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RESEARCH LETTER

Potential Diagnostic Role for a Combined Postmortem DNA and RNA Sequencing for Brugada Syndrome

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Postmortem genetic testing (molecular autopsy) is an important tool to identify genetic risk in family members following an unexplained sudden death (sudden arrhythmic death syndrome). However, exome sequencing is currently informative in only 13% to 30% of cases.[1] RNA sequencing (RNAseq) has been shown to aid genetic diagnosis where DNA sequencing (DNAseq) is uninformative. Formalin-fixed paraffin-embedded (FFPE) heart tissue is retained routinely for histopathologic examination after sudden cardiac death. Unfortunately, FFPE processing can lead to fragmentation, DNA crosslinks, and deamination leading to false positives variant calling in the subsequent sequencing. Brugada Syndrome (BrS), a heritable arrhythmia syndrome, is the most common underlying cause of death in sudden arrhythmic death syndrome.[1] One gene, sodium voltage-gated channel alpha subunit 5 (*SCN5A*), has definitive evidence for disease causation but only underlies ≈20% of clinical cases,[2] hampering the potential role of molecular autopsy as a diagnostic tool. We aimed to demonstrate that the combination of the DNAseq and RNAseq in postmortem tissue can successfully identify putative causative variants and establish whether there may be a distinctive functional expression profile in the right ventricular outflow tract of BrS decedents.

Six BrS cases with an antemortem diagnosis retrieved from the medical record employing expert consensus and Shanghai scoring criteria,[3] and 5 age- and sex-matched controls with anon-cardiac death were selected for this study. FFPE heart tissue from the RVOT, and where available, suitable samples of spleen tissue, were obtained from the Cardiac Risk in the Young Center for Cardiac Pathology at St George's, University of London. DNA and RNA were extracted from the FFPE heart samples following manufacturer's instructions, and their integrity was determined with Agilent Tape Station. DNAseq and RNAseq were undertaken on an Illumina HiSeq instrument. Sequence adapters were removed from 2×150 paired-end RNA sequencing with Trimmomatic v0.39. Alignment was undertaken with STAR-2.7.3a onGRCh38. QC metrics were assessed by FastQC, QualiMap, RNA-SeqMetrics, and PICARD. FeatureCounts generated counts for each gene. To call variants, SplitNCigarReads, BaseRecalibrator, ApplyBQSR, and HaplotypeCaller were applied to aligned DNAseq and RNAseq in accordance with germline short variant discovery GATK (v4) guidance. Overall,198 unique genes were investigated including sudden cardiac death (n=87), BrS (n=23), and trusight cardio (n=172). Differential expression between BrS cases and Control subjects was assessed using

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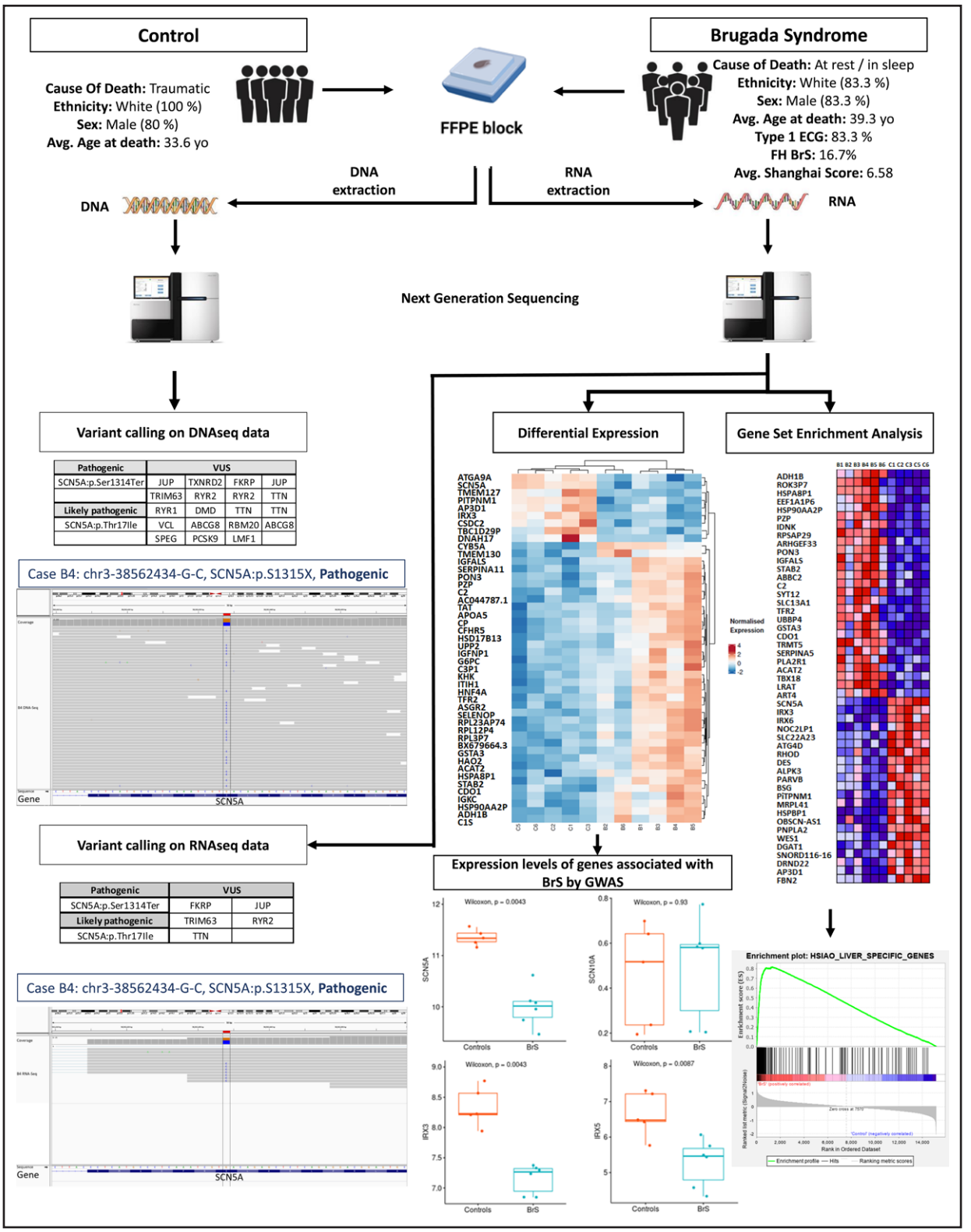

Figure. Combined DNA-sequencing (DNAseq) and RNA-sequencing (RNAseq) analysis approach as a potential diagnostic tool in Brugada syndrome (BrS).

Formalin-fixed paraffin-embedded (FFPE) heart tissue from the right ventricular outflow tract from 6 patients with BrS and 5 age- and sex-matched controls with a noncardiac death, were selected for this study. DNA and RNA from all samples were extracted and sequenced on an Illumina HiSeq instrument. Alignment was undertaken with STAR-2.7.3a on GRCh38. QC metrics were assessed by FastQC, QualiMap, RNASeqMetrics, and PICARD. FeatureCounts generated counts for each gene. To call variants, SplitNCigarReads, BaseRecalibrator, (*Continued*)

Figure Continued. ApplyBQSR, and HaplotypeCaller were applied to aligned DNAseq and RNAseq in accordance with germline short variant discovery GATK (v4) guidance. A total of 198 genes were investigated. Differential expression between BrS cases and Control subjects was assessed using DeSeq2.27 (false discovery rate cutoff at 0.01). Gene set enrichment analysis was performed using all genes ranked by their differential mRNA expression. SCN5A indicates sodium voltage-gated channel alpha subunit 5; and VUS, variants of uncertain significance.

DeSeq2.27 (false discovery rate cutoff at 0.01). Gene set enrichment analysis was performed using all genes ranked by their differential mRNA expression Ethical approval was granted by the London Stanmore National Health Service Research Ethics Committee (reference: 10/H0724/38).

Variant calling on DNAseq data revealed 2 SCN5A variants, p.S1315X in case B4 and p.T17I in case B6 classified as pathogenic and likely pathogenic, respectively, by ACMG criteria. Additionally, we observed 19 variants of uncertain significance in 14 different genes (gnomAD allele frequency $\leq 10^{-4}$, popmax filtering allele frequency $<1.85\times10^{-4}$ and reads ≥20; Figure).

Variant calling on RNAseq data (gnomAD allele frequency $\leq 10^{-5}$, CADD >20, and RNA reads ≥20) confirmed the presence of SCN5A: p.S1315X and SCN5A: p.T17I in the transcriptome. Only 5 out of the 19 VUSs in genes *FKRP*, *JUP*, *TRIM63*, *RYR2*, and *TTN* identified in DNAseq data were detected in the RNAseq data set. The subjects were heterozygous for all the variants identified in this study. Three genes at loci previously demonstrated as genome-wide significant associated with BrS, SCN5A, IRX3, and IRX5, showed significant differential expression between BrS and controls, regardless of the presence of a SCN5A variant (Figure).

Gene set expression analysis revealed 50 novel genetic associations with BrS. Interestingly,13 of these 50 new associations are present in 2 liver-specific gene sets (NES=4.06 and 3.30 with FDR=0; Figure).

The data that support the findings of this study are available from the corresponding author on reasonable request.

By merging variant calling data from DNAseq with RNA-seq from the same tissue source, an improved diagnostic accuracy of variant calling can be achieved in molecular autopsy. Gene expression analysis of RNAseq data from FFPE heart samples demonstrated reduced SCN5A expression levels in all BrS patients, regardless of SCN5A genotype. Furthermore, we associated 50 novel genes, including liver-specific gene sets, with BrS that could be used as an expression profile of the disease with the potential for improving the diagnostic accuracy and yield in SADS decedents. Interestingly, the most strongly associated liver gene, alcohol dehydrogenase 1B (*ADH1B*), has been previously associated with arrhythmic events after alcohol drinking in a BrS cohort.[4]

The transcriptional and post-transcriptional regulation of SCN5A in myocardial tissue may determine the penetrance and expressivity of associated diseases such as BrS. IRX3 and IRX5, 2 well-known regulators of the expression of different ion channels in the adult heart showed reduced expression in BrS decedents. SCN5A, IRX3, and IRX5 have proximal SNP variants associated with BrS with genome-wide significance, suggesting that the regulation of these genes is important in BrS risk.[5]

This study, therefore, supports the potential utility of combining RNAseq with DNAseq of FFPE tissue of sudden cardiac death decedents in a novel approach to molecular autopsy that requires further prospective investigation. It also unveils genomic pathways adding to the risk of BrS.

ARTICLE INFORMATION

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Disclosures

Dr Behr has undertaken consulting for Boston Scientific in the last 3 years. The other authors report no conflicts.

REFERENCES

1. Ben-Haim Y, Behr ER. Genetics of sudden cardiac death. *Curr Opin Cardiol*. 2022;37:212–218. doi: 10.1097/HCO.0000000000000946
2. Kapplinger JD, Tester DJ, Alders M, Benito B, Berthet M, Brugada J, Brugada P, Fressart V, Guerchicoff A, Harris-Kerr C, et al. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm*. 2010;7:33–46. doi: 10.1016/j.hrthm.2009.09.069
3. Zeppenfeld K, Tfelt-Hansen J, de Riva M, Winkel BG, Behr ER, Blom NA, Charron P, Corrado D, Dagres N, de Chillou C, et al; ESC Scientific Document Group. 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. *Eur Heart J*. 2022;43:3997–4126. doi: 10.1093/eurheartj/ehac262
4. Wu Q, Hayashi H, Hira D, Sonoda K, Ueshima S, Ohno S, Makiyama T, Terada T, Katsura T, Miura K, et al. Genetic variants of alcohol-metabolizing enzymes in Brugada syndrome: insights into syncope after drinking alcohol. *J Arrhythm*. 2019;35:752–759. doi: 10.1002/joa3.12227
5. Barc J, Tadros R, Glinge C, Chiang DY, Jouni M, Simonet F, Jurgens SJ, Baudic M, Nicastro M, Potet F, et al; KORA-Study Group. Genome-wide association analyses identify new Brugada syndrome risk loci and highlight a new mechanism of sodium channel regulation in disease susceptibility. *Nat Genet*. 2022;54:232–239. doi: 10.1038/s41588-021-01007-6