BIOPHYSICAL AND PHARMACOLOGICAL RESPONSE OF ATP-SENSITIVE POTASSIUM CHANNELS IN TRANSGENIC CANTU MICE HET KIR6.1V65M (KIR6.1WT/V65M) IN FIBERS FROM FAST-TWITCH AND SLOW-TWITCH SKELETAL MUSCLES, DERMAL SKIN FIBROBLASTS AND OSTEOBLASTS FROM LONG BONES AND CALVARIA

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Introduction: The rare disorder Cantu syndrome (CS) arises from gain-of-function mutations in KCNJ8or ABCC9, the genes encoding the Kir6.1and SUR2subunits of ATP-sensitive potassium (KATP) channels. Current information about genotype-phenotype correlation is limited. KATP over-activity and loss-of-response to glibenclamide (Glib) has already been reported for cardiac and smooth muscle cells. We investigate on the biophysical and pharmacological responses of KATP channel in WT and kir6.1$^{wt/V65M}$ mutated mice on flexor digitorum brevis (FDB) and soleus (SOL) muscle fibers, skin fibroblasts and long bones/calvaria-derived osteoblasts by patch clamp technique.

Material and method: Novel transgenic heterozygotes mice Kir6.1[V65M] mirroring the human CS were generated through CRISPR/Cas9gene editing. Isolated fibers and fibroblasts were obtained by enzymatic digestion from FDB/SOL muscles and ventral skin, respectively; osteoblasts from long bones/calvaria were obtained after migration from bone chips and cultured in DMEM containing 10% FBS, 1% L-glutamine, 1% antibiotics and 50 μg/mL ascorbic acid. KATP channel currents on skeletal muscle fibers were recorded using inside-out macropatches during pulses of -60 mV (Vm), in the presence of KCl on both sides of the membrane patches. Currents in skin fibroblasts were recorded in whole-cell configuration (ATP in pipette= 1mM), in osteoblasts in cell-attached configuration, using a depolarization protocol in physiological conditions.

Results: Higher KATP current amplitude at -60 mV (Vm) was observed in kir6.1$^{wt/V65M}$ FDB fibers with respect to the WT: WT -848.42±86(n patches=11); kir6.1$^{wt/V65M}$: -942.66±110 (n patches=20). Gaussian distribution analysis showed a tendency to higher current amplitude (7% of patches in WT showed a current amplitude higher than -1500 pA vs 15% in kir6.1$^{wt/V65M}$). In WT FDB fibers, 100 nM Glib caused a reduction of KATP current amplitude by -86.76±3% (n patches=20). By contrast, kir6.1$^{wt/V65M}$ mutant showed less sensitivity to Glib; 100 nM and 100 μM Glib reduced the current by -36.29±15% and by -82.43±3% (n patches=9), respectively. Similar KATP current reduction was observed in response to increasing MgATP concentration (10 μM-5mM) in WT and kir6.1$^{wt/V65M}$ fibers (n patches=3-9). No differences were observed in the current amplitude at -60 mV (Vm) in SOL fibers of WT vs kir6.1$^{wt/V65M}$ mice. A marked loss of sensitivity to Glib was observed in SOL fibers; in WT 100 nM Glib reduced the current by -41.90±6% (n patches=2); in kir6.1$^{wt/V65M}$ 100 nM and 10 μM Glib reduced it by -12.12±2% and by -14.07±1% (n patches=2), respectively. In long bones-derived osteoblasts the kir/KATP current amplitude at -180 mV (Vm) was -142.82±10 in WT (n patches=15) and -184.91±10 in kir6.1$^{wt/V65M}$ mice (n patches=28) ($p<0.05$, Student t test). 100 nM Glib reduced kir/KATP current by -54.75±13% (n patches=3) in WT mice cells. 100 nM and 10 μM Glib, respectively, reduced the current by -18.65±8% and -41.02±10% (n patches=6) in kir6.1$^{wt/V65M}$ mice cells. The same loss of sensitivity was observed with 200 μM repaglinide. Similar response was observed in calvaria-derived osteoblasts (n patches=6). In skin fibroblasts, the kir/KATP current amplitude at -160 mV (Vm) was -279.29±72in WT (n patches=9) and -381.76±92in kir6.1$^{wt/V65M}$ mice (n patches=9), respectively. 100 nM Glib reduced kir/KATP by -13.73±4% at -60 mV (Vm) (n patches=2) in WT mice cells. 100 nM and 10 μM Glib, respectively, reduced the current by -7.15±4% and -70.81±6% (n patches=2) in kir6.1$^{wt/V65M}$ mice cells.

Discussion and conclusion: The mutation kir6.1$^{wt/V65M}$ is conserved also in different tissues above cardiac and smooth muscle cells. The characteristic gain-of-function associated with the mutation was observed also in FDB fibers, skin fibroblasts and long bones/calvaria derived osteoblasts. Right shift of sensitivity to Glib was observed in FDB/SOL fibers, long bones-derived osteoblasts but not in skin fibroblasts.