Supplementary Material

Association of rare deleterious variants in *KCNQ1* potassium channel gene with large-vessel ischemic stroke in Polish population

PIOTR K. JANICKI, CEREN EYILETEN, VICTOR RUIZ-VELASCO, JUSTYNA PORDZIK, ANNA CZLONKOWSKA, IWONA KURKOWSKA-JASTRZEBSKA, SHIGEKAZU SUGINO, YUKA IMAMURA-KAWASAWA, DAGMARA MIROWSKA-GUZEL and MAREK POSTULA

Patients and methods supplemental information

1.1 Sequencing and analysis. The enriched libraries were sequenced using 101 bp paired-end mode on an Illumina HiSeq 2500 sequencer. Sequenced samples (consisting of FASTQ files) were aligned using the BWA-MEM aligner (BaseSpace Labs, Illumina, https://basespace.illumina.com) to a human reference genome version 19, using following tools: BWA version 0.7.13 (https://github.com/lh3/bwa), SAMtools version 1.3 (https://github.com/samtools/samtools), Picard version 2.1.1 (https://github.com/broadinstitute/picard). Aligned reads were recalibrated, sorted, marked for read duplication, and realigned near insertion/deletion (indels) using Genome Analysis ToolKit (GATK 3.7-0, http://www.broadinstitute.otg.gatk). Subsequent variant calling and filtrations were performed using generated from bam files (without removal of duplicate reads, as recommended for pooled sequencing) using Galaxy platform (https://usegalaxy.org). Firstly, bam files were sliced using the Samtools (Galaxy version 2) by the genomic regions containing exonic targets. Secondly, the genetic variants in the genomic targets were annotated using MPileup call variants (Galaxy Version 2.1.1) routine. The resulted pileup files were processed by VarScan2 software (Galaxy version version 0.1) for the final detection of variants. After final reads assembly by VarScan software and passing quality filters the subsequent screening of sequenced exons (in vcf format) was performed against dbSNP138 using wAnnovar software (14,15). The output files (one for each pool) from previous step was annotated by the dbNSFP version 3.2a software (https://sites.google.com/site/jpopgen/dbNSFP). The dbNSFP is an integrated database of functional annotations from multiple sources for the comprehensive collection of human non-synonymous single variants polymorphisms (nsSNVs). Its current version includes a total of 83,422,341 nsSNVs and splice site SNVs (ssSNVs). It compiles prediction scores from 17 prediction algorithms. In addition, each file was analyzed by the companion dbscSNV database, which includes all potential human SNV within splicing consensus regions (-3 to +8 at the 5' splice site and -12 to +2 at the 3' splice site), i.e. splicing consensus regions (scSNVs), and predictions for their potential of altering splicing.

Quality control and prioritization. Pooled sequencing, the initial quality analysis allow only variants with the quality read depth of bases with Phred score ≥30 and average per-pool sample depth of bases with Phred score ≥30 were subjected for further analysis. Low variant counts per pool (<12) were also filtered out. All variants were then additionally filtered using a Forward/Reverse stand balance between 10-90% (strand bias). Because of the study focus on the low frequency variants (MAF <5%) all variants with MAF ≥5% were removed from further analysis.

1.2 Cell cultures and membrane potential measurements. Cell culture. 1% penicillin/streptomycin/glutamine and 10% non-essential amino acids. The CHO-M1 cells were incubated in a humidified atmosphere (5% CO_2/95% air) at 37˚C. Twenty-four hrs prior to the start of the fluorescence assay, the CH-M1 cells were co-transfected with wild-type or mutant KCNQ1 cDNA constructs employing electroporation with the NEON Electroporator (Thermo Fisher Scientific) with three 1125 V pulses for 15 msec duration. Each electroporation solution contained 2 μg of KCNQ1 construct. Following the electroporation, 60,000 cells were plated onto each well (96-well format) in a final volume of 90 μl containing supplemented F12 and placed in the incubator overnight.

The Fluorescence Imaging Plate Reader (FLIPR) on FlexStation 3 (Molecular Devices) was employed to measure the fluorescence changes. The blue FLIPR membrane potential dye (Molecular Devices) was reconstituted in non-supplemented DMEM (without phenol red). Thereafter, the DMEM was aspirated from the wells and the cells were loaded with 90 μl of dye solution and 90 μl of DMEM (without phenol red) for at least 30 min in the cell incubator. After the cells were loaded, the plate with the cells and plate containing OxoM (10 nM) were inserted into the plate reader. Data acquisition was performed with SoftMax Pro software (Molecular Devices) in which the dye was excited at a wavelength of 530 nm and emitted at 565 nm. Fluorescence readings were acquired every 1.6 sec and OxoM was applied to each well 20 sec after the start of the recording.

Correspondence to: Marek Postula, MD, PhD: mpostula@wum.edu.pl