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Defining the pathogenic autoantibodies in congenital heart block

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Objectives: Maternal autoantibodies directed against the amino acids (aa) 200–239 of Ro52 (Ro52-p200) is associated with risk of foetal AV-block. The objective of this study was to identify the epitope specificity of human anti-Ro52-p200 antibodies.
Methods: Sera from 19 mothers of foetuses with CHB and 8 babies with CHB were analysed for binding to synthetic peptides representing variants of human and rodent Ro52-p200 and overlapping peptides by ELISA. Competition experiments were performed to confirm specificity and affinity of the binding. Secondary structure of the peptides was analysed by circular dichroism spectroscopy (CD).
Results: Analysis of autoantibody-binding to human p200 and a highly overlapping peptide including aa 197-232 (p197), demonstrated significantly higher reactivity towards p200 than to p197 ($p<0.0001$). The relevance of C-terminal aa of p200 was confirmed using truncated human p200 peptides, demonstrating that deletion of aa in the C-terminal of p200 completely abolished antibody binding. Furthermore, taking advantage of the fact that human sera do not bind rat p200 (r-p200), peptides based on the rat p200-sequence, with selected crucial residues mutated into the human counterparts were generated. C-terminal mutations including a glutamic acid substitution for an aspartic acid in position 233 reestablished binding of sera, while there was no gain of reactivity by substitutions in the N-terminal or mid part of the peptide. Analysis of the peptides by CD confirmed correct folding of the peptides. Finally, we generated peptides with alanine substitutions of each residue from position 233 to 239 (pA233-pA239). Substitution of the aspartic acid 233 abolished antibody binding, while the other mutations did not affect binding. Preincubation of the sera with the rat-to-human 233 mutated peptide blocked antibody reactivity to p200 to the same degree as the p200 peptide itself.
Conclusions: Our study suggests that the aspartic acid at aa residue position 233 of the Ro52-p200 peptide is crucial in forming the main epitope of the Ro52-p200 peptide bound by CHB-related human Ro52 antibodies. This specificity might be used as a tool to identify high risk pregnancies for CHB, and for identification of the cross-reactive target in the fetal heart bound by the maternal autoantibodies.