Alloantigens on Plasma Proteins

POLYMORPHISMS OF IMMUNOGLOBULINS: Gm, Am and Km TYPING

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Allotypes of immunoglobulins form the most polymorphic genetic system of human serum proteins. Allotypes have been shown on the heavy chains of IgG1, IgG2, IgG3, IgA2 and IgE and on kappa light chains, which are coded by the $\gamma 1$, $\gamma 2$, $\gamma 3$, $\alpha 2$, ε and κ genes respectively. They are called G1m, G2m, G3m, A2m, Em and Km allotypes (Table 1). Most allotypes have originated from the genes by mutation of only one or a few nucleotides resulting in the difference of one or two amino acids. Until now 24 allotypes have been described: four G1m, one G2m, thirteen G3m, two A2m, one Em and three Km allotypes (ref 1).

G1m

The four allotypes that may be present on the $\gamma 1$ chains of IgG1 molecules are G1m(z), G1m(a), G1m(x) and G1m(f). A maximum of three of these allotypes may be present on the same chain because G1m(z) and G1m(f) are mutually exclusive. Therefore these two allotypes cannot be present on the same chain. The four main combinations of allotypes or so called allelic forms, in which the $\gamma 1$ gene occurs, are: $G1m^{za}$, $G1m^{zax}$, $G1m^{f}$ and $G1m^{fa}$. The $G1m^{f}$ allele is found in Caucasians only, whereas $G1m^{fa}$ is a typical Oriental allele.

G2m

Because the G2m(n) allotype is up to now the only detectable allotype on $\gamma 2$ chains, the $\gamma 2$ gene will occur in the two alleles $G2m^{n}$ and G2m'' or $G2m^{n+}$ and $G2m^{n-}$. If a serum is G2m(n) positive, it can be homozygous, that is $G2m^{n}/G2m^{n}$, or heterozygous, that is $G2m^{n}/G2m''$.

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G3m

The 13 G3m allotypes give rise to a large number of IgG3 alleles, of which the main ones are: $G3m^{g1, g5, u, v}$, $G3m^{b0, b1, b3, b4, b5, u, v}$, $G3m^{b0, b1, c3, c5, u}$, $G3m^{b0, b1, c3, b4, b5, u, v}$, $G3m^{b0, b3, b5, s, v}$ and $G3m^{b0, b3, b5, s, t, v}$. (Table 2)

A shortened notation of these alleles can be used and is called respectively $G3m^{g}$, $G3m^{b}$, $G3m^{c3\ c5}$, $G3m^{c3}$, $G3m^{s}$ and $G3m^{st}$. In Caucasians $G3m^{g}$ and $G3m^{b}$ are the most frequent alleles. $G3m^{c3c5}$, $G3m^{c3}$ and $G3m^{s}$ are typical negroid alleles, although $G3m^{b}$ is frequent in Blacks, too. $G3m^{st}$ is shown to be present in Orientals and in Eskimos.

A2m

The IgA2 allotypes, named A2m(1) and A2m(2), form in practice a two-allelic system encode by the $\alpha 2$ gene. The occurence of the alleles $A2m^{1}$ and $A2m^{2}$ varies a lot between and withing most races (Table 3). As the $A2m^{2}$ allele is rare in Caucasians, the A2m system is not very informative in Whites. In most of the other populations the A2m system is much more informative.

Em

The only IgE allotype discovered, is named Em(1), and has been described by Van Loghem et al in 1984 (ref. 2). Typing for the allotype is not very informative, because it is very common in all races.

Km

Three allotypes of K light chains have been described, named Km(1), Km(2), and Km(3). Before the WHO meeting of 1974, these allotypes were called Inv(1,a,b) or Inv(1,2,3). (ref. 3)

The Km allotypes occur mainly in three alleles: $Km^{1,2}$, Km^{1} and Km^{3}. Km^{3} is the most frequent one in almost all populations (ref. 4). Km^{1} is very rare. Km(1) positive samples almost always are Km(2) positive as well. Therefore Km(2) typing in addition to typing for Km(1) and Km(3) does not give much additional information.

Nomenclature

In 1974 an international exchange of Ig allotype reagents took place followed by a WHO meeting in Rouen, France. At this meeting the members

agreed to accept an alphameric as well as a numeric nomenclature (table 1) (ref. 3). A disadvantage of the numeric nomenclature is the extensive and therefore complicated way of expressing the numbers, if there has been typed for more than a few allotypes. Another disadvantage is the fact that for some allotypes two different numbers are still in use for the same allotype, although it has been agreed in 1974 to use the lowest number. More than 10 years later, we still have the situation that the "American" G1m(3) is equal to the "German" G1m(4).

Gm-Am-Em haplotypes

The different heavy chain genes of Ig are linked because they are located close to each other on chromosome 14. Therefore the G1m, G2m, G3m, A2m and Em alleles are inherited in haplotypes, with a low number of crossing-overs within this region. Knowledge of the haplotypes has been achieved by familystudies and extensive population studies. The genotypes, which consists of the two haplotypes, can be deduced from the phenotype by familystudy.

An example of a pedigree of a family with their Gm-Am phenotypes and deduced genotypes is shown in figure 1. For example, it can be deduced that the propositus, marked with an arrow, is homozygous $G2m^{n}$, because both parents are G2m(n) positive and his eldest sister is G2m(n) negative. The same way it can be deduced that the eldest daughter, with phenotype zax;..;g;1,2, has two different G1m alleles, namely $G1m^{za}$ and $G1m^{zax}$.

By population studies it has been shown that some haplotypes are characteristic for a particular race although other haplotypes occur in lower frequencies (Table 3). In Blacks and Orientals G1m(a) acts as an isotypic marker because it is present in all individuals. However in Whites the frequency of G1m(a) positive haplotypes is lower than that of G1m(a) negative ones. On the other hand G1m(f) has never been found in Blacks.

Determination of Ig allotypes

The commonly used method for the detection of Ig allotypes is the haemagglutination inhibition (HAI) test. Human erytrocytes coated with allotype positive Ig are used in this assay with anti-allotype specific

antibodies. By addition of a serum sample to the anti-allotype antiserum followed by the Ig coated erythrocytes, the agglutination of the antiserum with the coated cells will be inhibited, in cases where the test sample contains the allotype. If the test sample does not contain that particular allotype, the antiserum will agglutinate the erytrocytes (Table 4). The method has been improved by using microtitre plates with V-shape bottom and thin cells suspensions. The sensitivity of the assay could be increased in a way that less test sample and less or weaker antiserum can be used.

Usefulness of Ig allotyping in paternity and other forensic analysis

Because the Gm system is a very polymorphic one, it is very useful in forensic medicine (table 5). The G1m, G2m, G3m and A2m genes are closely located on chromosome 14 and therefore these alleles are inherited as haplotypes. The Km allotypes can be determined with the haemagglutination inhibition method in the same way, but these allotypes are inherited independently of the other allotypes as the κ gene is located on chromosome 2. An advantage for bloodstain analysis is, that immunoglobulins are very strong. Several years old bloodstains can be used for Ig allotyping by dissolving of the stain in saline and using this solution in the HAI assay.

Pitfalls of Ig allotyping

As in most typing systems there are pitfalls to be watched. This can be due to technical errors, but also wrong conclusions can be drawn, due to misinterpretation of the typing results. To prevent the first type of errors it is necessary to check the typing system extensively, with typing reference sera and to determine the right dilution of each antiserum (table 5).

It is also necessary to test the samples in more than one dilution. It is possible that a testserum contains agglutinating antibodies against the Ig coated cells. This has to be checked by incubation of the same dilutions of the testsample with the coated cells. To get rid of these disturbing antibodies the serum can be heated for 10 minutes at 65°C or has to be absorbed with the coated cells before retyping.

Wrongly drawn conclusions can be prevented by typing more than just a few Gm allotypes. As, for example, G1m(z) and G1m(f) are mutually exclusive it is very important to type for both, instead of typing for G1m(a) and G1m(f). When G1m(a) and G1m(f) are present it is possible that these allotypes are located on the same molecule.
Another wrongly drawn conclusion can be made after typing of very young children. Children under the age of one year, still possess remnants of maternal IgG, which can inhibit in the assay. In those cases it is advisable to titrate the serumsample, to determine the amount of the different allotypes.

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Table 2 SHORTENED NOTATION OF THE MAIN G3m ALLELES WITH THE DISTRIBUTION OF THE G3m ALLOTYPES ON γ3Fc

G3m alleles	G3m allotypes							
	CH2 domain				CH3 domain			
g	g1	-	-	u	-	-	g5	v
b	-	b1	b4	u	b0	b3	b5	v
c3c5	-	b1	-	u	b0	c3	-	c5
c3	-	b1	b4	u	b0	c3	b5	v
s	-	-	-	s	b0	b3	b5	v
st	-	-	-	st	b0	b3	b5	v

Table 1

ALLOTYPES OF HUMAN IMMUNOGLOBULINS

Light chains	Heavy chains				
κ	γ1	γ2	γ3	α2	ε
Km	G1m	G2m	G3m	A2m	Em
1	z	n	g1,g5	1	1
2	a		b0,b1	2	
3	x		b3,b4		
	f		b5,		
			s,t		
			c3,c5		
			u,v		

Numerical nomenclature for Gm:

G1m	G2m	G3m		
1=a	23=n	5=b1	14=b4	24=c5
2=x		6=c3	15=s	26=u
3=f		10=b5	16=t	27=v
17=z		11=b0	21=g1	28=g5
		13=b3		

Table 3

FREQUENCIES OF Gm HAPLOTYPES AND A2m GENES IN REPRESENTATIVES OF FIVE CONTINENTS

G1m;G2m;G3m	AFRICA Yorub.	AMERICA Trio	ASIA Taiwan	AUSTRALIA Balimo	EUROPE the Neth
f;n;b	-	-	-	-	0.450
f;..;b	-	-	-	-	0.249
za;..;g	0.007	0.627	0.201	0.091	0.187
zax;..;g	-	0.338	0.043	-	0.098
za;..;b	0.678	-	-	0.164	0.004
za;..;bob1c3c5u	0.203	-	-	-	-
za;..;bob1b4b5c3uv	0.061	-	-	-	-
za;..;bob3b5sv	0.051	-	-	-	-
za;n;b	-	-	-	0.745	-
za;..;bob3b5stv	-	0.035	-	-	-
fa;n;b	-	-	0.755	-	-
others	-	-	0.001	-	0.012
A2m 1	0.174	0.960	0.248	0.646	0.982
A2m 2	0.826	0.040	0.752	0.354	0.018

Fig.1

Pedigree of a family with their Gm–Am phenotypes and deduced genotypes

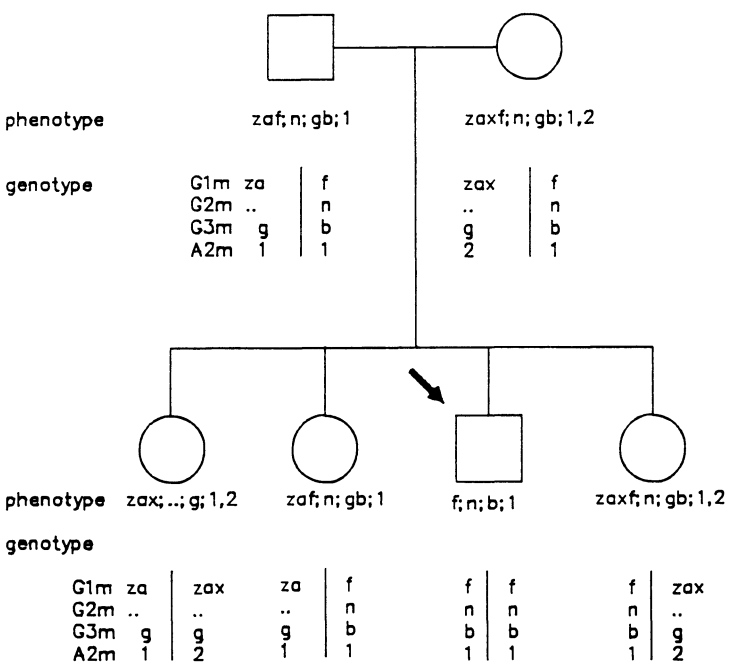

Table 4

TYPING FOR Gm, Am AND Km ALLOTYPES BY HAI TEST

1. Ig coated erythrocytes
   - for G1m and G3m typing: O R2R2 cells coated with selected anti-Rh antibodies
   - for G2m and A2m typing: O cells coated with selected IgG2 or IgA2 myeloma protein by CrCl3

2. Specific anti-Ig allotype antibodies

	advantages	disadvantages
- human antisera	specific	limited, weak
- polyclonal animal antisera	higher titres	pure imm.ag, abs
- mouse monoclonal antibodies	unlimited high titres	labour intensive

3. Reference sera
   - completely allotyped control sera, which discriminate between the allotypes under investigation

4. Test sample
   - serum, plasma, bloodstain or Ig preparation diluted in saline

Table 5

TYPING FOR Ig ALLOTYPES IS VERY USEFUL IN PATERNITY AND BLOODSTAIN ANALYSIS,

because

- the system is very polymorphic
- G1m - G2m - G3m - A2m form haplotypes
- Gm and Km are independently inherited, as their genes are located on different chromosomes
- Ig molecules are strong

WRONGLY DRAWN CONCLUSIONS CAN BE PREVENTED

by

- determining the right dilution of the antisera
- checking the typing system with reference sera
- testing the samples in more than one dilution
- typing for more than just a few Gm allotypes