure XII-G) and T.J. (Figure XII-E), found independently in the supposedly random collection, demonstrated another rare phenotype. A fresh sample of S.J. was obtained and rerun, showing no change in the pattern which is interpreted as a C7*1 allele with a variant whose bands appear at a more acidic pH than those of C7*1 or C7*2 and are seeminly of weaker intensity. It is believed that this allele is the C7*3 described by Hobart et al. (1978). Noting that the two individuals shared the same surname, it was possible to trace and establish their relationship as brothers from the

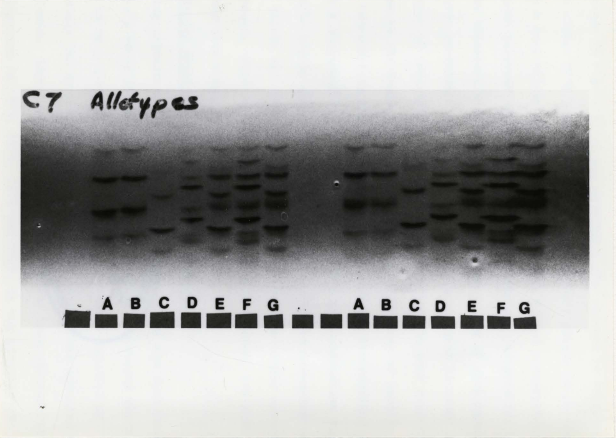


Figure XII. C7 phenotypes observed in Newfoundland. Tracks A and B = C7*1, track C = C7*3, tracks D and F = C7 2-1, tracks E and G = C7 3-1 (shown in duplicate).

Eagle River area of Labrador. A single individual, M.M., a 29 year old female from Goose Bay, Labrador, was hospitalized here at the Health Sciences Centre, St. John's, Newfoundland, for surgery to repair a torn meniscus and luckily was available for rebleeding. Confirmation of the pattern shown in Figure XII-C was possible. The pattern is believed to be due to either C7*3 homozygosity or else the C7*3 allele is in combination with a null allele as C7 3-0. Through M.M.'s kind cooperation and her own keen interest in her ancestry, a 4 generation pedigree for her family was obtained, with some detail as to the origin of each branch in the first generation (see Figure XIII). Of particular interest is the ancestor of individual I-2, a Norwegian who had the same unusual given name as the Eagle River individual, T.J.. The use of family names is customary in the communities of Labrador and Newfoundland and with such a distinctive given name, typical only in Scandinavian countries, it is highly probable that the three individuals demonstrating the C7*3 allele are descended in some manner from this common ancestor or his spouse. This family is to be investigated further, but this is ou side the time frame of this thesis.

C7 Typing Problems - Due to an Aging Artifact?

During attempts to produce C7 allotyping data for the West Coast community, a variation in pattern in some sera was discovered which appeared to be a heterozygote patten. The extra band occurred at approximately the position that published data showed was occupied by a C7*3 allele. Since the West Coast community had been visited for blood collection in 1974, 1975, 1976 and 1979, it was possible to confirm this pattern on three different bleeds from the same individual. On retyping, it was found that the variant pattern was not al-

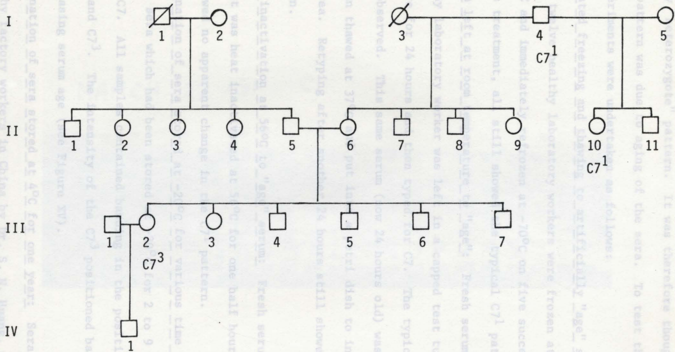


Figure XIII. Labrador family having a C7 *3 phenotype (individual III-2). Ancestors for each branch of the first generation are as follows: I-1, Isle of Barra, Scotland; I-2, Norway; I-3, Liverpool, England and I-4, England. Individuals I-4, II-10 and III-2 have been C7 typed.

ways present and was sometimes barely visible (Figure XIV). Furthermore, when many sera collected in 1974 were examined, all showed this supposedly "heterozygote" pattern. It was therefore thought likely that the pattern was due to aging of the sera. To test this idea, several experiments were undertaken as follows:

Repeated freezing and thawing to artificially "age" serum: Fresh sera from twelve healthy laboratory workers were frozen at -70°C, thawed at 37°C and immediately refrozen at -70°C on five successive days. After this treatment, all still showed the typical $C7^1$ pattern.

Serum left at room temperature to "age": Fresh serum from a single healthy laboratory worker was left in a capped test tube at room temperature for 24 hours and then typed for C7. The typical $C7^1$ pattern was observed. This same serum (now 24 hours old) was frozen at -70°C, then thawed at 37°C and put into a petri dish to increase the surface area. Retyping after another 24 hours still showed the typical $C7^1$ pattern.

Heat inactivation at 56°C to "age" serum: Fresh serum from a healthy student was heat inactivated at 56°C for one half hour. Subsequent typing showed no apparent change in the $C7^1$ pattern.

Examination of sera stored at -20°C for various time periods: Thirty-one sera which had been stored at -20°C for 2 to 9 years were typed for C7. All samples contained banding in the positions for alleles $C7^1$ and $C7^3$. The intensity of the $C7^3$ positioned bands increased with increasing serum age (see Figure XV).

Examination of sera stored at 4°C for one year: Sera collected from healthy factory workers in China by Dr. S. N. Huang, were carried at room temperature back to Newfoundland and stored thereafter at 4°C.

Figure XIV. Progressive appearance of C7*3 positioned artifacts in the West Coast sera. For each serum number shown, samples from 1974, 1976 and 1979 have been C7 allotyped in tracks above (A = 1974, B = 1976, C = 1979 for serum 1119; D = 1974, E = 1976, F = 1979 for serum 1126; etc.).

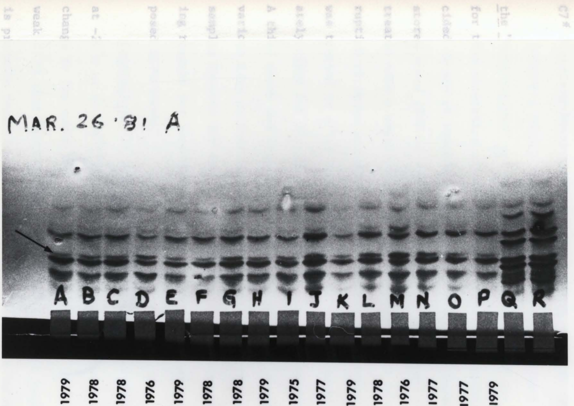


Figure XV. C7 patterns of sera stored at -20°C for 2-7 years. Tracks A-P contain sera stored at -20°C since date indicated. Tracks Q and R contain control C7 2-1. Intensity of the artifact bands (noted by arrow) increases with increasing serum age.

Typing of 20 of these sera showed patterns unlike any C7 pattern seen before and comparison with Caucasian sera kept at 4°C for one year was not possible. Some signs of underlying polymorphism were noted in the Chinese sera. Five of the 20 samples (Figure XVI-A,C,L,N,P) show the C7* 2 pattern found in the heterozygous sample T.N. (Figure XVI-J).

Treatment with a reducing agent of naturally aged serum showing the "heterozygote" pattern: Since oxidation of C7 was a possible cause for this alteration, by causing di-sulfide bond breakage, it was decided to try to reverse it with reducing agents. Serum which had been stored since 1978 at -20°C and showing a "heterozygote" pattern was treated with a small amount of dithiotreitol which caused total disruption of serum proteins. No C7 pattern was observed. A second serum was treated in a 3:1 ratio with 0.2 M mercaptoethanol and was immediately typed for C7. No change was seen in the "heterozygote" pattern. A third serum was treated with an equal volume of mercaptoethanol for various time periods. Disruption of all serum proteins occurred in samples treated for more than 8 minutes. Those treated with the reducing reagent for less than 8 minutes showed no disappearance of the supposed C7 *3 bands.

Conclusion: It seems certain that aging of frozen sera, either at -20°C or at -70°C for more than one year, produces an artifactual change in the serum such that it types for C7 as C7 3-1, with a very weak C7 *3 line initially which gets stronger with longer storage. It is probable that storage at 4°C for a year allows further changes to occur such that a complex multi-lined pattern appears. No physico-chemical basis for these changes is yet apparent.

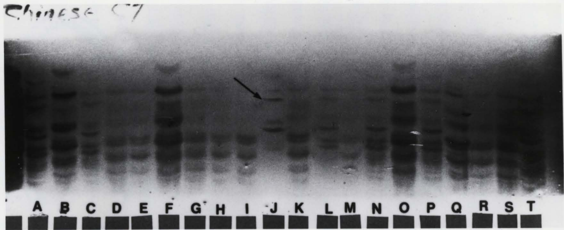


Figure XVI. C7 patterns observed in aged Chinese sera. Track J contains control C7 2-1. *2 band is noted by the arrow. Although there is extensive artifactual banding, C7*2 bands are thought to exist in sera of tracks A, C, L, N, P.

CHAPTER V DISCUSSION

The Genetics of Human C6

Allele frequencies: The frequencies for the common alleles, C6*A and C6*B, found in the present study are similar to those found in other populations previously studied as reviewed in Chapter I. The West Coast community includes a large number of related individuals, 85% of the population being genealogically traced back to a single ancestral pair, yet, the C6*A (0.6321) and C6*B (0.3670) frequencies are not significantly different from those found in the rest of the Newfoundland population in which all known related members were collected into family units before calculation of the allele frequencies, C6*A (0.6947) and C6*B (0.2980). This is probably due to two factors, the first of which is that this is basically a two allele balanced polymorphism and secondly, the number of founders in the second and third generations was quite large.

Rare alleles C6*A1 and C6*B1 occurred infrequently in the West Coast community study, having a combined frequency of 0.001 and no family data were obtained to show that these alleles were inheritable. The Newfoundland families plus random individuals collection had a slightly larger combined frequency (0.0073) of rare alleles, the C6*A1 (frequency = 0.0058) occurring more frequently than C6*B1 (frequency = 0.0015). These rare patterns were shown to be consistant with Mendelian inheritance of a rare allele by family study and there was no evidence of selection (unfavourable transmission ratio) against them.

An excess of C6BB homozygotes and a deficit of C6AB heterozygotes were noted in the Newfoundland families plus random individuals collection. Examining the sub-populations of this collection, the excess

could be attributed primarily to the C6 phenotypes of the random individuals. Considering the possibility of technical error, it was thought that any bias would slant toward the misreading of a C6AB as a homozygous C6BB (possibly caused by protein overload or overdevelopment of the plates before reading, see Methods). Therefore, the random individuals previously typed as C6BB were re-typed. The correction changed the prior calculations minimally. Therefore, this deviation of observed from expected phenotypes is thought probably due to chance.

Inheritance of C6 alleles: An initial study of the segregation of C6 alleles (Hobart et al. 1977) noted a slight increase in the number of C6AB progeny from C6AB X C6AB mating. Since there is evidence of duplication of the C6 locus in dogs (Hobart, personal communication) and of the C4 locus in humans (O'Neill et al. 1978a; O'Neill et al. 1978b); this asymmetry of Mendelian ratios suggested the possibility of duplication at the C6 locus (Hobart, personal communication). Collection of further data on this topic was of interest in this study. Matings of heterozygotes in the West Coast community and the Labrador myotonic dystrophy families gave results which were compatible with regular Mendelian inheritance at a single locus. Parent combinations other than C6AB X C6AB in the Newfoundland families revealed no significant deviation of the observed and expected offspring frequencies. However, C6AB X C6AB matings gave an excess of C6AA offspring. This is the opposite finding to that of Hobart et al. (1977) and does not support the idea of duplication of the C6 locus. The excess of C6AB children noted in the first study (Hobart et al. 1977) can be given two possible explanations. It may have been due to chance but may also have resulted from a storage effect. Older serum has a tendency to be more difficult

to allotype, the banding less crisp, generally thickened in all positions (see Allotyping of "older" serum, Methods); this results in difficulty in distinguishing C6AA homozygotes from C6AB heterozygotes. The storage effect would presumably not result in an excess of homozygote offspring and therefore it is probable that the excess found in the Newfoundland families study was due to chance. Taking the results together, there is no reason to suggest a more complicated genetics than one locus.

No abnormal phenotypes were evident in any matings which would indicate the presence of a null allele.

Linkage studies with C6: Data compiled in this thesis for testing for linkage between the C6 locus and the HLA complex reiterated previous efforts in that, at no recombination fraction was there a positive value. Although the attempt to link the C6 and SOD loci was uninformative, the C6 data of this thesis is to be used in a major effort to link a susceptibility gene for Hodgkin's disease and acquired immunodeficiency thought to be in the West Coast community, with various markers (E.A. Thompson, 1980) and in other genetic investigations of disease ongoing in this laboratory (see Samples, Materials).

The Genetics of Human C7

Data in this thesis reveal the perplexing presence of an artifact, in sera stored for prolonged periods, which mimics a C7*3 pattern. Moreover, the gradual appearance of the artifact may suggest the presence of an allele with reduced function. This artifact problem has not been noted by previous investigators. To suggest that a true C7*3 allele may therefore not exist would be at the very least hasty and in fact, the opposite is suggested; three sera studied in this thesis appear to have

alleles, whose products migrate to the $C7^3$ position. The heterozygote pattern, $C7^{3,1}$ observed in two samples from the supposedly random individuals collection, was confirmed on a fresh sample available from one of them. Upon further investigation, it was discovered that the two individuals were brothers and therefore it appears that they share a genuine $C7^3$ allele. Similarly, the patient who only had a $C7^3$ pattern, due to either $C7^3$ homozygosity or to the $C7^3$ allele in combination with a null allele, was rebled and an identical pattern was obtained on typing performed immediately. It happens that these $C7^3$ patterns all appeared to have somewhat reduced activity when compared with bands of the $C7^1$ or $C7^2$ alleles, but this reduction is slight. Of the nine $C7^3$ allele patterns described by Hobart et al. (1978), five were also confirmed on fresh sera, three were not able to be traced, and one was not confirmed on fresh serum. This single non-confirmation might be explained by the presence of artifactual banding. Thus, it appears that there are genuine $C7^3$ alleles which code for proteins having somewhat weaker activity than those of the $C7^1$ or $C7^2$ alleles; as well there is an age artifact band resembling $C7^3$ whose intensity may vary from very weak to fully developed.

The nature of this artifact is unclear and it is not known whether factors other than storage and age may cause changes in the $C7^1$ protein such that its migration mimics that of $C7^3$. As was seen in the Chinese sera examined, there is a possibility of further aging, resulting in other artifactual patterns which are still detectable by the hemolytic method. Therefore, it would seem that sera being examined for C7 polymorphism should not have been stored for prolonged periods and all $C7^3$ allele patterns observed should ideally be confirmed on fresh sera.

The fact that the $C7^{2,1}$ patterns described in this thesis were found in Asian persons raised suspicions that the $C7^{2}$ allele might occur with a greater frequency in Asian populations than in Caucasian populations. Although the Chinese sera from mainland China revealed some banding thought due to aging, a distinct band in the $C7^{2}$ position in approximately 40% of the individuals allotyped strongly suggests that the $C7^{2}$ allele frequency is indeed increased. Confirmation on fresh samples from a Chinese community is required, (and is currently underway), but it would appear a $C7^{2}$ allele of Chinese origin may have reached balanced polymorphism due to some selective pressure in the Asian environment.

The major difficulty in confirming linkage of the C6 and C7 loci lies in the fact that in Caucasian populations variation in C7 is rare. If these preliminary observations in Chinese are confirmed, then study of Chinese families could quickly add a great deal more data for measuring recombination between C6 and C7.

The identification and availability of the Labrador family described in this thesis as having a genuine $C7^{3}$ allele may also provide information for C6/C7 linkage studies and is to be pursued in the next few months.

In conclusion, the results on C6 and C7 compiled for this thesis not only represent the first Canadian data, but also provide the basic information for future efforts in this laboratory to map the C6 locus by classical linkage methods. In addition, the results on Chinese sera and on a Labrador family offer two avenues by which the linkage of the C6 and C7 loci may be confirmed and the distance between them estimated by recombination. Finally, the identification of artifactual banding

tifactual banding of C7 may clarify confusing results that might be obtained by future investigators.

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APPENDIX

West Coast community results, pages 99-120.

Newfoundland families plus random individuals results, pages 121-144.

Labrador myotonic dystrophy family results, pages 145-147.

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
1001	AB	1039	AA
1004	AB	1040	AA
1006	AA	1041	AB
1008	AA	1042	BB
1009	AB	1043	AB
1010	AB	1044	BB
1011	AA	1045	AB
1012	AB	1046	AA
1013	AA	1047	AB
1014	AA	1049	AA
1015	AA	1051	AB
1016	AB	1052	AA
1018	AA	1053	AA
1020	AA	1054	AB
1021	AA	1055	AB
1022	AA	1056	BB
1023	AA	1057	AB
1029	AA	1058	AA
1030	AA	1059	AA
1033	AB	1060	AB
1034	AB	1062	AB
1035	AB	1063	AA
1036	AB	1064	AA
1037	AB	1065	AB
1038	AA	1066	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
1067	AB	1099	AB
1068	AB	1100	AA
1069	AB	1101	AB
1070	AB	1102	AA
1071	AA	1103	AB
1072	AB	1105	AB
1077	AA	1106	AB
1078	AA	1112	AB
1079	AA	1116	AB
1080	AA	1117	AB
1081	AA	1118	AA
1082	AB	1119	AB
1083	AA	1123	BB
1084	AA	1124	AB
1085	AA	1125	BB
1086	AB	1126	AA
1087	AB	1127	BB
1088	AB	1129	BB
1092	AB	1130	AB
1093	AB	1131	AB
1094	AB	1132	AB
1095	AB	1133	AA
1096	AB	1134	AB
1097	BB	1135	BB
1098	AB	1136	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
1137	AA	1166	AA
1138	AA	1167	AB
1139	AB	1168	AB
1141	AB	1169	AA
1142	AB	1170	AA
1143	BB	1171	AB
1144	AA	1172	AA
1145	AB	1175	AB
1146	AA	1177	AA
1147	AA	1178	AA
1148	AA	1179	AB
1149	AB	1180	AB
1150	AA	1183	AB
1151	AA	1185	AA
1152	AB	1186	AB
1153	AB	1187	AB
1155	AA	1188	AB
1157	BB	1191	BB
1159	AA	1199	AB
1160	AB	1201	AB
1161	AA	1202	AA
1162	AA	1204	AA
1163	AA	1205	AB
1164	BB	1206	AB
1165	AB	1207	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
1208	AA	1247	AB
1209	AA	1248	AB
1210	AA	1250	AA
1212	AA	1251	BB
1213	BB	1253	BB
1217	AA	1254	AA
1218	AA	1255	AA
1220	AA	1256	AA
1221	AB	1257	AA
1222	AA	1258	AA
1223	AB	1259	AA
1225	AA	1343	AB
1226	AB	1344	AA
1229	AA	1345	AA
1230	AA	1346	AA
1233	AB	1347	AA
1235	AA	1348	AA
1237	AB	1349	AA
1238	AA	1350	AA
1239	AA	1351	AA
1240	AA	1503	AB
1241	AA	2001	AB
1242	AA	2002	AA
1243	AA	2003	AA
1245	AB	2004	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
2005	AB	2036	AA
2006	BB	2037	AA
2007	BB	2039	AA
2008	AB	2040	AB
2009	AA	2041	AB
2010	AB	2044	AB
2012	AA	2045	AB
2013	AA	2046	AB
2014	BB	2047	AB
2015	BB	2048	AB
2016	AB	2049	AB
2017	AB	2053	AA
2018	AB	2054	AB
2019	AA	2055	AB
2020	AB	2058	AA
2021	AB	2060	AA
2022	AB	2061	AA
2025	AB	2063	AA
2029	AB	2064	BB
2030	AB	2066	AA
2031	AA	2067	AA
2032	AB	2068	AA
2033	AA	2069	AB
2034	AA	2070	AB
2035	AB	2071	A1B

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
2072	BB	2102	AB
2073	BB	2103	AB
2074	BB	2104	AB
2075	AB	2105	AB
2076	AA	2107	AA
2077	AA	2108	AB
2078	AA	2109	AB
2080	AA	2111	AA
2081	AB	2112	AB
2082	AA	2114	AA
2084	AA	2115	AA
2085	AA	2194	AA
2086	AA	2196	AA
2087	AB	2197	AA
2088	AA	2199	AB
2089	AA	3001	AB
2090	AA	3004	AA
2091	AB	3006	AB
2092	AB	3007	AA
2093	AB	3009	AA
2094	AA	3010	AB
2096	AA	3011	AB
2097	AB	3012	AB
2100	AB	3013	AB
2101	AB	3015	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3016	AA	3054	AB
3017	BB	3055	BB
3018	AB	3056	BB
3019	BB	3057	AB
3020	AB	3058	AB
3021	AB	3060	AB
3023	AA	3061	AB
3026	AA	3063	AB
3027	AA	3064	BB
3029	AA	3065	AB
3031	AA	3066	AB
3032	AA	3067	AA
3034	AA	3068	AB
3035	AA	3069	AB
3038	AB	3072	AB
3039	AB	3073	AB
3040	AB	3074	AA
3042	AB	3075	BB
3043	AB	3076	BB
3045	AA	3078	AB
3046	AB	3080	AB
3047	AB	3081	AB
3048	AB	3082	AB
3049	AA	3083	AB
3050	AB	3084	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3086	AB	3127	BB
3088	AA	3128	AB
3090	AA	3129	AA
3091	AB	3130	AA
3092	AB	3133	BB
3093	BB	3135	AA
3095	AB	3143	AB
3096	AB	3146	AB
3099	BB	3148	BB
3101	BB	3149	AB
3102	AB	3150	AA
3103	AB	3151	AB
3104	AB	3152	BB
3105	AB	3153	BB
3106	BB	3155	AB
3112	AB	3157	AB
3114	AA	3158	AB
3115	BB	3159	BB
3116	AB	3162	AA
3117	BB	3163	AA
3120	AA	3164	AB
3122	AB	3165	AA
3124	AB	3166	AB
3125	AB	3169	AB
3126	AB	3170	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3171	AA	3208	AB
3172	AA	3209	BB
3173	AB	3210	AB
3175	AB	3212	AB
3176	AB	3213	AB
3177	BB	3214	AB
3178	BB	3215	AA
3179	AB	3219	AA
3180	AB	3220	BB
3181	AA	3221	BB
3183	AB	3222	AA
3186	AB	3223	AA
3188	AA	3224	AB
3189	AA	3226	AA
3190	AB	3227	AA
3191	AB	3228	AB
3194	AA	3229	AA
3195	AA	3230	AA
3197	AA	3231	AB
3199	AA	3232	AB
3202	AA	3233	BB
3203	AB	3234	AB
3204	AA	3235	AB
3205	AB	3236	AB
3206	AB	3237	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3238	AB	3275	AB
3244	AB	3276	AA
3246	AA	3279	AB
3247	AA	3280	BB
3250	AB	3281	AB
3251	AA	3286	AB
3252	BB	3287	AA
3255	AB	3288	AA
3256	BB	3289	AB
3257	AA	3294	AB
3258	BB	3296	AB
3259	BB	3297	AB
3260	AB	3298	AA
3261	BB	3300	AA
3262	AB	3301	AB
3263	AB	3302	BB
3265	AB	3304	AB
3266	AB	3306	BB
3267	AB	3308	AA
3269	BB	3310	AB
3270	BB	3312	BB
3271	AA	3316	AB
3272	AB	3317	AB
3273	AB	3319	AA
3274	AB	3321	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3322	AA	3365	AB
3325	AA	3367	AA
3327	AA	3369	AB
3329	AA	3370	AB
3330	AA	3371	AB
3332	AB	3372	AB
3335	AA	3373	AB
3336	BB	3374	AA
3337	BB	3375	BB
3340	AB	3377	BB
3342	AB	3378	BB
3344	AB	3379	BB
3347	AB	3384	AA
3348	AB	3385	AA
3349	AA	3386	AB
3352	AA	3387	AA
3354	AA	3388	AB
3355	AA	3389	BB
3356	AA	3390	AA
3359	AA	3392	AA
3360	AA	3395	AB
3361	AA	3396	AA
3362	AB	3397	AB
3363	AB	3398	AA
3364	AB	3399	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3400	AA	3436	BB
3401	AA	3437	AA
3402	AB	3438	AB
3403	BB	3439	AB
3404	AB	3441	BB
3405	AB	3442	BB
3406	AA	3443	BB
3407	AA	3444	AB
3409	BB	3445	AA
3410	AB	3446	AB
3412	AB	3448	AB
3414	AB	3449	AA
3416	BB	3451	AB
3417	AB	3452	AA
3418	AB	3453	AA
3420	BB	3454	AB
3421	AA	3456	AB
3425	AB	3457	AB
3427	BB	3458	AB
3429	AA	3460	AB
3430	AB	3461	AA
3431	AB	3462	AA
3433	BB	3463	AB
3434	BB	3464	AB
3435	BB	3465	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3466	AA	3498	AA
3468	AB	3499	AB
3469	AB	3500	AB
3471	AA	3501	AA
3472	AB	3505	AB
3473	AA	3506	AA
3475	AA	3509	AB
3476	AA	3510	AB
3477	AA	3511	AB
3478	AA	3512	AA
3479	AB	3514	AA
3480	AB	3516	AB
3481	AB	3517	AB
3482	BB	3518	AB
3483	AB	3519	AB
3485	BB	3520	AB
3486	AB	3522	AB
3487	AA	3527	AA
3489	AB	3528	AB
3490	AB	3529	AB
3491	AB	3530	AB
3492	AA	3531	AB
3495	AB	3532	AB
3496	AB	3533	AA
3497	AA	3534	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3535	AB	3567	AB
3536	AB	3568	AA
3537	AB	3569	AB
3538	AB	3570	AA
3539	BB	3571	AA
3540	AB	3572	AB
3543	AA	3573	AB
3545	AA	3575	BB
3546	AA	3577	AB
3547	AA	3580	AB
3548	AB	3581	AB
3550	BB	3582	AA
3553	AB	3583	AA
3554	AB	3584	AB
3555	AA	3585	AA
3556	AA	3587	AA
3557	AA	3588	AB
3559	BB	3589	AB
3560	AB	3590	AA
3561	AA	3591	AB
3562	AA	3592	AA
3563	AA	3593	AB
3564	AB	3594	AB
3565	AB	3595	AB
3566	AB	3596	AA

West Coast Community Study Results

Number	C6 Genotype
3597	AB
3598	AA
3599	AA
3601	AB
3605	AA
3606	AA
3607	AA
3609	AA
3612	AB
3615	BB
3617	AB
3618	AA
3620	AA
3632	AB
3634	AB
3637	AB
3638	AA
3640	AA
3641	AB
3645	AB
3646	AA
3647	AA
3648	BB
3649	AA
3658	AA

Number	C6 Genotype
3659	AA
3663	AB
3664	AB
3667	AA
3668	AA
3669	AB
3671	AA
3675	AB
3676	AA
3679	AA
3680	AB
3684	AA
3686	AA
3687	AA
3689	AB
3690	AA
3691	AB
3692	AB
3693	AB
3694	AA
3695	AA
3697	AA
3698	AB
3699	AB
3700	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3701	AB	3738	AB
3703	AB	3739	AA
3704	AA	3742	AA
3708	AB	3743	AB
3709	AB	3744	AA
3710	AB	3745	AA
3711	AA	3751	BB
3712	AB	3752	BB
3717	BB	3753	AB
3718	AB	3756	AA
3719	BB	3758	AB
3721	AB	3759	AA
3722	AB	3761	AA
3723	AB	3766	AA
3724	AB	3768	AB
3725	AA	3769	AA
3726	AB	3770	AB
3728	AB	3772	BB
3729	AA	3774	AB
3730	AB	3775	AB
3731	AB	3777	AB
3732	AA	3779	AB
3734	AB	3780	AB
3736	AA	3781	AB
3737	AB	3783	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3786	BB	3822	AA
3789	AB	3825	AA
3790	BB	3826	AA
3791	AB1	3827	AA
3792	BB	3828	AB
3793	AB	3829	AB
3795	AB	3830	AA
3796	AA	3832	AB
3799	AB	3833	AB
3801	AA	3834	AB
3802	AB	3835	AA
3804	AB	3836	AB
3805	AB	3837	AB
3806	AB	3838	AA
3807	AB	3840	AB
3808	AA	3841	BB
3809	AB	3842	BB
3812	BB	3843	AB
3813	BB	3844	AB
3816	AB	3847	AA
3817	AB	3848	AA
3818	AB	3849	AA
3819	AB	3850	AB
3820	AA	3851	AB
3821	AA	3853	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3854	AB	3901	AB
3856	BB	3902	AA
3858	AA	3904	AB
3859	AA	3906	AB
3860	AA	3907	AB
3861	AA	3908	AB
3862	AA	3909	AB
3863	AB	3911	BB
3865	AA	3912	AB
3868	AB	3913	AB
3869	AB	3915	AA
3871	AB	3916	AB
3874	AA	3918	AA
3875	BB	3920	AA
3876	BB	3922	AB
3878	AA	3923	AB
3882	AB	3924	AB
3883	AB	3927	AA
3888	AA	3928	AA
3889	AA	3931	AB
3890	AA	3932	AA
3891	AA	3933	AB
3892	AA	3934	AA
3895	AA	3935	AA
3900	AA	3937	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
3938	AA	3987	AB
3940	AA	3988	AB
3943	AB	3990	AB
3944	BB	3991	AB
3945	AB	3992	AA
3946	BB	3993	AB
3947	BB	3994	AB
3950	AA	3996	AB
3959	AB	3997	AA
3960	AB	3998	AA
3961	AB	3999	AA
3965	AB	4000	AA
3967	AA	4001	AB
3969	AA	4002	BB
3970	AA	4003	AA
3971	AB	4079	BB
3973	AB	4097	AB
3974	AA	4135	BB
3976	AA	4141	BB
3977	BB	4142	AB
3979	AB	4200	AA
3982	AA	4240	AA
3983	AA	4311	AB
3985	AB	4440	BB
3986	AB	4454	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
4493	AB	5157	AB
4495	AB	5167	AB
5004	BB	5170	AA
5005	BB	5177	AA
5506	AA	5182	AA
5007	AB	5187	AA
5009	AB	5189	AB
5015	AB	5190	AB
5017	AA	5191	AA
5028	AA	5195	AA
5029	BB	5198	AA
5032	AA	5199	AA
5034	AB	5201	AA
5037	AB	5202	AA
5039	AA	5204	AA
5053	AB	5208	AA
5063	AB	5212	AA
5097	AB	5230	AB
5108	BB	5239	AA
5112	AA	5246	BB
5139	AA	5250	AB
5140	AB	5257	AB
5142	AB	5275	AA
5148	AB	5280	AA
5149	AA	5291	AA

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
5304	AB	5523	AA
5305	AB	5524	AA
5309	BB	5525	AB
5320	AB	5526	AB
5330	AB	6022	AB
5405	AB	6212	AA
5413	AA	6302	BB
5415	AA	6305	AB
5419	AA	6306	AB
5438	AB	6308	BB
5439	AA	6309	AB
5450	AB	6500	AA
5452	AA	6527	AB
5457	AA	6563	AB
5468	AA	6586	AB
5469	AB	6587	AA
5473	AA	6639	BB
5515	BB	6640	BB
5516	AA	6641	AB
5517	BB	6649	BB
5518	AB	6651	AB
5519	AB	6652	AA
5520	AB	6732	AA
5521	AB	6733	AA
5522	AB	6836	AB

West Coast Community Study Results

Number	C6 Genotype	Number	C6 Genotype
7154	AA		
7156	AB		
7219	AB		
7220	AA		
7225	AB		
7244	AB		
7318	AB		
7319	AB		
8024	BB		
8026	BB		
8038	AA		
8212	AA		
9001	AB		
9002	BB		
9003	AB		
9004	AA		
9005	AB		
9006	AA		
9007	BB		
9008	AB		
9009	AB		
9010	BB		
9011	BB		
9996	AB		

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
BA 1001	BB	BA 1133	AA
BA 1004	AA	BA 1135	AA
BA 1005	AB	BA 1138	BB
BA 1019	BB	BA 1139	AB
BA 1021	BB	BA 1141	AB
BA 1025	AA	BA 1142	AA
BA 1030	BB	BA 1154	AA
BA 1037	AB	BA 1162	AA
BA 1040	AA	BA 1165	AB
BA 1043	AA	BA 1167	A1A
BA 1047	AA	BA 1177	AA
BA 1050	AA	BA 1180	AA
BA 1054	AA	BA 1181	AA
BA 1070	AA	BA 1190	AA
BA 1091	BB	BA 1191	AA
BA 1096	AA	BA 1197	AA
BA 1098	AB	BA 1199	AB
BA 1100	AB	BA 1200	AA
BA 1104	AB	BA 1207	BB
BA 1105	AB	BA 1214	AA
BA 1108	AB	BA 1224	AA
BA 1110	AB	BA 1239	AB
BA 1121	AA	BA 1240	AA
BA 1126	AA	BA 1262	AA
BA 1128	AB	BA 1268	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
BA 1269	AB	BA 1415	AB
BA 1302	AA	BA 1416	AA
BA 1304	AA	BA 1417	BB
BA 1307	AB	BA 1418	AB
BA 1308	AB	BA 1420	AB
BA 1309	AB	BA 1421	AA
BA 1327	AB	BA 1422	AB
BA 1335	BB	BA 1423	AB
BA 1336	AB	BA 1424	AA
BA 1338	AB	BA 1425	AA
BA 1358	AA	BA 1426	AA
BA 1362	AB	BA 1427	AB
BA 1366	AB	BA 1428	BB
BA 1370	AA	BA 1429	AB
BA 1373	AA	BA 1430	AB
BA 1387	AB	BA 1431	AB
BA 1389	AB	BA 1432	AB
BA 1390	AA	BA 1433	AA
BA 1392	AB	BA 1434	AA
BA 1393	AA	BA 1435	AA
BA 1396	AB	BA 1437	BB
BA 1408	AB	BA 1439	AA
BA 1410	A1A	BA 1440	AA
BA 1411	AB	BA 1441	AA
BA 1414	AA	BA 1442	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
BA 1443	AA	BB 1140	AA
BA 1444	AB	BB 1142	BB
BA 1445	AA	BB 1163	AB
BA 1446	AA	BB 1190	AB
BA 1447	AB	BB 1194	AA
BA 1448	BB	BB 1200	AA
BA 1449	AB	BB 1207	AB
BA 1450	AB	BB 1231	AA
BA 1451	BB	BB 1235	BB
BA 1452	AB	BB 1271	AA
BA 1453	AB	BB 1273	AA
BA 1454	AA	BB 1321	AA
BA 1455	AA	BC 1018	AA
BA 1456	AB	BC 1045	AB
BA 1457	AA	BC 1050	AB
BA 1458	AA	BC 1051	AB
BA 1459	AA	BC 1122	AB
BB 1023	AB	BC 1163	AA
BB 1045	AA	BC 1185	AB
BB 1086	BB	BC 1186	AB
BB 1097	BB	BC 1187	AA
BB 1115	AA	BC 1189	AB
BB 1116	AA	BC 1190	AB
BB 1135	AA	BC 1191	AB
BB 1138	AB	BC 1209	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
BC 1210	AB	FS 1000	AA
BC 1211	AA	FS 1001	BB
BC 1212	AB	FS 1002	AB
BD 1003	AB	FS 1003	AB
BD 1040	AA	FS 1004	AB
BD 1056	AA	FS 1006	AB
BD 1061	AA	FS 1007	AB
BD 1062	AA	FS 1008	AA
BD 1063	AA	FS 1012	BB
BD 1078	AB	FS 1014	BB
BD 1080	BB	FS 1019	AB
BD 1126	AA	FS 1020	AB
BD 1144	AA	FS 1025	AB
BD 1175	AB	FS 1026	AA
BD 1229	AB	FS 1029	BB
BD 1230	AA	FS 1032	AA
BD 1231	AB	FS 1033	AA
BD 1232	AA	FS 1034	AB
BD 1233	AB	FS 1035	AA
BE 1002	AB	FS 1036	AA
BE 1009	AB	FS 1037	AA
BE 1032	AA	FS 1038	AB
BE 1096	AA	FS 1039	AB
BE 1114	AB	FS 1040	AB
BE 1115	AA	FS 1041	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1042	AB	FS 1102	AA
FS 1049	BB	FS 1109	AA
FS 1050	AB	FS 1113	AB
FS 1051	BB	FS 1114	AB
FS 1052	AB	FS 1116	A1A
FS 1053	AB	FS 1117	AB
FS 1054	AB	FS 1126	AA
FS 1056	AA	FS 1129	BB
FS 1057	AB	FS 1130	BB
FS 1059	AA	FS 1131	AB
FS 1060	AA	FS 1132	AA
FS 1061	AA	FS 1136	AA
FS 1062	AA	FS 1137	AB
FS 1065	AA	FS 1138	AA
FS 1066	BB	FS 1139	AB
FS 1069	AA	FS 1140	AA
FS 1070	AA	FS 1142	AA
FS 1073	AA	FS 1144	AA
FS 1074	AB	FS 1156	AB
FS 1081	AA	FS 1160	AA
FS 1083	AB	FS 1171	AB
FS 1084	BB	FS 1176	AA
FS 1085	AB	FS 1186	AA
FS 1096	AA	FS 1188	AB
FS 1098	AB	FS 1193	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1096	AA	FS 1278	AA
FS 1197	AB	FS 1281	AA
FS 1206	AB	FS 1287	AA
FS 1207	AB	FS 1295	AB
FS 1229	AB	FS 1300	AA
FS 1238	AB	FS 1302	AA
FS 1239	AA	FS 1303	AA
FS 1242	AA	FS 1304	AB
FS 1243	AA	FS 1305	AB
FS 1244	AB	FS 1306	AB
FS 1245	AB	FS 1313	BB
FS 1250	AA	FS 1315	AA
FS 1251	AA	FS 1316	AA
FS 1256	AB	FS 1317	AA
FS 1257	AB	FS 1319	AB
FS 1258	AA	FS 1320	AB
FS 1259	AB	FS 1321	AB
FS 1266	AA	FS 1322	AA
FS 1267	AB	FS 1323	AB
FS 1270	AA	FS 1324	AB
FS 1271	AA	FS 1325	BB
FS 1272	AA	FS 1326	AA
FS 1273	AA	FS 1327	AA
FS 1274	AA	FS 1328	AA
FS 1277	AA	FS 1331	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1333	BB	FS 1426	AA
FS 1341	AA	FS 1427	AB
FS 1346	AA	FS 1428	AA
FS 1347	AA	FS 1429	AB
FS 1348	AB	FS 1430	AB
FS 1349	AA	FS 1440	AA
FS 1350	AA	FS 1441	AA
FS 1351	AA	FS 1442	AA
FS 1353	AB	FS 1443	AB
FS 1355	AB	FS 1444	BB
FS 1366	AA	FS 1445	AB
FS 1367	AA	FS 1447	AB
FS 1368	AA	FS 1448	AA
FS 1369	AA	FS 1451	AB
FS 1370	AA	FS 1452	AA
FS 1371	AB	FS 1453	AA
FS 1379	AB	FS 1454	AA
FS 1386	BB	FS 1455	AB
FS 1406	BB	FS 1456	AB
FS 1407	AB	FS 1457	AA
FS 1414	AB	FS 1458	AA
FS 1415	AB	FS 1459	AA
FS 1416	AA	FS 1460	AB
FS 1417	AA	FS 1461	AA
FS 1418	AA	FS 1462	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1463	AA	FS 1497	AA
FS 1464	AB	FS 1498	AA
FS 1465	AA	FS 1504	AA
FS 1466	AB	FS 1506	AA
FS 1467	AA	FS 1508	AB
FS 1470	AB	FS 1509	AA
FS 1471	AA	FS 1514	AA
FS 1472	BB	FS 1515	AA
FS 1473	AB	FS 1516	AB
FS 1474	AA	FS 1519	AA
FS 1476	AA	FS 1520	AB
FS 1477	AA	FS 1524	AB
FS 1479	AA	FS 1532	AA
FS 1480	AA	FS 1542	AA
FS 1481	AA	FS 1543	AB
FS 1482	AA	FS 1545	AA
FS 1484	AB	FS 1546	AA
FS 1485	AA	FS 1547	AB
FS 1488	AA	FS 1548	AB
FS 1489	AA	FS 1549	AB
FS 1490	AA	FS 1550	AB
FS 1491	AA	FS 1551	AB
FS 1492	AA	FS 1552	AA
FS 1495	BB	FS 1553	AA
FS 1496	AA	FS 1554	BB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1555	AA	FS 1588	AB
FS 1556	AB	FS 1589	AA
FS 1557	AA	FS 1590	AA
FS 1558	AB	FS 1591	AA
FS 1559	AB	FS 1592	AA
FS 1560	BB	FS 1595	AB
FS 1561	AA	FS 1596	BB
FS 1562	AA	FS 1597	BB
FS 1563	AA	FS 1599	BB
FS 1564	BB	FS 1603	BB
FS 1565	AA	FS 1606	AA
FS 1566	AA	FS 1607	BB
FS 1567	AA	FS 1608	BB
FS 1568	AB	FS 1610	AA
FS 1569	BB	FS 1612	AA
FS 1570	BB	FS 1613	BB
FS 1572	AB	FS 1614	AB
FS 1573	AB	FS 1615	AA
FS 1574	AB	FS 1616	AA
FS 1575	AB	FS 1617	AA
FS 1579	AA	FS 1618	AB
FS 1580	AB	FS 1620	AA
FS 1581	BB	FS 1622	AA
FS 1582	AA	FS 1623	AA
FS 1583	AA	FS 1626	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1627	AA	FS 1659	AA
FS 1628	AA	FS 1660	AB
FS 1629	AA	FS 1661	AB
FS 1630	AA	FS 1662	AA
FS 1631	AB	FS 1663	AB
FS 1632	AB	FS 1664	BB
FS 1635	AA	FS 1665	AA
FS 1636	AA	FS 1666	AB
FS 1637	AA	FS 1667	AB
FS 1638	AA	FS 1669	AB
FS 1639	BB	FS 1670	AB
FS 1640	AB	FS 1673	AA
FS 1642	AA	FS 1674	BB
FS 1643	AB	FS 1676	BB
FS 1644	AA	FS 1681	AA
FS 1645	BB	FS 1686	AB
FS 1646	BB	FS 1689	AA
FS 1647	AA	FS 1690	AA
FS 1651	AA	FS 1691	AA
FS 1652	AB	FS 1692	AA
FS 1653	AB	FS 1693	AA
FS 1654	AA	FS 1694	AA
FS 1655	AB	FS 1695	AA
FS 1656	AA	FS 1696	AA
FS 1657	AA	FS 1697	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1698	AA	FS 1766	AB
FS 1699	BB	FS 1767	AA
FS 1700	AA	FS 1772	AA
FS 1704	BB	FS 1776	AA
FS 1705	AB	FS 1779	AA
FS 1706	BB	FS 1780	AA
FS 1719	AA	FS 1781	AB
FS 1720	BB	FS 1782	AB
FS 1723	AA	FS 1784	AA
FS 1727	AA	FS 1786	AA
FS 1728	AA	FS 1787	AA
FS 1733	AA	FS 1788	AA
FS 1734	AB	FS 1792	AB
FS 1739	AA	FS 1793	AB
FS 1742	AA	FS 1794	AB
FS 1745	AB	FS 1795	AA
FS 1746	AB	FS 1796	AA
FS 1748	AA	FS 1797	AB
FS 1749	BB	FS 1798	AA
FS 1758	AB	FS 1799	AA
FS 1761	AB	FS 1800	AA
FS 1762	AA	FS 1801	AA
FS 1763	AB	FS 1802	AA
FS 1764	AA	FS 1806	AB
FS 1765	AB	FS 1807	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1808	AB	FS 1842	AA
FS 1809	AB	FS 1843	AA
FS 1811	AB	FS 1844	AA
FS 1812	AB	FS 1845	AA
FS 1814	AA	FS 1846	AB
FS 1815	AA	FS 1847	AB
FS 1816	AA	FS 1848	AB
FS 1817	AA	FS 1849	BB
FS 1819	AB	FS 1850	AB
FS 1820	AA	FS 1851	AB
FS 1821	AB	FS 1858	AA
FS 1822	BB	FS 1860	AA
FS 1823	AB	FS 1863	BB
FS 1824	AA	FS 1864	AB
FS 1825	AB	FS 1865	AB
FS 1826	AB	FS 1866	AB
FS 1827	BB	FS 1871	BB
FS 1828	BB	FS 1872	BB
FS 1829	AB	FS 1874	AA
FS 1830	BB	FS 1881	AA
FS 1834	AB	FS 1882	AA
FS 1835	AB	FS 1885	BB
FS 1836	AB	FS 1886	AB
FS 1837	AB	FS 1887	AB
FS 1839	AA	FS 1889	BB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1891	AB	FS 1966	AA
FS 1892	AB	FS 1967	AA
FS 1893	AA	FS 1968	AA
FS 1894	AA	FS 1969	AA
FS 1897	AB	FS 1970	AA
FS 1899	BB	FS 1971	AA
FS 1911	AB	FS 1973	AB
FS 1915	AA	FS 1974	AB
FS 1916	AB	FS 1975	BB
FS 1917	AB	FS 1975	BB
FS 1923	AA	FS 1976	BB
FS 1929	AA	FS 1981	AA
FS 1930	AB	FS 1982	AB
FS 1931	AB	FS 1983	AB
FS 1932	AB	FS 1984	AA
FS 1933	BB	FS 1985	AA
FS 1934	AB	FS 1986	BB
FS 1935	AB	FS 1988	AB
FS 1936	BB	FS 1989	AA
FS 1941	AA	FS 1992	AA
FS 1942	AA	FS 1993	AA
FS 1952	AB	FS 1995	AA
FS 1953	AB	FS 1996	AB
FS 1954	AB	FS 1997	AA
FS 1955	AB	FS 1998	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 1999	AA	FS 2050	AA
FS 2000	AB	FS 2051	AA
FS 2003	AA	FS 2052	AA
FS 2004	BB	FS 2053	AB
FS 2005	AB	FS 2054	AA
FS 2011	AA	FS 2055	AA
FS 2012	AB	FS 2056	AA
FS 2013	BB	FS 2057	AA
FS 2014	AA	FS 2061	AA
FS 2015	AA	FS 2062	AA
FS 2017	AB	FS 2063	AA
FS 2018	AA	FS 2064	AA
FS 2019	AB	FS 2065	BB
FS 2020	AB	FS 2066	AB
FS 2029	AA	FS 2067	BB
FS 2030	AB	FS 2069	AB
FS 2033	AA	FS 2072	AB
FS 2035	AA	FS 2073	AA
FS 2036	AA	FS 2074	AA
FS 2039	AB	FS 2075	AB
FS 2040	AA	FS 2076	AB
FS 2041	AA	FS 2077	AB
FS 2042	AB	FS 2078	AB
FS 2044	BB	FS 2081	BB
FS 2048	AA	FS 2088	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2089	AA	FS 2132	BB
FS 2090	AB1	FS 2133	BB
FS 2091	AA	FS 3134	BB
FS 2092	AA	FS 3135	BB
FS 2098	AA	FS 2136	BB
FS 2099	AB	FS 2137	BB
FS 2100	AA	FS 2139	AB
FS 2102	AA	FS 2140	AB
FS 2106	BB	FS 2141	AB
FS 2107	AB	FS 2156	AB
FS 2108	BB	FS 2157	AB
FS 2110	AB	FS 2158	AB
FS 2111	AB	FS 2159	AA
FS 2114	AA	FS 2160	AA
FS 2115	AB	FS 2161	BB
FS 2116	AA	FS 2162	AA
FS 2118	AA	FS 2163	AA
FS 2119	BB	FS 2168	AA
FS 2120	AA	FS 2169	AA
FS 2121	AA	FS 2170	AB
FS 2122	AA	FS 2171	AA
FS 2123	A1B	FS 2172	AA
FS 2125	AA	FS 2173	AB
FS 2126	AA	FS 2174	AB
FS 2131	BB	FS 2175	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2176	AA	FS 2238	AB
FS 2179	BB	FS 2239	AA
FS 2183	AA	FS 2240	AA
FS 2189	BB	FS 2243	AA
FS 2190	AA	FS 2244	AA
FS 2191	AA	FS 2245	AB
FS 2192	AB	FS 2246	AB
FS 2193	AA	FS 2247	A1A
FS 2194	AA	FS 2248	AA
FS 2195	BB	FS 2249	AB
FS 2206	BB	FS 2250	AA
FS 2207	AA	FS 2251	A1A
FS 2208	AB1	FS 2252	AA
FS 2219	AB	FS 2254	AB
FS 2222	AA	FS 2256	AB
FS 2225	AB	FS 2257	AB
FS 2226	AB	FS 2258	AA
FS 2227	BB	FS 2259	AB
FS 2228	AA	FS 2260	AA
FS 2232	AA	FS 2261	AA
FS 2233	BB	FS 2262	AA
FS 2234	AA	FS 2266	AA
FS 2235	BB	FS 2272	AA
FS 2236	AA	FS 2277	AA
FS 2237	AA	FS 2285	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2286	AA	FS 2319	AB
FS 2287	AB	FS 2320	AA
FS 2288	A1A	FS 2321	AA
FS 2289	A1A	FS 2322	AA
FS 2295	AA	FS 2325	AB
FS 2297	AB	FS 2327	AA
FS 2298	AA	FS 2335	AA
FS 2299	AA	FS 2336	AA
FS 2300	AA	FS 2337	AB
FS 2301	AB	FS 2338	AA
FS 2302	AA	FS 2339	AA
FS 2304	AB	FS 2340	AB
FS 2305	AA	FS 2348	AB
FS 2306	AA	FS 2350	AA
FS 2307	AA	FS 2353	AA
FS 2308	AA	FS 2357	AA
FS 2309	AA	FS 2359	AA
FS 2310	AA	FS 2360	AA
FS 2311	AB	FS 2361	AA
FS 2312	A1A	FS 2362	AA
FS 2313	AA	FS 2364	AA
FS 2314	AA	FS 2365	AA
FS 2316	AA	FS 2366	AA
FS 2317	AB	FS 2368	AB
FS 2318	AA	FS 2369	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2370	AA	FS 2402	AB
FS 2371	AA	FS 2403	AA
FS 2372	AA	FS 2404	AA
FS 2373	AA	FS 2405	AB
FS 2374	AA	FS 2406	AA
FS 2375	AA	FS 2407	AA
FS 2376	AA	FS 2408	AA
FS 2377	AA	FS 2409	BB
FS 2379	AA	FS 2410	BB
FS 2384	AA	FS 2411	BB
FS 2385	AB	FS 2414	BB
FS 2386	AA	FS 2415	AB
FS 2387	AB	FS 2417	BB
FS 2388	AB	FS 2418	AB
FS 2389	AA	FS 2419	BB
FS 2390	AB	FS 2420	BB
FS 2391	AB	FS 2421	AB
FS 2392	AA	FS 2422	AA
FS 2393	AA	FS 2423	AB
FS 2396	AA	FS 2424	BB
FS 2397	AB	FS 2425	AB
FS 2398	AB	FS 2427	AA
FS 2399	AA	FS 2428	AA
FS 2400	AB	FS 2431	AA
FS 2401	AA	FS 2432	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2433	AA	FS 2503	BB
FS 2435	AB	FS 2504	AA
FS 2436	AA	FS 2508	AA
FS 2441	AA	FS 2513	AA
FS 2443	AB	FS 2514	AA
FS 2445	AA	FS 2515	AA
FS 2446	AA	FS 2516	AA
FS 2447	AA	FS 2518	AA
FS 2448	BB	FS 2520	AB
FS 2449	AA	FS 2521	AB
FS 2450	BB	FS 2522	AB
FS 2455	BB	FS 2523	AB
FS 2456	BB	FS 2534	A1A
FS 2464	AB	FS 2535	AA
FS 2465	AA	FS 2536	A1A
FS 2476	AB	FS 2541	AB
FS 2477	AA	FS 2542	AB
FS 2478	BB	FS 2548	AB
FS 2486	BB	FS 2554	AB
FS 2487	AB	FS 2559	AB
FS 2495	AA	FS 2560	AB
FS 2499	BB	FS 2561	AB
FS 2500	BB	FS 2562	AA
FS 2501	AB	FS 2563	AB
FS 2502	AA	FS 2564	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2565	AB	FS 2622	AA
FS 2566	AA	FS 2623	AB
FS 2567	AA	FS 2624	BB
FS 2571	AA	FS 2625	AB
FS 2573	AB	FS 2628	AA
FS 2574	AA	FS 2632	AB
FS 2575	AA	FS 2633	BB
FS 2585	AB	FS 2634	AB
FS 2587	AA	FS 2635	BB
FS 2588	BB	FS 2639	AB
FS 2593	AB	FS 2640	BB
FS 2594	AA	FS 2641	AB
FS 2595	BB	FS 2642	AB
FS 2598	AA	FS 2643	BB
FS 2600	AB	FS 2644	AB
FS 2603	AA	FS 2646	AB
FS 2613	AB	FS 2647	AB
FS 2614	AB	FS 2648	BB
FS 2615	AA	FS 2649	AB
FS 2616	AA	FS 2650	AB
FS 2617	AA	FS 2651	AA
FS 2618	AA	FS 2664	AB
FS 2619	AA	FS 2665	AA
FS 2620	AA	FS 2666	AA
FS 2621	AA	FS 2667	AA

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2679	AA	FS 2727	AA
FS 2680	AB	FS 2732	AA
FS 2681	AB	FS 2748	AB
FS 2686	AA	FS 2749	AA
FS 2687	AA	FS 2750	AB1
FS 2691	AB	FS 2751	AA
FS 2692	AB	FS 2752	AB1
FS 2694	BB	FS 2753	AB1
FS 2698	AB	FS 2756	AA
FS 2704	AA	FS 2770	BB
FS 2705	AA	FS 2771	AB
FS 2706	AA	FS 2772	AA
FS 2707	AA	FS 2773	AB
FS 2708	AA	FS 2783	AA
FS 2709	AB	FS 2784	AA
FS 2710	AA	FS 2785	AA
FS 2711	AB	FS 2788	AB
FS 2712	AB	FS 2791	AA
FS 2713	AA	FS 2792	AA
FS 2715	AB	FS 2793	AA
FS 2716	AA	FS 2794	AA
FS 2717	AB	FS 2795	AA
FS 2718	AA	FS 2796	AA
FS 2723	AA	FS 2797	AB
FS 2724	AA	FS 2798	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2799	AA	FS 2842	AA
FS 2800	BB	FS 2843	AA
FS 2801	AA	FS 2844	AA
FS 2803	AA	FS 2848	BB
FS 2804	AA	FS 2855	AA
FS 2805	BB	FS 2856	AA
FS 2806	AA	FS 2857	AA
FS 2807	BB	FS 2859	AB
FS 2808	AB	FS 2860	AA
FS 2809	AA	FS 2861	AA
FS 2810	AB	FS 2862	AA
FS 2811	AA	FS 2863	AA
FS 2812	AB	FS 2864	AA
FS 2813	BB	FS 2865	AA
FS 2830	AA	FS 2866	AA
FS 2831	AA	FS 2867	AB
FS 2832	AB	FS 2868	AB
FS 2833	AB	FS 2869	AB
FS 2834	AB	FS 2870	AA
FS 2835	AA	FS 2872	AA
FS 2836	BB	FS 2873	AA
FS 2837	BB	FS 2874	AA
FS 2838	AB	FS 2875	AB
FS 2839	AB	FS 2876	AB
FS 2841	AB	FS 2877	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2878	AA	FS 2921	BB
FS 2879	AB	FS 2923	AA
FS 2880	AB	FS 2924	AA
FS 2881	AA	FS 2925	AA
FS 2882	AA	FS 2926	AA
FS 2883	AB	FS 2929	AB
FS 2884	AA	FS 2930	AB
FS 2889	AA	FS 2933	AA
FS 2890	BB	FS 2934	AA
FS 2891	AB	FS 2935	AA
FS 2892	AA	FS 2936	AB
FS 2893	AB	FS 2937	AA
FS 2894	AA	FS 2948	AA
FS 2895	AA	FS 2949	AB
FS 2897	AA	FS 2952	AA
FS 2902	AB	FS 2953	AA
FS 2903	AB	FS 2954	AB
FS 2904	AA	FS 2955	AB
FS 2905	AA	FS 2956	AB
FS 2906	AA	FS 2957	AB
FS 2907	BB	FS 2958	AA
FS 2911	BB	FS 2959	AA
FS 2912	AA	FS 2960	AA
FS 2913	BB	FS 2962	AA
FS 2920	AB	FS 2963	AB

Newfoundland Families Plus Random Individuals Results

Number	C6 Genotype	Number	C6 Genotype
FS 2964	AB	FS 3001	AB
FS 2965	AA	FS 3106	AA
FS 2966	AA		
FS 2967	AA		
FS 2968	AA		
FS 2969	AA		
FS 2970	AA		
FS 2971	AA		
FS 2972	AB		
FS 2973	AA		
FS 2974	AB		
FS 2975	AA		
FS 2976	BB		
FS 2978	AB		
FS 2984	AA		
FS 2986	AA		
FS 2987	AA		
FS 2988	AA		
FS 2989	AA		
FS 2992	AB		
FS 2993	AB		
FS 2994	AB		
FS 2997	AA		
FS 2998	AA		
FS 2999	AA		

Labrador Myotonic Dystrophy Family Results

Number	C6 Genotype	Number	C6 Genotype
L100	AB	L127	AA
L101	AB	L128	AA
L102	AB	L129	AA
L103	AB	L130	AA
L104	AB	L131	AA
L105	AB	L132	AA
L106	AB	L133	AA
L107	AB	L134	AA
L108	AB	L135	AA
L110	AA	L136	AA
L111	AA	L137	AA
L112	AB	L138	AA
L113	AA	L139	AA
L114	AB	L140	AA
L115	AB	L141	AA
L116	AB	L142	AA
L117	AA	L143	AA
L118	AA	L145	AA
L119	AA	L146	AA
L120	AA	L147	AB
L121	AA	L148	AA
L122	AA	L149	AA
L123	AB	L150	AA
L125	AA	L151	AB
L126	AA	L152	AB

Labrador Myotonic Dystrophy Family Results

Number	C6 Genotype	Number	C6 Genotype
L155	AB	L181	AA
L156	AA	L182	AA
L157	AB	L184	AA
L158	AA	L185	AB
L159	AB	L186	AA
L160	AB	L187	AA
L161	AB	L188	AA
L162	AA	L190	AA
L163	AA	L191	AA
L164	AA	L192	AA
L165	AB	L193	AA
L166	AA	L194	AA
L168	AA	L195	AB
L169	AA	L196	AB
L170	AA	L197	AA
L171	AA	L198	AA
L172	AA	L199	BB
L173	AA	L200	AB
L174	AA	L201	AB
L175	AA	L203	AA
L176	AA	L204	AA
L177	AA	L205	AB
L178	AA	L207	AB
L179	AA	L209	AB
L180	AA	L210	AB

Labrador Myotonic Dystrophy Family Results

Number	C6 Genotype	Number	C6 Genotype
L214	AA		
L215	AB		
L219	AA		
L223	AA		
L226	AA		
L227	AB		
L231	AA		
L241	AB		
L243	AB		
L246	AA