Proteins in Plasma and Urine

GENETIC POLYMORPHISMS OF COMPLEMENT COMPONENTS AND OTHER PLASMA PROTEINS

G. Mauff

Hygiene - Institut, Universität zu Köln, Federal Republic of Germany

More than thirty plasma proteins were described to be genetically polymorphic; only a limited number has been applied to forensic haemogenetics on the basis of genetic variability, known mode of inheritance and technological feasibility. The present review will therefore consider, with the exception of immunoglobulins, those plasma proteins which are well established or appear to be most informative. Special emphasis will be placed on new developments in the field of complement genetics.

Nine plasma proteins are commonly used in forensic haemogenetics and have gained attention for their subtypes or were introduced in recent years (Tabel 1). For some of them subtyping by isoelectric focusing or immobiline techniques has resulted in a considerable increase of information as indirectly expressed in the increment of their single exclusion chance for non-fathers (SENF) in paternity expertises, as calculated according to SPIELMANN & KÜHNL (53). This has especially been observed for haptoglobin (HP) (40,56), the vitamin D-binding protein (DBP, formerly GC) (28), transferrin (TF) (12) and alpha-1-antitrypsin (proteinase inhibitor, PI) (2). The regulatory proteins of the coagulation system plasminogen (PLG) (30,51), factor XIIIA (F13A) (53) and its carrier protein factor XIIIB (F13B) (33,53) were more recently applied to forensic haemogenetics. Prospective candidates for future applications are orosomucoid (ORM) (58,63), and alpha-2-HS glycoprotein (A2HS) (55) polymorphisms. Silent alleles were discovered for most of these proteins, recently also for PLG (52), and may lead to erroneous assumption of inverse homozygosity. Besides, partial or total nucleotide sequences were described for the majority of these proteins, but restriction fragment length polymorphisms (RFLP) seen only for HP (42), PI (11), and DBP (13), the BamH I-RFLP being obviously the most useful in the latter.

Chromosomal assignment either from somatic cell hybrids or recombinant DNA has shown the structural genes of TF and A2HS to be located on chromosome 3q, the DBP gene on chromosome 4q (13), the F13A gene distal to HLA-A on chromosome 6 (5,60), and the ORM, PI, and HP genes to be located on chromosomes 9, 14, and 16 respectively (15; for review cf. also 14). Despite repeated attempts the F13B locus is still unknown and localization of the PLG gene must remain doubtful. Two ORM loci were recently identified, ORM1 encoding for the known alleles, ORM2 being monomorphic (63).

Complement components and regulatory proteins of the complement system are outstanding for their extensive genetic heterogeneity, structural polymorphisms having been discovered for nearly all components except C1 and C9, and for most of the regulatory proteins, including the C3b receptor (CR1) (see Table 2). The many complement polymorphisms though exhibit a higher degree of variability only in five proteins with a potential for practical application; these are C3, BF, C4, C6, and C81. All other components possess limited polymorphic features. Factor D and C5, possibly also factor I (C3b inactivator), were found polymorphic only in certain ethnic groups (reviewed in 34,45).

Table 1: **Plasma protein polymorphisms in forensic haemogenetics**

	electrophoresis		isoelectric focusing/ immobilines					
	no. alleles*)	SENF (%)	no. alleles*)	SENF (%)	silent alleles	recomb. DNA	RFLP	chromosomal assignment
HP	2 (>10)	12.9	4 (2 + 10?)	30.6	+	+	+	16
GC(DBP)	2 (>10)	15.5	3 (>36)	29.8	+	+	+	4q
TF	1 (>21)	1	3 (>25)	19.5	+	+		3q
PI	3 (>24)	3.9	5 (>27)	27.0	+	+	+	14
ORM	2	18	3	21	?	+		9
PLG	2 (2)	16.1	2 (>11)	19.8	+	+		?
F13A	2 (3)	13.7	-	-	+	+		6p
F13B	3 (>8)	22.7	3 (>8)	22.7	?			-
A2HS	-	-	2 (5)	17.8	?			3q

*) No. of rare alleles in parenthesis; SENF : single exclusion chance for non-fathers; RFLP : restriction fragment length polymorphism

Most complement components have also been assigned for the chromosomal location of their structural genes by linkage studies, segregation analysis in families or recombinant DNA methods. Two linkage groups, C4 binding protein (C4BP), factor H (ß1H-globulin or H), and C3b receptor (CR1) on chromosome 1 (29,46), as well as C4, BF, and C2 on chromosome 6 (9) have advanced the knowledge on functional similarities and common ancestral genes of the regulatory protein group (C4BP/H/CR1) and the C3 activators (C4/BF/C2).
The formal genetics of the majority of components exhibits one or two common alleles with a limited number of rare variants, such as in C4BP, H, CR1, C81 or C82. In contrast the spectrum of variants has reached a considerable degree in C3, C4, BF, and C6. Polymorphisms of C8, and of C4 (as to be shown later), are complex with two or more structural loci encoding for each of these proteins (34,45).

Inherited complement deficiencies have been described for all complement proteins, many with disease manifestation, some without. Silent or inactive genes are most common in C1INH, leading to hereditary angioedema, where RFLP may now aid diagnosis. Silent genes are also common in C2 and C4, with systemic lupus erythematosus (SLE) or SLE-like diseases in homozygously deficient individuals (26) and many other associated diseases in heterozygous carriers (34,41,43). Furthermore, in individuals with complete C3, C5, C6 or C7 deficiency multiple episodes of infectious complications were seen (cf. also 48).
The structural gene for C3, the first complement polymorphism to be discovered, now widely used in population and forensic genetics, and also first to be located by recombinant DNA, is found on chromosome 19 (18,61). For five components chromosomal locations are still unknown, including factor I, newly described to be genetically determined in a Japanese population (37). C6 and C7 form a separate linkage group (LG4), which remains unassigned in spite of several linkage studies and cloned nucleotides (36).

Table 2 : **Complement polymorphisms**

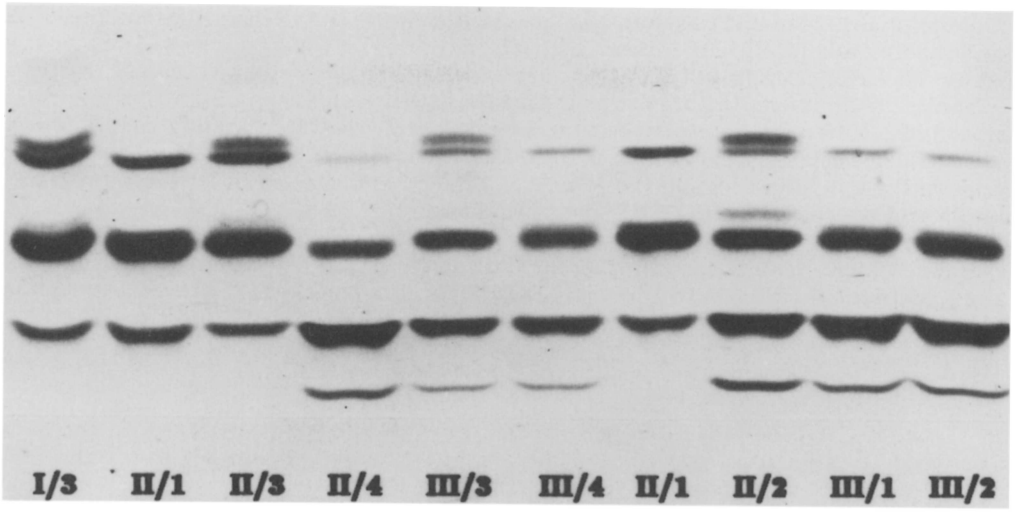
	Alleles : common	rare	silent	recomb. DNA	RFLP	no. of fragments
CHROMOSOME 1 :						
C4BP	1	1	+	+		
H	2	1	+	+		
CR1 (C3BR)	2	1	+	+		
C81 (α / γ -chain)	2	3	+			
C82 (ß-chain)	1	2	+	+		
C1Q	-	-	(+)	+		
CHROMOSOME 6 :						
C4 α -chain: C4A	4	>9	+	+	BamH I	2
C4B	3	>18	+	+	Bgl II	2
C4ß-chain	2	+			Kpn I	4
					Taq I	4
					Xba I/BamH I	3
					Nlq IV	A/B genes
					Eco0 109	A/B genes
C2	1	3	+	+	BamH I	multiple
					Taq I	2
BF	2	>16	+			
F-subtypes	2			+	Taq I	2
S-subtypes (?)	2	2				
CHROMOSOME 11 :						
C1INH	-	-	+	+	HgiA I	?
CHROMOSOME 12 :						
C1R	-	-	+	+		
C1S	-	-	(+)	+		
CHROMOSOME 19 :						
C3	2	>26	+	+	Bgl II	2
					Sst I	3
					Taq I	2
UNASSIGNED :						
C5*)	1	1	+	+		
C6	2	>12	+	+		
C7	1	2	+	+		
D*)	1	1	+	+		
I	2	-	+	+		

*) C5 : only polymorphic in Negroids; D: factor D only polymorphic in Melanesians; C3BR : C3b receptor; LG4 : linkage group 4

The amount of information on the major histocompatibility complex (MHC) class III proteins C2, BF, C4A, and C4B has reached a remarkable level. In the context of this review the available data may only be highlighted and the reader referred to more extensive reviews and original publications (4,7,8,9,34,44,45). Among the three class III proteins *C2* is least polymorphic with the common allele C and the rarer B and A alleles. *BF* polymorphism, consisting of the two common alleles F and S and a large collection of rare variants with different electrophoretic migration characteristics, has also been supplemented with the detection of subtypes (17,21,57). *C4* polymorphism, in short, consists of the two proteins C4A and C4B, which differ in general in their electrophoretic mobility, relative haemolytic activity in regard to their receptor binding properties (27), their antigenicity, and the size of their alpha chains in the reduced molecule. C4A*3 and B*1 are the most common alleles, A*6, A*4, A*2, B*3, and B*2 less common; a multitude of rare variants , exceeding nine for C4A and 18 for C4B, has been described (31,35).

Close linkage of class III components, presumptively having arisen by gene duplication is considered to reflect their structural similarities and/or functional interactions. The orientation of their genes between the MHC class I and II regions was long at variance (9). ABBAL and coworkers (1) through analysis of three unrelated French families with the very rare BF variant *S*11* have postulated the C4 genes to be oriented toward HLA-DR. Identical C4 haplotypes segregated with BF and HLA-A,B,C,DR,DQW in two families, but with a different C4 and DR haplotype in a third family. Assuming one common ancestral point mutation for the generation of *BF*S11* the recombination between BF and C4A must have been a later event. It would imply the following order of genes: HLA-B - C2 - BF - C4A - 21-OHA - C4B - 21-OHB - HLA-DR. This order has recently been confirmed through recombinant DNA methods (20).
Within the class III gene region also the structural genes for 21-hydroxylase (21-OHA and 21-OHB) have been located. The deficiency of 21-OHB is the cause of the salt-wasting form of adrenal hyperplasia (10). Furthermore, tumor necrosis factor (TNF), a lymphokine, has now been located within 260 kb to HLA-B (RAGOUSSIS, J., BLÖMER, K., WEISS, E., ZIEGLER, A., personal communication).

C2 phenotyping on the basis of functional assays, seemingly complicated to workers outside the complement field, has now been faciliated and made available to many laboratories using the "Western blot" technique which also detects phenotypes in the C2a fragment of decayed C2 (19,59).
Whereas phenotyping of BF S-subtypes was not successfully confirmed in most laboratories, doubts on the existence of BF F-subtypes are unjustified as shown in Figure 1 where segregation of BF*FA and BF*FB is demonstrated in a three generation family. In the Ba fragment of converted factor B distinction between BF FB with a single band and BF FA with one more pronounced anodal major and one cathodal minor band poses no problems; two bands of about equal strength are found in heterozygous FAFB carriers.
Phenotyping of C4 may require in many instances beyond the standard methods of immunofixation agarose-gel electrophoresis and haemolysis (3,31) the determination of Rodgers (Rg) and Chido (Ch) antigens (22,23,24), the identification of rare or aberrant phenotypes in "Western blots" with C4A or C4B specific monoclonal antibodies (6,25,54) and the distinction of C4-alpha A or B chains (47), and C4ß H or L chains (32) by SDS-PAGE for the analysis of haplotypes, silent and aberrant alleles. Monoclonal antibodies recognize two different forms of C4 proteins in the C4A*1/B*3/4 region with low haemolytic activity and C4B epitopes. One has been designated C4BI (54) or C4BHI (6), the other C4"A1" on the basis of its alpha-A-chain property in reduced C4 (6).
Allotypic identification of major bands through treatment of C4 with carboxypeptidase B (50) on one hand and TaqI-C4-RFLP (49) on the other hand are newly introduced methods for the recognition of haplotype constellations in C4 as shown in a family with five remarkable C4 characteristics: *1.* lack of C4A but apparent duplication of B3/B1 on haplotype b) as deduced from individual II,2 with three haemolytically active C4B



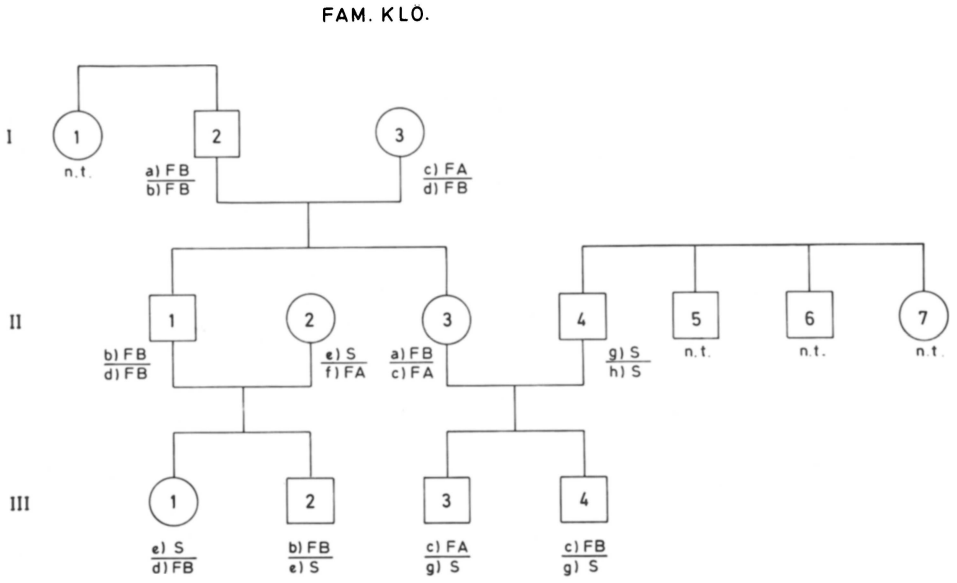


Figure 1: BF*F subtypes : Segregation in *Family KLÖ.*

(a) Immunofixation of the factor Ba fragment with goat anti-B serum (ATLANTIC ANTIBODIES) after inulin treatment of samples, separation by agarose-gel isoelectric focusing, pH 4.5-5 (anode at top). Designation of individuals and BF phenotypes correspond to pedigree.
(b) Pedigree of Family KLÖ.
(From : G. MAUFF, I. SIEMENS, G. GESERICK, K. BENDER, G. PULVERER : The BF*F subtypes are detectable in the Ba fragment of factor B; in preparation).

proteins; *2.* the C4A*4,B*6 haplotype in conjunction with HLA-BW55,DRW6 on haplotype c) in individuals I,2, II,1, II,2, and III,2, B6 migrating in the C4A region; *3.* the linkage group HLA-B35/C4A*3,A*2,B*Q0 on haplotype d) in individuals I,2, and II,5, the duplicated "A2" obviously possessing the RFLP properties of a long 22 kb C4B gene; *4.* a second long C4B gene on haplotype g) as revealed in the RFLP, obviously coding for the so called "A*2" allele in duplication with A*6 in individuals II,4 and III,3; *5.* a short C4B gene on the C4A deleted haplotype h) seen in the RFLP of individual II,4 with the 6.4 kb intron as described by SCHNEIDER and coworkers (49).
From the most probable interpretation of results in this family it may be concluded that specifically the HLA-B35 associated duplicated A*3,A*2,B*Q0 haplotype contains a B-like A*2 gene, as first reported by PALSDOTTIR and coworkers (39) but in contradiction to a comparable haplotype recently described in family ST. by GILES and coworkers (25).

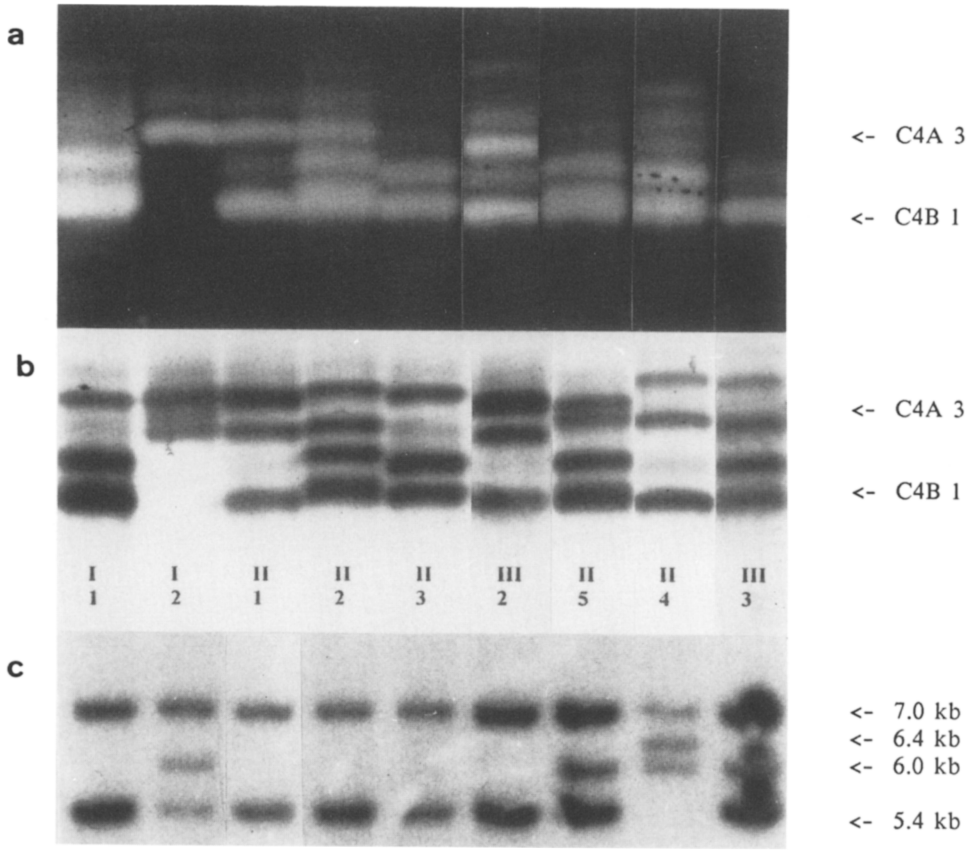


Figure 2 : **C4 phenotypes and restriction fragment length polymorphism (RFLP) of *Family BO.*** **For the designation of individuals and typing results cf. to pedigree in fig. 3. (a) Haemolytic overlay, (b) immunofixation with goat anti-C4 (ATLANTIC ANTIBODIES) after agarose-gel electrophoresis of carboxypeptidase B and neuraminidase treated samples (anode at top).**
(c) TaqI-C4-RFLP (anode at bottom); BamHI/KpnI fragment from full length cDNA-probe pAT-A (a gift from Dr. M. CARROLL, Boston).
(From: G. MAUFF, K. BENDER, M. BRAUN, M. BRENDEN, W. BOKSCH, E. DUTOIT, C.GILES, S. GOLDMANN, R. NEUMANN: C4-TaqI-RFLP in families with duplicated and aberrant C4 allotypes; in preparation)

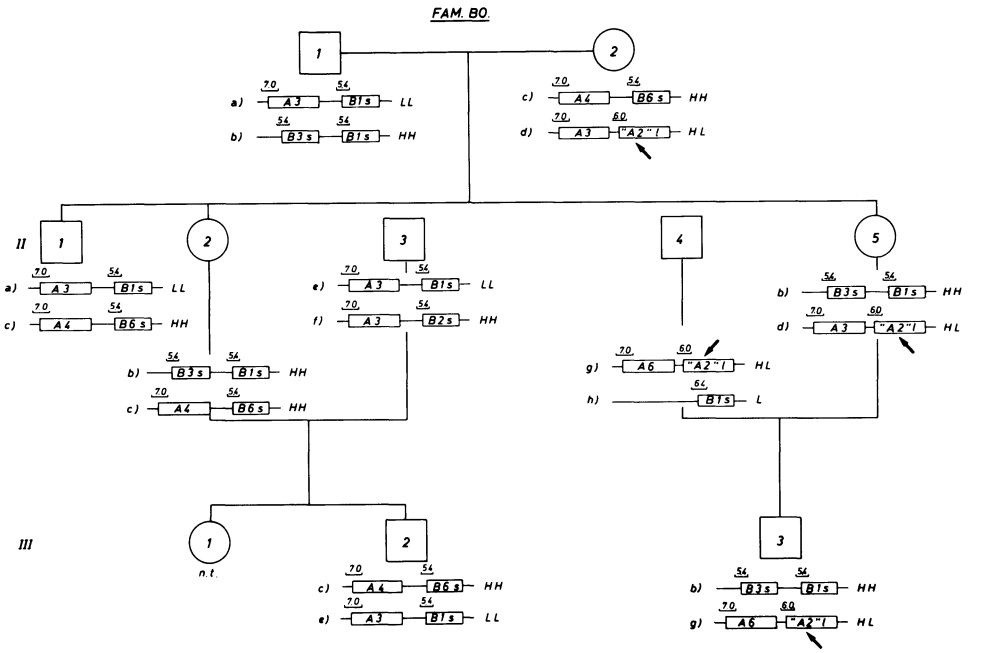


Figure 3 : **Pedigree of *Family BO*** **(for results of C4 phenotyping and RFLP cf. fig.2).**
Segregation of C4 genes according to C4 phenotyping, C2, BF, C4ß-chain (HH, LL, HL), and HLA data (not shown). Probe fragments (indicated above genes) recognize the 22 kb C4A gene (7.0 kb), the 22 kb long (l) C4B gene (6.0 kb fragment), the 16 kb short (s) C4B gene (5.4 kb fragment) and in individual II/4 the short C4B gene (6.4 kb fragment) in combination with a deleted C4A gene. (From: G. MAUFF, K. BENDER, M. BRAUN, M. BRENDEN, W. BOKSCH, E. DUTOIT, C.GILES, S. GOLDMANN, R. NEUMANN: C4-TaqI-RFLP in families with duplicated and aberrant C4 allotypes; in preparation)

This B-like A*2 seems also present in the A*6,A*2,B*Q0 haplotype g) in individuals II,4 and III,3.
In general it will therefore appear that C4 haplotypes with assumed deletions of one gene in fact may contain so called duplicated alleles in many instances which might have arisen from gene conversion (62). This may at length reduce the present estimate on silent and duplicated alleles but increase the frequency of aberrant genes. In addition, it must be mentioned that on the basis of Taq I-RFLP a considerable number of individuals seem to carry B*1 genes but do not express C4 protein (49).

Turning to the application of protein polymorphisms and RFLPs in forensic haemogenetics, as mentioned earlier, for three non-complement plasma proteins and five complement components RFLPs have been reported. They include *EcoR I*, *Hind III*, and *Pst I* polymorphism for HP, *BamH I* for DBP, and *EcoR I*, *Sph I*, and *Taq I* for PI (14). Restriction enzymes used for complement genes are listed in Table 2. The number of fragments obtained vary from two to a multitude of bands, such as in PI or C4, C4 having most extensively been investigated. Over all, *Taq I* RFLPs are most widely applied, although e.g. *BamH I* has proven to be useful in C4 and C2 RFLP (16), or *Bgl II* for the recognition of the C4A6 gene fragment (38) as well as for C3 RFLP (18). *Nlq IV* and *Eco0 109* in C4 distinguish only between C4A and B resp. Rg and Ch of the C4d fragment.

Table 3 : **Application of complement polymorphism**

	SENF (%)	technology[1)]	in forensic haemogenetics[2)]
C3	14.4	*	+
BF[3)]	14.6 (15.5)	*	+
C6	19.7	*	(+)
C81	20.8	***	(+)
C82	5.1	***	(+)
C4αA	24.3	***	-
C4αB	24.0	***	-
C2	4.9	**	-
C7	12.8	**	-
C5	6.1	***	-
C4BP	1.7	**	-
D	-	***	-
I	8.6	**	-
H	17.4	***	-

SENF : single exclusion chance for non-fathers
1) : * easy; ** moderately difficult; * difficult;**
2) : + commonly in use; (+) occasionally in use; - currently not in use
3) : in parenthesis including BF*F subtypes

From reported frequencies of polymorphic fragments single exclusion chances are in the range of those seen in protein polymorphisms. With the rapid explosion of experimental evidence from molecular genetics the data may not be complete. It appears, that however useful RFLPs have been for the fine characterization of the genome, in view of complex genetics and laborious technologies in forensic genetics they seem of limited value at present. It might therefore be expected that their application will remain for some time the domaine of a few specialized laboratories.

Reviewing the data on complement *protein* polymorphisms according to single exclusion chances, laboratory demand on technology and applicability to forensic haemogenetics (Table 3), they may be classified into three groups: *1.* polymorphisms with a high exclusion chance and frequent or occasional application in the expertise; *2.* proteins with complex genetics, variing exclusion chance and moderate or difficult demands on laboratory technology; *3.* polymorphisms with little gain in information or difficult technology. Among the two commonly used genetic systems, BF*F subtyping might potentially be introduced, as well as phenotyping of C6 on "Western blot" or immunofixation plates.

From the data presented before obviously C4 polymorphism will not be eligible for the paternity expertise, and little or moderate gain is to be expected from C2, C7, C4BP or factor I typing, whereas C5 and factor D are not polymorphic in most populations. More convenient methods in the future could well see factor H and C81 , due to their rather high single exclusion chances, as possible new candidates for the expertise in forensic haemogenetics.

Acknowledgements

I am especially indebted to Dr. M.Carroll, Boston, for donating the pAT-A probe, and to Dr. P. Schneider, Mainz, for most helpful advice.
The author's experimental work presented here was in part supported by DFG grant Ma 848/2-1.

References

1 **ABBAL M,** MOENNARD C, CAMBON-THOMSON A, TKACZUK J, OHAYON E, MAUFF G (1987): A new BF variant (BFS11): an information for MHC-class III gene orientation. Immunogenet (in press)

2 **ARNAUD P,** ALLEN RC (1981): Alpha1-antitrypsin deficiency and disease. In: Allen RC, Arnaud P (eds) Electrophoresis 81., 495-504 .de Gruyter Berlin

3 **AWDEH ZL,** ALPER CA (1980): Inherited structural polymorphism of the fourth component of human complement. Proc Natl Acad Sci USA 77: 3576-3580

4 **BENTLEY DR,** PORTER RR (1984): Isolation of cDNA clones for human complement component C2. Proc Natl Acad Sci USA 81: 1212-1215

5 **BOARD PG,** REID M, SERJEANTSON S (1984): The gene for coagulation factor XIIIA subunit (F13A) is distal to HLA on chromosome 6. Hum Genet 67: 406-408

6 **BOKSCH W,** BRAUN M, BRENDEN M, DU TOIT E, GILES C, HASBACH H, HITZERORTH H, KLEEBERG H, KLEIN A, KOK S, MAUFF G, O NEILL G, OUDSHOORN M, SCHLAPHOFF T, VD BERG I (1986a): "C4A" proteins with C4B antigenic epitopes. Immunobiol 173: 451

7 **CAMPBELL RD,** PORTER RR (1983): Molecular cloning and characterization of the gene coding for human complement protein factor B. Proc Natl Acad Sci USA 80: 4464-4468

8 **CARROLL MC,** PORTER RR (1983): Cloning of a human complement component C4 gene. Proc Natl Acad Sci USA 80: 264-267

9 **CARROLL MC,** CAMPBELL RD, BENTLEY DR, PORTER RR (1984): A molecular map of the human major histocompatibility complex class III region linking complement genes C4, C2 and factor B. Nature 307: 237-241

10 **CARROLL MC,** PALSDOTTIR A, BELT KT, PORTER RR (1985): Deletion of complement C4 and steroid 21-hydroxylase genes in the HLA class III region. EMBO J 4: 2547-2552

11 **CHANDRA T,** STACKHOUSE R, LEICHT M, LONG G, KURACHI K, DAVIE EW, WOO SLC (1982): DNA polymorphism in the human chromosomal alpha-1-antitrypsin gene. DNA 1: 210

12 **CONSTANS J,** KÜHNL P, VIAU M, SPIELMANN W (1980): A new procedure for the determination of transferrin C (Tf C) subtypes by isoelectric focusing. Existence of two additional alleles, TF C4 and TF C5. Hum Genet 55: 111-114

13 **COOKE NE,** WILLARD HF, DAVID EV, GEORGE DL (1986): Direct regional assignment of the gene for vitamin D binding protein (Gc-globulin) to human chromosome 4q11-q13 and identification of an associated DNA polymorphism. Hum Genet 73: 225-229

14 **COOPER DN,** SCHMIDTKE J (1984): DNA restriction fragment length polymorphisms and heterozygosity in the human genome. Hum Genet 66: 1-16

15 **COX DW,** FRANCKE U (1985): Direct assignment of orosomucoid to human chromosome 9 and alpha-2HS-glycoprotein to chromosome 3 using human fetal liver x rat hepatoma hybrids. Hum Genet 70: 109-115

16 **CROSS SJ,** EDWARDS HJ, BENTLEY DR, CAMPBELL RD (1985): DNA polymorphism of the C2 and factor B genes. Immunogenetics 21: 39-48

17 **DAVID V,** FAUCHET R, PHENGSAVATH H, GUENET L, LEGALL JY (1983): Properdin factor B (Bf) polymorphism: Subtyping of SS phenotypes. Hum Genet 64: 189-190

18 **DAVIES KE,** JACKSON J, WILLIAMSON R, HARPER PS, BALL S, SARFARAZI M, MEREDITH L, FEY G (1983): Linkage analysis of myotonic dystrophy and sequences on chromosome 19 using a cloned complement 3 gene probe. J Med Genet 20: 259-263

19 **DOXIADIS G,** DOXIADIS I, GROSSE-WILDE H (1985): Polymorphism of human C2 detected by immunoblotting. Hum Genet 70: 355-358

20 **DUNHAM I,** SARGENT CA, TROWSDALE J, CAMPBELL RD (1987): The orientation and location of the complement genes in the human MHC by pulsed field gel electrophoresis. XII Int. Complement Workshop, Chamonix, Sept. 87 (Abstract)

21 **GESERICK G,** PATZELT D, SCHRÖDER H, NAGAI T (1983): Isoelectrofocusing in the study the Bf system: Existence of two common serotypes of the BfF allele. Vox Sang 44: 178-182

22 **GILES CM** (1984): "Partial inhibition" of anti-Rg and anti-Ch reagents. I. Assessment for Rg/Ch typing by inhibition. Vox Sang 48: 160-166

23 **GILES CM** (1984): "Partial inhibition" of anti-Rg and anti-Ch reagents. II. Demonstration of separable antibodies for different determinants. Vox Sang 48: 167-173

24 **GILES CM,** BATCHELOR JR, DODI IA, FIELDER AHL, RITTNER CH, MAUFF G, BENDER K. LEVENE C, SCHREUDER I, WELLS L. (1984): HLA and C4 haplotypes associated with partial inhibition of anti-Rg and anti Ch. Immunogenet 21: 305-317

25 **GILES CM,** URING-LAMBERT B, BOKSCH W, BRAUN M, GOETZ J, NEUMANN R, MAUFF G, HAUPTMANN G (1987): The study of a French family with two duplicated C4A haplotypes. Hum Genet (in press)

26 **HAUPTMANN G,** GOETZ J, URING-LAMBERT B, GROSSHANS E. (1986): C4 deficiency. In: ROTHER K (ed) Hereditary and acquired complement deficiencies. Progr Allergy 39 (in press)

27 **ISENMAN DE,** YOUNG JR (1986): Covalent binding properties of the C4A and C4B isotypes of the fourth component of human complement on several C1-bearing cell surfaces. J Immunol 136: 2542-2550

28 **KAMBOH MI,** FERRELL RE (1986): Ethnic variation in vitamin D-binding protein (GC): a review of isoelectric focusing studies in human populations. Hum Genet 72: 281-293

29 **KRISTENSEN T,** WETSEL RA, TACK BF (1986): Structural analysis of human complement protein H: 1. Homology with C4b binding protein beta-2-glycoprotein I, and the Ba fragment of BF. J Immunol 136: 3407-3411

30 **MAUFF G,** ERFURDT U, WEGRZYNOWICZ, PULVERER G, HUMMEL K (1981): Genetic polymorphism of human plasminogen. In: Hummel K, Gerchow J (eds): Biomathematical Evidence of Paternity, p 89-96. Springer Berlin

31 **MAUFF G,** ALPER CA, AWDEH ZL, BACHTELOR JR, BERTRAMS J, BRUUN-PETERSEN G, DAWKINS RL, DEMANT P, EDWARDS JH, GROSSE-WILDE H, HAUPTMANN G, KLOUDA P, LAMM L, MOLLENHAUER E, NERL C, OLAISEN , O'NEILL G, RITTNER C, ROOS MH, SKANES V, TEISBERG P, WELLS L (1983): Statement on the nomenclature of human C4 allotypes. Immunobiol 164: 184-191

32 **MAUFF G,** STEUER M, WECK M, BENDER K (1983): The C4 beta-chain. Evidence for a genetically determined polymorphism. Hum Genet 64: 186-188

33 **MAUFF G,** SCHILLING A, PULVERER G (1984): Coagulation factor XIII: Determination of the genetic polymorphism of subunit B (F13B) by immunoblotting in a West German population sample. Proc 10 Int Congr Soc Forens Haemogenet, p 453-457, München

34 **MAUFF G** (1985): The complement system: genetics and function. In: KOSCIELAK W, WIKTOR-JEDRZEJCZAK W, KOTELBA-WITOWSKA B, TRACZYK Z (eds): Trends in Haematology I, p. 4-30, Inst Haematol, Warsaw

35 **MAUFF G,** (1986): Application of the MHC-class III complement markers to population genetics. In: OHAYON E & CAMBON-THOMSON (eds): Human Population Genetics, p. 143-166, INSERM, Paris

36 **MCALPINE PJ,** SHOWS TB, MILLER RL, PAKSTIS AJ (1985): The 1985 catalog of mapped genes and report of the nomenclature committee, p 8-49

37 **NAKAMURA S,** ABE K (1985): Genetic polymorphism of human factor I (C3b inactivator). Hum Genet 71: 45-48

38 **PALSDOTTIR A,** CROSS SJ, EDWARDS JH, CARROLL MC (1983): Correlation between a restriction fragment length polymorphism and C4A6 protein. Nature 306: 615-616

39 **PALSDOTTIR A,** FOSSDAL R, ARNASON A, EDWARDS HJ, JENSSON O (1987): Heterogeneity of human C4 gene size. Immunogenetics 25: 299-304

40 **PATZELT D,** SCHRÖDER H (1986): Simple method for haptoglobin subtyping. In: Brinkmann B, Henningsen K: Advances in Forensic Haemogenetics 1, p 141-145. Springer Berlin

41 **PORTER RR** (1983): Complement polymorphims, the major histocompatibility complex and associated diseases: A speculation. Mol Biol Med 1: 161-168

42 **RAUGEI G,** BENSI G, COLANTUONI V, ROMANO V, SANTORO C, COSTANZO F, CORTESE R (1983): Sequence of human haptoglobin cDNA: evidence that the alpha and ß subunits are coded by the same mRNA . Nucl Acids Res 11: 5811-5819

43 **RITTNER CH,** BERTRAMS J (1981): On the significance of C2, C4 and factor B polymorphisms in disease. Hum Genet 56: 235-247

44 **RITTNER CH,** MAUFF G (1984): C4 Polymorphism In: ALBERT ED, BAUR MP; MAYR WR(eds): Histocompatibility Testing 1984, p. 318-324, Springer, Heidelberg

45 **RITTNER CH,** SCHNEIDER P (1987): Genetics and polymorphism of the complement components. In: Rother K, Till (eds): The Complement System. (in press)

46 **RODRIGUEZ DE CORBIDA S,** DYKMAN TR, FELLNER FG, ERCILLA G, AQUA M, ATKINSON JP, RUBINSTEIN P. (1984): Evidence for linkage between the loci coding for the binding protein for the fourth component of human complement (C4BP) and for the C3b/C4b receptor. Proc Natl Acad Sci USA 81: 7890-7892

47 **ROOS MH,** MOLLENHAUER E, DEMANT P, RITTNER C (1982): A molecular basis for the two locus model of human complement component C4. Nature 298: 854-856

48 **RYNES R,** PICKERING RJ (1985): Inherited complement component deficiencies in man. In: Whaley K (ed) Methods in Complement for Clinical Immunologists. Churchill Livingstone Edinburgh

49 **SCHNEIDER P,** CARROLL MC, ALPER CA, RITTNER CH, WHITEHEAD AS, YUNIS EJ, COLTEN HR (1986): Polymorphism of the human complement C4 and steroid 21-hydroxlase genes. J Clin Invest 78: 650- 657

50 **SIM E,** CROSS SJ (1986): Phenotyping of human complement C4, a class-III HLA antigen. Biochem J 239: 763- 767

51 **SKODA U,** BERTRAMS J, DYKES D, EIBERG H, HOBART M, HUMMEL K, KÜHNL P, MAUFF G, NAKAMURA S, NISHIMUKAI H, RAUM D, TOKUNAGA K, WEIDINGER S (1986): Proposal for the nomenclature of human plasminogen (PLG) polymorphism. Vox Sang 51: 244-248

52 **SKODA U,** GOLDMANN SF, HÄNDLER CH, HUMMEL K, LECHLER E, LÜBCKE I, MAUFF G, MEYER-BÖRNECKE D, PESCH S, PULVERER G (1987): Plasminogen hemizygosity: Detectection of a silent allele in seven members of a family by determination of plasminogen (PLG) phenotypes, antigenic levels and functional activity. (submitted)

53 **SPIELMANN W,** KÜHNL P (1982): Erfahrungen mit neueren Blutgruppensystemen für die Vaterschaftsbegutachtung: FXIIIA und FXIIIB, PLGN, AMY2, GAA, GDH, PGM1-Thermostabilitätsvarianten. Ärztl Lab 28: 367-377

54 **SUZUKI K,** O'NEILL GJ, MATSUMOTO H (1986): A product of the C4B locus lacking hemolytic activity. Hum Genet 73: 101-103

55 **TARKKALA MENDNER T,** KÜHNL P (1986): Alpha-2-HS glycoprotein polymorphism. In: Brinkmann B, Henningsen K: Advances in Forensic Haemogenetics 1, p 167-173. Springer Berlin

56 **TEIGE B,** OLAISEN B, PEDERSEN L (1986): Subtyping of haptoglobin. In: Brinkmann B, Henningsen K: Advances in Forensic Haemogenetics 1, p 136-140. Springer Berlin

57 **TENG YS,** TAN SG (1982): Subtyping of properdin factor B (Bf) by isoelectric focusing. Hum Hered 32: 362-366

58 **THYMANN M,** EIBERG H (1986): Orosomucoid polymorphism: Determination of separator isoelectric focusing and demonstration of ORM*F subtypes. In: Brinkmann B, Henningsen K: Advances in Forensic Haemogenetics 1, p 162-166. Springer Berlin

59 **URING-LAMBERT B,** GAS S, GOETZ J, MAUFF G, GOLDMANN S, FRÖSSLER M, BENDER K, HAUPTMANN G (1986): Detection of the genetic polymorphism of human C2 (native protein and C2a fragment) by immunoblotting after polyacrylamide gel isoelectric focusing. Complement 2: 185-192

60 **WEISBERG LJ,** SHIU DT, GREENBERG CS, KAN YW, SHUMAN MA (1987): Localization of the gene for coagulation factor XIIIa-chain to chromosome 6 and identification of sites of synthesis. J Clin Invest 79: 649-652

61 **WHITEHEAD AS,** SOLOMON E, CHAMBERS S, BODMER WF, POVEY S, FEY G (1982): Assignment of the structural gene for the third component of human complement to chromosome 19. Proc Natl Acad 79: 5021-5025

62 **YU YC,** BELT KT, GILES CM, CAMPBELL RD, PORTER RR (1986): Structural basis of the polymorphism of human complement components C4A and C4B: gene size, reactivity and antigenicity. The EMBO Journal 5: 2873-2881

63 **YUASA I,** UMETSU K, SUENAGA K, ROBINET-LEVY M (1986): Orosomucoid (ORM) typing by isoelectric focusing: evidence for two structural loci ORM 1 and ORM 2. Hum Genet 74: 160-161