SUPPLEMENTAL MATERIAL

Methods

Study Subjects

From eight hospitals in the region of Copenhagen, patient records with the ICD-10 diagnose code I48.9 (atrial fibrillation and flutter) were collected and read. Clinical characteristics of the patients are shown in Table S1.

Table S1. Clinical data of patient and control populations

	Lone AF	**AF**	**Controls**
N	358	157	750
Median age of AF onset, y (IQR)	34.5 (28-39)	-	
Median age, y (IQR)	-	66.2 (32-86)	66 (52-76)
Male gender	297 (83%)	108 (68%)	385 (52%)
BMI	26	27.1	26
AF type			
Paroxysmal	224 (62.6%)	19 (12%)	
Persistent	113 (31.6%)	93 (58.9%)	
Permanent	21 (5.9%)	46 (29.1%)	
First degree relatives with AF	127 (35.5%)	-	
Hypertension	-	70 (44.3%)	430 (58%)
Diabetes	-	17 (10.8%)	30 (4%)

BMI is given as mean values. AF, atrial fibrillation; BMI, body mass index (calculated as weight [kg]/height2[m2]; IQR, interquartile range.

SCN10A Screening

Genomic DNA was isolated from blood samples using the ReliaPrep™ Blood gDNA Miniprep System (Promega, USA). Using intronic primers the entire coding sequence and splice junctions of SCN10A [NM_006514.2] were amplified and analyzed using high-resolution melting curve

analysis (Light Scanner, Idaho Technology, Salt Lake City, UT, USA). The control cohorts and publicly available databases (Exome sequencing project database (n=6,503, ESP6500 and dbSNP) were screened for all rare variants identified in the patients. In probands with non-synonymous variants, bidirectional sequencing of genes previously associated with AF was performed.

All primers were designed with M13 tail sequences. Fragments with different melting curves than wild-type DNA were directly sequenced using Big Dye chemistry (DNA analyzer 3730, Applied Biosystems, Foster City, CA, USA). All identified non-synonymous variants were validated by resequencing in an independent polymerase chain reaction.

In probands with non-synonymous variants bidirectional sequencing of SCN1-3B (NM_001037.4, NM_004588, NM_018400.3), SCN5A (NM_00035), KCNQ1 (NM_000218.2), KCNH2 (NM_000238), KCNN3 (NM_002249.5), KCNN2 (NM_021614.2), KCNA5 (NM_002234.2), KCNE1/2/3/5 (NM_001127668, NM_172201, NM_005472.4, NM_012282.2), KCNJ2/3/5 (NM_000891.2, NM_002239.3, NM_000890.3), ANP (NM_006172.3), Cx40/43 (NM_005266.5, NM_000165.3) and LMNA (NM_005572) was performed.

SNP genotyping

The DNA was extracted from whole blood, using the ReliaPrep™ Blood gDNA Miniprep System (Promega, USA). The SNP genotype for rs6795970 was determined using fluorescence-based real-time polymerase chain reaction (PCR) (ABI PRISM 7900 Sequence Detection System; Applied Biosystems, Foster City, CA) and a predeveloped TaqMan assay (Applied Biosystems). An allelic discrimination run was performed allowing for discrimination between the allele compositions of each sample. For genotype and allele frequencies please see Table S2.

Table S2. The Genotype and Allele Frequencies of rs6795970.

	Genotype Frequency (%)			*Allele Frequency (%)*	
	Minor Allele Homozygote	***Heterozygote***	***Major Allele Homozygote***	***Allele***	***Allele***
rs6795970	**AA**	**AG**	**GG**	**A**	**G**
Total AF cases	58	208	243	324	694
n = 508	(11,4)	(40,9)	(47,7)	(31,8)	(68,2)
Lone AF cases	45	136	173	226	482
n = 354	(12,7)	(38,4)	(48,9)	(31,9)	(68,1)
Controls Holter	68	256	190	392	636
n=514	(13,2)	(49,8)	(37,0)	(38,1)	(61,9)
Controls	27	107	82	131	249
n=216	(12,5)	(49,5)	(38,0)	(34,5)	(65,5)
Total Controls	95	363	272	553	907
n=730	(13,0)	(49,7)	(37,3)	(37,9)	(62,1)
Danish Control	899	2808	2454	4606	7716
n=6161	(14.6)	(45.6)	(39.8)	(37.4)	(62.6)
Controls ESP	676	2072	1552	3424	5176
n = 4300	(15.7)	(48.2)	(36.1)	(39.8)	(60.2)
Controls Danish Cohort+ESP	1575	4880	4006	8030	12892
	(15.1)	(46.6)	(38.3)	(38.4)	(61.6)

The genotype distributions and allele frequencies of rs6795970 in different cohorts are presented

Bioinformatics and Statistical Analysis

We used 4 in silico tools to predict whether the variants were disease causing (PolyPhen-2, SIFT, Grantham Prediction and Conservation). Co-segregation analyses of the variants within the family members were also done. The variants were checked for whether they were located in conserved genomic sequence of the family members of hSCN1A–hSCN10A. Species alignment

across eukaryotic species was performed.

Cloning of Sodium Channel Subunits

For the site directed mutagenesis, PCR with the variant selective primers was performed with the following PCR parameters: 1) 96°C/2 min; 2) 96°C/30 sec; 3) 55°C/15 sec; 4) 72°C/7 min; 5) 72°C/10 min, with the total number of cycles (Step 2-4) being 20. The PCR reaction was subsequently digested with DpnI (Fermentas, Denmark) and transformed into and amplified in SURE cells (Agilent Technologies, Denmark).

Cell Culture and transfection

The neuroblastoma cell line, Neuro-2A cells (ATCC, USA), were maintained in 90% Dulbecco's Modified Eagles Medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin in a 5% CO2 incubator at 37°C. Neuro-2A cells were trypsinized, diluted in culture medium, and grown in 35-mm dishes. When grown to 30–50% confluence, cells were transiently co-transfected with 1 μg wild-type (WT) or mutant pc-hSCN10A and 0.2 μg of pcDNA3-EGFP as a reporter gene, using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions.

Molecular Cloning of Sodium Channel

The genetic variations in hSCN10A (GenBank Acc. No. [NM_006514.2]), were introduced into the pCMV6-AC-GFP-hSCN10A plasmid (Origene, USA), via site-directed mutagenesis using a full plasmid overlap PCR strategy (PfuTurbo DNA Polymerase, Stratagene, Denmark). The variants V94G (GTG->GGG), A1073 (GTC->GCC), P1092 (CTA->CCA), and R1588Q (CGA->CAA) were introduced by overlapping oligonucleotides designed in Vector NTI Advanced 10 (Invitrogen, Denmark) and constructed by Eurofins MWG Operon (Germany). All of the plasmid constructs were verified by complete DNA sequencing of the hSCN10A cDNA and flanking

regions (Macrogen Inc., Seoul, Republic of Korea).

In vitro Electrophysiology

Patch-clamp experiments were performed 2–3 days after transfection. Whole-cell currents were measured at room temperature (20–22 °C). The internal pipette solution consisted of (in mM) CsCl 60, Cesium aspartate 70, CaCl2 1, MgCl21, HEPES 10, EGTA 11, MgATP 5 (pH 7.2 with CsOH); the external solution consisted of (in mM): NaCl 130, CsCl 5, CaCl2 2, MgCl2 1.2, HEPES 10, glucose 5 (pH 7.4 with CsOH). Measurements were made with Pulse software and using an EPC-9 amplifier (HEKA Elektronik, Germany). Borosilicate glass pipettes were pulled on a DPZ-Universal puller (Zeit Instrument, Germany). The pipettes had a resistance of 1.5–2.5 MΩ when filled with intracellular solution. The series resistances recorded in the whole-cell configuration were 2–5 MΩ and were compensated (80 %). As the Neuro-2A cells endogenously express TTX sensitive sodium currents, cells where incubated with 300 nM TTX prior to measurement. Recordings were performed between minute 5 and 10 after obtaining a whole cell configuration. Electrophysiological data were analyzed using Igor Pro (Wavemetrics, USA) and GraphPad Prism (GraphPad Software Inc., USA).

LuCamp Partner List

Professor, LuCamp Centre Director, Oluf B. Pedersen
1) Hagedorn Research Institute, Gentofte, Denmark
2) Marie Krogh Center for Metabolic Research, Section of Metabolic Genetics, Faculty of Health Sciences, University of Copenhagen, Denmark

Research Manager, Daniel R. Witte, MD, PhD
Steno Diabetes Center, Gentofte, Denmark

Professor Torben Hansen
1) Marie Krogh Center for Metabolic Research, Section of Metabolic Genetics, Faculty of Health Sciences, University of Copenhagen, Denmark
2) Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

Gitte Andersen, PhD
Hagedorn Research Institute, Copenhagen, Denmark

Professor Jun Wang
BGI-Shenzhen, Shenzhen, China

Professor Lars Bolund
Department of Human Genetics, University of Aarhus, Denmark

Professor Torsten Lauritzen
Faculty of Health Sciences, University of Aarhus, Denmark

Professor, Head of Department, Karsten Kristiansen
Department of Biology, University of Copenhagen, Denmark

Professor Torben Jørgensen
Research Centre for Prevention and Health, Glostrup University Hospital, Glostrup, Denmark

Professor Arne Astrup
Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark.

Professor Thue W. Schwartz
1) Marie Krogh Center for Metabolic Research, Metabolic Receptology and Enteroendocrinology, Faculty of Health Sciences, University of Copenhagen, Denmark
2) Laboratory for Molecular Pharmacology, University of Copenhagen, Copenhagen, Denmark

Professor Rasmus Nielsen
1) Department of Biology, University of Copenhagen, Copenhagen, Denmark
2) Department of Statistics, University of California Berkeley, Berkeley, California, USA

Anders Albrechtsen, PhD
1) Department of Biology, University of Copenhagen, Copenhagen, Denmark
2) Department of Statistics, University of California Berkeley, Berkeley, California, USA