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The cardiac blood transcriptome predicts de novo onset of atrial fibrillation in heart failure

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ABSTRACT

Heart failure (HF) increases the risk of developing atrial fibrillation (AF), leading to increased morbidity and mortality. Therefore, better prediction of this risk may improve treatment strategies. Although several predictors based on clinical data have been developed, the establishment of a transcriptome-based predictor of AF incidence in HF has proven to be more problematic. We hypothesized that the transcriptome profile of coronary sinus blood samples of HF patients is associated with AF incidence. We therefore enrolled 192 HF patients who were selected for biventricular cardioverter defibrillator implantation. Both coronary sinus and peripheral blood samples were obtained during the procedure. Patients were followed-up during two years and AF occurrence was based on interrogation of the defibrillator. A total of 96 patients stayed in sinus rhythm (SR) during follow-up, 13 patients developed AF within 1 year and 10 patients developed AF during the second year of follow up. Gene expression profiling of coronary sinus samples led to the identification of 321 AF predictor genes based on their differential expression between patients developing AF within 1 year of blood sampling and patients remaining in SR. The expression levels of these genes were combined to obtain a molecular atrial fibrillation prediction score for each patient which was significantly different between both patient groups (Mann-Whitney, $p = 0.00018$). We conclude that the cardiac blood transcriptome of HF patients should be further investigated as a potential AF risk prediction tool.

1. Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia worldwide and poses a substantial health and economic burden [1]. The presence of AF is associated with a five-fold increased risk of stroke after adjusting for age [2]. In addition, up to 35 % of the disease remains undiagnosed, accounting for 15 % of stroke incidence [3]. This fact is underlined by the ASSERT trial (Asymptomatic Atrial Fibrillation and Stroke Evaluation in Pacemaker Patients and the Atrial Fibrillation Reduction Atrial Pacing Trial), which showed that asymptomatic subclinical AF detected by implanted devices is associated with a 2.5-fold increased risk of stroke [4]. Improvement of AF detection

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could therefore lead to prevention of stroke by appropriate anti-coagulation treatment. AF is also closely related to heart failure (HF), with an AF prevalence of up to 50 % depending on NYHA class. This coexistence leads to increased morbidity and mortality (reviewed in [5]). Therefore, the identification of a population at risk for AF development has important implications on an individual level, since it may allow the selection of high-risk individuals for clinical trials of primary prevention as well as for more recent "-omics" tests (reviewed in [5]).

Examples of existing risk scores for AF prediction are the Framingham AF risk score [6] and the $CHADS_2$ and CHA_2DS_2-VASc scores [7]. Since these scores are based on commonly- and readily-collected clinical individual data, they are easily applicable. However, they do suffer from multiple weaknesses: First, the scores were developed to predict AF in mid to long term follow-up (3 to 10 years), whereas short term prediction (1 year) is of greater clinical interest because of the potential to adjust risk factors and to adapt treatment planning. Second, the identification of AF was based on standard ECG recording, which implies a significant under-estimation of the disease, especially in asymptomatic patients. Finally, genetic factors have not been considered in these scores whereas familial and heritable AF have been widely described [8]. AF prediction based on an omics approach of circulating biomarkers has also been attempted in several studies (reviewed in [9]). The use of proteomics has identified biomarkers associated with AF incidence [10]. However, adjusting for age, sex and AF risk factors eliminated six of the eight identified biomarkers, leaving only ADAMTS13 and N-terminal pro-B-type natriuretic peptide (NT-proBNP). In addition, only long-term prediction was analyzed ($\geq$ 4 years). Another study focused on inflammatory proteomics [11]. Again, after risk factor-adjusted analysis, only NT-proBNP predicted development of AF. The authors hypothesized that the lack of association between circulating cytokines and AF incidence may be due to the fact that the inflammatory processes related to AF are localized and not systemic. Based on this hypothesis, the use of peripheral blood for the identification of predictive biomarkers may not facilitate their identification. The use of transcriptomics has been successful in the identification of biomarkers associated with AF prevalence but not with AF incidence (also reviewed in [12]). One study used blood samples from the Framingham Heart Study. Even though they used a large cohort, no AF prediction biomarkers were identified [13]. Again, the focus was on long-term prediction. Asymptomatic AF patients may have been missed because of the set-up of the study (12-lead ECG every 4–8 years). In addition, since the study is based on a community-based observational cohort, clinical characteristics of the cohort are heterogeneous.

We here describe a novel approach for the identification of predictive AF biomarkers in blood samples. We conducted a multicenter prospective study in a population of HF patients already known to be at risk for the development of AF. All patients were implanted with a cardiac resynchronization therapy defibrillator, allowing for continuous monitoring of cardiac arrhythmias. We obtained both local (cardiac blood) and systemic (peripheral blood) samples from all patients and performed transcriptome analysis using clinical data obtained after short-term (1–2 years) follow-up.

2. Methods

2.1. Patients and samples

A total of 192 heart failure patients were enrolled at the start of the study. The clinical characteristics of selected patients are presented in Table 1. Cardiac whole blood samples were collected using an ACUITY™ Pro guide catheter (Boston Scientific) inserted into the coronary sinus during biventricular cardioverter defibrillator implantation. Peripheral blood samples were collected from the left or right subclavian vein during the same intervention. The LeukoLOCK™ Fractionation & Stabilization Kit (ThermoFisher) was used to isolate leukocytes and stabilize RNA. Patients were followed up during two years and paroxysmal or persistent atrial fibrillation occurrence was based on interrogation of the defibrillator.

Table 1
Clinical characteristics of patients selected for analysis.

	AF (n = 13)	Matched SR (n = 13)	All SR (n = 96)	AF vs matched SR P-value	AF vs all SR P-value
Age (y)	66 (2)	65 (2)	61 (1)	0.61	0.09
Gender (M/F)	12 / 1	12 / 1	67/29	1.00	0.11
BMI (kg/m^2)	27.3 (0.9)	28.5 (1.0)	27.7 (0.5)	0.36	0.78
IHD (%)	85	85	41	1.00	<0.01
NYHA >2 (%)	67	27	44 *	0.1	0.55
SBP (mmHg)	117 (5)	120 (4)	120 (2) *	0.65	0.39
DBP (mmHg)	70 (3)	71 (2)	74 (1) *	0.79	0.30
HR (/min)	74(3)	67(5)	69 (1) *	0.28	0.17
RASi (%)	92	85	85	1	0.69
BB (%)	77	92	94	0.34	0.07
MRA (%)	54	38	47	0.70	0.77
Cardiac glycoside (%)	15	0	2	0.24	0.07
Amiodarone (%)	38	27	18	0.67	0.13
NT-proBNP (ng/l)	2471 (606) *	1871 (625) *	2043 (402) *	0.49 #	0.67
LVEDD (mm)	73 (2)	71 (3)	71 (1) *	0.46	0.36
LVEF (%)	27 (2)	24 (2)	26 (1)	0.30	0.42
MR > 2/4 (%)	15	15	13 *	1	1

* NYHA (New York Heart Association classification): n = 92 for all SR (sinus rhythm group); SBP (systolic blood pressure): n = 95 for all SR; DBP (diastolic blood pressure): n = 95 for all SR; HR (heart rate): n = 95 for all SR; NT-proBNP n = 10, 9, and 64 for AF (1-year atrial fibrillation group), matched SR, and all SR, respectively; LVEDD (left ventricular end diastolic diameter): n = 91 for all SR; MR (mitral regurgitation): n = 89 for all SR. M – male, F – female, BMI - body mass index, IHD - ischemic heart disease, RASi - Renin Angiotensin system inhibitors, BB - beta blockers, MRA - Mineralocorticoid receptor antagonists. For quantitative data, values are provided as mean and standard error of the mean (). Values for categorical data are shown as percentages. Statistical tests: Paired t-test for quantitative data between matched groups, standard t-test for NT-proBNP (#) because of missing data and for the AF vs all SR comparison, Fisher's exact test for categorical data.

Total RNA was isolated using the LeukoLOCK™ Alternative Protocol following manufacturer's instructions (ThermoFisher) from half of the cell homogenate. The other half was used for DNA extraction [14]. RNA quality was assessed using either an Agilent 2100 Bioanalyzer or an Agilent TapeStation System.

2.2. Ethics statement

This study has been approved by a local ethics committee (GNEDS; Groupe Nantais d'Ethique dans le Domaine de la Santé).

2.3. Gene expression analysis

The full method for gene expression analysis is detailed in the Supplementary Methods.

2.4. Digital cytometry

The CIBERSORTx tool [15] was used to provide an estimation of the abundances of cell types in our samples based on our gene expression data and the LM22 gene signature matrix. The input consisted of the filtered data matrix after deletion of replicate gene symbols.

2.5. NEURL1 sequencing

DNA samples from selected patients were subjected to sequence analysis by Eurofins Genomics Europe to detect the presence of the rs11598047 variant. Selected patients were those that developed AF at 1 year follow-up and an equal number of patients that stayed in SR throughout the study and displayed highest NEURL1 expression levels.

2.6. Quantitative PCR

Microarray gene-expression results were verified using Taqman® Gene Expression Assays (Life Technologies) on the StepOnePlus™ System (Life Technologies) according to the manufacturer's protocol. The relative expression levels of BMP8B, EPS15, LDHAL6A, NEURL1, RNU1–5, SPEG and TRPM5 were analyzed in 48 patient samples. These genes were selected based on three criteria: 1) they have been linked to HF and/or AF; 2) they were among the genes with highest absolute log2 (FC) values; 3) the sequence of the target of the Taqman Gene Expression Assay overlapped with the sequence of the Agilent oligonucleotide. The housekeeping gene HPRT1 was used as a reference gene.

2.7. Test of existing AF prediction scores

Three existing AF prediction scoring algorithms were tested on our patient cohort: The Framingham AF risk score [6] and the $CHADS_2$ and CHA_2DS_2-VASc scores [7]. Statistical significance was tested using a Mann Whitney test and Pearson correlation coefficients were calculated for all comparisons.

3. Results

3.1. Patient selection

A total of 192 patients were enrolled (Fig. 1A). Of these, six patients were excluded because of missing clinical data or RNA quality issues and 51 patients were excluded because of the presence of AF in their medical history. Of the remaining patients, five developed arrhythmias other than AF and four patients died during the first year of follow-up (three of these deaths were HF-related). Therefore, at 1-year follow-up, a total of 113 patients remained in sinus rhythm (SR) and 13 patients developed AF (the 1-year AF group). During the second year of follow-up, seven patients were excluded because of the development of arrhythmias other than AF or death. An additional 10 patients developed AF at 2-year follow-up (the 2-year AF group), whereas 96 patients remained in SR (the SR group).

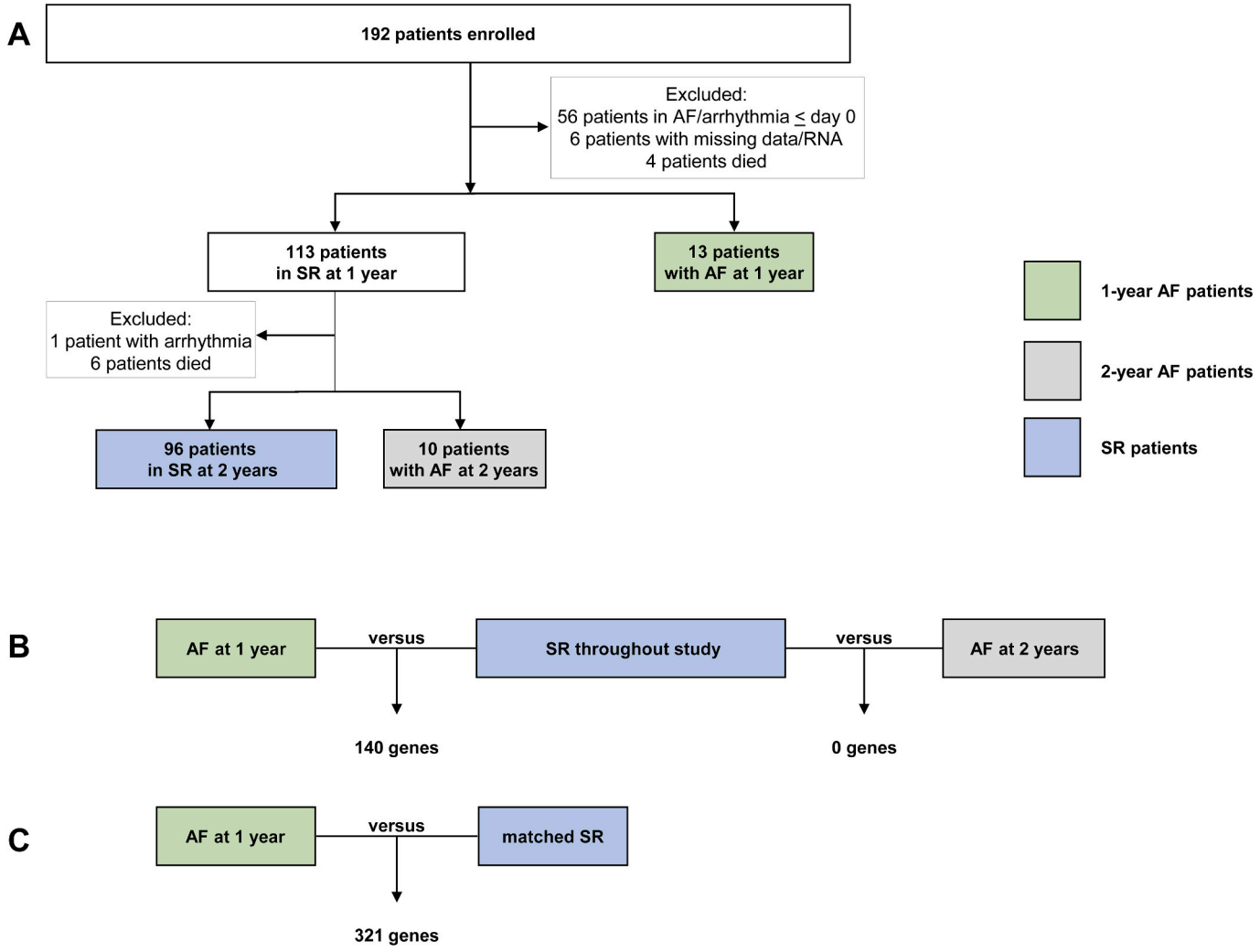

Fig. 1. Flow-chart and set-up of the study.
A. Flow-chart of the study. B. Comparison between 1-year and 2-year AF groups to the SR group C. Comparison between the 1-year AF group and the matched SR group. AF - atrial fibrillation, SR - sinus rhythm.

3.2. AF transcriptome predictor identification

The transcriptome of the 1-year AF and the 2-year AF groups were individually compared to that of the SR group using two-class unpaired SAM. No differentially expressed genes (DEGs) were identified in the 2-year AF vs. SR comparison, whereas 140 genes were found to be differentially expressed between the 1-year AF and the SR groups (Fig. 1B). Based on these results, further analysis was focused on 1-year AF prediction. Hierarchical clustering of the 1-year AF and the SR patients using the 140 DEGs showed an enrichment of 1-year AF patients in sub-branches (right side of Fig. 2A) and a depletion in other branches. The discriminating value of these 140 predictor genes was further evaluated by calculating a 'Molecular Atrial-fibrillation Prediction Score' (MAPS) for each sample. As shown in Fig. 2B, MAPS values were significantly different between both patient groups, underlining the validity of the predictor genes. When performing ROC curve analysis on these values, we obtained an Area Under the Curve (AUC) of 0.906 with a 95 % confidence interval of 0.8418–0.9695 (Fig. 2C). No significant interaction of AF risk factors age, body mass index, gender and presence of ischemic heart disease with the MAPS value was identified using logistic regression (Supplementary Table 2).

However, alongside unbalanced group sizes, the observation nature of the study meant that groups were unmatched in age, gender and underlying cardiopathy: In the SR group the mean age was 62 ± 9 years, 72 % were male patients and 39 % had an ischemic cardiopathy. In the 1-year AF group, the mean age was 66 ± 8 years, 92 % were male patients and 85 % had an ischemic heart disease. Therefore, AF predictor gene identification was repeated using a thirteen-patient SR group for which each patient was matched to an AF patient for age (+/− 5 years), gender and absence/presence of ischemic heart disease. In this thirteen-patient SR group, the mean age was 65 ± 7 years, 92 % were male patients and 85 % had an ischemic heart disease (Table 1). Using this approach, two-class unpaired SAM identified 321 DEGs, representing putative AF predictor genes (Fig. 1C). Hierarchical clustering using these genes resulted in an almost perfect separation between (matched) SR and 1-year AF patients (Fig. 3A) and MAPS values were significantly different (Fig. 3B). The discriminative power of the 321 AF predictor genes was further tested by ROC curve analysis on MAPS values. As shown in Fig. 3C, the AUC was 0.935, with a 95 % confidence interval between 0.8064 and 1. When comparing both strategies (Fig. 3D, left), pearson correlation indicated that there is a significant large positive overlap between the obtained log2(Fold Change) values ($r = 0.911$, $p \leq 0.001$), with 60 DEGs identified by both the global (un-matched) and the case-control (matched) approaches (Fig. 3D, right). Further AF predictor gene analysis was performed on the 321 AF predictor genes from the matched comparison. All DEGs are listed in Supplementary Table 3. A cross-validation strategy was employed to account for data over-fitting due to reclassification of the samples used to define the predictors. The gene lists obtained after SAM analysis of the thirteen learning datasets all contained the 321 AF predictor genes described above. The

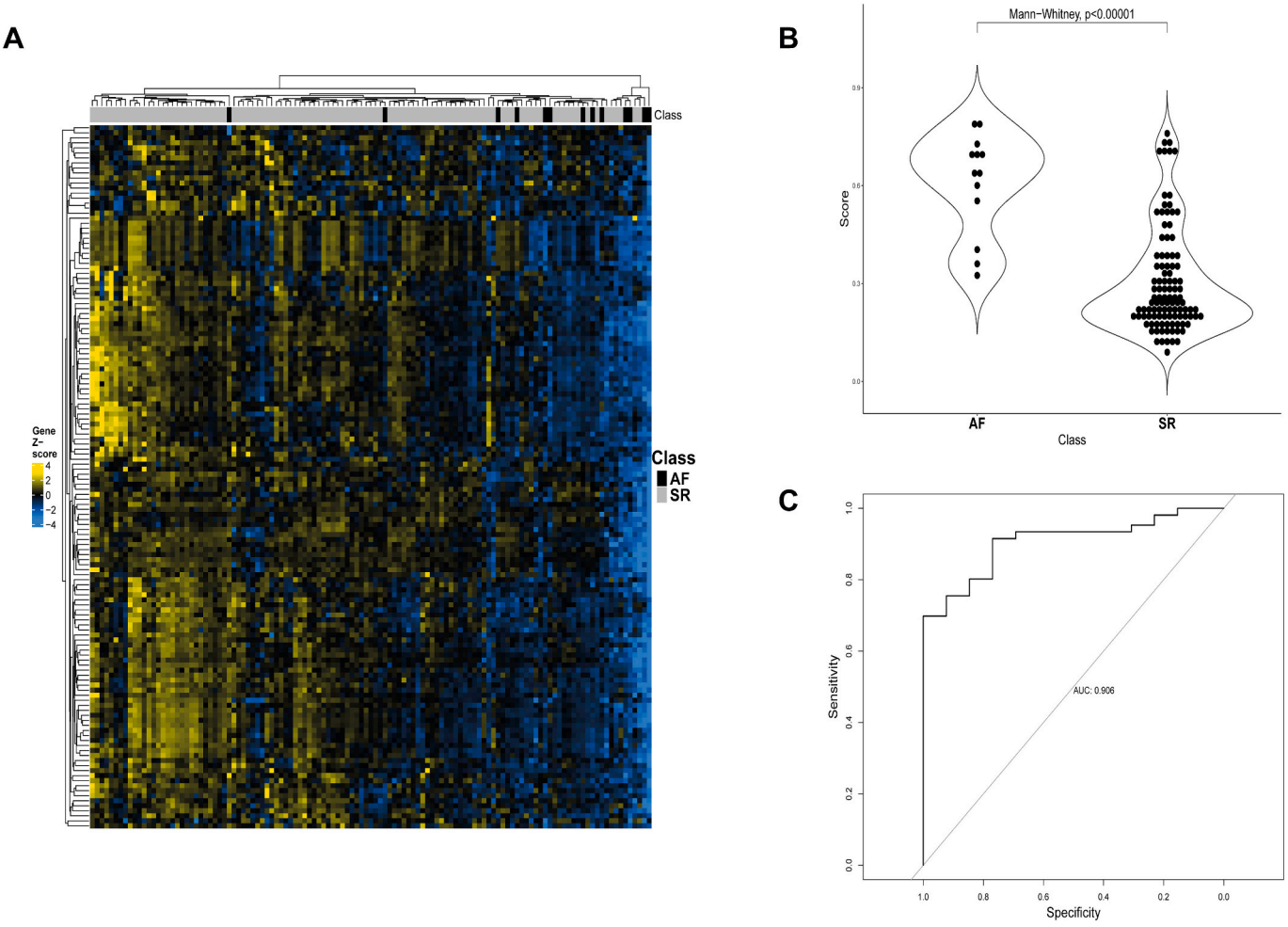

Fig. 2. Visualization of differential gene expression between 1-year AF and SR patients.
A. Heatmap of two-way hierarchical clustering of 140 genes differentially expressed between thirteen 1-year AF patients (black boxes in the patient tree) and 96 SR patients (grey boxes in the patient tree) (global comparison). B. Violin plot of the MAPS (Molecular Atrial fibrillation Prediction Score) of each patient. AF - atrial fibrillation, SR - sinus rhythm. C. ROC curve based on the 140 differentially expressed genes in the global comparison.

MAPS obtained from the thirteen learning datasets all distinguished the 1-year AF from the SR patients (Mann-Whitney, $0.00016 < p \leq 0.00022$). In addition, ROC analysis of the MAPS values of the test samples obtained from the leave-one-out strategy, led to an AUC of 0.941 (95 % confidence interval between 0.8234 and 1) (Supplementary Fig. 1). We also tested the case-control-based AF predictor on the patients from the global analysis. MAPS values were significantly different between 1-year AF and SR patients (Mann-Whitney, $p = 0.00014$), and ROC analysis of these values led to an AUC of 0.826 with a 95 % confidence interval between 0.6986 and 0.9531 (Supplementary Fig. 2).

3.3. Digital cytometry

The relative abundance of 22 cell types in our samples is shown in Fig. 4A. For each cell type, the relative percentage between the 1-year AF and the matched SR group was not statistically significant (Mann-Whitney). This indicates that the gene expression differences observed between both groups were not a consequence of a change in cell type populations. We also tried to determine to what cell type correspond the AF predictor genes. Among these 321 genes, 60 were present in the LM22 gene signature matrix. Two-way hierarchical clustering of the LM22 gene signature matrix using the 60 overlapping genes is shown in Fig. 4B and reveals that all cell types from this matrix seem to contribute to the prediction expression profile.

3.4. AF predictor gene analysis

3.4.1. NEURL1 sequencing

Interestingly, one of the AF predictor genes (NEURL1, Neuralized E3 Ubiquitin Protein Ligase 1) has been associated with AF susceptibility loci [16]. We verified the presence of the NEURL1 variant most frequently associated with AF (rs11598047) in the thirteen 1-year AF patients and in thirteen SR patients selected for highest NEURL1 expression. The variant was detected in two of the 1-year AF group patients as well as in two of the SR patients. These results do not indicate an association between the presence of the variant and the NEURL1 expression level.

3.4.2. Least absolute shrinkage and selection operator (LASSO) regression analysis

LASSO was performed on the AF predictor genes and identified a combination of 11 transcripts with non-zero coefficients: AHSA2, ANKRD20A1, DB153536, DOCK9, KCP, KLF17, LOC729852, OR10A5, SLC9A3R2, XLOC_002997 and an unknown transcript (Agilent oligonucleotide A_33_P3377274). The MAPS values of these transcripts separated the 1-year AF from the SR patients (Mann-Whitney, $p \leq 0.0001$) (Supplementary Fig. 3 A) and ROC analysis of the corresponding discriminative power showed an AUC of 1 (Supplementary Fig. 3B).

3.4.3. Enrichment analysis

A first enrichment analysis of the 321 AF predictor genes did not lead to the identification of statistically significant enriched categories, based on adjusted p values. The five highest ranking categories based on p values are visualized in Supplementary Fig. 4. Among these five categories, four are related to synaptic signaling and one is related to skeletal muscle tissue regeneration. Functional enrichment associated with either the AF or the SR phenotype was further evaluated using GSEA and all GO BP genesets as input. GO terms significantly enriched in the SR phenotype were either related to intermediate filaments or “sensory perception”. Using single genesets as input, enrichment of GO terms “synapse assembly”, “regulation of synapse structure or activity” and “regulation of synapse assembly” was also validated. GO terms significantly enriched in the AF phenotype were related to the following functions: “antigen processing and presentation”, “megakaryocyte development”, “T cell receptor signaling”, “T cell proliferation” and “fatty acyl CoA metabolism”. Complete results are shown in the data supplement (Table 4 and Fig. 5).

3.5. Weighted gene co-expression network analysis (WGCNA)

As the identified deregulated function may be not (solely) related to the presence or absence of AF but to other variables such as other clinical characteristics, WGCNA was used to analyze relationships between gene expression and clinical characteristics of the patients. The clinical characteristics were selected based on their relevance to HF and AF and their availability in the case report forms. The aim of this strategy was to further restrict our AF predictor gene list. The gene expression matrix used as input was filtered to 10,000 genes based on highest variance. As shown in Fig. 5A, no outliers were identified among the 26 patients and all were included in the analysis. 25 clinical characteristics were selected to be included in the analysis. As shown in Fig. 5B, three gene modules associated with AF prediction were identified based on a correlation coefficient < -0.6 (or > 0.6) and $p < 0.0001$: MEblack, MEturquoise and MEdarkred. Neither of these modules was associated with any of the other clinical characteristics, based on these criteria. The distribution of the 10,000 genes among the different modules is shown in the left part of Fig. 5C. The right part of Fig. 5C shows the distribution of the AF predictor genes among the modules. These figures clearly show that the proportion of genes belonging to the AF-prediction-associated modules MEblack, MEturquoise and MEdarkred (indicated by arrows), was increased among the AF predictor genes compared to the WGCNA input genes. When directly comparing the genes from the three selected modules to the 321 AF predictor genes, a substantial overlap of 257 genes was found (Fig. 6A). Fig. 6B-D shows the relationship between the Gene Significance for AF prediction (i.e. the absolute value of the correlation between the gene and the AF-prediction trait) and the module membership of the gene (i.e. the absolute value of the correlation of the module eigengene and the gene expression profile) for the three selected modules. The overlapping AF predictor genes (DEGs) are indicated in the scatterplots. Gene Significance and Module Membership cutoffs of 0.6 are displayed in the figures, highlighting genes in the upper-right quadrants with highest Gene Significance and Module Membership. These genes may be considered as the hub-genes of the modules. Visual inspection of the data shows us that all but two of the AF predictor genes present in these modules fall within the hub-genes fields, and may therefore be considered as the best candidates for AF prediction (see Supplementary Table 3).

3.5.1. Enrichment analysis

Gene Ontology Biological Process enrichment analysis of the 257 gene-overlap between the AF predictor genes and the three selected modules did not identify statistically significant enriched categories based on adjusted p values. As shown in Fig. 7A, the five highest ranking categories based on p values contain two categories related to synapses, two categories related to muscle and one category related to reactive oxygen species. Enrichment analysis of the turquoise module (Fig. 7B) identified several significantly enriched categories: Keratinization (p-adjusted = 0.002), cell junction organization/assembly (three categories, p-adjusted = 0.03–0.11), and epithelial cell differentiation (p-adjusted = 0.11). Of these GO terms, “keratinization” was also identified as associated with the SR phenotype in GSEA. In the black module (Fig. 7C), enrichment (p-adjusted <0.16) was found for response to lead ion, forebrain and head development and two categories related to alpha-beta T cell activation. Enrichment analysis of the darkred module (Fig. 7D) did not identify statistically significant enriched categories. The five highest ranking categories based on p values contain two categories related to thyroid hormone, as well as cellular response to carbohydrate stimulus, apoptotic mitochondrial changes and forebrain cell migration. Enrichment analysis based on Disease Ontology provided interesting results for the turquoise module. Although these results did not reach statistical significance based on the adjusted p-value, ranking based on lowest p-value did show an enrichment of congestive-heart-

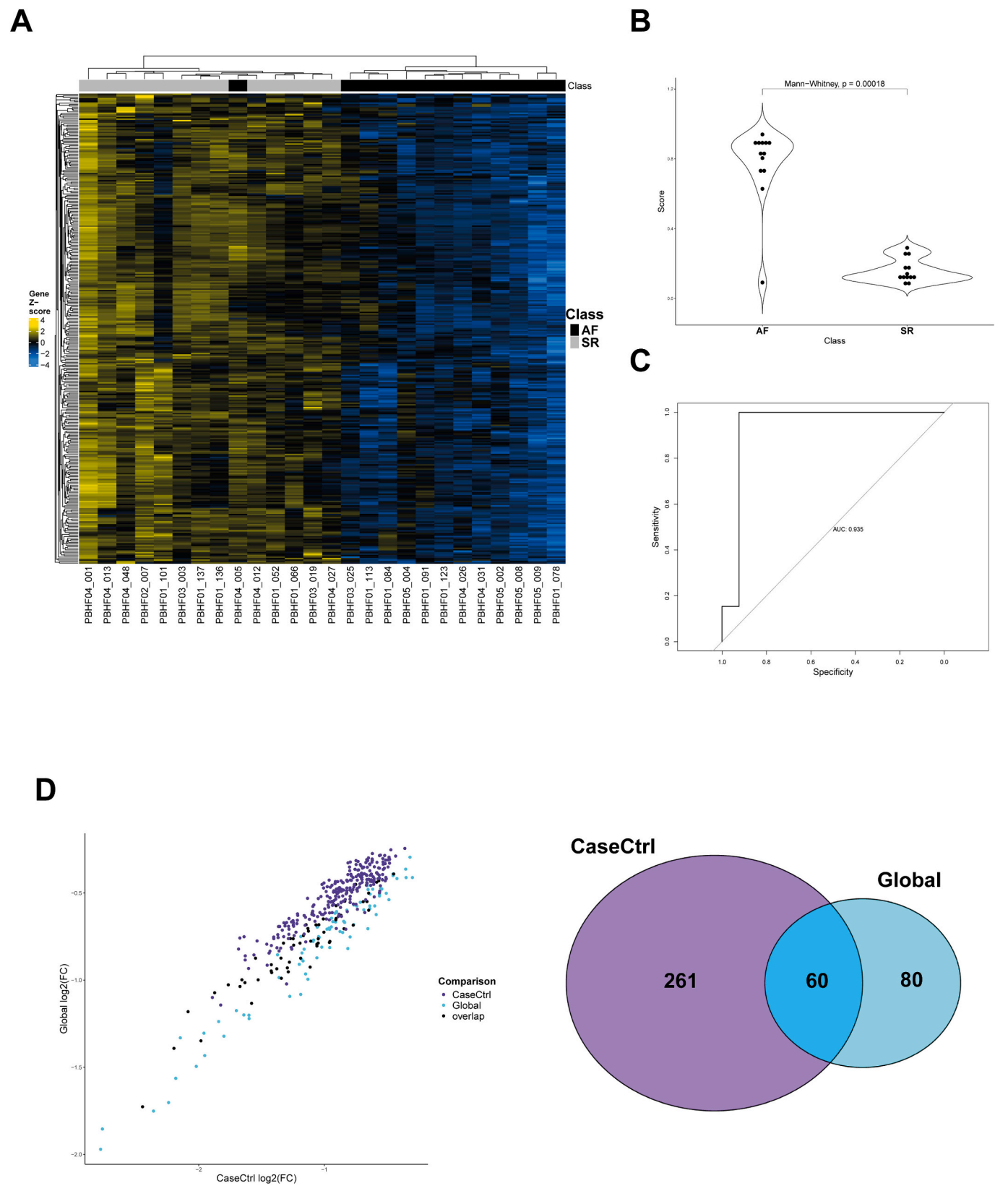

Fig. 3. Visualization of differential gene expression between 1-year AF and matched SR patients.
A. Heatmap of two-way hierarchical clustering of 321 genes differentially expressed between thirteen 1-year AF patients (black boxes in the patient tree) and thirteen matched SR patients (grey boxes in the patient tree). B. Violin plot of the MAPS (Molecular Atrial fibrillation Prediction Score) of each patient from the case-control comparison. C. ROC curve based on the 321 differentially expressed genes in the case-control comparison. D. Left; comparison between the log2(FC) values obtained by the global and the case-control comparisons. Right; Venn diagram showing the overlap between the number of genes differentially expressed using either the global or the case-control comparison. AF - atrial fibrillation, CaseCtrl - case-control, SR - sinus rhythm, FC - Fold change, AUC - area under the curve.

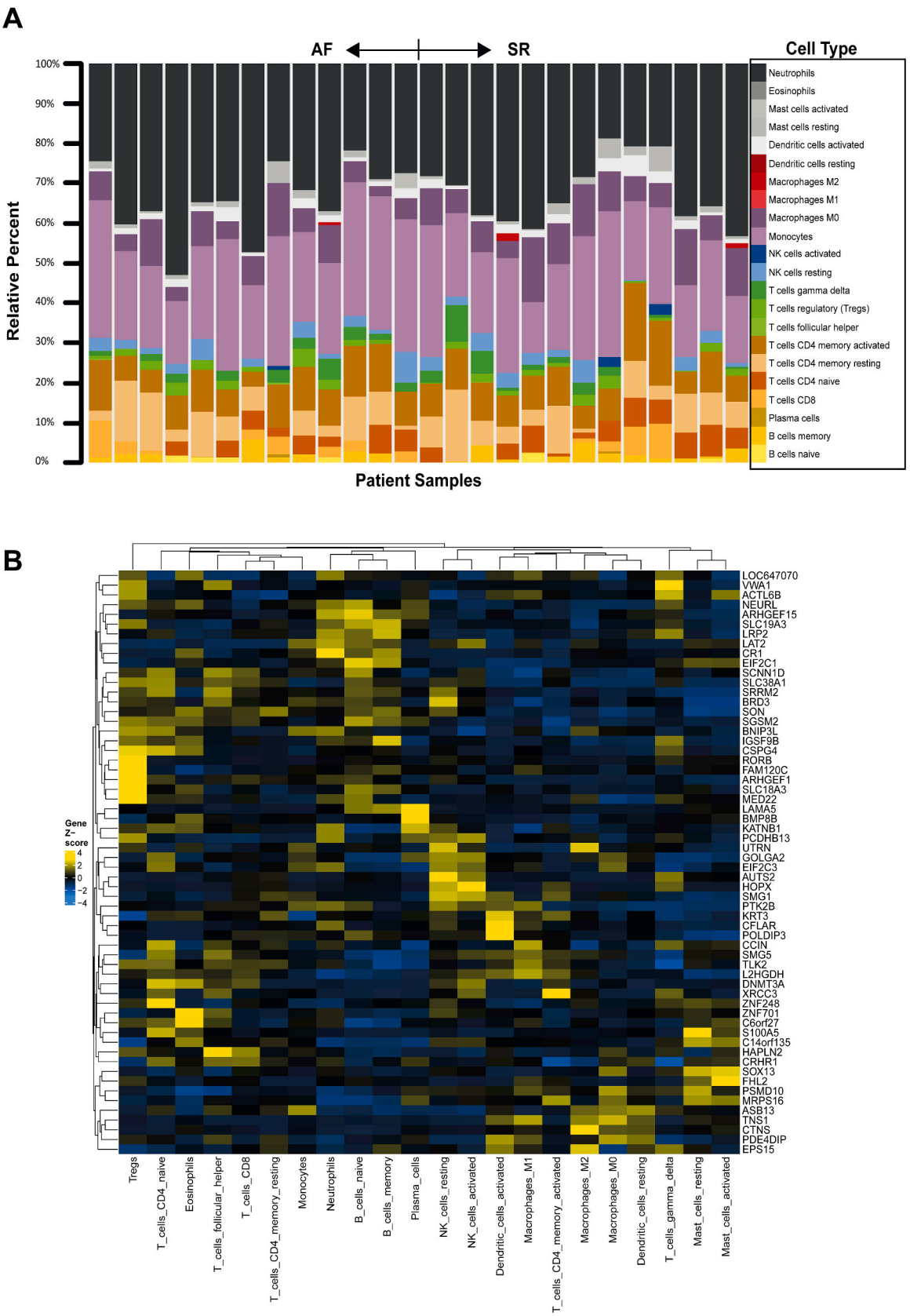

Fig. 4. Digital cytometry results of the case-control patient samples.
Visualization of the relative percentage of 22 immune cell types in the 26 blood samples of the case-control patients, based on digital cytometry. B. Heatmap of two-way hierarchical clustering of 60 genes from the AF predictor in 22 immune cell types.

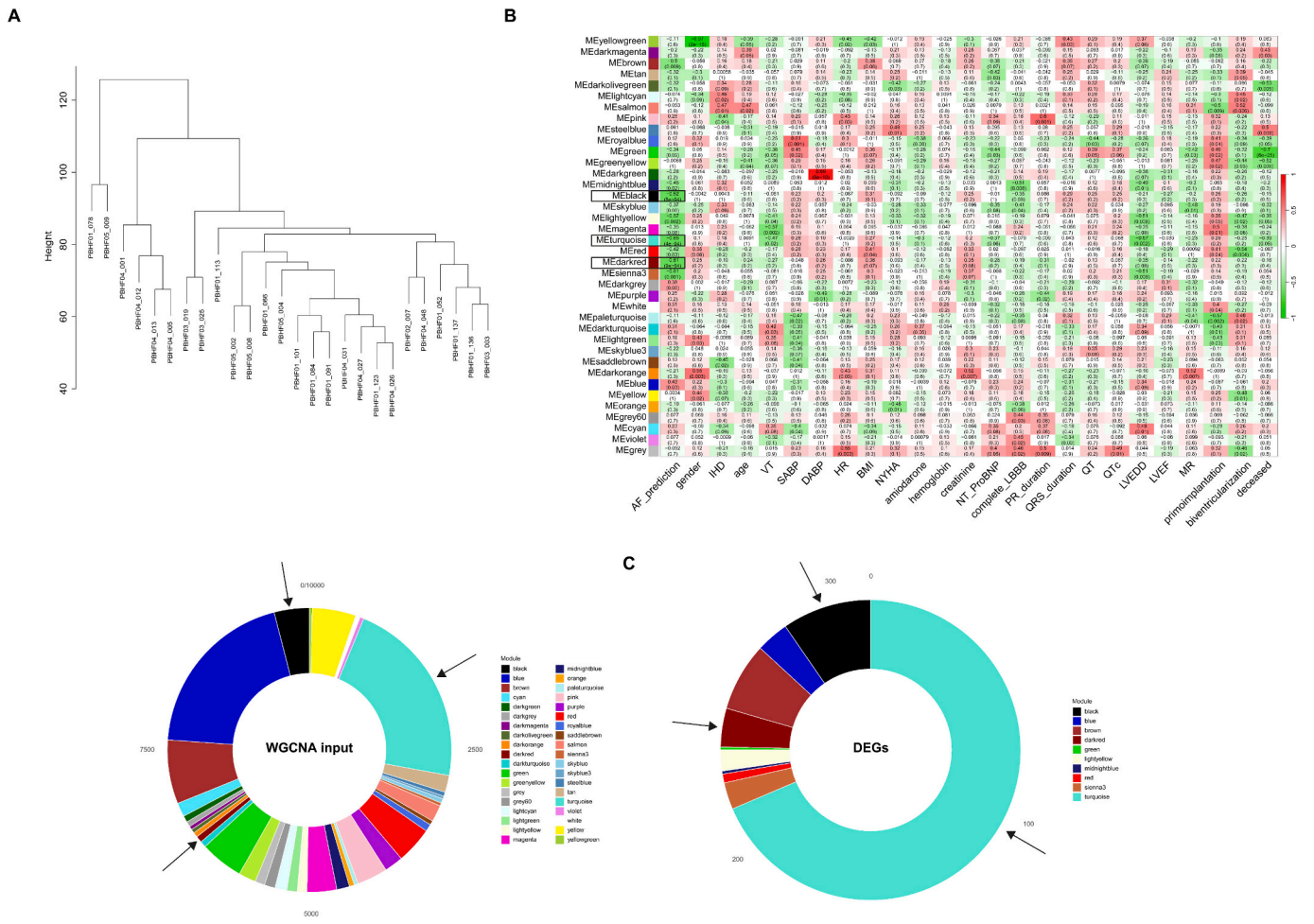

Fig. 5. Weighted gene co-expression network analysis (WGCNA).
A. Dendrogram depicting hierarchical clustering to detect outlier samples. B. Heatmap of correlations between sample traits and WGCNA module eigengenes. The selected modules are indicated by black boxes C. Left side: Donut chart showing the distribution of the 10,000 input genes over the different modules. Right side: Donut chart showing the distribution of the AF predictor genes over the different modules. Arrows indicate the selected modules MEturquoise, MEdarkred and MEblack. BMI - body mass index, HR – heart rate, DABP - diastolic arterial blood pressure, DEGs - differentially expressed genes, IHD - ischemic heart disease, LBBB - left bundle branch block, LVEDD - left ventricular end diastolic diameter, LVEF - left ventricular ejection fraction, MR - mitral regurgitation >2/4, NYHA - New York Heart Association class, SABP - systolic arterial blood pressure, VT - ventricular tachycardia.

failure-related genes (Fig. 7E).

3.6. Quantitative PCR

To validate microarray-based AF predictor gene identification, quantitative PCR (qPCR) was performed on a selection of seven genes; BMP8B, EPS15, LDHAL6A, NEURL1, RNU1–5, SPEG and TRPM5. Six of these genes were among the hub-genes of the turquoise module (Fig. 6B). HPRT1 was used as a reference gene. qPCR was performed on twelve 1-year AF patients and twelve SR-patients (from the matched comparison). Down-regulation of cardiac blood gene expression in the 1-year AF group was confirmed for five genes (BMP8B, LDHAL6A, NEURL1, RNU1–5 and TRPM5) (Supplementary Fig. 6). EPS15 and SPEG were down-regulated in the 1-year AF group, but this did not reach statistical significance. Next, we checked whether down-regulation of these genes could also be detected in peripheral blood samples from the same patients. qPCR could be performed on ten 1-year AF patients and twelve SR patients (from the matched comparison). BMP8B and SPEG were indeed down-regulated in peripheral blood samples from 1-year AF patients. LDHAL6A was expressed at a lower level in 1-year AF patients than in SR patients, but this did not reach statistical significance. Peripheral blood gene expression levels of EPS15, NEURL1, RNU1–5 and TRPM5 were not concordant with those identified in cardiac blood.

3.7. Comparison to existing AF prediction scores

AF prediction scores based on the Framingham AF risk score [6] and the $CHADS_2$ and CHA_2DS_2-VASc scores [7] were calculated for the 1-year AF group and the matched SR group. Differences between both patient groups were evaluated using a Mann Whitney test. None of the existing AF prediction algorithms distinguished our 1-year AF patients from the matched SR patients (Framingham AF risk score: $p = 0.49$, $CHADS_2$: $p = 0.32$, CHA_2DS_2-VASc: $p = 0.24$). In addition, we analyzed the correlation between the different predictors on the same patient groups. None of the existing prediction scores correlated with our AF gene predictor: Framingham AF risk score vs. MAPS: $r = 0.0105$, $p = 0.959$; $CHADS_2$ vs MAPS: $r = 0.00544$, $p = 0.979$; $CHAD_2DS_2$-VASc vs. MAPS: $r = 0.0924$, $p = 0.654$. The Framingham AF risk score was not correlated with both $CHADS_2$ and $CHAD_2DS_2$-VASc predictors: $r = 0.0243$, $p = 0.906$ and $r = 0.223$, $p = 0.273$ resp. Not surprisingly, $CHADS_2$ and $CHAD_2DS_2$ were correlated: $r = 0.791$, $p = 0.001$.

4. Discussion

The main findings of our study are: 1) The cardiac blood transcriptome can predict de novo onset of AF in HF patients; 2) in the context of our study, the cardiac blood transcriptome is a better

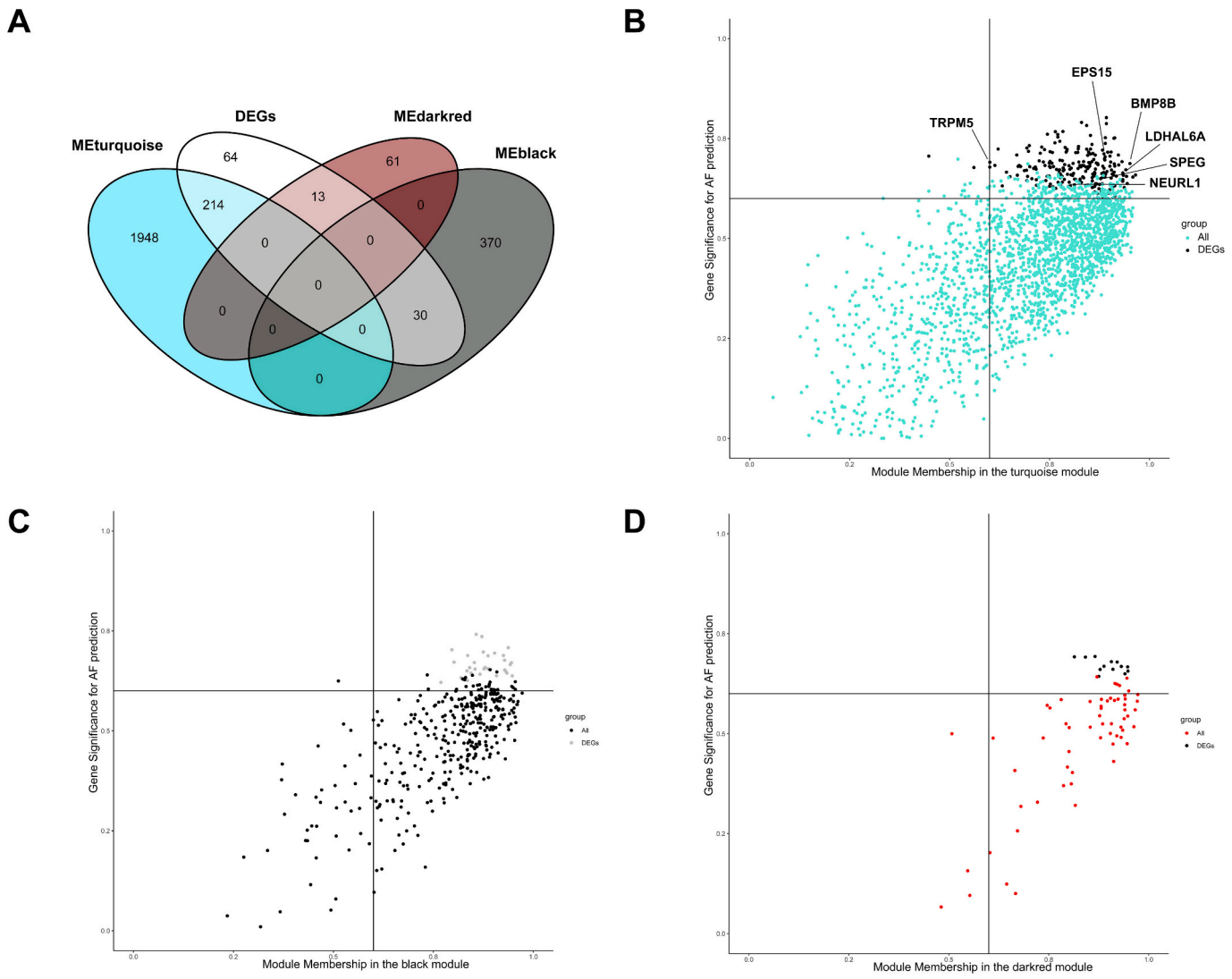

Fig. 6. Comparison of the AF predictor genes to the genes from the AF-prediction-associated WGCNA modules MEturquoise, MEdarkred and MEblack.
A. Venn diagram showing the overlap between the AF predictor genes (DEGs) and genes from the MEturquoise, MEdarkred and MEblack modules. B—D. Scatterplots displaying the relationship between the Gene Significance for AF prediction and the module membership of the gene for the three selected modules (MEturquoise, MEblack and MEdarkred resp.). The overlapping AF predictor genes (DEGs) are indicated by black (B and D) or grey (C) dots. Genes verified by quantitative PCR are identified in (B). DEGs - differentially expressed genes.

predictor than the peripheral blood transcriptome; 3) Our AF gene predictor is associated with short term (1 year) development of AF.

To the best of our knowledge, this is the first study that shows that de novo onset of AF can be predicted from the blood transcriptome. We believe that two factors that distinguish our study from others were essential for this result. The first is the fact that AF detection during clinical follow-up was not based on temporally spaced ECG recordings as used in other studies, but on the continuous recording of the atrial electrical activity (electrogram) by the patients' cardioverter defibrillator. Therefore, AF-detection was not hindered by the intermittent nature of the arrhythmia and asymptomatic episodes [17]. The second distinguishing factor is the use of cardiac blood from coronary sinus sampling as a source for transcriptome analysis. The search for prognostic biomarkers in plasma and/or serum from HF patients has already shown that coronary sinus sampling may lead to better results than the use of peripheral venous blood [18–20]. In concordance with these findings, we obtained a better separation between patient groups based on cardiac blood than on peripheral blood based on quantitative PCR results. This underlines the importance of selecting the right tissue source in the search for biomarkers. We have to add that, since the aims of our study did not include a comparison of cardiac and peripheral blood using large-scale transcriptome analysis, our data do not allow the identification of an enrichment or depletion of functional categories in localized (cardiac) vs. systemic (peripheral) blood samples. We also checked whether existing predictors [6,7] based on clinical data could predict AF in our cohort and found no distinction between the 1-year AF group and the SR group. This underlines the importance of our results. Besides the fact that we relied on continuous recording of the atrial electrical activity and not on standard ECG recording for AF detection (as stated above), three additional reasons may explain these differences: First, our score was developed for short term (1-year) AF prediction, whereas the existing AF predictors were developed for longer term prediction (10 years in the Framingham AF score and 3 years in the CHADS2 and CHA2DS2-VASc predictors). Second, the existing prediction algorithms were based on the general population, whereas our prediction score was developed based on a population of HF patients selected for biventricular cardioverter defibrillator implantation. Finally, our score was based on the cardiac blood transcriptome, whereas the existing algorithms relied on clinical parameters only.

Among the hub-genes of the AF-associated gene modules, several

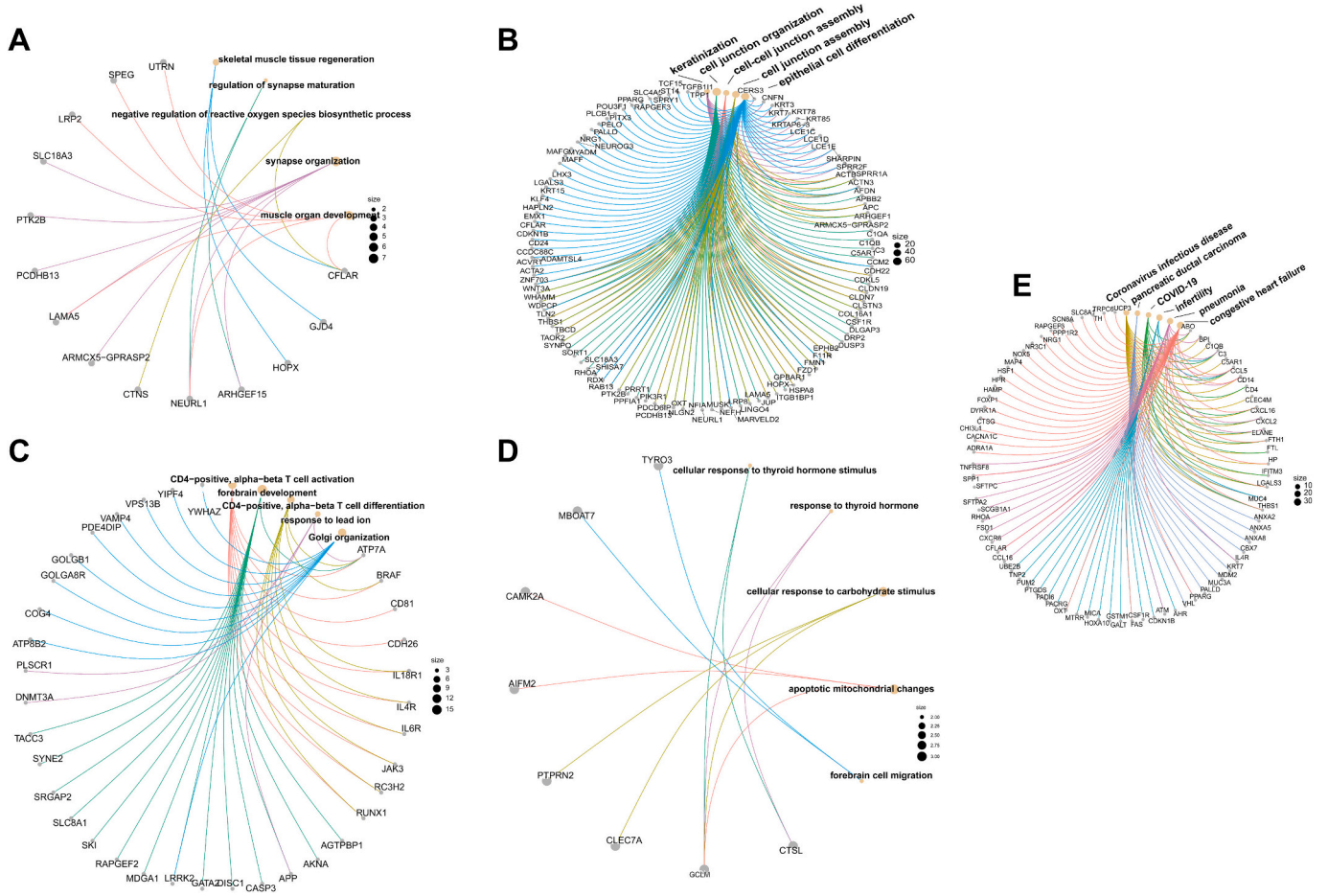

Fig. 7. Concept network of Gene Ontology Biological Processes and Disease Ontology enrichment analysis.
A-D. Gene Ontology Biological Processes enrichment analyses of (A) the overlap between the AF predictor genes and either the MEturquoise or the MEdarkred or the MEblack genes; (B) the turquoise module; (C) the black module and (D) the darkred module. E. Disease Ontology enrichment analysis of the turquoise module. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

genes with known links to HF and/or AF were identified: SPEG, Striated Muscle Enriched Protein Kinase, regulates phosphorylation of Ryanodine Receptor Type 2 (RyR2) and reduces its activity [21]. Loss or down-regulation of SPEG, and therefore loss of its inhibitory phosphorylation of RyR2 in atrial tissue, has been associated with promotion of AF. In addition, SPEG has been found to be down-regulated in failing human hearts as a whole [22]. We here show that, within our HF group, distinct circulating levels of SPEG transcripts correlate with different AF susceptibility. Genetic variation of NEURL1, Neutralized E3 Ubiquitin Protein Ligase 1, has been associated with susceptibility to AF and postoperative AF in different patient populations [16,23–27]. Down-regulation of NEURL1 expression in AF patients has not yet been described. However, knockdown of NEURL1 in Zebrafish resulted in prolongation of atrial action potential durations [16]. In addition, an in vitro protein interaction between NEURL1 and AF-associated transcription factor PITX2 has been detected [16]. HOP Homeobox (HOPX) has been identified as a regulator of cardiac development and is highly expressed in the adult murine cardiac conduction system [28]. It has been shown to be down-regulated in human failing hearts [29] and predicted to be involved in AF using an orthologous-phenotypes-based algorithm [30]. Although the SCNN1D gene codes for a subunit of an epithelial sodium channel, it is also expressed in nonepithelial tissue including heart and blood [31]. In addition, it has been shown to be down-regulated in ischemic cardiomyopathic hearts [32]. Another ion-channel-encoding gene among our hub-genes was Potassium Sodium-Activated Channel Subfamily T Member 1 (KCNT1). This gene is widely expressed in the nervous system and mutations have been associated with epilepsy of infancy [33]. However, since there is a high prevalence of sudden unexpected death among these patients, involvement of the cardiac electrical system is suspected, especially since mutation of KCNT1 has been found in a Brugada syndrome patient [34] and in a patient with intermittent QTc prolongation in addition to other cardiovascular and neurological abnormalities [35]. Like KCNT1, Protein Tyrosine Kinase 2 Beta (PTK2B) is highly expressed in the central nervous system. In this tissue, PTK2B-related signaling may be involved in the regulation of ion channel activity [36]. Another study showed protein expression in human failing and nonfailing ventricles and suggested that PTK2B activation may be involved in cardiac protection against arrhythmia in nonischemic cardiomyopathy [37]. PDE4DIP (Phosphodiesterase 4D Interacting Protein) is highly expressed in cardiac tissue and has been linked to the phosphorylation of proteins involved in cardiac contractile function and conduction. In addition, mutations in PDE4DIP have been associated with familial AF [38]. DNA Methyltransferase 3 Alpha (DNMT3A) is the gene most commonly mutated in Clonal Hematopoiesis of Indeterminate Potential (CHIP), defined as the presence of an expanded somatic blood cell clone in persons without other hematological abnormalities [39]. In addition, CHIP carriers with DNMT3A mutations were shown to have impaired long-term survival and increased progression among HF patients [40]. Finally, an interesting hub gene also identified by LASSO regression analysis was Kielin Cysteine Rich BMP Regulator (KCP). KCP enhances BMP signaling [41] and has been shown to be up-regulated in cardiac

tissue from HF patients [42]. Down-regulation of KCP (as in our study) has been shown to be deleterious: KCP KO mice displayed aggravated aging-related cardiac dysfunction and remodeling [43] and in mice deficient for KCP, cardiac dysfunction and remodeling induced by pressure overload was worsened [44]. The expression of genes related to cardiac functioning in blood samples has already been shown. Liew et al. hypothesized that blood cells can act as sentinels of disease, containing a pool of gene transcripts that respond to changes in the macro- and micro-environments [45]. In addition, Seiler et al. identified expression of genes relevant to cardiomyocyte excitability or contractility in peripheral blood [46]. Furthermore, they detected an increased transcription level of the Na/Ca exchanger in blood of HF patients in line with the known upregulation in myocardial tissue from this type of patients. Therefore, the deregulation of gene expression in blood may be a reflection of what happens in myocardial tissue.

We recognize that there are limitations in this study. First, the main finding that the cardiac - but not the peripheral - blood transcriptome may be used to predict de novo onset of AF, limits its application in a clinical setting. Coronary sinus sampling can only be performed during invasive cardiac procedures. However, these procedures are not uncommon in HF patients with left bundle branch block and blood sampling during device implantation does not add risk for the patient. In addition, coronary sinus blood can be obtained without difficulty through femoral vein access. It is therefore possible that, if our approach will be further developed, specific patients beyond those analyzed in our study may benefit from better informed decision making. Second, our patient cohort was too small to divide into a test and a validation cohort. Therefore, further studies are necessary to validate our results. Finally, our AF gene predictor was developed on a specific group of patients and may not be valid for patients with different characteristics.

In conclusion, the cardiac blood transcriptome of HF patients is a promising tool for the prediction of de novo onset of AF. In line with precision medicine development, the hereby identified high risk patients may benefit from more aggressive clinical management.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmccpl.2024.100077.

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CRediT authorship contribution statement

Guillaume Lamirault: Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Imen Fellah-Hebia:** Resources, Project administration. **Catherine Chevalier:** Methodology, Investigation, Formal analysis. **Isabelle Guisle:** Methodology. **Béatrice Guyomarc'h:** Methodology. **Aude Solnon:** Investigation. **Jean-Baptiste Gourraud:** Investigation. **Laurent Desprets:** Investigation. **Selim Abbey:** Investigation. **Christophe Leclercq:** Investigation. **Paul Bru:** Investigation. **Antoine Milhem:** Investigation. **Olivier Billon:** Investigation. **Frederic Anselme:** Investigation. **Arnaud Savouré:** Investigation. **Jean-Noël Trochu:** Investigation. **Rémi Houlgatte:** Validation, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Gilles Lande:** Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Marja Steenman:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Microarray data can be accessed in the GEO database through accession number GSE235307.

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Declaration of Generative AI and AI-assisted technologies in the writing process

The authors did not use generative AI or AI-assisted technologies in the development of this manuscript.

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