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THE P.P888L SAP97 POLYMORPHISM SHORTENS THE CARDIAC ACTION POTENTIAL DURATION AND THE QT INTERVAL

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The *DLG1* gene encodes for SAP97, an scaffolding protein that interacts with several cardiac ion channels including those underlying the fast Na (I_{Na}) and the transient outward (I_{to}) currents. By next generation sequencing we identified a common *DLG1* polymorphism (p.P888L) in a man and two sisters diagnosed with Brugada Syndrome (BrS). This work aimed to determine the electrocardiographic and the cellular electrophysiological consequences of the SAP97 polymorphism.

Native (WT) and p.P888L SAP97 tagged with ds-red were cotransfected or not together with the cDNA encoding the alpha and beta subunits underlying human I_{Na} and I_{to} in Chinese hamster ovary (CHO) cells. Two cardiac–specific transgenic-like mouse models on the basis of adeno-associated virus gene transfer were created expressing WT and p.P888L SAP97, respectively.

Co-expression of WT SAP97 significantly increased the I_{Na} and I_{to} recorded using patch-clamp in CHO cells and in ventricular myocytes from SAP97 overexpressing mice. The SAP97 polymorphism increased the I_{Na} similarly as WT SAP97 did, both in CHO cells and in transgenic-like mouse myocytes. Conversely, p.P888L SAP97 further increased the time constant of current inactivation and, thus, the I_{to} charge density in both CHO cells (from 18.6 ± 2.3 to 42.2 ± 6.1 pC/pF at +50 mV, $n \geq 8$, $P < 0.05$) and mouse myocytes (from 0.4 ± 0.04 to 0.7 ± 0.06 pC/pF at +50 mV, $n \geq 15$, $P < 0.05$). As a consequence, in p.P888L SAP97 mouse myocytes the AP duration (APD) measured at 20% and 50% of repolarization was significantly shortened. Furthermore, in transgenic-like mice p.P888L overexpression significantly shortened the QT interval compared with WT SAP97 (from 61.5 ± 2.7 to 52.5 ± 2.1 ms, $n = 6$, $P < 0.05$).

The SAP97 p.P888L polymorphism shortens the QT interval and the APD as a consequence of a marked increase of the Ito charge. Therefore, this polymorphism could contribute to the phenotypic manifestations of the BrS.

Keywords: Sodium current, transient outward potassium current, SAP97, cardiac

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PHARMACOLOGICAL AND BIOPHYSICAL PROPERTIES OF PLASMA MEMBRANE PORES INDUCED BY SILICA NANOPARTICLES

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Amorphous silica nanoparticles (SiNPs) are extensively used for their beneficial properties in cosmetics and food industry as an anti-caking, densifying and hydrophobic agent. However, the high levels of exposure have raised concerns about health hazards, since SiNPs can penetrate tissues and cells resulting in health problems. In this study we first evaluated the effects of commercial 9 nm SiNPs (Ludox®) on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in HEK293, CHO and mouse airway epithelial cells. We found that extracellular application of SiNPs at 25 °C increased $[Ca^{2+}]_i$ when cells were co-stimulated with arachidonic acid or LPS. These effects were not observed when Ca^{2+} was omitted in the extracellular solution, indicating that SiNPs induce a Ca^{2+} entry pathway through the plasma membrane. Similar results were obtained when cells were exposed to SiNPs and heating from 25 to 35 °C, suggesting that SiNPs are sufficient to induce the Ca^{2+} entry pathway at physiological temperatures. Whole-cell patch-clamp experiments revealed that SiNPs combined with arachidonic acid trigger large currents that could be blocked by the cation channel blocker ruthenium red in a voltage-dependent manner. Analysis of the selectivity properties of this current showed that they are mainly carried by cations. Taken together, our results demonstrate that SiNPs induce Ca^{2+}-permeable pores in the plasma membrane, and that this phenomenon is enhanced by factors that increase membrane fluidity. We propose that this Ca^{2+} entry pathway may be relevant for the toxicological properties of SiNPs.

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RETT-LIKE SEVERE ENCEPHALOPATHY CAUSED BY A *DE NOVO GRIN2B* MUTATION IS ATTENUATED BY D-SERINE DIETARY SUPPLEMENT

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N-Methyl-D-aspartic acid subfamily of glutamate ionotropic receptors (NMDARs) are activated during fast excitatory transmission and they have been proved to be key elements in synaptic plasticity, synaptogenesis and neuron survival. Several genetic studies have identified *de novo* NMDAR mutations in patients with neurodevelopmental diseases (including severe encephalopathies, autism, intellectual disability) as well as psychiatric disorders. In this work we report a case study of a 4 years-old Rett-like patient with a severe encephalopathy. The genetic studies of this patient (WES and Sanger sequencing) showed the presence of a missense *de novo* mutation of GRIN2B(p.P553T) coding for the GluN2B subunit of NMDARs.

Given the key role of GluN2B subunit in the very early stages of synaptogenesis, we hypothesized that this mutation could be leading to neuronal dysfunction and, subsequently, its normalization would potentially ameliorate the patient's symptomatology. In heterologous expression systems, GluN2B(P553T) mutant construct do neither affected NMDAR oligomerization nor their surface expression in primary neuronal cultures. However, electrophysiological studies showed that although functional, the mutant receptor displayed a significantly reduced channel conductance concomitant with a strong reduction of NMDA-evoked current density. These data are in agreement with our structural molecular model, and strongly suggest the hypo-functionality of mutant NMDARs that, potentially, could be rescued throughout the enhancement of their activity. In accordance with this hypothesis, *in vitro* administration of D-serine, a physiological NMDAR co-agonist, displayed a significant increase of NMDA-evoked currents of mutant receptors. Next, a clinical trial with dietary supplement of D-serine was performed. Importantly, after fourteen-months dietary supplement of D-serine, the patient showed an increase of serine plasma levels, together with a noteworthy clinical improvement. Our results show the possibility to enhance the hypofunctionality of glutamatergic transmission as a therapeutic approach to attenuate cognitive and motor impairment in early childhood.

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