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Using a computer model of human CYP21 to correlate structural features with clinical severity

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Short Abstract: A structural model of human CYP21 was calculated based on the crystal structure of CYP2C5, to better understand the molecular causes of CAH. 60 disease causing mutations and six normal variants, were analysed using this model. A structural explanation for the corresponding phenotype was found for all but two mutants.

Long Abstract:

A structural model of human 21hydroxylase, CYP21, was calculated based on the crystal structure of rabbit CYP2C5, in order to enhance our knowledge of structurefunction relationships of human CYP21 and to better understand the molecular causes of congenital adrenal hyperplasia (CAH) due to 21hydroxylase deficiency. A total of 60 disease causing mutations, and six normal variants, were analysed using this model.

To analyse the effects of the mutations, individual molecular models were calculated, using homology modelling techniques implemented in the ICM program (Molsoft LLC). For each mutant, the corresponding amino acid residue was exchanged and the structure subsequently energy minimized. The calculated protein stabilities of the modelled mutants were found to correlate inversely with the corresponding clinical severity. The stability of the protein together with the location, surrounding environment and amino acid conservation of the mutation made it possible to explain the corresponding phenotype for all but two mutants. Conservation was calculated using a multiple alignment between CYP21 from human, pig, dog, ox, sheep, mouse, and rat. We have also identified putative structurally important residues involved in haem and substrate binding, redox partner interaction and enzyme catalysis using docking calculations and homology with structurally determined cytochrome P450s. As the CYP2C5 contained a haemgroup we got its location directly from the homology modelling. The substrate location was inferred from a homologue by superimposing the two 3Dstructures followed by energy minimization. Residues were regarded as binding if they have side chains close enough to interact with their targets. Possible redox partner interaction site was mapped over from CYP2B4 where this interaction is well studied.

Functional and structural consequences of seven novel mutations, with suspected CAH of different severity, are predicted using the molecular modelling procedure. We confirm that

the structural features deduced from the models are in good correlation with clinical severity of CYP21 mutants, showing the applicability of a modelling approach in assessment of new CYP21 mutations.