163. *Studies on Lewis Blood Group System. II. Distribution and Heredity of Le^c Blood Group Factor*

By Shoei ISEKI, Shinju MASAKI, and Kunitake SHIBASAKI

Department of Legal Medicine, School of Medicine,
Gunma University, Maebashi, Japan

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Mourant¹⁾ discovered Lewis blood group factor, which was reported to be a hereditary character, having the frequency of 25%. According to Andresen,²⁾ the frequency of this factor is 21.17%, and its character is recessive, though this is not generally true for children under the age of about eighteen months. From later reports, the frequency of Lewis blood group factor is viewed to be near 22% for Europeans. Also for Japanese it is reported to be about 22%.³⁾ Then Andresen⁴⁾ found another antibody related to this blood group system. Accordingly, the antibody found by Mourant is designated as anti-Le^a antibody, and that by Andresen as anti-Le^b antibody. Andresen⁴⁾ investigated 238 persons of group O with these two antibodies, and obtained the result that 46 were Le(a+b-), 178 Le(a-b+), and 14 Le(a-b-). The later obtained frequencies of Le(a-b-) group for Europeans of group O ranged 4-10%. On the other hand, Grubb,⁵⁾ and Grubb and Morgan⁶⁾ reported that those with Le(a+) belonged to non-secretors and that those with Le(a-) belonged almost always to secretors with only a few exceptions to the non-secretors. It is Schiff and Sasaki^{7,8)} who for the first time found the distinction between the secretor and the non-secretor; the former secretes the great amount of blood group substance in the saliva, but the latter does not. And the non-secretor character is known to be inherited recessively. It is therefore very natural that the Le^a blood group factor is also recessive in character, since this is found in non-secretors. Now, those of Le(a+b-) group are generally known to be non-secretors, those of Le(a-b+) group secretors, and those of Le(a-b-) group mostly secretors, but rarely non-secretors. And further, in the red cells of Le(a-b-) group, the presence of Le^c blood group factor is presumed. Besides this presumption various hypotheses have been established concerning the heredity of the Lewis blood group system. Among others Pettenkofer^{9,10)} advanced a view that the gene Le^b is dominant against the gene Le^a, and that the gene Le^c is dominant against the genes Le^b and Le^a. We reported in our previous paper¹¹⁾ on the presence of Le^c blood group factor. In the present paper we report on the distribution and heredity of the Lewis blood group factors.

Materials and methods. 1) As Lewis antibodies, anti-Le^a, anti-Le^b, and anti-Le^c antibodies in rabbit sera immunized with human saliva were used. 2) For the determination of the Lewis blood group, papain-treated red cells were subjected to agglutination test, and for the examination for Lewis blood group substances in saliva, it was heated at 100°C for 30 minutes, and, after dilution, subjected to an agglutination inhibition test against each Lewis antibody.

Results. 1. The distribution of the Lewis blood group factors. Of 485 persons investigated, Le(a+b-c-) occupied 107(22.06%), Le(a-b+c-) 369(76.08%), and Le(a-b-c+) 9(1.86%). Sucklings, in whom Le^a substance is abundant, but in whom Le^b and Le^c substances have not yet perfectly developed, were excluded. As for the ABO, the MN, or the Q blood group system, no special relationship was observed between these and the Lewis blood group system as shown in Table I.

Table I. Distribution of Lewis blood group

Blood groups and saliva types		Le(a+b-c-)		Le(a-b+c-)		Le(a-b-c+)		Total
		No.	%	No.	%	No.	%	
	O	37	26.24	99	70.21	5	3.55	141
	A	45	22.73	150	75.75	3	1.52	198
	B	18	18.37	79	80.61	1	1.02	98
	AB	7	14.58	41	85.42	0	0	48
Blood groups	M	22	17.74	99	79.84	3	2.41	124
	N	14	16.28	71	82.56	1	1.16	86
	MN	71	25.82	199	72.36	5	1.82	275
	Q	22	17.46	100	79.37	4	3.17	126
	q	85	23.68	269	74.93	5	1.39	359
Saliva types	St	0	0	369	97.88	8	2.12	377
	sT	107	99.07	0	0	1	0.93	108
Total		107	22.06	369	76.08	9	1.86	485

However, those of Le(a+b-c-) group belonged to non-secretors (sT), those of Le(a-b+c-) group belonged to secretors (St), and those of Le(a-b-c+) group belonged to secretors except only one to non-secretor.

2. Heredity of Lewis blood group factors. Pedigrees concerning Lewis blood group, type of saliva, and Lewis blood group substance in the saliva are shown in the following figure.

In the family of T., from Le(a+b-c-) father and Le(a-b-c+) mother were born one Le(a-b-c+) child and two Le(a-b+c-) children, and in each of the families of S. and H., from Le(a-b-c+) father and Le(a+b-c-) mother were born two Le(a-b-c+) children

and one Le(a-b+c-) child. Since the gene Le^a is recessive, one of the parents in each of these three families must have the genotype Le^aLe^a, and consequently the gene Le^a must have exactly been inherited by all the children. Therefore the genotype of the Le(a-b-c+) child is considered to be Le^aLe^c. Further, in these families there are Le(a-b+c-) children, too. Since their genotype is considered to be Le^aLe^b, one of each parents must have the gene Le^b. Therefore the genotype of the mother of family T. and the fathers of families S. and H., all of whom have the phenotype Le(a-b-c+), is considered to be Le^bLe^c.

In this way, the comparison of the phenotypes and genotypes of the Lewis blood group in the above results demonstrates that the gene Le^c is dominant against the genes Le^b and Le^a, and the gene Le^b is dominant against the gene Le^a.

In each of the families of A. and K., from Le(a-b+c-) father and Le(a-b-c+) mother were born two Le(a-b-c+) children. It

is difficult to estimate the genotype for the members of these families, but nevertheless the dominance of the gene Le^c against the genes Le^b and Le^a can be presumed.

As to the relation between the Lewis blood group substances in the saliva and the genotypes of Lewis blood group, we found that in those of $Le(a+b-c-)$ group, whose genotype is considered to be Le^aLe^a , only Le^a substance was demonstrated in the saliva. As the genotype for $Le(a-b+c-)$, either Le^aLe^b or Le^bLe^b is considered. The two sons of family T., the daughter of family S., and the daughter of family H. are assumed to have the genotype Le^aLe^b , both Le^a and Le^b substances being demonstrated in their saliva. However, when anti- Le^a immune agglutinin is used for agglutination inhibition test, it appears that Le^a substance is always demonstrated, though slightly, in the saliva of those of the genotype Le^bLe^b . It is therefore difficult to determine the genotype by the secretion of Le^a substance in the saliva.

As the genotypes for $Le(a-b-c+)$, Le^aLe^c , Le^bLe^c , and Le^cLe^c are considered. The daughter of family T., the two sons of family S., and the two sons of family H. are all considered to have the genotype Le^aLe^c . However, while Le^c and Le^a substances were demonstrated in the saliva of the two sons of family S., Le^c was demonstrated besides Le^c in the saliva of the daughter of family T., and Le^a and a minute amount of Le^b were demonstrated besides Le^c in the saliva of the two sons of family H. Further, the mother of family T. and the fathers of families S. and H. are all considered to have the genotype Le^bLe^c . However, in the saliva of the mother of family T. and the father of family H., Le^c and Le^b substances were demonstrated, while in the saliva of the father of family S., Le^c and Le^a substances were demonstrated. It seems, therefore, difficult to distinguish the genotype by the secretion of Le^b substance in the saliva. No cases were observed whose genotype was confirmed to be Le^cLe^c .

The Lewis blood group of the mother of family K. was $Le(a-b-c+)$, and she belonged to the non-secretor type, Le^a substance being demonstrated besides Le^c in the saliva, though slightly when compared with the case of $Le(a+b-c-)$ group.

The above-described results of the family investigation on the Lewis blood group substances in red cells and saliva, and the fact that, in the suckling, Le^a substance is more developed than Le^b and Le^c lead us to presume that Le^a substance in the Lewis blood group system, similarly as O(H) substance in the ABO system, may be the basal substance, from which Le^b and further Le^c substance would be developed by the action of the gene.

3. Gene frequencies of Lewis blood group system. The gene Le^b

is dominant against the gene Le^a , and the gene Le^c is dominant against the genes Le^b and Le^a . On the ground of this assumption, the gene frequencies were calculated from the results of mass investigation, and the following values were obtained:

$$\begin{aligned} Le^a &= \sqrt{Le(a+b-c-)} = \sqrt{0.2206} = 0.4697 \\ Le^b &= \sqrt{Le(a+b-c-) + Le(a-b+c-) - \sqrt{Le(a+b-c-)}} \\ &= \sqrt{0.2206 + 0.7608 - \sqrt{0.2206}} = 0.5210 \\ Le^c &= \frac{Le(a-b-c+)}{1 + \sqrt{Le(a+b-c-) + Le(a-b+c-)}} \\ &= \frac{0.0186}{1 + \sqrt{0.2206 + 0.7608}} = 0.0093 \\ Le^a + Le^b + Le^c &= 1 \end{aligned}$$

The relation between the frequencies of the genotypes and the phenotypes, calculated from the above gene frequencies is shown in Table II.

Table II. Relation between the frequencies of genotypes and phenotypes

Genotypes	Gene frequencies	Phenotypes	Frequencies
$Le^a Le^a$	0.2206	$Le(a+b-c-)$	22.06%
$Le^a Le^b$ $Le^b Le^b$	0.4894 0.2714	} $Le(a-b+c-)$	76.08%
$Le^a Le^c$ $Le^b Le^c$ $Le^c Le^c$	0.0088 0.0097 0.0001		

Summary. Investigation of the Lewis blood group was carried out on 485 persons with anti- Le^a , anti- Le^b , and anti- Le^c antibodies which were obtained by immunizing the rabbit with human saliva, with the following results: $Le(a+b-c-)$ cases numbered 107(22.06%), $Le(a-b+c-)$ cases 369(76.08%), and $Le(a-b-c+)$ cases 9(1.86%). $Le(a+b-c-)$ cases belonged to non-secretors, and $Le(a-b+c-)$ cases secretors, whereas $Le(a-b-c+)$ cases belonged to secretors except one non-secretor.

The results of investigation of families containing $Le(a-b-c+)$ members indicated that the gene Le^b is dominant against the gene Le^a , and that the gene Le^c is dominant against the genes Le^b and Le^a . Consequently, the genotype for $Le(a+b-c-)$ is considered to be $Le^a Le^a$, and as the genotypes for $Le(a-b+c-)$, $Le^a Le^b$, and $Le^b Le^b$, and for $Le(a-b-c+)$, $Le^a Le^c$, $Le^b Le^c$, and $Le^c Le^c$ can be distinguished. The frequency of each gene for Japanese was as follows: Le^a , 0.4697; Le^b , 0.5210; and Le^c , 0.0093.

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