It is worthwhile filling in the remaining blank spots for blood group antigen frequencies

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In the middle of the 20th century, shortly after the detection of the Rhesus blood group system, numerous reports were published on the distribution of Rh antigens in various regions and in distinct populations. Mourant *et al*.[1] initially collated these reports and edited the data in large tables which gave a comprehensive overview on the distribution of the Rh antigens throughout the world, based on the techniques available at that time. Although hundreds of studies were performed and analysed, many blank spots regarding Rhesus blood groups remained on the geographical map. Contributions such as the study by Makroo and colleagues in this issue of Blood Transfusion[2] help to fill in these blank spots. Makroo *et al*. investigated more than 50,000 blood donors in a major tertiary care hospital in New Delhi in North India, and reported the Rh phaenotypes and calculated haplotype frequencies. Knowledge of the distribution of blood groups in a region allows the identification of rare phenotypes and provides valuable information for blood services organising donor recruitment and managing inventories.

According to early studies in Caucasian populations, up to 85% of D-negative recipients will produce an anti-D in response to transfusion with D-positive red blood cells[3]. The presence of an anti-D means that subsequent D-positive transfusions should not be given, even in the case of emergency. In addition, pregnant women with anti-D and their foetuses are at risk of developing haemolytic disease of the foetus and newborn. Thus, considerable effort is expended to supply D-negative patients with D-negative units. Blood services carefully manage their stock of D-negative blood units. D-negative blood is a rare and sought after phenotype, even in Caucasian populations in which up to 18% of donors are D negative.

The frequency of the D-negative phenotype differs significantly between populations. D-negative frequencies of about 29% were documented among Basques and in distinct populations living in the High Atlas Range of Morocco (Table I), which have the highest reported prevalence of D-negative phenotypes. Geographic or cultural isolation of these populations may have led to the positive selection of the D-negative phenotype, although some uncertainty about a statistical bias remains, because some of these studies encompassed fewer than 500 individuals. Furthermore, data obtained using the serological techniques of the 1960s may be not directly comparable with those obtained by more recent techniques. In large studies investigating other European populations, the frequency of D-negative phenotypes ranged from 11% in Greece up to 18% in England. Generally, Caucasian populations have the highest prevalence of D-negative individuals. Some of the studies investigated large donor populations, thereby eliminating the statistical bias of small study groups. In one of the largest, modern studies, Wagner *et al*.[4] recognized an excess of ccddee donors, because their frequency did not match the expected frequency based on the cde haplotype. This observation bias might have been caused by enhanced encouragement of D-negative donors[4] and possibly also affects many other donor population studies.

In Africa and Asia the D-negative phenotype is less common. For example, there are reports of a 6% rate of D negatives in Nigeria and only 1% in Madagascar. In India, several studies found prevalence rates of 4-6% and the publication by Makroo *et al*.[2] confirmed these numbers. However, only 0.6% D negatives were found in a recent small study[5] investigating the Kahira ethnic group in Central Eastern India, which underscores the need for additional data on the distribution of blood group phenotypes. In South-East Asia the D-negative phenotype is even rarer than in India. In China, Vietnam, and Indonesia, less than 1% of the population is D-negative.

At a genetic level, the most frequent cause of a D-negative phenotype is deletion of the *RHD* gene[6]. Up to the present many other variations, which cause very low expression or the absence of D antigen on the cell membrane, have been added[7]. The red blood cells of these individuals react negative when tested for D with routine serological methods. Typing with IgG anti-D in the indirect antiglobulin test (IAT) enables the detection of weak D expression[8]. The red blood cells of a few individuals will test D negative even in the IAT, because they express extremely small amounts of D antigen. The presence of D antigen can only be demonstrated by absorption and elution techniques, which enable these cells to be categorised as DEL phenotypes.

It turned out that the genetic background of D-negative phenotypes differs between populations. In

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Table I - Prevalence of the D-negative phenotype in populations.

Region / Population	Study*	Individuals tested	D-neg individuals	
		n	n	%
Europe				
Spain†	Goti Iturriaga 1958	386	110	29
	Goti Iturriaga *et al.* 1965	500	141	28
England	Kopeć, 1970	355,221	65,370	18
Germany	Wagner *et al.*[4]	624,163	124,744	17‡
Czechoslovakia	Vlčkova & Vlček 1962	80,518	13,707	17
France	Levy et al. 1967	92,285	14,688	16
Italy	Zotti et al. 1965	111,369	13,440	12
Greece	Constantoulis & Païdoussis 1958	15,922	1,788	11
Africa				
Morocco§	Messerlin & Lorho 1951	247	71	29
Tunisia	Ranque *et al.* 1961	584	45	7.7
Nigeria	Enosolease & Bazuaye[17]	160,431	9,674	6
Mauritania	Hamed *et al.*[18]	10,116	~584#	5.8
Guinea	Loua *et al.*[19]	59,452	~2,414#	4.1
Congo	Resseler 1963	3,000	122	4.1
Madagascar	Pigache 1969	22,514	240	1.1
Asia				
Turkey	Mizan et al. 1963	44,565	5,669	13
Pakistan	Mian & Farooq[20]	1,632	~147#	9.0#
Iran	Mohallatee & Haghshenas 1969	16,368	1,426	8.7
India, North	Makroo *et al.*[2]	51,857	~3,267#	6.3
India, South	Das *et al.*[21]	150,536	8,225	5.5
India, North	Jolly *et al.* 1969	6,204	225	3.6
China	Li *et al.*[12]	400,253	1,585	0.4
China	Shao *et al.*[13]	41,921	118	0.3
Japan	Furuhata *et al.* 1957	2,097	43	2.1
	Tsuchiya *et al.* 1964	1,633	2	0.12
Vietnam	Tran-Vy & Nguyen-Huynh-Thi-Lien 1963	114,022	21	0.02
Indonesia	Maruna 1959	48,964	0	-
U.S.A.				
White non-Hispanic	Garratty *et al.*[22]	2,215,623	~383,303#	17.3
North American Indian	Garratty *et al.*[22]	19,664	~1,907#	9.7
Hispanic	Garratty *et al.*[22]	259,233	~18,924#	7.3
Black non-Hispanic	Garratty *et al.*[22]	236,050	~16,760#	7.1
Asian	Garratty *et al.*[22]	126,780	~2,155#	1.7

Legend *: studies without references are found in Mourant et al.1; †: Basques; ‡: the observed frequency was 19.99 %. After correction for an observation bias the actual frequency was reported as 17.3%; §: the Ait Moghrad population lives in the High Atlas Range; #: as calculated here, as approximate figures, by using data from the original publication.

most studies more than 98% of D-negative Caucasians were homozygous for the *RHD* gene deletion (Table II). In Sub-Saharan Africa the *RHDψ* allele, containing a nonsense mutation and a 37 base pair duplication, and the hybrid allele *RHD-CE-D*^{s}[9], causing a D-negative phenotype with a weakly reacting C antigen [dubbed (C)ce^{s}], are found in many D-negative subjects. In most individuals these alleles were combined with the *RHD* deletion, which is the most frequent allele in D-negative Africans[10,11]. Due to the high frequency of these alleles, a considerable number of D-negative Africans were homozygous for *RHDψ* or for *RHD-CE-D*^{s}, or carried a combination of both[11].

Among Asians about 80% of individuals with an apparent D-negative phenotype are homozygous for the deletion of *RHD*. About 20% have DEL, in most cases caused by the 1227 G>A substitution (homozygous or in combination with the *RHD* deletion[12,13]).

Table II - Molecular basis of apparent D-negative subjects in populations.

Population	Author	D-negative individuals tested (n)	Weak D		DEL			D negative				
			Hybrid alleles	Other nucleotide changes	Hybrid alleles	1227G>A exchange	Other nucleotide changes	Homozygous for D deletion (D del)	RHDψ	RHD-CE-D^{s} (Phaenotype [C]ces)	Hybrid alleles	Other nucleotide changes
Germany	Flegel *et al.*[23]	46,133 (IAT)	-	-	1 (0.002)	4 (0.009)	42 (0.09)	46,037 (99.8)	14 (0.03)	-	21 (0.05)	14 (0.03)
Austria	Polin *et al.*[24]	23,330	-	6 (0.03)	9 (0.04)	1 (0.004)	58 (0.3)	23,236 (99.6)	3 (0.01)	-	16 (0.07)	1 (0.004)
Germany	Wagner *et al.*[14]	8,437 (IAT)	-	-	-	5 (0.06)	10 (0.1)	8,392 (99.5)	1 (0.01)	1 (0.01)	21* (0.3)	5 (0.07)
Central Europe	Gassner *et al.*[25]	1,700 D-, C/E+ (IAT)	-	4 (0.2)	-	-	15 (0.9)	1,611 (94.8)	-	-	59 (3.5)	11 (0.6)
Tunisia	Moussa *et al.*[26]	448 (no IAT)	-	3 (0.7)	-	-	2 (0.5)	437 (97.5)	2 (0.5)	2 (0.5)	2 (0.5)	-
Congo	Touinssi *et al.*[11]	110 (no IAT)	-	7† (6.4)	-	-	-	59 (53.6)	35‡ (31.8)	6‡ (5.5)	-	-
South Africans	Singleton *et al.*[9]	82+ (IAT)	-	-	-	-	-	15 (18.3)	54 (65.9)	12 (14.6)	-	-
China	Li *et al.*[12]	1,585	69§ (4.4)		3 (0.2)	268 (16.9)	8 (0.5)	1,237§ (78.0)				
China	Ye *et al.*[27]	163 (IAT; Abs./Elution)	-	-	-	-	-	129 (79.1)	-	-	27 (16.6)	7 (4.3)
China	Shao *et al.*[13]	102 (IAT; Abs./Elution)	-	-	-	26 (25.5)	-	64 (62.8)	-	-	9 (8.8)	3 (2.9)
Taiwan	Sun *et al.*[28]	118 (no IAT)	-	-	-	38# (32.2)	-	77# (65.3)	-	-	-	3# (2.5)
Japan	Okuda *et al.*[29]	130 (IAT; Abs./Elution)	-	-	-	-	-	92 (70.8)	38§ (29.2)			

Legend IAT: indirect antiglobulin test; *: for two out 23 individuals with a hybrid allele the D phenotype was not determined; †: one of the seven individuals carried a weak D type 4.0 allele and a RHD-CE-D^{s} allele, another one of the seven individuals carried a DAR allele and a RHD-CE-D^{s} allele; ‡: three additional D-negative individuals carried both alleles, RHDψ and RHD-CE-D^{s}; +: one D-negative sample could not be investigated further; §: the molecular mechanism was not investigated for these phenotypes; #: a real-time polymerase chain reaction-melting curve analysis at nucleotide 1,227 was performed to predict the D phenotypes.

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This substitution does not cause an amino acid exchange (K409K)[14], but its location within the splice site of exon 9 impairs the production of normal amounts of D protein. Nevertheless, transfusion of red cell units in subjects with this DEL phenotype might be capable of immunising truly D-negative recipients[15,16]. On the other hand, individuals carrying this DEL phenotype do not become immunised in the case of pregnancy or transfusion. For people with this phenotype anti-D prophylaxis is unnecessary, and transfusion therapy should not be delayed by time-consuming searches for D-negative blood units.

Thus, for these and for other individuals, such as pregnant women and patients, whose red blood cells react weakly positive in serological Rh-D typing (or discrepant, if typed with more than one anti-D reagent), well-founded decisions regarding anti-D-prophylaxis and Rhesus matched transfusions are increasingly based on the results of molecular typing. Blood grouping by serological methods, however, is the preceding step and will probably remain for a while. It is less elaborate in terms of technical equipment, methods, and training of staff. In addition, serological blood grouping is still the standard for typing blood donors, and many studies on the distribution of blood groups, such as that by Makroo and colleagues[2], came from blood services. Thus, despite the growing importance of molecular typing, many of the future population studies, which are still needed to fill in the blank spots regarding blood group antigen frequencies, will report on serological data.

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