Genetic Linkage Between Structural Loci for Albumin and Group Specific Component (Gc)

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A GENETIC VARIANT of albumin was first described by Knedel (1957), who demonstrated by serum electrophoresis the occurrence of a double albumin band in five members of three generations of one family. Also in 1957, Nennsteil and Becht independently demonstrated a splitting of the albumin band in three members of two generations. In a search of the literature, we have found a total of 21 families containing over 150 affected members (Ungari and Lopez, 1965, for review; Drachman *et al.*, 1965; Braend *et al.*, 1965; Sandor *et al.*, 1965; Adams, 1966). Inheritance has always followed the autosomal codominant pattern.

Cooke *et al.* (1961) reported in a brief note two new families with albumin variants and an examination for linkage with other loci. They did not find linkage with "any other genetically controlled plasma protein nor with phenylthiourea tasting . . . (nor with) . . . any of the common blood or saliva systems. . . ." The exact systems examined and the number of people included in the two families were not specified in their abstract.

We have had the privilege of examining two new families with variant albumins and a third family kindly referred by Dr. M. S. Adams (1966). Evidence for the genetic linkage of structural loci governing the production of albumin and group specific component (Gc) is presented. The members of these families have also been classified for haptoglobin, transferrin, acid phosphatase, phosphoglucomutase, ABO, Rh, Secretor, MNS, P, Kell, Duffy, Kidd, and Lewis as well as albumin and Gc types.

MATERIALS AND METHODS

The probands of families 1 and 3 were ascertained during admission to the University of Michigan Hospital (for mitral stenosis and nephrotic syndrome, respectively). The individual in family 2 was identified during a screening of prison volunteers for other purposes.

Vertical starch gel electrophoresis (Starch Hydrolyzed; Connaught Laboratories, Toronto) was performed on the sera of all individuals studied according to the modifications of the technique of Smithies (1959) and the buffer of Gahne (1963) as described by Bowman and Bearn (1965), except that the gels were run for 6–7 hours at 20–30 volts/cm. Amido Black 10B was used for

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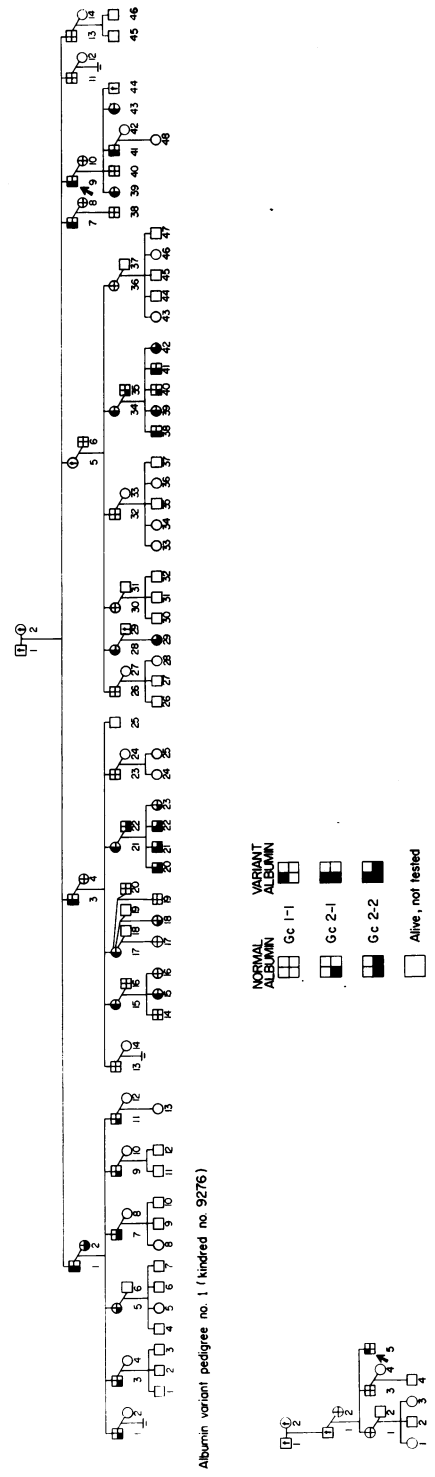

FIG. 1. Pedigrees showing segregation of genes for serum albumin and group specific component (Gc protein). Squares designate males, circles females, arrows propositi.

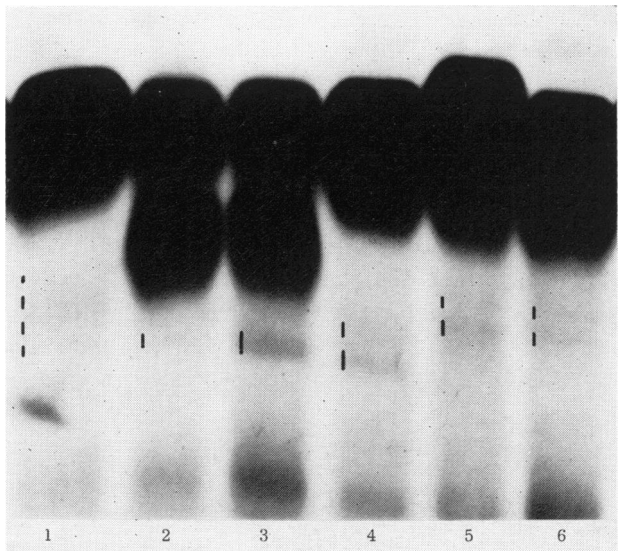

FIG. 2. Vertical starch gel electrophoresis showing albumin and Gc phenotypes:

1. Al^{n}; Gc 2-1
2. $Al^{Ann\ Arbor}$; Gc 2-1
3. $Al^{Oliphant}$; Gc 2-2
4. Al^{n}; Gc 2-2
5. Al^{Larson}; Gc 1-1
6. Al^{n}; Gc 1-1

staining. Agar immunoelectrophoresis on 8 × 10 cm glass slides was carried out using the buffer system and electrophoretic conditions described by Hirschfeld (1959) with No. 2 Ionagar (Consolidated Laboratories) as the supporting medium. A rabbit serum prepared against partially purified Gc protein was employed. Red cell acid phosphatase types were determined according to the method of Hopkinson *et al.* (1964) and phosphoglucomutase types by the method of Spencer *et al.* (1964).

RESULTS

The pedigrees of the three families are shown in Fig. 1. The variant serum albumin of family 1, of Danish descent, and of family 3, of German descent, migrates toward the anode at pH 8.2 more slowly than normal albumin. These are referred to as Albumin Ann Arbor ($Al^{Ann\ Arbor}$) and Albumin Oliphant ($Al^{Oliphant}$), respectively. The propositus in family 2 (descent unknown) has a variant albumin which migrates more rapidly than the normal (Al^{Larson}).

Figure 2 is a photograph of the electropherogram of sera containing fast and slow variants of albumin and the three Gc phenotypes. The slow albumin variants obscure the Gc 1 bands and the fast Gc 2 band of the same serum sample. The slow Gc 2 band migrates in the same position in the sera containing the variant albumins as in sera containing only normal albumin and is clearly separable from the slow albumin band. Note, however, that the slow Gc band of person 3 is more intensely staining than that of person 2, suggesting that the phenotype of the former is Gc 2-2 and of the latter Gc 2-1. Independent typing of 43 specimens by immunoelectrophoresis produced

agreement with the estimate of Gc type based upon the intensity of the slow 2 band in all but two instances. Complete agreement was obtained for the 1-1 phenotypes. The immunoelectrophoretic patterns for Gc in the presence of albumin variants were indistinguishable from comparable patterns in persons with only normal albumin. Thus, the possibility of an artifact in the Gc pattern as a result of the presence of an albumin variant seems unlikely.

In family 1, 20 of the 43 direct relatives of the propositus which were tested possessed the variant albumin. If in family 3 the propositus, his mother, and grandmother are discounted, there are 19 direct relatives who possess the variant and 15 who do not (including II-11 who was not tested by us but has been reported negative by Adams). Thus, the segregation ratio was 39 with the trait to 38 without. Among the 43 people who possessed the variant, 18 were male and 25 female.

There were 10 matings which were determined to be of the double backcross type with respect to Gc and Al by direct genotyping of both parents (family 1: II-1 × II-2, II-3 × II-4, II-7 × II-8, II-9 × II-10, III-15 × III-16, III-17 × III-20, III-21 × III-22; family 3: II-1 × II-2, II-3 × II-4, III-19 × III-20). Of the 39 offspring who were typed, 38 received either the genes for the albumin variant and Gc 2 or the genes for normal albumin and Gc 1 from the affected parent. There was one apparent recombinant (III-7, family 1) in whom illegitimacy could not be demonstrated with any of the genetic markers employed. Three of the seven offspring from two single backcross matings (family 1: IV-42; family 3: IV-9 and IV-10) were informative and also had received either the gene for the albumin variant and Gc 2 or the gene for normal albumin and Gc 1 from the affected parent. From these matings alone, it is clear that genetic linkage of the loci for albumin and Gc in families 1 and 3 is highly likely and that the phase of linkage in both families is coupling of Gc^2 with the variant albumin gene. In family 2, the gene for the electrophoretically fast albumin variant occurs in an individual with a Gc^1/Gc^1 genotype.

Dr. C. A. B. Smith has kindly estimated the recombination fraction for these pedigrees using the likelihood curve method (Haldane and Smith, 1947; Morton, 1955, 1956; Smith, 1959). It is presumed that II-5 in family 1 and I-1 in family 3 must have been heterozygous at the albumin and Gc loci. In the absence of known consanguinity, the probability of the mating of two albumin heterozygotes was considered insignificantly small. Note also that there are seven offspring whose informative value does not depend at all on the Gc type of the untested parent (III-6, IV-17, IV-29 in family 1; III-28, III-30, III-31, III-37 in family 3); the fact that III-26 is illegitimate (Appendix) therefore is not relevant to the probability calculation.

The "likelihood" (L) of a pedigree is the algebraic probability expression of obtaining such a pedigree. If the recombination fraction is called c, the nonrecombination fraction $b = 1-c$, and the population frequencies of the genes Gc^1, Gc^2 respectively p and q, then the log likelihood ($\log L$) for the three families is given by the following expressions:

Pedigree 1:

$$\log L_1 = 11 \log b_1 + \log(c_1p + b_1q) + \log[2qb_1^3c_1(b_1^2 + c_1^2)(b_1^5 + c_1^5)^2(b_1^6 + c_1^6)(b_1^7 + c_1^7) + 4qb_1^6c_1^8(b_1^3 + c_1^3)^2(b_1^4 + c_1^4)(b_1^5 + c_1^5) + 128\, p(b_1^{28}c_1 + b_1^{21}c_1^8) + b_1c_1^{21}]$$

Pedigree 2:

$$\log L_2 = \log(qb_2c_2 + p)$$

Pedigree 3:

$$\log L_3 = 23 \log b_3 + \log(b_3^5 + c_3^5) + \log(p + 2qb_3)$$

Since it is evident from inspection that there are few crossovers in pedigrees 1 and 3, the recombination fraction is certainly near zero; only the left-hand end of the likelihood curve matters. Here, taking b to be near 1 and c to be small, the following approximations for the likelihood curves in these families are obtained:

$$\log L_1 \simeq (40 - p/q)\log b + \log c + \text{constant}$$
$$\log L_3 \simeq [28 + 2q/(2q + p)]\log b + \text{constant}$$

That is, for all practical purposes, pedigree 1 can be counted as having $(40 - p/q)$ nonrecombinations and one recombination, and pedigree 3 as having $28 + 2q/(2q + p)$ nonrecombinations and no recombinations. If the *Gc* gene frequencies applicable to the untested individuals in this family are .74 for Gc^1 and .26 for Gc^2 (Hirschfeld, 1962), the effective number of nonrecombinants becomes 37.2 and 28.4, respectively, for families 1 and 2.

It is possible that all three albumin variants are controlled by genes at the same locus, so that the recombination fraction $c = c_1 = c_2 = c_3$ is the same for all three families. The data are clearly compatible with this hypothesis; pedigree 2 gives little information either to support or refute it. If this is assumed, the likelihood of the complete sample is the product $L = L_1L_2L_3$, or $\log L = \log L_1 + \log L_2 + \log L_3$. For small values of c, $\log L_2 \simeq -(q/p)\log b + \text{constant} \simeq -.3 \log b + \text{constant}$; family 2 behaves as if it had $-.3$ nonrecombinants and no recombinants. In the whole data, there are effectively $37.2 + 28.4 - .3 = 65.3$ nonrecombinants and one recombinant, i.e., the likelihood curve is of the form $L = \text{constant} \times b^{65.3}c^1$ at the left-hand end (and elsewhere the likelihood is negligibly small). The estimated recombination fraction is therefore $1/66.3 = 0.015$.

Since the recombination fraction c has a roughly uniform *a priori* distribution between 0 and 1/2 (Morton, 1955), the final probability distribution for c has (nearly enough) the same shape as the likelihood curve. A value C such that the recombination fraction has a probability .95 of falling between 0 and C can be determined thus:

$$\int_0^C c(1-c)^{65.3}\,dc = .95 \int_0^{.5} c(1-c)^{65.3}\,dc$$

A numerical solution of this equation gives $C \simeq .07$; there is a 95% chance that $c \leq .07$.

DISCUSSION

Good evidence exists for only a few autosomal linkages in man. The first three linkages described—Lutheran blood group and secretor (Mohr, 1951), Rh and elliptocytosis (Chalmers and Lawler, 1953), ABO and nail-patella (Renwick and Lawler, 1955)—are now well established (summarized in Race and Sanger, 1962). The hemoglobin β and δ structural loci appear to be closely linked (Ceppellini, 1959; Boyer *et al.*, 1963) as well as other less well defined inherited abnormalities related to hemoglobin (cf. Neel, 1961; Pearson and Moore, 1965). In addition, Renwick and Lawler (1963) have shown a probability of linkage between a congenital cataract locus and Duffy blood group; Hösli *et al.* (1957) have proposed linkage of ptosis and MNS; and El-Hefnawi *et al.* (1965) recently have presented evidence suggestive of linkage between xeroderma pigmentosum and ABO. In the present study, segregation of the secretor, Rh, ABO, MNS, and Duffy loci was such that close linkage with the *Al* locus should have been demonstrable. None was found. The *Gc* locus, furthermore, has not been shown to be linked with these groups (Mohr and Reinskou, 1963). Thus, except for the hemoglobin loci which were not tested, this new linkage group does not appear to be linked to any of the previously demonstrated linkage groups.

Although a map distance of 1.5% does not constitute close linkage at the molecular level, in view of the accumulating evidence for tandem loci governing related proteins in higher organisms, including man (Boyer *et al.*, 1963; Smithies *et al.*, 1962), the linkage demonstrated between loci for albumin and Gc raises the possibility that both loci phylogenetically are derived from a single locus, e. g., by unequal crossing over and duplication. The assessment of such a relationship, however, must await a comparison of the amino acid sequences of the albumin and Gc molecules, either in man or perhaps in other species in which proteins homologous to human albumin and Gc have been demonstrated.

SUMMARY

Evidence for genetic linkage between structural loci for human serum albumin and serum group specific component was presented. There appeared to be one recombinant in 42 informative offspring in which both parents were genotyped. Making maximum use of the available information, the likelihood of obtaining these pedigrees was calculated to be effectively that of one recombinant and 65.3 nonrecombinants, or a recombination fraction of 0.015. In two families, slow variants of albumin were in coupling with Gc^2; a single example of a fast variant albumin in an individual with the Gc^1/Gc^1 genotype was also presented. This new linkage group has not been shown to be associated with any of five blood groups previously suggested to have linkage relationships.

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APPENDIX

(See following page)

APPENDIX

DATA ON FAMILY 1 (KINDRED 9276)

Individual number	Parents	Sex	Age	Serum types			Red cell enzyme types		Blood types									Secretor types	
				Alb	Gc	Hp	Acid phos.	PGM	ABO	MNSs	P	CDE	Kell	Fy^a	Jk^a	Lewis a	Lewis b	ABH	Le^a
II-1	I-1, 2	M	61	variant	2-1	2-2	CB		B	MNS	−	CcDee	kk	+	+	−	+	+	+
II-2		F	56	N	2-2	1-1	B	1-1	A_1	MNs	−	CcDee	kk	−	+	−	−	−	+
II-3	I-1, 2	M	60	variant	2-1	2-1	B		A_1	MNS	−	CcDee	Kk	+	+	−	−		
II-4		F		N	1-1				A_1	Ns	−	ccDEe	kk	+	+	+	−		
II-6		M	57	N	1-1	2-1	CA		A_1	Ns	+	ccdee	kk	−	+	+	−		
II-7	I-1, 2	M	53	variant	2-1	2-2			B	MS	−	CcDee	kk	+	+	−	+		
II-8		F		N	1-1	2-1			A_1	MNs	−	ccDEe	kk	+	+	+	−		
II-9	I-1, 2	M	50	variant	2-1	2-1			A_1	NS	+	CcDee	kk	+	+	+	−		
II-10		F	44	N	1-1	2-2			0	MNs	−	ccdee	Kk	+	−	−	+		
II-11	I-1, 2	M	48	N	1-1	2-2			A_1	MS	+	CcDee	kk	+	+	−	+		
II-13	I-1, 2	M	36	N	1-1	2-1			A_1	NS	+	CcDee	kk	+	+	+	−		
III-1	II-1, 2	M	36	N	2-1	2-1	CB	2-1	A_1	MSs	−	CCDee	kk	+	−	+	−	−	+
III-3	II-1, 2	M	33	N	2-1	2-1	B		A_1	MSs	−	CcDee	kk	+	−	−	−		
III-5	II-1, 2	F	31	N	2-1	2-1	CB		0	MNSs	−	CCDee	kk	+	−	−	−		
III-7	II-1, 2	M	28	N	2-2	2-1	CB		B	MSs	−	CCDee	kk	+	+	−	+	+	+
III-9	II-1, 2	M	26	N	2-1	2-1	CB	2-1	B	MSs	−	ccdee	kk	+	−	−	+	+	+
III-11	II-1, 2	M	23	N	2-1	2-1	CB	1-1	0	NSs	−	CcDee	kk	+	+	−	+	+	+
III-13	II-3, 4	M	35	N	1-1	2-1	B		0	MNSs	−	ccDEe	Kk	+	+	−	+		
III-15	II-3, 4	F	33	variant	2-1	1-1	B		A_1	NSs	−	CcDee	Kk	+	+	+	−		
III-16		M	39	N	1-1	2-1	B		0	MNs	−	CcDee	kk	+	+	−	+		
III-17	II-3, 4	F	32	variant	2-1	2-1	BA		A_1	MNSs	+	CcDEe	Kk	+	+	+	−		
III-20		M	37	N	1-1				A_1	MNSs	+	ccDEe	kk	+	+	−	+		
III-21	II-3, 4	F	30	variant	2-1	2-1	B		A_1	MNSs	−	ccDEe	kk	+	+	−	+		
III-22		M	33	N	2-2	2-1	BA		A_1	MNSs	+	ccdee	kk	+	+	+	−		
III-23	II-3, 4	M	25	N	1-1	1-1	BA		0	NSs	−	ccdee	Kk	+	+	−	+		
III-26	II-5, 6	M	39	N	1-1	2-2	B*		A_2*	MNSs	+	ccdee	Kk	+	+	+	−		
III-28	II-5, 6	F	37	variant	2-1	2-1	CA		A_1	NSs	−	ccdee	Kk	+	+	+	−		
III-30	II-5, 6	F	36	N	1-1	2-1	BA		0	NSs	+	ccdee	Kk	+	−	+	−		
III-32	II-5, 6	M	33	N	1-1		CB		0	MNSs	+	CcDee	kk	+	+	+	−		
III-34	II-5, 6	F	32	variant	2-1	1-1	BA		B	MNSs	+	ccdee	kk	+	+	+	−		

*Exclusion.

APPENDIX 1 (Continued)

III-35		M	33	N	2-1	2-1	B	A_1	Ms	+	CCdee	kk	+	+	+	−
III-36	II-5, 6	F	31	N	1-1	2-1	A	A_1	NSs	−	CcDee	kk	+	+	−	−
III-38	II-7, 8	M	17	N	1-1	2-1		A_1B	MNSs	−	ccdee	kk	+	+	+	−
III-39	II-9, 10	F	26	variant	2-1	2-1		A_1	NSs	−	ccdee	Kk	+	−	+	−
III-40	II-9, 10	M	24	N	1-1	2-2		A_1	NSs	+	ccdee	kk	+	−	+	−
III-41	II-9, 10	M	20	variant	2-1	2-2		A_1	NSs	−	ccdee	kk	+	+	+	−
III-42	II-9, 10	F	16	variant	2-1	2-1		A_1	NSs	−	CcDee	kk	+	−	−	+
IV-14	III-15, 16	M	12	N	1-1	1-1	B	0	NSs	−	CcDee	Kk	−	+	−	+
IV-15	III-15, 16	F	11	variant	2-1	1-1	B	A_1	NSs	−	CcDee	Kk	+	−	−	+
IV-16	III-15, 16	F	7	N	1-1	2-1	B	0	Ns	−	ccdee	Kk	+	+	−	+
IV-17	III-17, 18	F	16	N	1-1	1-1	B	0	MNSs	−	ccDEE	Kk	+	+	+	−
IV-18	III-17, 19	F	14	N	2-1	2-1	B	A_1	MNSs	+	CCDee	kk	−	+	−	+
IV-19	III-17, 20	M	11	N	1-1	1-1	BA	A_1	MNSs	+	CcDEe	Kk	+	+	+	−
IV-20	III-21, 22	M	10	variant	2-2	2-1	BA	A_1	MNSs	+	ccdee	kk	+	+	+	−
IV-21	III-21, 22	M	7	variant	2-2	2-1	BA	A_1	MNSs	+	ccdee	kk	+	+	+	−
IV-22	III-21, 22	M	5	variant	2-2	2-1	B	A_1	MNSs	+	ccDEe	kk	+	+	−	+
IV-23	III-21, 22	F	3	N	2-1	1-1	B	A_1	Ns	+	ccdee	kk	+	+	−	+
IV-29	III-28, 29	F	15	variant	2-2	1-1	CB	A_1	MNSs	+	ccdee	kk	+	−	−	−
IV-38	III-34, 35	M	12	variant	2-1	1-1	B	A_2B	MSs	+	CcDee	kk	−	+	+	−
IV-39	III-34, 35	F	11	variant	2-1	1-1	B	A_1B	MSs	+	CcDee	kk	−	+	+	−
IV-40	III-34, 35	M	9	N	2-1	1-1	BA	A_2	MNs	+	CcDee	kk	+	+	+	−
IV-41	III-34, 35	M	8	variant	2-1		BA	A_2	MNs	+	CcDee	kk	+	+	+	−
IV-42	III-34, 35	F	6	variant	2-2		BA	A_2	MNs	+	CcDee	kk	+	+	+	−

DATA ON FAMILY 2 (KINDRED 9467)

Individual number	Parents	Sex	Age	Serum types			Red cell enzyme types		Blood types									Secretor types	
				Alb	Gc	Hp	Acid phos.	PGM	ABO	MNSs	P	CDE	Kell	Fy^a	Jk^a	Lewis a	Lewis b	ABH	Le^a
II-2		F	47	N	1-1	1-1			A_1	Ns	+	CCDee	kk	+	+	−	−		
III-1	II-1, 2	F	28	N	1-1	1-1			A_1	MNs	+	CCDee	kk	−	+	−	−		
III-3	II-1, 2	M	26	N	1-1	2-1			A_1	MNs	+	CcDee	kk	−	+	−	+		
III-5	II-1, 2	M	24	variant	1-1	2-1			A_1	MNSs	+	CcDee	kk	−	+	−	+		

DATA ON FAMILY 3 (KINDRED 9396)

Individual number	Parents	Sex	Age	Serum types			Red cell enzyme types		Blood types										Secretor types	
				Alb	Gc	Hp	Acid phos.	PGM	ABO	MNSs	P	CDE	Kell	Fy^a	Jk^a	Lewis a	Lewis b	ABH	Le^a
I-2		M	71	N	1-1	2-1	A	2-1	0	NSs	+	ccDEe	kk	−	+	−	+	+	+
II-1	I-1, 2	M	49	variant	2-1	2-1	BA	1-1	A_1	MNSs	+	ccDEe	kk	+	+	−	+	+	+
II-2		F	49	N	1-1	2-2		1-1	0	MNSs	−	$CcD^{u}ee$	kk	−	−	−	+	+	+
II-3	I-1, 2	M	47	variant	2-1	2-2	CA		0	MNS	+	ccDEe	kk	+	+	+	−	−	+
II-4		F		N	1-1	2-2	BA		0	Ns	+	CcDEe	kk	+	−	−	+	+	+
II-6	I-1, 2	F	44	variant	2-1	2-1	CA	2-1	A_1	MNS	+	ccDEE	kk	+	+	−	+	+	+
II-9	I-1, 2	F	42	variant	2-1	2-1	BA		0	MNSs	+	ccDEe	kk	−	+	+	−	−	+
II-14	I-1, 2	F	34	N	1-1	1-1	BA	1-1	0	MNS	+	ccDEE	kk	−	+	−	+	+	+
III-1	II-1, 2	F	29	N	1-1	2-2	BA		A_1	MNSs	−	ccdee	kk	+	+	−	+	+	+
III-4	II-1, 2	F	28	N	1-1	2-1	BA		0	Ns	−	$CcD^{u}ee$	kk	+	+	+	−	−	+
III-7	II-1, 2	M	25	variant	2-1	2-1	CB	1-1	A_1	Ns	+	ccdee	kk	−	+	−	+	+	+
III-8		F	22	N	2-1	2-2	B	2-1	0	MSs	−	CcDee	kk	−	+	−	+	+	+
III-9	II-1, 2	F	22	variant	2-1	2-1	B		0	Ns	−	ccdee	kk	−	+	−	+		
III-11	II-1, 2	M	19	N	1-1	2-1	B		A_1	MNSs	+	ccDEe	kk	+	+	+	−		
III-13	II-1, 2	F	18	variant	2-1	2-1	CB	1-1	A_1	Ns	−	$CcD^{u}ee$	kk	+	+	−	+	+	+
III-15	II-1, 2	F	13	N	1-1	2-2	BA	1-1	A_1	MNSs	−	CcDEe	kk	+	+	−	−	−	+
III-18	II-3, 4	F	25	variant	2-1	2-2	CB		0	MNSs	+	ccDEE	kk	+	−	+	−	−	+
III-19	II-3, 4	M	23	variant	2-1	2-2	CA		0	NSs	+	ccDEe	kk	−	−	+	−	−	+
III-20		F		N	1-1	2-2	BA		A_2	MNs	+	ccDEe	kk	+	+	−	+	+	+
III-21	II-3, 4	M	21	variant	2-1	2-2	BA		0	MNSs	+	CcDee	kk	+	+	−	−	+	+

III-23	II-3, 4	F	18	variant	2-1	2-2	A		0	MNSs	+	ccDEe	kk	+	+	−	+	+	+
III-25	II-3, 4	F	15	N	1-1	2-2	A		0	NSs	+	CcDee	kk	+	+	+	−	−	+
III-26	II-3, 4	F	13	variant	2-1	2-2	BA		0	MNSs	+	CcDEe	kk	−	−	−	+	−	+
III-27	II-3, 4	F	12	N	1-1	2-2	A		0	MNSs	+	CcDee	kk	−	+	−	+		
III-28	II-5, 6	F	26	variant	2-2	2-1	BA	1-1	A_1	MSs	+	ccDEe	kk	−	+	−	+	+	+
III-29		M	26	N	1-1	2-2	BA	2-1	0	MNSs	−	CcDee	kk	−	+	−	+	+	+
III-30	II-5, 6	M	21	variant	2-2	1-1	BA	2-1	0	MSs	+	ccDEe	kk	−	+	−	+	+	+
III-31	II-5, 6	M	20	variant	2-2	2-1	A	1-1	A_1	MNSs	+	ccDEe	kk	+	+	−	+	+	+
III-35	II-9, 10	F		N	2-1	2-2	BA		B	MNSs	+	ccdee	kk	+	+	−	+	+	+
III-37	II-9, 10	F	19	variant	2-2	2-2	BA	1-1	B	MNSs	+	ccDEe	kk	+	+	−	−	+	+
IV-9	III-7, 8	F	5	variant	2-2	2-1	B	1-1	A_1	MNSs	+	CcDee	kk	−	+	−	+	+	+
IV-10	III-7, 8	M	3	N	1-1	2-1	B	1-1	A_1	MNSs	−	ccdee	kk	−	+	−	+	+	+
IV-12	III-9, 10	M	5	N	1-1	2-2			0	Ns	−	ccDEe	kk	−	+	−	+		
IV-13	III-9, 10	F	3	N	1-1	2-2			0	Ns	−	ccdee	kk	−	+	−	+		
IV-14	III-19, 20	F	1	N	1-1	2-2	CA		0	MNSs	+	ccDEe	kk	−	−	−	+		
IV-15	III-28, 29	M	8	variant	2-1	2-2	B	1-1	A_1	MSs	+	CcDEe	kk	−	+	−	+	+	+
IV-16	III-28, 29	F	7	variant	2-1	2-2	BA	1-1	0	MNS	+	CcDEe	kk	−	+	−	+	+	+
IV-17	III-28, 29	F	5	variant	2-1	2-2	BA	2-1	0	Ms	+	CcDEe	kk	−	+	+	−	−	+
IV-18	III-28, 29	F	2	variant	2-1	2-2			A		+		kk	−					
IV-19	III-31, 32	M	2 mo.	N	2-1	2-1													
IV-25	III-37, 38	F	2	N	2-1	2-1	B	1-1	B	MNs	+	ccDEE	Kk	+	+	+	−		
IV-26	III-37, 38	F	7 mo.	variant	2-1	2-1	BA	1-1	A_2	MNs	+	ccDEE	kk	+	+	+	−		

All individuals tested were transferrin type C, positive for Kp^b, and negative for Kp^a, M^g, Vw, and Wr^a.
All P-negative individuals were Tj^a-positive.