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ORIGINAL ARTICLE

Human Connexin40 Mutations Slow Conduction and Increase Propensity for Atrial Fibrillation

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Background Patch clamping studies using non-cardiomyocytes revealed that the human connexin40 mutations P88S, G38D, and A96S are associated with reduced gap junction conductances compared to wild type connexin40 (wtCx40). Their effects within myocytes however are unclear. We aimed to characterise P88S, G38D, and A96S after expression in rat hearts and primary cardiomyocyte cultures.

Methods Adult Sprague-Dawley rat atria were transduced with a lentivector containing a transgene encoding wtCx40, P88S, G38D, A96S, or eGFP (n = 6 per transgene). Electrophysiology studies (EPS) were performed just prior to and seven days after surgery. Left atria were assessed for connexin expression, mRNA levels, inflammation and fibrosis. Primary cardiomyocyte cultures were also transduced with the abovementioned vectors (n = 6 per transgene) and monolayer conduction velocities (CV) and protein expression were assessed at 96 hours.

Results At day 7 EPS, P wave and induced atrial fibrillation (AF) durations were significantly longer in the mutant groups when compared to wtCx40 controls ($p < 0.05$). There were no significant differences in inflammation, fibrosis, or heart to body weight ratios. Monolayer CV's were reduced in the A96S group compared to the wtCx40 group. While similar to wtCx40 controls, P88S velocities were reduced compared to eGFP controls. G38D monolayers possessed spontaneous fibrillatory activity and could not be paced. Immunofluorescence revealed that P88S and G38D reduced native connexin43 myocyte coupling while A96S appeared to co-localise with connexin43 in gap junctions. Connexin43 mRNA levels were similar between groups.

Conclusions The A96S, G38D, and P88S Cx40 mutations slow conduction and increased the propensity for inducible AF.

Keywords Connexin40 • Gene mutation • Atrial fibrillation • Cardiac electrophysiology

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Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia, affecting 1% of the general population, 6% of people over the age of 65, and 9% of individuals over the age of 80 [1,2]. With a squaring of the population age distribution, the overall prevalence of AF and its impact on the health system is set to increase [3,4]. The effectiveness of both medical and catheter based therapy targeting rate and/or rhythm control and stroke risk reduction is modest at best and not without side effects. Hence, there is a desperate need for a greater understanding of the disease process.

Over the last two decades, there has been mounting evidence that connexins play a significant role in cardiac myocyte electrical coupling. Human atrial myocytes express the connexin isotypes 40 and 43 which form intercellular channels to facilitate the passive flow of intracellular ions associated with action potential propagation. Intercellular conductance is greatly influenced by the ratio of connexin isotypes in a channel and by the total number of channels expressed at a membranous gap junction. Connexin40 (Cx40) only channels possess higher conductances than connexin43 (Cx43) only channels. Mixed isotype channel conductances vary widely and are dependent on their isotype ratio. They are lower than Cx40 only channels and are often lower than that of Cx43 channels. Alterations in the levels and patterns of connexin expression in humans have been purported to provide the substrate required for the chronic form of AF [5]. In 2006, a germline (A96S) and two somatic mutations (P88S, G38D) of the gene encoding the gap junction protein Cx40 were identified in patients suffering from idiopathic AF [6]. Since this discovery, further connexin40 mutations have been identified and a number of functional studies performed [7–13]. One of the major limitations of these genetic discoveries, however, is the lack of a convincing, demonstrable, functional effect on electrical cardiac conduction that could mechanistically underpin the generation of AF. We aimed to assess the functional effects of the A96S, P88S, and G38D Cx40 mutations in their native context, that is the cardiomyocyte, with both *in vivo* and *in vitro* models. We hypothesised, that if pathogenic, each mutant should slow conduction and increase the propensity for atrial arrhythmia.

Methods

Study Protocol

For full details of methods, please see the Online Appendix. Briefly, the low level of endogenous cardiac Cx40 expression within Sprague Dawley adult rats and pups made this rodent an ideal platform to determine the phenotype of each of the Cx40 mutations. Five study groups (n = 6 each) were used. Groups were transduced to express enhanced green fluorescent protein (eGFP), wild-type connexin40 (wtCx40), A96S, G38D, or P88S. Non-transduced controls (n = 6) were used for ex vivo atrial tissue studies and all in vitro studies. For in vivo studies, 200 gram adult Sprague Dawley rats underwent a baseline electrophysiology study (EPS) followed by lentivector gene transfer. An EPS was re-performed seven days post transduction followed by removal of hearts. Body and heart weights were recorded. Left atria were studied ex vivo for Cx40 andCx43 expression and for the presence of inflammation and fibrosis. For in vitro studies, mono-layer conduction velocities and Cx40/Cx43 expression were assessed 96 hours following transduction of neonatal rat ventricular myocyte (NRVM) cultures.

Apart from AF duration, all continuous data in this work are expressed as means ± standard deviations and were analysed with t-tests for statistical significance. In the case of AF duration, data was non-parametric and hence medians with interquartile range are shown; Mann-Whitney tests were used for statistical significance. Categorical data was assessed with Fisher's exact test. A value of $p < 0.05$ was considered significant.

This study was approved by the Animal Research Ethics Committee of Westmead Hospital. All animals received humane care in accordance with the 'Statement on Animal Experimentation' by the National Health and Medical Research Council of Australia.

Results

Electrophysiology

Thirty rats received baseline electrophysiology studies before randomisation into five study groups (n = 6 each). Seven days after viral transduction, there was evidence of left atrial conduction slowing in the A96S, G38D, and P88S groups, when individually compared to wtCx40 rats, with a significant prolongation of mean P wave duration; bifid P wave formation and the absence of PR prolongation suggested conduction slowing was within the left atrium (Figure 1A). Atrial fibrillation (Figure 1B) was inducible in all study groups except for the wtCx40 transduced. Conduction slowing was associated with a significantly prolonged median duration of burst pacing induced atrial fibrillation within all three mutant Cx40 groups when individually compared to the wtCx40 group ($p < 0.01$ for each mutant group; Figure 1C). Transduced NRVM cultures were used to obtain corroborative evidence that each of the Cx40 mutations effect conduction slowing. It was possible to produce isochronal maps and calculate monolayer conduction velocities in only five of the six study groups (Figure 1D). The A96S group possessed a markedly slower mean conduction velocity when compared to wtCx40 transduced monolayers (84 ± 21 mm/s vs 141 ± 12 mm/s respectively; $p < 0.05$). Mean conduction velocity of the P88S cultures (138 ± 16 mm/s) while being similar to that of the wtCX40 group ($p = 0.72$) was reduced when compared to the eGFP group (183 ± 21 mm/s; $p < 0.01$). All G38D transduced cultures possessed spontaneous fibrillatory type myocyte contractile activity and could not be paced with stimulation even after escalating the current amplitude slowly up to a maximum of 1 mA and pulse duration up to 2 ms. Hence conduction velocities were not calculable and isochronal maps not produced for this group.

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