Identification of a loss-of-function polymorphism in the human P2X4 receptor

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The P2X4 receptor is a ligand-gated ion channel activated by extracellular ATP. The *P2RX4* gene lies adjacent to the highly polymorphic *P2RX7* gene on chromosome 12q24.3. To date four non-synonymous single nucleotide polymorphisms (SNPs) have been found in *P2RX4* however the functional effects associated with these mutations in the receptor are unknown. Site directed mutagenesis was used to introduce mutations into a GFP-tagged human P2X4 plasmid and functional P2X4 responses were measured using whole cell patch clamp electrophysiology in transfected HEK-293 cells. The Tyr 315>Cys mutation showed a dramatic loss-of-function with a response of only 10.9% of wild-type P2X4 receptors ($p= 0.0002$, n=4-8 cells). This tyrosine residue is predicted to contribute to ATP binding in the extracellular domain and the Tyr 315>Cys mutant displayed a reduced sensitivity to ATP (EC_{50} of 192 μM compared to wild-type P2X4 EC_{50} of 5 μM). The Ala 6>Ser, Ile 119>Val and Ser 242>Gly mutations showed no significant difference in ATP sensitivity. We genotyped 200-500 Caucasian subjects at four SNPs in the *P2RX4* gene to determine allele frequencies in the population. We found the Tyr 315>Cys polymorphism was rare with a frequency of 1.1% (n=416 subjects) and was only found in heterozygous dosage.