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Specific amino acid substitutions cause distinct expression of JAL (RH48) and JAHK (RH53) antigens in RhCE and not in RhD

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Westhoff and colleagues[1] and Hustinx and coworkers[2] have recently reported a total of three molecular causes for the low-prevalence Rh antigen JAL (RH48). Quantitative weakening and qualitative effects are documented for these variant RhCE proteins. Similar serological effects have been observed in the JAHK+ (RH53),[3] ceSL,[4] and RhCe(S122P)[5] variants of the RhCE protein. We have observed 4 RhD variants harboring similar amino acid substitutions at positions 114 or 122 (Table 1).

Most Rh antigens are known to be associated with molecular variants of either the *RHD* or *RHCE* gene. However, distinct Rh antigens, like c (RH4), G (RH12), Rh32 (RH32), Evans (RH37) and FPTT (RH50), can be expressed by RhD and RhCE variants (Table S1, available online). We collated all low-prevalence Rh antigens caused by single nucleotide substitutions and identified pairs of *RHD* and *RHCE* alleles that harbor identical substitutions (Table S2, available online).[1–4,6] Only amino acid substitutions at positions 114 and 122 were currently found to fulfill both criteria. In RhCE these substitutions cause JAL or JAHK antigen expression. Hence, we assessed the possible expression of JAL and JAHK by RhD proteins harboring similar amino acids at positions 114 or 122. A DVII sample harboring a substitution at 110 was used as negative control.

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Authorship contribution: PS analyzed and interpreted the data, and wrote the manuscript; IvZ and FFW collected samples and analyzed data; EAS collected the anti-JAHK sera; WAF designed the study, collected samples, interpreted the data, and wrote the manuscript.

Supplementary material. Additional information in Tables S1 to S3 can be found in the online version at doi: [to be inserted by the publisher]

All RhD samples with amino acid substitutions at position 114, weak D type 17, type 25 and type 47, were negative for the JAL antigen (Table 2). The CeMA and Rhce(R114Q) control samples were JAL positive, as expected. Because the antigen density of weak D type 17 is very low (Table 1), we corroborated its lack of JAL expression by a negative adsorption-elution test (not shown).

The weak D type 54 sample with an amino acid substitution at position 122 was negative for the JAHK antigen (Table 2). The RhCe(S122L) control sample was positive with the 3 anti-JAHK sera, as expected.

ceSL has been reported to be JAHK negative.[4] We confirmed this result with the original red blood cell (RBC) samples and the anti-JAHK P4.53A serum used previously. However, the two anti-JAHK sera of greater titer revealed that ceSL samples were in fact JAHK positive (Table 2). Both RhCe(S122P) samples were JAHK negative with all 3 sera.

The two sera P4.53B and P4.53C were identified from a series of 138 samples collected from pregnant women with a positive antibody screen.[7] Both women had neither been transfused nor were their newborns or the putative fathers positive for the JAHK antigen.[7] Hence, anti-JAHK is possibly a rather frequent, naturally occurring antibody associated with pregnancy.

The titers of anti-JAL (Table 2) determined with CeMA and Rhce(R114Q) differed in congruence with the known weaker expression of JAL in the Rhce variants Rhce(R114Q) and ce^{s}(340) compared to the RhCe variant CeMA.[2] In contrast to Rhce(R114Q) harboring Gln114, ce^{s}(340) and CeMA are caused by mutations encoding for Trp114.[1,2] The model presented by Westhoff et al.[1] suggests conformational changes due to Trp114 that contribute to the expression pattern of the JAL antigen. The weak JAL expression in Rhce(R114Q) may be due to the different biochemical properties of Trp (W) and Gln (Q). However, the JAL expression in ce^{s}(340), which also harbors Trp114, is distinctly weaker than in CeMA and, hence, requires a different molecular explanation (Table S3, available online):[1,2,8] The additional Leu245Val substitution, known to cause a weak expression of the e antigen,[8] could also induce the weak expression of the JAL antigen. Currently observed alleles cannot exclude the possibility that the weak JAL expression in both Rhce variants is caused by the 4 amino acid residues at positions 48, 60, 68, and 103, which typically differ between the RhCe and Rhce proteins.

We conclude that JAL and JAHK antigens are expressed by Ce and ce variants of the RhCE protein. We found that neither antigen was expressed by any of the 4 RhD protein variants possessing similar amino acid substitutions at positions 114 and 122.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

RHD alleles encoding amino acid substitutions at positions 114 and 122

Trivial name	Allele	Nucleotide change	Exon involved	Effect on protein sequence	Membrane localization*	Phenotype	Probable haplotype	Antigen density†	Number of probands	Reference‡
Weak D type 17	*RHD*(R114W)	340C>T	3	Arg to Trp at 114	TM	CcDee	*CDe*	172	1	Wagner et al.[6]
Weak D type 25	*RHD*(R114Q)	341G>A	3	Arg to Gln at 114	TM	CcDee ccDEe	*CDe* *cDE*	835 and 916 1,163	2 1	This study
Weak D type 47	*RHD*(R114G)	340C>G	3	Arg to Gly at 114	TM	ccDee	*cDe*	432	1	This study
Weak D type 54	*RHD*(S122L)	365C>T	3	Ser to Leu at 122	TM	ccDEe	*cDE*	3,241	1	This study

*TM - transmembraneous

†D antigens per RBC was determined as previously described, when the antigen density of the weak D type 17 sample was published.[6]

‡The nucleotide sequence data were deposited under GenBank accession numbers AJ548430 for weak D type25, AM157176 for weak D type 47, and AM396583 for weak D type 54.

Table 2

Serologic results of RhD and RhCE variants with amino acid substitutions at positions 114 and 122

				Titer with polyclonal antisera*				
					anti-JAHK			
Amino acid position	**Protein**	**Trivial name†**	**Amino acid substitution**	**anti-JAL Pas**	**P4.53A**	**P4.53B**	**P4.53C**	**Number of samples**
114	RhCE	CeMA	R114W	512	0	0	0	1
		Rhce(R114Q)	R114Q	16	0	0	0	1
	RhD	Weak D type 17	R114W	0	0	0	0	1
		Weak D type 25	R114Q	0	0	0	0	3
		Weak D type 47	R114G	0	0	0	0	1
122	RhCE	Ce(S122L)	S122L	0	8	32	32	1
		ceSL	S122L	0	0	64	16 – 64	7
		Ce(S122P)	S122P	0	0	0	0	2
	RhD	Weak D type 54	S122L	0	0	0	0	1
110	RhD	DVII	L110P	0	0	0	0	1

*Anti-JAL (Pas) was kindly provided by Hein Hustinx.[2] The anti-JAHK antisera P4.53A,[3] P4.53B and P4.53C[7] were reported previously. Isoagglutinins and the known anti-E antibody in P4.53C were absorbed when needed; a titer of at least 8 was confirmed in all sera after absorption. Hemagglutination was performed in gel matrix technique (Liss-Coombs Scangel; Bio-Rad, Munich, Germany).

†Initial deposition of the nucleotide sequences for JAL+ *RHCe*(R114W)[1,2] and *RHce*(R114Q)[2] variants under GenBank accession numbers AJ548431 and AJ548432. The ceSL samples were identical with the 7 previously reported RBC[4] including one sample with the additional W16C substitution.

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