The Tyr-315 to Cys polymorphism in the P2X4 receptor causes loss of function and is associated with increased pulse pressure in humans

L. Stokes,[1] K. Scurrah,[2] J. Ellis,[3] B. Cromer,[4] K. Skarratt,[1] S. Harrap[2] and J. Wiley,[1,4] [1]Sydney Medical School Nepean, University of Sydney, Level 5 South Block Nepean Hospital, Penrith, NSW 2751, Australia, [2]Centre for Molecular, Environmental, Genetic and Analytic Epidemiology, University of Melbourne, Parkville, VIC 3052, Australia, [3]Murdoch Childrens Research Institute, Royal Children's Hospital, Flemington Road, Parkville, VIC 3052, Australia and [4]Florey Neurosciences Institute, University of Melbourne, Level 3 Alan Gilbert Building, 161 Barry St,, South Carlton, VIC 3053, Australia.

The P2X4 receptor is expressed in endothelial cells where it is involved in shear stress-induced calcium signalling and generation of nitric oxide. We mutated an EGFP-tagged human P2X4 plasmid and expressed the constructs in HEK-293 cells. Electrophysiological studies showed that the Tyr315Cys mutation significantly reduced the peak amplitude of the ATP-induced inward current to 10.9% of wild-type P2X4 receptors (n = 4-8 cells, p = 0.0002). Co-transfection experiments to mimic a heterozygous state reduced the response to ATP to 40% of wild-type alone. Concentration-response curves for ATP showed that the Tyr315Cys P2X4 mutant caused a rightward-shift of the EC50 compared to the wild-type receptor. Further investigation using BzATP, a partial agonist at human P2X4 receptors, also showed a right-shifted dose-response and an increase in EC50 in the Tyr315Cys P2X4 receptor compared to the wild-type. Cell surface expression of the Tyr315Cys-P2X4 mutant was significantly reduced compared to wild-type P2X4 receptor. A single nucleotide polymorphism representing the Tyr315Cys P2X4 mutant was tested against supine systolic, diastolic and pulse pressures in 2864 subjects from the Victorian Family Heart Study. The mutant allele frequency was 1.4% (89 heterozygotes and 1 homozygote) and showed no evidence of population stratification (p = 0.73). In a variance components analysis with adjustments for age, sex and their interaction in parents and offspring separately we found significant association with pulse pressure (p = 0.023 for total association) such that 1 minor allele increased pulse pressure by 2.84 mmHg (95% CI 0.41 to 5.27). These data suggest that Tyr315Cys disrupts ATP binding to the P2X4 receptor and this is supported by modelling data using the recently published crystal structure of the zebrafish P2X4 receptor. The associated increase in pulse pressure might reflect reduced large arterial compliance as a result of impaired endothelium-dependent vasodilation in large arteries.