non-sustained polymorphic ventricular tachycardia (VT), bi-directional VT, syncope, and mild QTc prolongation. The proband displayed dysmorphic features including micrognatia, clinodactylia and syndactyly. The patient's symptoms continued following administration of propranolol, but subsided after treatment with flecainide. Molecular genetic screening revealed a novel heterozygous mutation (c.779G>C/p R260P) in KCNJ2. Whole-cell patch-clamp studies conducted in TSA201 cells transfected with wild type human KCNJ2 cDNA (WT-KCNJ2) yielded robust IK1, but no measurable current in cells expressing the R260P mutant. Co-expression of WT and R260P-KCNJ2 (heterozygous expression) yielded a markedly reduced inward IK1 compared with WT alone (-36.5 ± 9.8 pA/pF vs. -143.5 ± 11.4 pA/pF, n=8, p>0.001, respectively at -90 mV) indicating a strong dominant negative effect of the mutant. The outward component of IK1 measured at -50 mV was also markedly reduced with the heterozygous expression vs. WT (0.52 ± 5.5 pA/pF vs. 23.4 ± 6.7 pA/pF, n=8, p>0.001, respectively). Conclusion: We report a novel KCNJ2 mutation associated with classical phenotypic features of Andersen-Tawil syndrome and CPVT mimicry. The R260P mutation produced a strong dominant negative effect leading to marked suppression of the inward rectifier potassium current.

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Ready-to-use CHO-NaV1.5 And Hek-hERG Instant Cells: Study On Frozen Cells Thawed And Immediately Patched At Manual And Automatic Patch Clamp Devices

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Well characterised cell lines and constant high cell quality are prerequisites for reliable data in electrophysiological studies. We developed the cell culture system ''Instant Cells'' that enables quality control of frozen cell batches and guarantees a constant cell quality. To show that the Instant Cells are suited for pharmacological studies, we have adapted CHO-K1 cells stably expressing hNaV1.5 and HEK 293 cells stably expressing hERG to the Instant Cells system.

The frozen Instant Cells were thawed, spun down and resuspended in a physiological buffer. Afterwards, the cell suspension was kept for four hours in the Cell Reservoir, a bench-top cell storage device. During this time span the cells were taken from the Cell Reservoir to be evaluated on a conventional and on an automated patch clamp device, the CytoPatchTM instrument.

We show that both types of Instant Cells have the same characteristics in terms of electrophysiological and pharmacological properties compared to permanently cultured cells:

- Trypan-blue tests showed 95 % vital cells after preparation.
- The mean peak current of the NaV1.5 Instant Cells was 12.5 nA, the mean tail current of the hERG Instant Cells was 1.2 nA.
- More than 80 % of the cells sealed (above 1 GOhm), more than 60 % were stable for 15-25 min (Rm was above 500 MOhm).
- The isochronal I/V relationship of the hERG activation and tail current and the NaV1.5 activation current were similar to freshly prepared cultured cells.
- No difference was observed in the dose-response relationship for blocking compounds between the Instant cells and the running culture of both cell types.

This proves that the Instant Cells are well suited to investigate ion channel pharmacology.

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Dual Variations in SCN5A and CACNB2b Underlie Cardiac Conduction Disease without Brugada Syndrome

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Introduction: Inherited loss of function mutations in *SCN5A*, the gene that encodes the α-subunit of the human cardiac sodium channel ($hNa_v1.5$), have been linked to overlapping syndromes including cardiac conduction disease (CCD) and Brugada syndrome (BrS). The mechanisms responsible for the development of one without the other are poorly understood.

Methods: Direct sequencing analysis was performed in a family with CCD. Wild type (WT) and variant channels were co-expressed with CD_8 cDNA in TSA201 cells for electrophysiological study. Green fluorescent protein (GFP)-fused WT or mutant *SCN5A* genes were used for confocal microscopy to assess channel trafficking.

Results: A novel *SCN5A* missense mutation, P1008S, was identified in all family members displaying 1st degree AV block, but not in unaffected family members nor in 430 reference alleles. Peak P1008S current was 11.77% of WT ($p<0.001$). Confocal microscopy showed that WT channels tagged with GFP were localized on the cell surface, whereas GFP-tagged P1008S channels remained trapped in intracellular organelles. P1008S current and trafficking could be rescued by incubation at room temperature, but not by incubation with mexiletine (300μM) at 37°C. We also identified a novel polymorphism (D601E) in CACNB2b. The variation in the β subunit of the calcium channel caused a slowing of inactivation of the L-type calcium channel current (ICa), significantly increasing total charge, when co-expressed with the α1 and $\alpha_2\delta$ subunits of the calcium channel in TSA201 cells.

Conclusions: Our results suggest that variations leading to a loss of function in INa coupled with a gain of function in ICa may underlie the development of cardiac conduction disease without Brugada syndrome.

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Expression and Distribution of Voltage Gated Ion Channels in Ferret SA Node

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Spontaneous diastolic depolarization in the sinoatrial (SA) node enables it to serve as pacemaker of the heart. The combination of variation of cellular morphology within the SA node and heterogeneity of ion channel expression in the atrium predict that ion channel expression would be different and more heterogeneous than in the atrium. To evaluate ion channel heterogeneity within the SA node, we used fluorescent in-situ hybridization to examine ion channel transcript expression in the ferret SA nodal region and atrial appendage. We analyzed transcripts for 24 voltage-gated K^+ channel alpha subunits, 4 hyperpolarization-activated cation channels, 3 voltage-gated Ca^{2+} channels and 6 voltage-gated Na^+ channels and 3 ancillary subunits. Immunofluorescence was used to verify localization patterns of voltage-dependent K^+ channels. Co-localizations were performed to observe any preferential patterns. Neuronal antibodies were used in association with K^+ channel transcripts and antibodies to segregate the associated patterns in cardiac tissue. There were some overlapping and non-overlapping binding patterns observed. As positive controls, oligonucleotide probes from Troponin I slow and Troponin I cardiac sequences were used. Measurement of different K^+ channel transcripts showed heterogeneous expression with many different patterns of expression, attesting to the complexity of electrical activity in the SA node. This study enabled us for the first time to analyze the microscopic distribution of different transcripts in contiguous images and in a continuous manner over a cross-section of the SA nodal region. Such information provides a better understanding of the role that ion channel heterogeneity might play a role in SA node pacemaker activity.

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The Anchoring Protein SAP97 Is Crucial For The Surface Expression Of *Shal* Kv Potassium Channels And Their Regulation By CaMKII In Cardiac Myocytes

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The *Shal*-type Kv channels (Kv4.x) account for a large part of the outward potassium current, I_{to}, in heart. Membrane-associated guanylate kinase proteins are major determinants of the organization of several ion channels however, few are known on the interaction between Kv4.x channels and cardiac MAGUK, SAP97 in the heart. Here using pulldown assays we found a direct interaction via the VSAL amino acid motif between the Kv4.x C-terminus and the SAP97 in rat and human myocardia. In Kv4.3-KChIP stable CHO cell line and using the whole cell patch clamp technique, SAP97 increased the Kv4.3 encoded current by a factor 2 (145 ± 19pA/pF *vs* 300 ± 52 pA/pF; n=11; $p<0.001$) without changes of current gating properties. SAP97 had no effect on Kv4.3 encoded current when channel were deleted of the VSAL motif (ΔSAL-Kv4.3). Suppression of SAP97 by using shRNA inhibited I_{to} in cardiac myocytes. In CHO cells ΔSAL-Kv4.3 channel-encoded current showed a marked acceleration of its time-dependent inactivation and was insensitive to CaMKII inhibition achieved by intracellular application of the CaMKII inhibitor KN93, or of inhibitory peptide. In HEK293 cells, SAP97 silencing reproduced the effects of CaMKII inhibition on the current kinetic and suppressed the interaction between Kv4.x C-terminus and CaMKII studied by pull down assay. Conclusion: The anchoring protein SAP97 enhances the functional expression of Kv4.x channels and facilitates its regulation by the CaMKII in cardiac myocytes.