European Heart Journal (2009) **30**, 487–496
doi:10.1093/eurheartj/ehn520

Transcriptional profiling of ion channel genes in Brugada syndrome and other right ventricular arrhythmogenic diseases

Nathalie Gaborit[1,2,3†], Thomas Wichter[4,5†], Andras Varro[6,7], Viktoria Szuts[7], Guillaume Lamirault[1,2], Lars Eckardt[4], Matthias Paul[4], Günter Breithardt[4], Eric Schulze-Bahr[4], Denis Escande[1,2,3], Stanley Nattel[8], and Sophie Demolombe[1,2,3*]

[1]INSERM, UMR915, l'institut du thorax, Nantes F-44000, France; [2]CNRS, ERL3147, Nantes F-44000, France; [3]Université de Nantes, Nantes F-44000, France; [4]Department of Cardiology and Angiology, Hospital of the University of Münster and Leibniz Institute for Arteriosclerosis Research (LIFA), Münster, Germany; [5]Department of Internal Medicine and Cardiology, Marienhospital Osnabrück, Osnabrück, Germany; [6]Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary; [7]Division for Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary; and [8]Department of Medicine and Research Center, Montreal Heart Institute and Université de Montréal, Montréal, Quebec, Canada

Received 3 April 2008; revised 30 September 2008; accepted 23 October 2008; online publish-ahead-of-print 23 November 2008

Aims Brugada syndrome is an inherited sudden-death arrhythmia syndrome. Na^+-current dysfunction is central, but mutations in the *SCN5A* gene (encoding the cardiac Na^+-channel Nav1.5) are present in only 20% of probands. This study addressed the possibility that Brugada patients display specific expression patterns for ion-channels regulating cardiac conduction, excitability, and repolarization.

Methods and results Transcriptional profiling was performed on right-ventricular endomyocardial biopsies from 10 unrelated Brugada probands, 11 non-diseased organ-donors, seven heart-transplant recipients, 10 with arrhythmogenic right-ventricular cardiomyopathy, and nine with idiopathic right-ventricular outflow-tract tachycardia. Brugada patients showed distinct clustering differences vs. the two control and two other ventricular-tachyarrhythmia groups, including 14 of 77 genes encoding important ion-channel/ion-transporter subunits. Nav1.5 and K^+-channels Kv4.3 and Kir3.4 were more weakly expressed, whereas the Na^+-channel Nav2.1 and the K^+-channel TWIK1 were more strongly expressed, in Brugada syndrome. Differences were also seen in Ca^{2+}-homeostasis transcripts, including stronger expression of RYR2 and NCX1. The molecular profile of Brugada patients with *SCN5A* mutations did not differ from Brugada patients without *SCN5A* mutations.

Conclusion Brugada patients exhibit a common ion-channel molecular expression signature, irrespective of the culprit gene. This finding has potentially important implications for our understanding of the pathophysiology of Brugada syndrome, with possible therapeutic and diagnostic consequences.

Keywords Gene expression • Humans • Ion channels • Ventricular tachycardia

Introduction

Brugada syndrome, first described in 1992, has a high risk of sudden cardiac death.[1] The syndrome associates a typical ECG pattern (ST-segment elevation in the right precordial leads) with an increased risk of life-threatening ventricular arrhythmias.[2] Inherited as an autosomal dominant trait, Brugada syndrome is associated with mutations in the *SCN5A* gene encoding the pore-forming α-subunit of the cardiac Na^+-channel (Nav1.5) in about 20% of cases.[3] The diagnosis of Brugada syndrome may be difficult because penetrance is age-dependant, gene-carriers are often asymptomatic, and the ECG pattern can be dynamic or concealed, although pharmacologic challenge can unmask the phenotype.[2]

† These authors contributed equally.

* Corresponding author: INSERM UMR915, 1, rue G. Veil, 44035 Nantes cedex, France. Tel: +33 240 41 11 11, Fax: +33 240 41 29 50, Email: sophie.demolombe@nantes.inserm.fr

Since only a minority of Brugada patients has detectable mutations, we considered alternative disease mechanisms involving a consistent pattern of abnormalities in cardiac conduction and arrhythmogenesis. We specifically hypothesized that a common pattern of cardiac ion-channel and transporter gene-expression might contribute to the pathophysiology of Brugada syndrome, by producing a phenotypic background that facilitates the expression of disease characteristics in the context of appropriate genotypic and/or environmental factors. To test this notion, we applied high-throughput real-time PCR to obtain full profiling of ion-channel subunit expression in right-ventricular septal endomyocardial biopsies from patients with Brugada syndrome, in comparison with results from two control groups and two patient-groups with other types of arrhythmogenic right-ventricular disorders.

Methods

Samples

All protocols for sample procurement and handling were approved by local institutional research boards and informed consent was obtained. From October 2004 to December 2005, endomyocardial biopsies from 10 unrelated consecutive Brugada-syndrome patients were collected at the Department of Cardiology and Angiology, Hospital of the University of Münster, Germany. The final diagnosis of Brugada syndrome was made following presently accepted standards, including type-I ECGs in all.[2] Patients were genotyped by direct sequencing of all coding exons of the *SCN5A* gene.[3]

A minimum of five endomyocardial biopsies (1–2 mm^3) were collected per patient from the right-ventricular septum, at least 7 days after the last arrhythmic episode to exclude the effects of the acute episode and/or its treatment. Three biopsies were fixed for histology to detect structural heart disease, in particular arrhythmogenic right-ventricular cardiomyopathy (ARVC). Histological examination showed non-specific fatty-tissue infiltration in one of the three samples from patient V, mild focal interstitial fibrosis in patient III, and focal interstitial fibrosis with borderline myocyte hypertrophy in adjacent areas in patient IV. Histology was normal in other cases. No patient showed signs of inflammation or ARVC. The remaining samples were immediately frozen in liquid-N_2 and stored at $-80^{\circ}C$ for research purposes.

Cardiac material from two distinct control groups, both age- and sex-matched to Brugada-syndrome patients, was also used (Supplementary material online, *Table S1*). Control group 1 (Ctl-1; 10 males, one female; mean age 39.5 $\pm$ 3.7 years) consisted of non-diseased endomyocardial tissue from hearts explanted to collect pulmonary and aortic valves for transplant surgery at the University of Szeged, Hungary. Subjects from whom hearts were explanted received no medication except dobutamine, furosemide, and plasma expanders. All experimental protocols for Ctl-1 sample procurement and handling were approved by the Ethical Review Board of the Medical Center of the University of Szeged (No. 51-57/1997 OEJ) and conformed to the Helsinki Declaration of the World Medical Association.[4] Cardiac tissue was stored in cardioplegic solution at $4^{\circ}C$ for 4–8 h. Endomyocardial slices, 1 mm thick, were carefully cut from the right-sided ventricular septum and frozen in liquid nitrogen.[5] Tissue preservation and dissection procedure in this group differed from Brugada patients, so we included an additional control group (Ctl-2; five males, two females; mean age 37.8 $\pm$ 2.9 years), from whom biopsies were obtained during routine post-transplantation follow-up (in the absence of any signs of rejection) $<$1 month after heart-transplantation in an identical fashion to Brugada-syndrome samples. These control groups allowed us to control for tissue-procurement and handling methods (identical for Brugada and Ctl-2 patients) and to control for potential post-transplant changes (absent in both Brugada and Ctl-1 subjects).

To determine whether the ion-channel expression profile of Brugada patients is different from patients with other forms of right-ventricular tachyarrhythmia, we examined endomyocardial samples obtained with the same biopsy-catheter protocol used for Brugada patients and Ctl-2, from 10 patients with ARVC and nine patients with idiopathic right-ventricular outflow-tract tachycardia (RVOT), at the University Hospital in Münster, Germany. The diagnosis was established in all patients by detailed non-invasive and invasive investigations according to established diagnostic criteria.[6] Endomyocardial biopsies in patients with RVOT were obtained within a broader research protocol related to the pathophysiological assessment of patients with inherited ventricular tachyarrhythmias. The protocol was approved by the Institutional Review Board and Ethics Committee of the University Hospital in Münster, Germany.

RNA preparation

Two snap-frozen endomyocardial biopsies per patient were pooled (constituting one sample set) for RNA extraction. Total RNA was isolated using TRIzol (Life Technologies) and DNase treated with the RNeasy Fibrous Tissue Micro Kit (Qiagen). RNA quality was assessed by polyacrylamide-gel micro-electrophoresis (Agilent 2100 Bioanalyzer). Lack of genomic DNA contamination was verified by PCR.

TaqMan real-time reverse-transcriptase–polymerase chain reaction

TaqMan low-density arrays (TLDA; Applied Biosystems) were used in a two-step reverse-transcriptase–polymerase chain reaction (RT–PCR) process, as previously reported.[7,8] First-strand cDNA was synthesized from 200 ng total RNA using the High-Capacity cDNA Archive Kit (Applied Biosystems). PCR reactions were then performed on TLDA with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). The 384 wells of each card were pre-loaded with 96 $\times$ 4 pre-designed fluorogenic TaqMan probes and primers. Probes were labelled with the fluorescent reporter 6-carboxyfluorescein (FAM®; Applera Corp.) at the 5′-end and with non-fluorescent quencher on the 3′-end. The genes selected (see Supplementary material online, *Table S2*) encode 67 α- and β-ion channel subunits, 17 Na^+/K^+-ATPase isoforms, and proteins involved in Ca^{2+}-homeostasis, eight markers (ANF, BNP, β-MHC, CD4, CNN1, COL6A1, IL6, and UCHL1) and four reference genes. Data were analysed by the threshold cycle (Ct) relative-quantification method.[9] TaqMan gene expression assays average 100% efficiency ($\pm$10%, Applied Biosystems application note). We selected the hypoxanthine guanine phosphoribosyl transferase (HPRT) gene for data normalization, as the most uniformly distributed gene. The relative expression of each gene vs. HPRT was calculated for each sample set ($2^{-\Delta Ct}$ indicates normalized data). Medians $\pm$ median absolute deviation (MAD) in each group are listed in Supplementary material online, *Tables S3A–E*. Seven genes (Cavγ4, Cavγ6, CFTR, Kv1.1, Kv1.3, Kv4.2, and Kir3.1) were eliminated because of below detection-threshold signals (>50% undetermined data). Among remaining genes, 67 exhibited no undetermined data points and 10 exhibited a maximum of 7% undetermined data.

Data analysis

Data were analysed independently by two procedures: (i) two-way hierarchical agglomerative clustering was applied to the gene-expression matrix consisting of biopsies and the ΔCt for each of the 77 electrical-signalling genes. We applied average linkage-clustering with uncentred correlation using Cluster software.[10] Clusters were visualized using Treeview software; (ii) gene-expression values in control and right-ventricular disease groups were first compared with the Wilcoxon rank-sum test, followed by the Significance Analysis of Microarrays (SAM version 2023A)[11] to minimize false-positive gene identification by using repeated random-sample permutations. The false-discovery rate was set at 0.5% so that less than 1 out of the 77 analysed genes would be falsely identified per inter-group comparison as differentially expressed. To assess the over or under expression of each gene in the Brugada group, we calculated the variation of gene expression defined as: (absolute gene-expression value minus median gene-expression value in control group)/median control gene-expression value ($\times 100\%$).

Results

Clinical characteristics

Ten patients (nine males, one female: mean age 36.4 $\pm$ 3.3 years) with a clinical diagnosis of Brugada syndrome entered the study (*Table 1*). Seven were symptomatic with cardiac arrest ($n = 1$), syncope ($n = 5$), or attacks of dizziness ($n = 1$). A spontaneous type-1 Brugada ECG was present in all three asymptomatic patients and five symptomatic patients. In the remaining two symptomatic patients, type-1 ECGs were provoked by ajmaline challenge (1 mg/kg i.v.).[12] Ten male patients with ARVC (mean age 48.4 $\pm$ 3.3 years) and nine patients with idiopathic right-ventricular outflow-tract tachycardia (two males, seven females; mean age 50.4 $\pm$ 4.6 years) were enrolled (*Table 2*).

Hierarchical clustering

Hierarchical clustering analysis was applied to the 77 genes and 28 samples from Brugada syndrome and control subjects, and grouped samples according to gene-expression similarities, with the most similar expression patterns located closest to each other and the most different furthest apart. For visual appreciation, samples are linked to a tree: the size of tree branches indicates the distance between clusters, larger branch sizes indicating greater cluster-separation (*Figure 1*). There was a clear separation between Brugada and control (Ctl-1; Ctl-2) samples. Ctl-1 samples grouped on a distinct branch from those containing Brugada and Ctl-2 samples. The separation of Ctl-1 from Brugada and Ctl-2 samples may be due to the different methods use to obtain tissue-samples from Ctl-1 patients (scalpel biopsies on tissue stored in cardioplegic solution for up to several hours) vs. Ctl-2 and Brugada groups (fast-frozen catheter biopsies). When the branch containing only the biopsies is considered, Brugada samples grouped on a single sub-branch, distinct from Ctl-2. Thus, the Brugada samples have distinct transcriptional expression patterns for ion-channel and transporter subunits for a sufficient number of genes to endow a specific transcriptional signature. Among Brugada patients, the five

Table 1 Clinical characteristics of patients with Brugada syndrome

Patient	Sex	Age (years)	SCN5A mutation	Nucleotide alteration	VF	Symptoms	ECG findings during sinus rhythm		QRS duration (ms)	Electrophysiology	
							Type-1 ECG at baseline	Type-1 ECG after ajmaline challenge		HV interval (ms)	Induced VF
BrS I	Male	31	No	No	+	Cardiac arrest	No	Yes	100	35	Yes
BrS II	Male	51	V1764F	5290 G>T		Asymptomatic	Yes	Yes	100	75	Yes
BrS III	Male	26	F861WfsX90	2581–2582delTT		Syncope	Yes	n.a.	100	60	No
BrS IV	Male	21	1493delK	4477–4479delAAG		Syncope	Yes	Yes	120	50	No
BrS V	Male	41	No	No		Syncope	No	Yes	110	50	No
BrS VI	Male	35	E1574K	4720 G>A		Syncope	Yes	Yes	110	60	No
BrS VII	Male	50	No	No		Asymptomatic	Yes	Yes	110	36	No
BrS VIII	Male	43	No	No		Pre-syncope	Yes	Yes	120	35	Yes
BrS IX	Female	26	No	No		Asymptomatic	Yes	Yes	110	35	No
BrS X	Male	40	P1002HfsX25	3005–3012 delCCAGCTGC		Syncope	Yes	No	110	60	No

VF, ventricular fibrillation; HV, bundle of His to ventricle; n.a., non-applicable.

Table 2 Clinical characteristics of patients with arrhythmogenic right-ventricular cardiomyopathy and right-ventricular outflow-tract tachycardia

Patient	Gender	Age (years)	VT	RV-dysfunction	QRS duration (ms)	HV interval (ms)	Induced arrhythmia
ARVC I	Male	55	sVT	Moderate	90	40	sVT
ARVC II	Male	45	nsVT	Moderate	100	50	NI
ARVC III	Male	34	sVT	Moderate	100	50	sVT
ARVC IV	Male	51	sVT	Severe	100	40	sVT
ARVC V	Male	58	sVT	Moderate	90	40	sVT
ARVC VI	Male	65	sVT	Moderate	100	40	sVT
ARVC VII	Male	42	sVT	Moderate	75	34	sVT
ARVC VIII	Male	58	sVT	Severe	90	55	sVT
ARVC IX	Male	43	sVT	Severe	80	50	sVT
ARVC X	Male	34	sVT	Severe	105	30	nsVT
RVOT I	Female	65	nsVT	Normal	70	40	NI
RVOT II	Female	54	nsVT	Normal	80	35	NI
RVOT III	Female	65	sVT	Normal	100	45	nsVT
RVOT IV	Female	53	sVT	Normal	65	40	NI
RVOT V	Female	48	nsVT	Normal	80	40	NI
RVOT VI	Female	23	nsVT	Normal	80	45	NI
RVOT VII	Male	51	nsVT	Normal	95	40	NI
RVOT VIII	Female	32	nsVT	Normal	90	45	NI
RVOT IX	Male	63	nsVT	Normal	90	40	NI

ARVC, arrhythmogenic right-ventricular cardiomyopathy; RVOT, right-ventricular outflow-tract tachycardia; VT, sustained ventricular tachycardia; NI, non-inducible; nsVT, non-sustained ventricular tachycardia; RV, right-ventricular.

carrying *SCN5A*-gene mutations (patients II, III, IV, VI, and X) did not cluster separately from the five patients without *SCN5A* mutation. Five gene clusters discriminated Brugada patients from controls (*Figure 1*). Gene groups A and B had a lower level of expression in Brugada patients vs. controls, whereas a higher level of expression was found for Brugada patients in gene groups C, D, and E.

To challenge the specificity of the ion-channel transcriptional signature of Brugada patients, we examined biopsies from patients with right-ventricular tachycardia due to conditions other than Brugada syndrome (*Figure 2*). The Brugada patients were grouped on one branch, distinct from ARVC and RVOT patients. Three gene clusters that discriminated Brugada patients are indicated. Gene groups A and B were expressed at a higher level in Brugada patients, whereas the opposite was true for group C. In contrast, the ion-channel expression profile of the ARVC patients intermingled with the RVOT-patient profile (as vs. the both control groups; Supplementary material online, *Figure S1*). Of interest, many of the same gene-expression variations that distinguished Brugada patients from controls also differentiated them from right-ventricular arrhythmia patients.

Differentially expressed genes in Brugada-syndrome tissue

Since we used two distinct control groups and two right-ventricular tachyarrhythmia groups, four collections of genes that were differentially expressed in Brugada-syndrome hearts were identified: one reached significance when compared with Ctl-1 ($n = 54$ genes), one reached significance vs. Ctl-2 ($n = 25$ genes), one was significant compared with ARVC ($n = 33$ genes), and the other was significant vs. RVOT ($n = 48$ genes). Detailed gene-expression values are provided in Supplementary material online, *Tables S3A–E*. We then focused on the 14 genes that showed consistent differential expression between Brugada patients and each of the four other groups (Ctl-1, Ctl-2, ARVC, and RVOT; *Figures 3–5*). As expected, all of the 14 genes are included in the gene clusters that discriminate Brugada patients (*Figures 1* and *2*). Brugada-syndrome samples showed consistently lower expression levels of Nav1.5-transcripts, whereas Nav1.3 (a neuronal-type Na^+-channel) and Nav2.1 (a Na^+-channel with unknown function) transcripts had higher expression levels (*Figure 3*). Among the five patients positive for *SCN5A*, two with a premature protein-truncation (patients III and X) had clearly reduced Nav1.5-transcripts (−48% and −41% vs. Ctl-2; −52% and −46% vs. ARVC), whereas the three others (patients II, IV, and VI), all without a premature protein-truncation, had normal Nav1.5 expression (+10%, −0.1%, and −0.4% vs. Ctl-2; +2%, −8%, and −8% vs. ARVC). Interestingly, the two patients with Brugada syndrome and reduced Nav1.5-expression exhibited markedly increased Nav1.3-expression (+62% and +54% vs. ARVC), whereas the three patients with normal Nav1.5-expression had a smaller increase in Nav1.3 (+20%, +20%, and +5%). Of note, ankyrin-B was not differentially expressed in Brugada-syndrome biopsies. T-type Ca^{2+}-channel Cav3.1 and HCN3 transcripts were more strongly expressed in

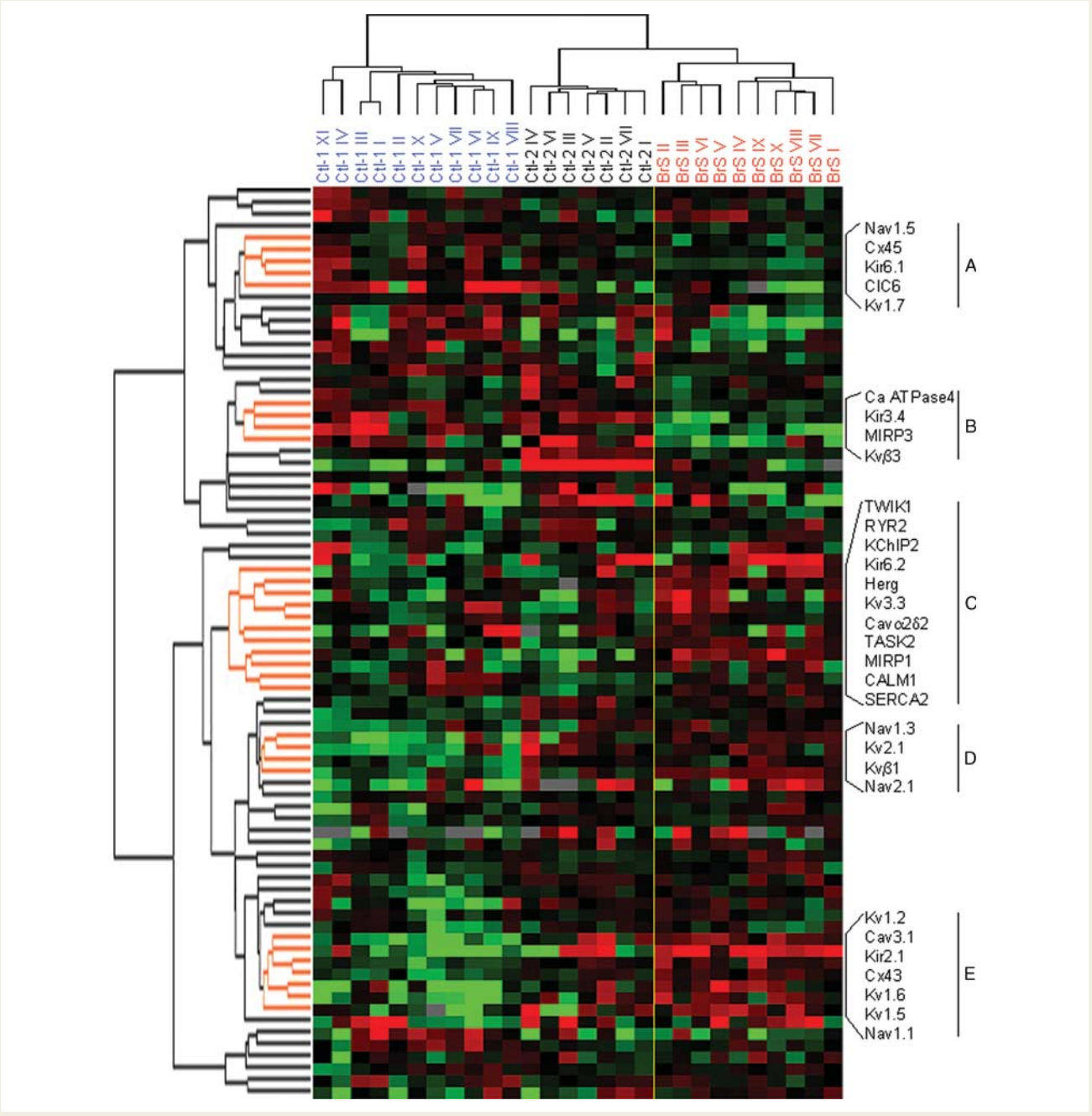

Figure 1 Two-way hierarchical agglomerative clustering applied to 77 genes (vertically) and to 11 normal tissues from donor hearts (Ctl-1), seven biopsies from transplanted hearts (Ctl-2), and biopsies from 10 patients with Brugada syndrome (BrS; horizontally). The input consisted of the ratio for each patient and gene vs. the reference gene. Each gene is represented by a single row of coloured boxes and each patient by a single column. Each colour patch in the map represents the gene expression level in one sample, with expression levels ranging from bright green (lowest) to bright red (highest). Missing values are colour-coded silver. Ctl-1 consists of non-diseased human tissues (1 mm slices of right-ventricular septal endomyocardium). Ctl-2 consists of endomyocardial biopsies from the right-ventricular septum of transplanted patients obtained via biopsy-catheter procedure. BrS consists of endomyocardial biopsies from the right-ventricular septum obtained from patients having Brugada syndrome (see Methods for more details). Five clusters containing genes that discriminate Brugada patients from controls are shown on the right (*A*–*E*).

Brugada patients. Among the K^+-channel genes (*Figure 4*), Brugada subjects showed greater expression of the 2P-domain gene TWIK1 and reduced expression of Kv4.3, involved in the transient-outward K^+-current I_{to}. Similarly, the Kir3.4 subunit of the acetylcholine-dependent K^+-channel (IK_{ACh}) was reduced. Kv4.3, in particular, showed extremely low-level expression in Brugada patients, over an order of magnitude smaller than in the other groups.

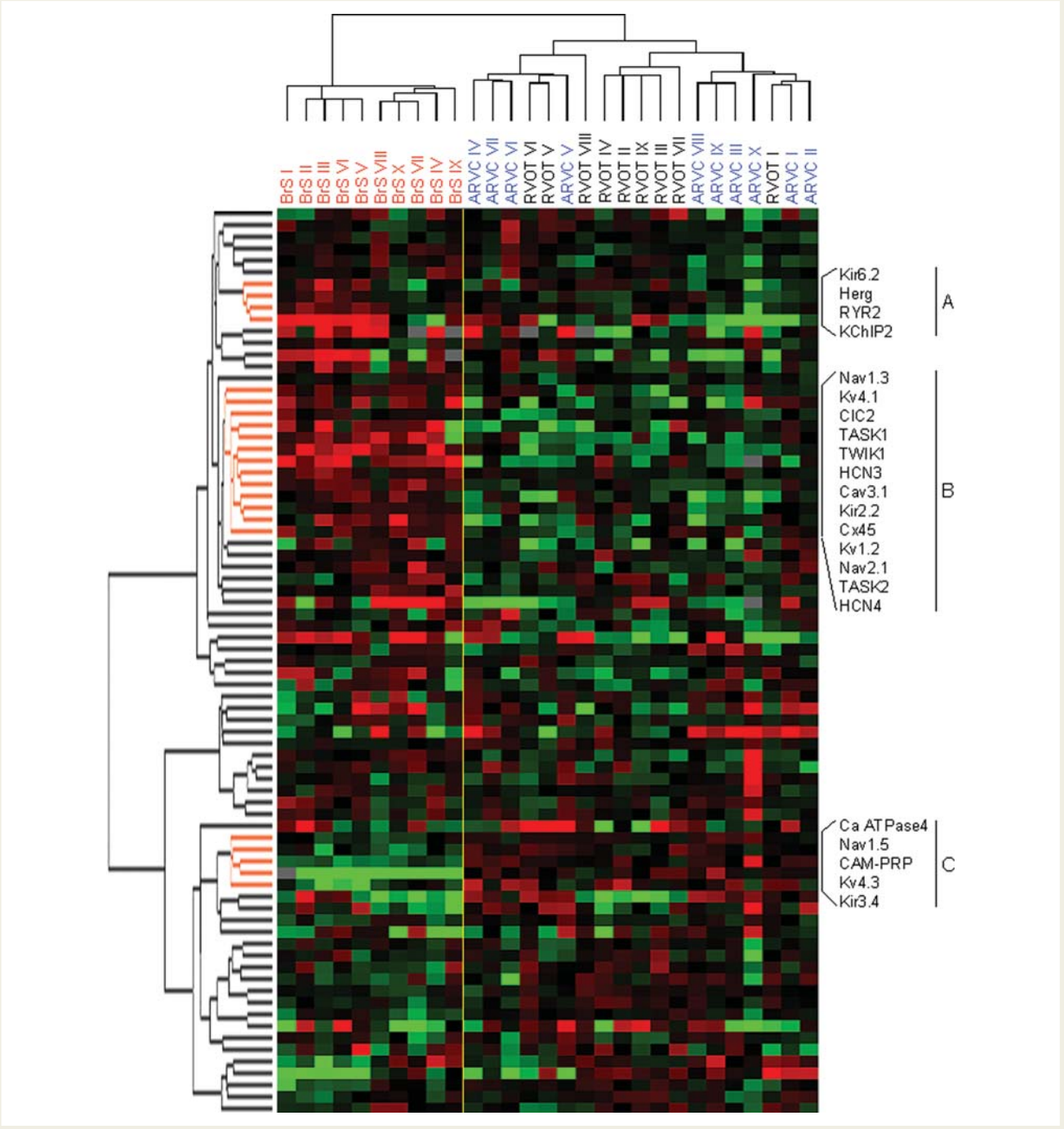

Figure 2 Two-way hierarchical agglomerative clustering applied to 77 genes (vertically) and to biopsies from 10 patients with arrhythmogenic right-ventricular cardiomyopathy (ARVC), biopsies from nine patients with idiopathic right-ventricular outflow-tract tachycardia (RVOT), and biopsies from 10 patients with Brugada syndrome (BrS; horizontally). Same format as in *Figure 1*. Three gene-clusters that discriminate Brugada patients from ARVC and RVOT patients are shown on the right (*A*–*C*).

Substantial differences were also observed for genes involved in Ca^{2+}-homeostasis. Expression of the ryanodine-receptor gene, RYR2, and Na^{+}/Ca^{2+}-exchanger, NCX1, were enhanced in Brugada-syndrome samples, whereas Ca^{2+}-ATPase type 4 and calmodulin-dependent protein phosphatase (CAM-PRP) showed slightly lower-level expression (*Figure 5*).

Figure 6 illustrates relative expression levels in individual control, ARVC, RVOT, and Brugada patients, as well as median group-values, for the 14 genes showing differential expression in Brugada syndrome. The most strongly expressed are ion-transporters and Ca^{2+}-homeostasis genes, with Na^{+}-channel subunits representing the most strongly expressed ion-channel genes.

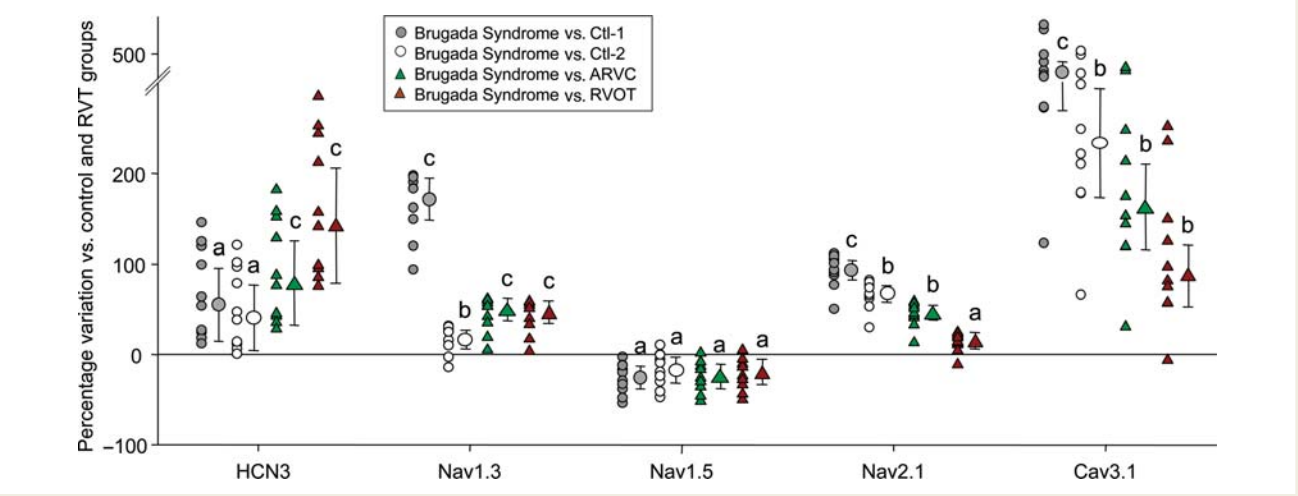

Figure 3 Cardiac ion-channel remodelling associated with Brugada syndrome. Percentage differences in expression for Brugada patients compared with the two control groups and the two arrhythmogenic groups (*y*-axis) evaluated by TaqMan low density arrays for Na^+-channels, HCN3, and Cav3.1 (*x*-axis). Data points (circles and triangles) indicate individual measurements for each patient. Larger circles and triangles represent median values for each gene ($\pm$MAD). Only genes with statistically significant differential expression in patients with Brugada syndrome compared with the other four groups are illustrated (a: $P < 0.05$, b: $P < 0.01$, c: $P < 0.001$ vs. the group of interest).

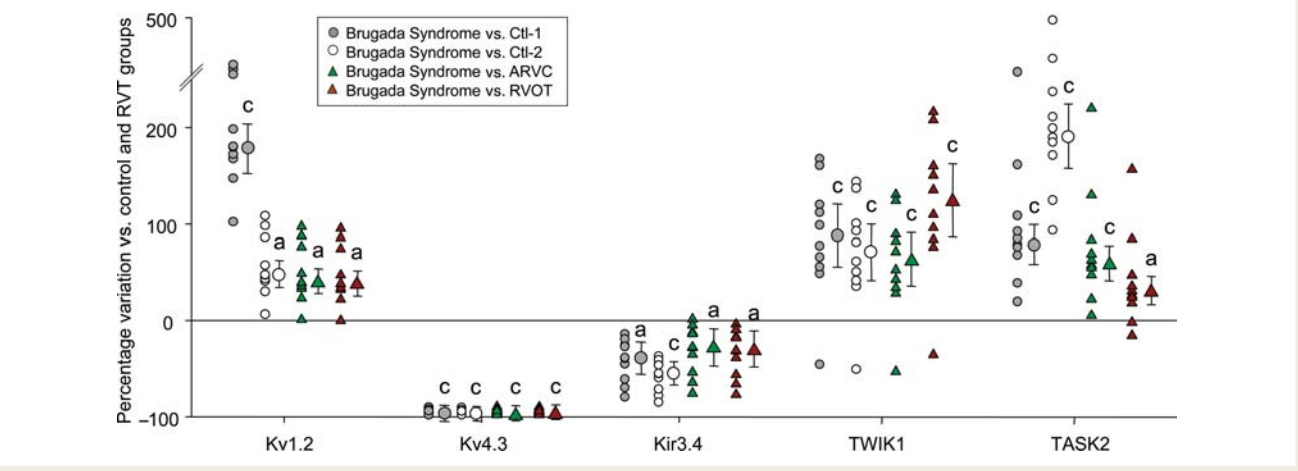

Figure 4 Cardiac potassium ion-channel remodelling associated with Brugada syndrome. Same format as in *Figure 3* (a: $P < 0.05$, c: $P < 0.001$ vs. the group of interest).

The interindividual variability appears homogenous through the five groups, excepted for TWIK1 and HCN3, which is more variable in Brugada patients than in other groups.

Discussion

Major findings

In this first report of systematic transcriptional profiling of Brugada-syndrome patients, their cardiac ion-channel expression pattern was found to be distinct from controls and from patients with other types of right-ventricular tachyarrhythmia. Differences were noted in the expression of Na^+-channels, K^+-channels, and Ca^{2+}-channel and homeostasis genes.

Pathophysiological aspects and potential significance

The most commonly accepted construct to explain Brugada-syndrome electrophysiology consists of reduced Na^+-current, which in the face of a large epicardial I_{to} causes loss of epicardial action-potential domes and marked action-potential abbreviation, generating a difference current relative to the normally repolarizing endocardium.[13] Brugada syndrome related to *SCN5A* dysfunction exhibits conduction anomalies resulting from the deficiency of the major cardiac Na^+-channel,[14] and an alternative explanation of Brugada-syndrome pathophysiology relates it to delayed activation of the right-ventricular outflow tract due to conduction abnormalities.[15] It is unclear why the penetrance of the syndrome

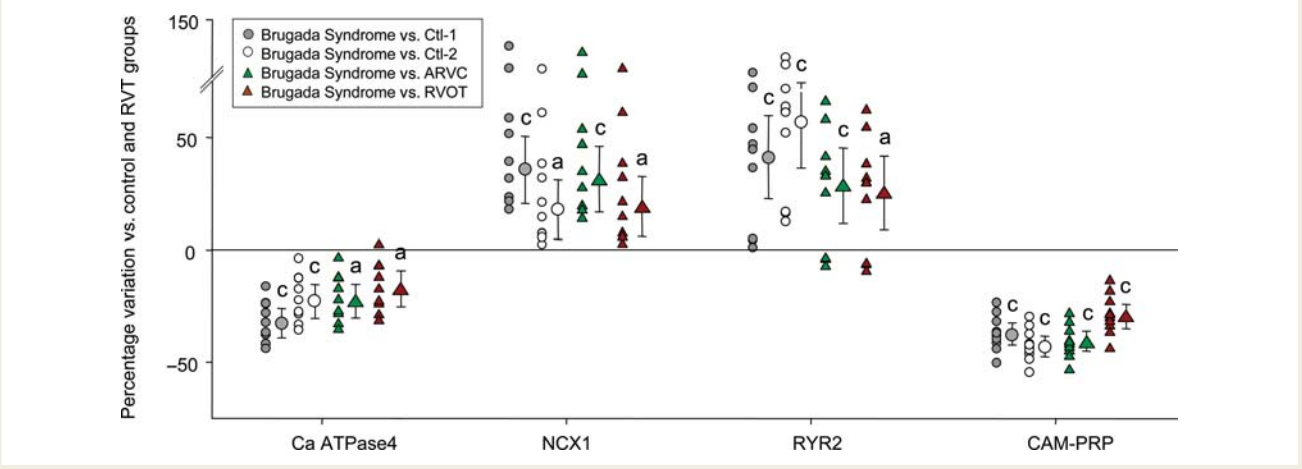

Figure 5 Cardiac calcium regulators remodelling associated with Brugada syndrome. RYR2, ryanodine receptor; NCX1, Na^+/Ca^{2+} exchanger; CAM-PRP, calmodulin-dependent protein phosphatase. Same format as in *Figures 3* and *4*. (a: $P < 0.05$, c: $P < 0.001$ vs. the group of interest).

is so variable, and why so few Brugada patients have defined Na^+-channel mutations.[15] Our finding of consistent cardiac ion-channel expression patterns in Brugada patients may have pathophysiological significance. It is possible that the occurrence of Brugada-syndrome manifestations requires not only a suitable genetic substrate, but also a distinct background ion-channel expression pattern. Alternatively, a distinct ion-channel expression pattern may be sufficient to produce Brugada-syndrome manifestations, in the face of appropriate electrophysiological modifiers such as dietary, neurohormonal, physical, or other environmental factors. Finally, the ion-channel expression variations that differentiate Brugada patients from the other groups could result from an adaptive phenomenon to a common pathophysiological alteration, resulting from yet unknown primary defects in the non-*SCN5A* Brugada patients.

Our findings could have potential value in developing novel testing for Brugada patients. The identification of distinct transcriptional profiling differences from other populations could eventually lead to molecular diagnostic procedures.

Specific ion-channel variations characteristic of Brugada patients

The reduced Nav1.5-expression characteristic of our Brugada samples may reflect the pathophysiological role suggested by the recognized importance of *SCN5A* mutations.[13–15] Nav2.1 (*SCN6A* or *SCN7A*) channels were identified for the first time in human hearts,[16] where they are expressed at comparable levels to Nav1.5-channels.[8] This atypical Na^+-channel is only 50% identical to Nav1.x-channels.[17] Attempts to express functional Nav2.1 channels using heterologous expression systems such as *Xenopus* oocytes, Chinese hamster ovary cells, and human embryonic kidney-293 cells have been unsuccessful,[18,19] suggesting the requirement of an as-yet unidentified partner. The increased transcript expression of Nav2.1 in Brugada patients could be a compensatory mechanism for the under-expression of Nav1.5. We also observed a dramatic reduction in the expression of Kv4.3, encoding the principal I_{to} subunit.[20] If the Kv4.3 under-expression we detected in endomyocardial biopsies corresponds to a large generalized decrease in I_{to}-density, this could be a way of attenuating the effects of the loss of Na^+-current. However, in Brugada syndrome, amplification of transmural dispersion of repolarization is related to large epicardial I_{to}.[21] If our samples reflect an endocardial-restricted Kv4.3/I_{to}-reduction, endocardial–epicardial I_{to}-differences could be amplified, contributing to Brugada manifestations. Under-expression of Kir3.4, involved in IK_{ACh}, could reduce the deleterious effects of the loss of Na^+-current. Indeed, Yan and Antzelevitch[22] demonstrated that parasympathetic agonists such as acetylcholine can contribute to loss of action potential dome.

In humans, the 2P-domain K^+-channels TWIK1 and TASK2 are more strongly expressed in Purkinje fibres than in right ventricle, with higher-level expression of TWIK1 than TASK2.[8] Preferential expression of a 2P-channel in Purkinje fibres also occurs in mice.[23] Increased 2P-channel expression might also compensate for reduced Na^+-channel function.

If molecular modifications in genes controlling Ca^{2+}-homeostasis, particularly over-expression of RyR2 and NCX1, translate to the functional level, Ca^{2+}-homeostasis would be significantly disturbed in right-ventricular tissue of patients with Brugada syndrome. Disruptions to Ca^{2+}-homeostasis are important contributors to the occurrence of cardiac arrhythmias.[24] A recent publication reports loss-of-function mutations in genes encoding the cardiac L-type Ca^{2+}-channel associated with a familial sudden cardiac death syndrome, in which a Brugada-syndrome phenotype is combined with shorter than normal QT intervals.[25]

Potential limitations

Because of ethical and practical considerations that limit access to myocardial-tissue samples from patients with rare arrhythmia syndromes, our sample sizes are relatively small. No significant difference was observed in the transcriptional-expression patterns of ion-channel and transporter genes between Brugada patients

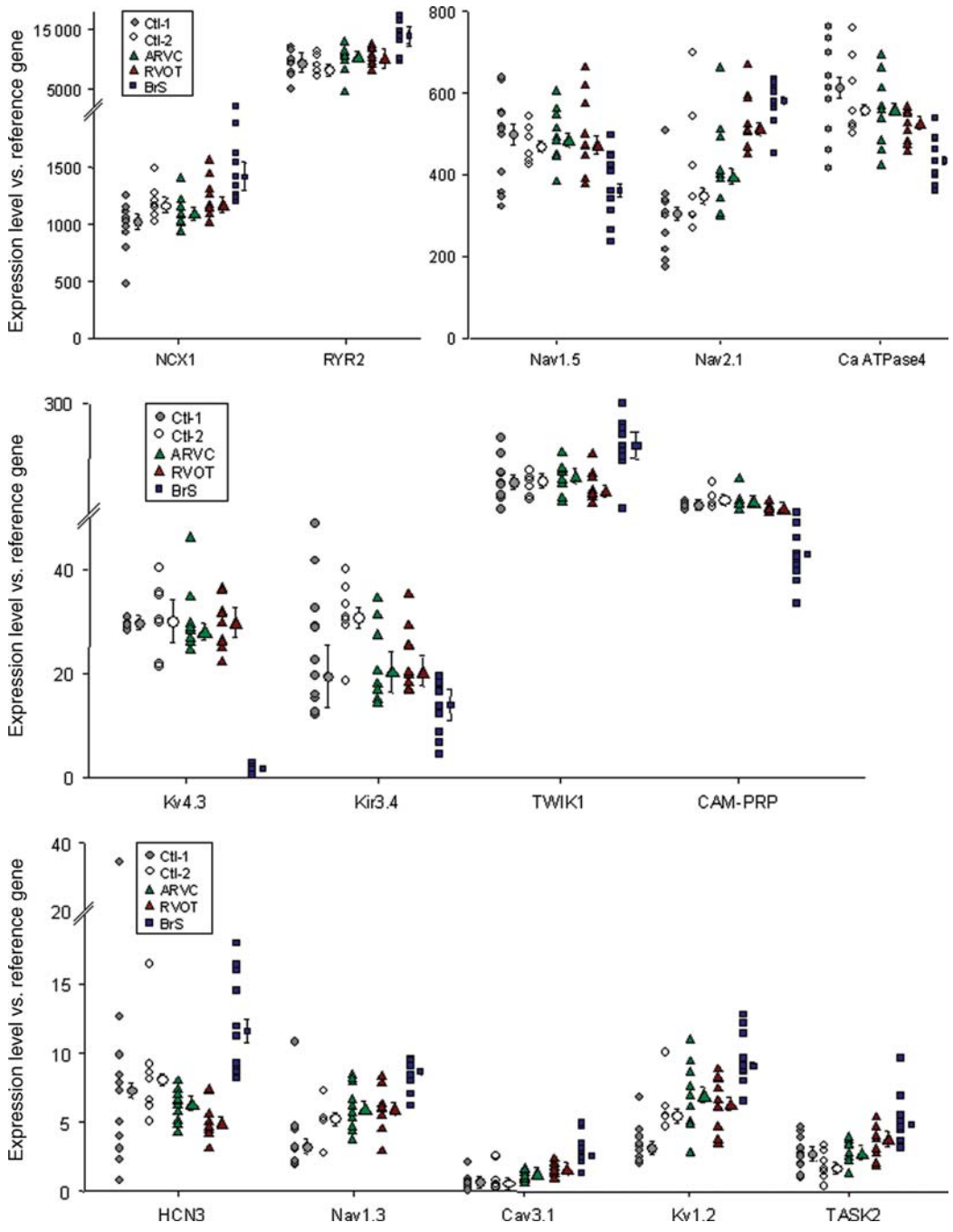

Figure 6 Relative abundance and variability of selected genes in the control and arrhythmogenic groups. The graphs show the relative quantification (*y*-axis) of genes with statistically significant differential expression in BrS patients vs. Ctl-1, Ctl-2, ARVC, and RVOT groups (*x*-axis). Data points indicate individual measurements for each patient from Ctl-1 (grey circles), Ctl-2 (empty circles), ARVC (green triangles), RVOT (red triangles), and Brugada (blue squares) groups. Larger circles, triangles, and squares represent the median ($\pm$MAD). Data are expressed as ratio vs. HPRT ($\times 100$).

carrying an *SCN5A*-gene mutation and patients without an *SCN5A*-gene mutation. However, we cannot exclude the differences that might have been detected with larger patient-populations. Ion-channel function depends on proper functioning of the expressed gene-product proteins, which we were unable to study because of the small size of our tissue-samples. In addition, tissue-sampling is of necessity limited to the right ventricle, and to an endomyocardial region of the septum. We cannot comment on potential regional differences that could play a significant pathophysiological role. Finally, because of the available patient populations, there were some differences in mean age and sex distribution, particularly for the cardiac-disease groups.

However, sex differences cannot account for the differences between Brugada and ARVC or Ctl-1/Ctl-2 patients, since these groups were predominantly male. In addition, we have examined sex-based differences in ion-channel subunit expression (unpublished observations) and virtually none of the Brugada-differential genes were identified. With respect to age issues, we have repeated the clustering with ARVC and RVOT patient-subsets age-matched to Brugada subjects (Supplementary material online, *Figure S2*) and virtually the same clustering was seen as for the entire groups.

Conclusions

In this study, we applied high-throughput ion-channel transcriptome-analysis approaches to identify novel pathways potentially involved in the pathophysiology of Brugada syndrome. Our work demonstrates clear differences between patients with Brugada syndrome and controls, and between Brugada syndrome and other right-ventricular arrhythmic diseases. Our results provide the first evidence that genomic techniques identify specific expression-profiles in inherited arrhythmia syndromes, with a potential to improve pathophysiological understanding and eventually perhaps molecular diagnostic practice.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Funding

Support was received from Agence Nationale de la Recherche (ANR-05-PCOD-037-01), and from the Leducq Foundation (Transatlantic network of excellence 'Preventing sudden cardiac death'; 05 CVD 01). The work was further supported by grants from the German Research Foundation to E.S.-B. (DFG Schu1082/3-1 and 3-2 and Ki653/13-1 and 13-2) and to T.W. (DFG SFB-556, project C4), by a KPI grant (OTKA NI-61902) to A.V., by a Canadian Institutes of Health Research Award (MOP 68929) to S.N., and by the Peter-Osypka foundation for L.E.

Conflict of interest: none declared.

References

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol* 1992;**20**:1391–1396.
2. Wilde AA, Antzelevitch C, Borggrefe M, Brugada J, Brugada R, Brugada P, Corrado D, Hauer RN, Kass RS, Nademanee K, Priori SG, Towbin JA. Study Group on the Molecular Basis of Arrhythmias of the European Society of Cardiology. Proposed diagnostic criteria for the Brugada syndrome: consensus report. *Circulation* 2002;**106**:2514–2519.
3. Schulze-Bahr E, Eckardt L, Breithardt G, Seidl KH, Wichter T, Wolpert C, Borggrefe M, Haverkamp W. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. *Hum Mutat* 2003;**21**:651–652.
4. World Medical Association Declaration of Helsinki. Recommendations guiding physicians in biomedical research involving human subjects. *Cardiovasc Res* 1997;**35**:2–3.
5. Zicha S, Xiao L, Stafford S, Cha TJ, Han W, Varro A, Nattel S. Transmural expression of transient outward potassium current subunits in normal and failing canine and human hearts. *J Physiol* 2004;**561**:735–748.
6. McKenna WJ, Thiene G, Nava A, Fontaliran F, Blomstrom-Lundqvist C, Fontaine G, Camerini F. Diagnosis of arrhythmogenic right ventricular dysplasia/cardiomyopathy. Task Force of the Working Group Myocardial and Pericardial Disease of the European Society of Cardiology and of the Scientific Council on Cardiomyopathies of the International Society and Federation of Cardiology. *Br Heart J* 1994;**71**:215–218.
7. Marionneau C, Couette B, Liu J, Li H, Mangoni ME, Nargeot J, Lei M, Escande D, Demolombe S. Specific pattern of ionic channel gene expression associated with pacemaker activity in the mouse heart. *J Physiol* 2005;**562**:223–234.
8. Gaborit N, Le Bouter S, Szuts V, Varro A, Escande D, Nattel S, Demolombe S. Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. *J Physiol* 2007;**582**:675–693.
9. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 2001;**25**:402–408.
10. Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 1998;**95**:14863–14868.
11. Tusher VG, Tibshirani R, Chu G. Significance analysis of microarrays applied to the ionizing radiation response. *Proc Natl Acad Sci USA* 2001;**98**:5116–5121.
12. Rolf S, Bruns HJ, Wichter T, Kirchhof P, Ribbing M, Wasmer K, Paul M, Breithardt G, Haverkamp W, Eckardt L. The ajmaline challenge in Brugada syndrome: diagnostic impact, safety, and recommended protocol. *Eur Heart J* 2003;**24**:1104–1112.
13. Antzelevitch C, Brugada P, Brugada J, Brugada R, Towbin JA, Nademanee K. Brugada syndrome: 1992–2002: a historical perspective. *J Am Coll Cardiol* 2003;**41**:1665–1671.
14. Smits JP, Eckardt L, Probst V, Bezzina CR, Schott JJ, Remme CA, Haverkamp W, Breithardt G, Escande D, Schulze-Bahr E, LeMarec H, Wilde AA. Genotype–phenotype relationship in Brugada syndrome: electrocardiographic features differentiate SCN5A-related patients from non-SCN5A-related patients. *J Am Coll Cardiol* 2002;**40**:350–356.
15. Meregalli PG, Wilde AA, Tan HL. Pathophysiological mechanisms of Brugada syndrome: depolarization disorder, repolarization disorder, or more? *Cardiovasc Res* 2005;**67**:367–378.
16. George AL Jr, Knittle TJ, Tamkun MM. Molecular cloning of an atypical voltage-gated sodium channel expressed in human heart and uterus: evidence for a distinct gene family. *Proc Natl Acad Sci USA* 1992;**89**:4893–4897.
17. Goldin AL, Barchi RL, Caldwell JH, Hofmann F, Howe JR, Hunter JC, Kallen RG, Mandel G, Meisler MH, Netter YB, Noda M, Tamkun MM, Waxman SG, Wood JN, Catterall WA. Nomenclature of voltage-gated sodium channels. *Neuron* 2000;**28**:365–368.
18. Felipe A, Knittle TJ, Doyle KL, Tamkun MM. Primary structure and differential expression during development and pregnancy of a novel voltage-gated sodium channel in the mouse. *J Biol Chem* 1994;**269**:30125–30131.
19. Akopian AN, Souslova V, Sivilotti L, Wood JN. Structure and distribution of a broadly expressed atypical sodium channel. *FEBS Lett* 1997;**400**:183–187.
20. Kaab S, Dixon J, Duc J, Ashen D, Nabauer M, Beuckelmann DJ, Steinbeck G, McKinnon D, Tomaselli GF. Molecular basis of transient outward potassium current downregulation in human heart failure: a decrease in Kv4.3 mRNA correlates with a reduction in current density. *Circulation* 1998;**98**:1383–1393.
21. Antzelevitch C. Role of spatial dispersion of repolarization in inherited and acquired sudden cardiac death syndromes. *Am J Physiol Heart Circ Physiol* 2007;**293**:H2024–H2038.
22. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. *Circulation* 1999;**100**:1660–1666.
23. Graham V, Zhang H, Willis S, Creazzo TL. Expression of a two-pore domain K+ channel (TASK-1) in developing avian and mouse ventricular conduction systems. *Dev Dyn* 2006;**235**:143–151.
24. Ter Keurs HE, Boyden PA. Calcium and arrhythmogenesis. *Physiol Rev* 2007;**87**:457–506.
25. Antzelevitch C, Pollevick GD, Cordeiro JM, Casis O, Sanguinetti MC, Aizawa Y, Guerchicoff A, Pfeiffer R, Oliva A, Wollnik B, Gelber P, Bonaros EP Jr, Burashnikov E, Wu Y, Sargent JD, Schickel S, Oberheiden R, Bhatia A, Hsu LF, Haissaguerre M, Schimpf R, Borggrefe M, Wolpert C. Loss-of-function mutations in the cardiac calcium channel underlie a new clinical entity characterized by ST-segment elevation, short QT intervals, and sudden cardiac death. *Circulation* 2007;**115**:442–449.