

NEW *RHD* ALLELES WITH WEAK HEMAGGLUTINATION AND GENETIC EXON 9 DIVERSITY: weak D TYPES 45.1, 75, AND 76.

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Background: Weak agglutination of RhD positive samples may be observed for most category and partial Ds, which are prone to anti-D development, and among weak Ds, usually tolerating confrontation with RhD upon pregnancy, or transfusion, without developing anti-D. Still, new weak D alleles are discovered on a regular basis and are then ideally described by molecular variant *RHD* allele analysis, complemented by detailed characterization of the associated D phenotype.

Study design and methods: Sample 45.1 originated from Labor Wisplinghoff, Cologne, Germany, and samples 75 and 76 were both patients from the General Hospital and University Clinics in Innsbruck, Austria. Variant D types were characterized using PCR-SSP based molecular typing, *RHD* sequencing, extended serologic D antigen investigations, and flow cytometric D antigen quantification.

Results: Three novel weak D types termed weak D Types 45.1, 75, and 76 with *RHD* nucleotide substitutions coding for amino acid exchanges in predicted intracellular RhD polypeptide stretches were discovered by standard serological methods. Flow cytometric analysis determined their antigen densities to be approximately 1.990, 900, and 240 D sites per red blood cell, respectively. Adsorption-elution-technique–supported D epitope mapping of these three weak D types demonstrated the expression of all tested D epitopes. All three novel weak D Types 45.1, 75, and 76 alleles share a remarkable common feature, in that all of them typed negative for coding nucleotide A1193 located in Exon 9 of the *RHD* gene. This could clearly be shown by *RHD* gene exon scanning polymerase chain reaction using sequence-specific priming and was explainable by specific mutations for weak D Types 45.1 (C818T, G1195A), 75 (G1194C), and 76 (A1215C).

Conclusion: All novel weak D types expressed all tested D epitopes. Therefore, in respective carriers, immunization-tendency upon challenge may be small, but can't be excluded in general. It is of interest that for weak D Types 45.1, 75, and 76, similar alleles with a maximal divergence of one amino acid only, that is, weak D Types 45, 41, and 68, respectively, have been reported so far.