ESTIMATED NEED FOR D NEGATIVE BLOOD WHEN TRANSFUSING *RHD*DAU*-POSITIVE INDIVIDUALS

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Background

Variant *RHD* alleles and anti-D immunization are more prevalent in Africans as compared to Europeans. The aberrant *RHD* alleles of the *DAU* (in German: "D Afrikanischen Ursprungs") cluster are considered to be one of the major causes of *RHD* polymorphism in the African population, with *DAU-0* (*RHD*10.00*) being the most prevalent one. As suggested by a relatively high RhD similarity index of 0.74 for *DAU-0* (1), D-positive transfusions may be considered for *DAU-0* carriers. All other members of the *DAU* cluster, e.g. *DAU-1* to *DAU-7* (*RHD*10.01-07,* Fig.1) may behave like partial Ds and are prone to rise anti-D upon transfusion of RhD positive blood. In order to prevent unnecessary RhD negative transfusion to *DAU* positive individuals, both parental *RHD*-haplotypes and type of the *DAU* allele present need to be assessed.

Exon	2	4	5				6	7	8
Nucleotide	209 G>A	579 G>A	667 T>G	689 G>T	697 G>C	697 G>A	835 G>A	998 G>A	1136 C>T
*RHD*DAU0*									■
*RHD*DAU0.1*		■							■
*RHD*DAU1*				■					■
*RHD*DAU2*	■	■						■	■
*RHD*DAU3*							■		■
*RHD*DAU4*						■			■
*RHD*DAU5*			■		■				■
*RHD*DAU6*								■	■
*RHD*DAU7*							■	■	■

Fig. 1:
Nucleotide changes characteristic for all various *RHD*DAU* alleles. Note that all alleles share one common nucleotide change in exon 8 (1136C>T) suitable for identification of *RHD*DAU* in general by routine PCR-SSP genotyping of donors and patients.

Results

Genetic frequency of *RHD* 1136C>T was roughly measured to be about 1 among 1,000 regular blood donors in the Zurich area of Switzerland ("Caucasians", allele frequency: 0.0006). 16 of 17 individuals (94%) with *DAU* positivity (1136C>T) could have been transfused with RhD positive blood, given the most prevalent *DAU-0* genotype is considered to behave like a "regular" RhD positive. PCR-SSP analysis and sequencing of *RHD* exons 4 to 8 of all 17 samples revealed various *RHD* genotypes: Five samples were heterozygous *DAU-0/RHD*, three were *DAU-0/RHd*, and one was homozygous for *DAU-0/DAU-0*. Sequencing displayed *DAU-0* (1136C>T), in heterozygous combination with *DAU-3 (n=3), RHD*null (other than the deletional type, n=2), and partial *RHD* (n=1), respectively. Genotyping of the remaining four samples revealed *DAU-0.1/RHD*, *DAU-5/RHD, DAU-3/RHD* and *DAU-3/RHd* genotypes, respectively (Fig. 2).

Methods

17 *DAU*-positive (1136C>T, all *DAU*-positive individuals available at our institute) individuals have been identified using commercial kits or by high throughput genotyping based on MALDI-TOF mass spectrometry. All samples were analysed for the presence of "Rhesus boxes" and *RHD* null alleles using commercial kits. *RHD*-sequencing was carried out for exons 4 to 8, allowing identification of all known *DAU* alleles. The origin of all 17 samples was heterogenous: Eleven of the individuals were patients, nine of them were assigned to presumptive African ancestry based on other *RHD* alleles (e.g. *RHD*03N.01*) or blood group antigens (e.g. *FY*02N.01)*. Another six samples were identified by high throughput genotyping of approximately 5,500 regular blood donors of various areas of Switzerland.

Sum	Patients (p.A.a.)	Blood donors	1. GT based on PCR-SSP and/or mass spectrometry	D	R	2. GT based on sequencing results	D	R
5	4(3)	1	*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU0* | *RHD*01*	+	+
3	1(1)	2	*RHD*DAU* | *RHD*01N.01*	+	-	*RHD*DAU0* | *RHD*01N.01*	+	+
1	1(1)		*RHD*DAU* | *RHD*03N.01*	+	-	*RHD*DAU0* | *RHD*03N.01*	+	+
1		1	*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU0* | *RHD(545delCTGT)*	+	+
1	1(1)		*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU0* | *RHD(Part)*	+	+
1	1(1)		*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU0* | *RHD*DAU0*	+	+
1	1(1)		*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU0* | *RHD*DAU3*	+	+
1	1(0)		*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU0.1* | *RHD*01*	+	+
1	1(1)		*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU3* | *RHD*01*	+	+
1		1	*RHD*DAU* | *RHD*01N.01*	+	-	*RHD*DAU3* | *RHD*01N.01*	+	-
1		1	*RHD*DAU* | *RHD*01*	+	+	*RHD*DAU5* | *RHD*01*	+	+
17	**11(9)**	**6**						

Fig. 2: Seventeen *DAU* positive (1136C>T) individuals were analysed in detail to determine RhD status (D=Donor, R=Recipient). Eleven of the samples were patients identified by routine genotyping at our laboratory. Nine of them were assigned to presumptive African ancestry (p.A.a.). Remaining six samples were identified among regular blood donors in the Zurich area of Switzerland. *RHD* genotype (GT) of all individuals were determined by PCR-SSP using commercial kits and/or MALDI-TOF mass spectrometry. Subsequent sequencing allowed distinction between various *RHD*DAU* alleles and additionally, identification of further partial *RHD* alleles. According to sequencing results, RhD status of four samples would have been changed and allowed transfusion of these individuals with RhD positive blood.

Conclusion

In most *DAU* positive cases it seems appropriate to transfuse RhD positive blood, provided the complete *RHD* genotype is extensively assessed. E.g. in the presented study, demand for RhD negative supply would have been reduced from 5 of 17 (29%) to 1 of 17 (6%) by the presented analysis in *DAU* positive individuals. However, some *DAU* proposita (e.g. *DAU-3/RHd*, or homozygous *non-DAU-0/non-DAU-0*) still require RhD negative blood in order to prevent anti-RhD immunization. For decision making, besides genotyping of *RHD* zygosity status in *DAU* positive individuals, the second parental allele may sufficiently be assessed by excluding the common SNPs of *non-DAU-0* alleles in exon 5, 697G>C/A (*DAU-4*, *-5*), in exon 6, 835G>A (*DAU-3*, *-7*) and in exon 7, 998G>A (*DAU-2*, *-6*, *-7*) of the *RHD* gene by respective and yet to establish PCR-SSPs.

References

(1). Wagner et al. (2002) The *DAU* allele cluster of the *RHD*gene. Blood, 100:306-11