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Research paper

GSTM3 variant is a novel genetic modifier in Brugada syndrome, a disease with risk of sudden cardiac death ☆

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ABSTRACT

Background: Brugada syndrome (BrS) is a rare inherited disease causing sudden cardiac death (SCD). Copy number variants (CNVs) can contribute to disease susceptibility, but their role in Brugada syndrome (BrS) is unknown. We aimed to identify a CNV associated with BrS and elucidated its clinical implications.

Methods: We enrolled 335 unrelated BrS patients from 2000 to 2018 in the Taiwanese population. Microarray and exome sequencing were used for discovery phase whereas Sanger sequencing was used for the validation phase. HEK cells and zebrafish were used to characterize the function of the CNV variant.

Findings: A copy number deletion of *GSTM3* (chr1:109737011-109737301, hg38) containing the eighth exon and the transcription stop codon was observed in 23.9% of BrS patients versus 0.8% of 15,829 controls in Taiwan Biobank ($P < 0.001$), and 0% in gnomAD. Co-segregation analysis showed that the co-segregation rate was 20%. Patch clamp experiments showed that in an oxidative stress environment, *GSTM3* down-regulation leads to a significant decrease of cardiac sodium channel current amplitude. Ventricular arrhythmia incidence was significantly greater in *gstm3* knockout zebrafish at baseline and after flecainide, but was reduced after quinidine, consistent with clinical observations. BrS patients carrying the *GSTM3* deletion had higher rates of sudden cardiac arrest and syncope compared to those without (OR: 3.18 (1.77–5.74), $P<0.001$; OR: 1.76 (1.02–3.05), $P = 0.04$, respectively).

Interpretation: This *GSTM3* deletion is frequently observed in BrS patients and is associated with reduced I_{Na}, pointing to this as a novel potential genetic modifier/risk predictor for the development of the electrocardiographic and arrhythmic manifestations of BrS.

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1. Introduction

Brugada Syndrome (BrS) is a

Research in Context section

Evidence before this study

Unexpected sudden cardiac death in the young (SCDY) always make people, especially parents, grieve over their lost tremendously. Brugada Syndrome (BrS) is one of SCDY, an inheritable cardiac channelopathy associated with an increased risk of young sudden death or syncope, and a distinct electrocardiogram (ECG) pattern consisting of a right bundle-branch block with ST segment elevation in V1, and V2 in the absence of any structural heart disease. The average age at diagnosis is 40 ± 22 years, and it is male-predominant. The prevalence of BrS is estimated to be 1–5 per 10,000 people in Caucasians but is higher in Southeast Asians (12 per 10,000). Over 25 years, the pathophysiologic mechanism of BS still remains elusive because of limited genetic information. *SCN5A* is the major BrS-causing gene responsible for approximately 20% of BrS cases in Caucasians and 7.5–8% of cases in Han Chinese. Although several susceptibility genes have been identified, a genetic cause remain unknown in approximately 80% of BrS patients. Copy number variants (CNVs) can contribute to disease susceptibility, but their role in Brugada syndrome (BrS) is unknown.

Added value of this study

In this study, we enrolled 335 unrelated BrS patients from 2000 to 2018 in the Taiwanese population using a 2-stage approach with extreme phenotype sampling strategy. We used microarray and exome sequencing for discovery phase whereas Sanger sequencing were used for validation phase. We performed patch clamp study using HEK293 cells and *gstm3* knockout zebrafish experiments to characterize the CNV function. We identified a diallelic deletion of *GSTM3* contains the eighth exon and the transcription stop codon, and functional studies showed that this *GSTM3* deletion is associated with reduced cardiac sodium channel current. In this Taiwanese BrS patient cohort, the frequency of a copy number deletion of *GSTM3* was observed in 23.9% of 301 BrS patients without *SCN5A* mutations versus 0.8% of 15,829 ancestry-matched healthy controls in Taiwan Biobank. Intriguingly, the *GSTM3* deletion is not reported in the large dataset based on whole-genome sequences (>10,000 individuals), suggesting that it is closely associated with BrS. We also found that BrS patients carrying the *GSTM3* deletion had higher rates of sudden cardiac arrest and syncope compared to those without.

Implications of all the available evidence

We propose that our finding have both diagnostic and risk stratification clinical impacts for patients with BrS. Our study identified a deletion of *GSTM3* in BrS patients, which is associated with reduced I_{Na}, suggesting that the deletion could be a genetic modifier of the BrS phenotype. This study drives the understanding of this disease forward. This variant may be a novel genetic modifier/risk predictor for the development of the electrocardiographic and arrhythmic manifestations of BrS. It could be used as a risk predictor in patients with BrS for clinical practice. The gene could also be a potentially future therapeutic target and clinical genetic testing for patient care.

cardiac channelopathy associated with an increased risk of sudden death. The prevalence of BrS is estimated to be 1–5 per 10,000 people in Caucasians but is higher in Southeast Asians (12 per 10,000) [1,2]. The average age at diagnosis is 40 ± 22 years, and it is male-predominant [3].

SCN5A, which encodes the cardiac sodium channel, is the major BrS-causing gene responsible for approximately 20% of BrS cases in Caucasians and 7.5–8% of cases in Han Chinese [4,5]. Although several susceptibility genes have been identified [1,6,7], a genetic cause remains unknown in approximately 80% of BrS patients. One primary reason for this lack of causal certainty is the fact that a large proportion of patients is likely to represent non-Mendelian cases with oligogenic inheritance [8]; phenotyping errors, inadequate sensitivity of screening methods, and mutations in non-coding regions or in unknown genes are additional sources of causal uncertainty. Another possibility may be the presence of copy number variations (CNVs) in genes affecting the onset of BrS, which are known to play a role in cardiovascular diseases [9,10]. However, there is limited evidence regarding whether CNVs are important in BrS.

Here, we conducted a genome-wide CNV study in BrS patients without *SCN5A* mutations using a multi-stage study design with extreme phenotype sampling strategy [11,12]. We initially used genome-wide microarray to screen CNV regions in a case-control design, then used whole exome sequencing (WES) to fine-tune the length of candidate CNV regions in BrS patients because the candidate CNV regions were too long for Sanger sequencing technology. Thereafter, we validated the candidate CNV region in an independent BrS patient cohort using Sanger sequencing. We compared the frequency of identified CNV regions in the healthy populations using in-house controls from Taiwanese, the Taiwan Biobank (TWB), and the gnome aggregation database (gnomAD). Finally, we used cell and zebrafish models to investigate the functional role of the identified CNVs in BrS patients.

2. Materials and methods

2.1. Study subjects

We consecutively recruited 335 unrelated patients with BrS from 2000 to 2018 in the Taiwanese population in Taiwan; 76 were identified via symptoms of sudden cardiac arrest (SCA) or syncope early in the study period (2000–2010) and 259 more, both symptomatic and asymptomatic, were identified later (2011–2018), after the SADS-TW BrS registry increased awareness of BrS [13]. Aborigines were excluded from this study. BrS was diagnosed by 2 independent cardiologists using established criteria (Shanghai BrS Score ≥ 3.5)[14]. Since *SCN5A* is the major BrS-causal gene [4], we screened it first. Peripheral blood samples were collected from all participants. Mutations or SNPs in the *SCN5A* gene were screened using direct sequencing. We followed the primers and PCR conditions published by Wang et al. [15] to perform genotyping in all amino acid-coding exons and intron borders of *SCN5A*. Amplicons were purified by solid-phase extraction and were bidirectionally sequenced using a PE Biosystems Taq DyeDeoxy terminator cycle sequencing kit (PE Biosystems, Foster City, CA, USA). Sequencing reactions were separated on a PE Biosystems 373A/3100 sequencer, and the results were compared with a reference sequence from GenBank and the TWB. The pathogenicity of a mutation was defined by American College of Medical Genetics and Genomics guidelines [16]. Exclusion of 34 BrS patients with *SCN5A* mutations left 301 unrelated BrS patients enrolled in this CNV study.

The prevalence of BrS is lower (0.05–0.1%) [1,17] than that of common diseases (e.g., hypertension (28–31%) [18]). To overcome the small case number, we used a 2-stage study design with extreme phenotype sampling strategy and 2 independent cohorts

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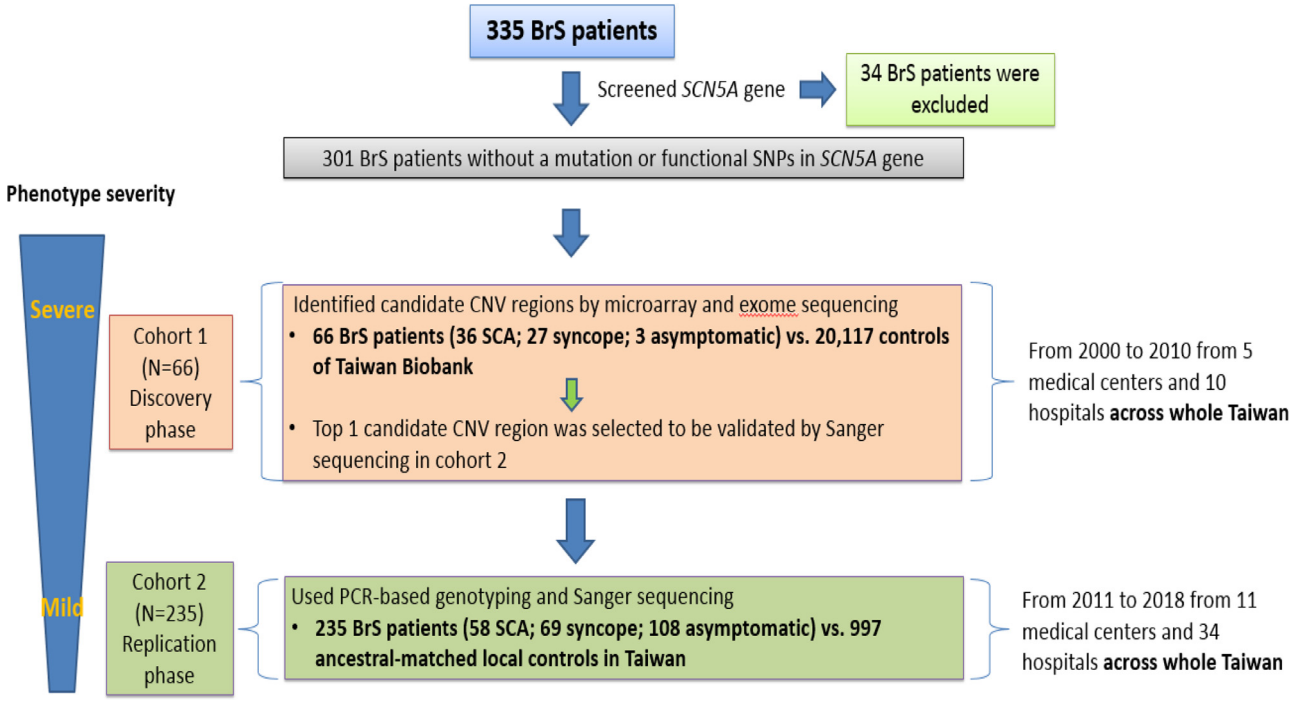

Fig. 1. An illustration of the timeline and experimental workflow of this study. This study used 2-stage design with extreme phenotype sampling strategy with two independent BrS cohorts.

with increasing sample size which attempted to maximize power and efficiency [11,12] (Fig. 1). In stage I, we initially selected 66 BrS patients (cohort 1, discovery cohort) to discover candidate CNV regions by microarray and WES. In stage II, cohort 2 (replication cohort, 235 unrelated BrS) were used to confirm the significant CNVs using PCR-based genotyping assay and Sanger sequencing. This study was approved by the ethics committee of National Taiwan University Hospital. All participants gave informed consent before participating in the study.

2.2. Microarray experiments, exome sequencing and CNV analysis

Omni1-Quad BeadChip microarrays (Illumina, USA) were performed according to the manufacturer's instructions. The data were submitted to the Gene Expression Omnibus (accession number GSE46348). To evaluate whole-genome CNV regions, raw intensity data from 1.14 million SNPs and CNV probes were imported into Partek Genomics Suite software (Partek Inc., USA) to perform CNV analysis. The criteria used for identifying CNV regions are shown in the Supplementary Note and the accuracy of the segmentation algorithm has been discussed in a previous study [19]. Lastly, Ingenuity Pathway Analysis (Ingenuity Systems, Inc., USA) was performed to characterize the biological functions of genes located in CNV regions. We used WES to fine-tune the length of candidate CNV regions. The detailed procedures of WES are described in the Supplementary Note. The CNVkit (v0.9.4) algorithm was used to obtain genome-wide CNV regions of each sample while the flat reference was set as the identical coverage in all samples [20]. A CNV region was defined as a deletion if its copy number was less than 1.2.

2.3. Validation of identified CNV regions

To further minimize the chance that false associations arose as a result of technical genotyping artefacts [21], different platforms using PCR-based genotyping and Sanger sequencing were used to validate the results of microarray and WES. To confirm the region containing the deletion of *GSTM3* identified by genome-wide microarray and WES, we designed PCR primers for the region with the lowest copy number of *GSTM3*. Forward and reverse primers used to amplify target regions are listed in **Table S1**, and the detailed procedures are given in the Supplementary Note.

2.4. Investigation of the identified CNV region in local controls and a public control

To evaluate the CNV frequency of *GSTM3* in relatively healthy populations, we used 2 local controls and 1 public control. For the first local controls, we first performed PCR and Sanger sequencing in 997 ancestral-matched in-house controls. In-house controls were ancestral-matched individuals with no arrhythmia-related symptoms, normal coronary arteries by angiography, normal 12-lead electrocardiogram (ECG), and no family history of sudden cardiac death (SCD), BrS, or heart failure. For the second local controls, we analysed genotyping data from the TWB (20,117 participants; https://taiwanview.twbiobank.org.tw/search, Supplementary Note). The design of the TWB array was a joint effort of the TWB, the National Center of Genome Medicine (NCGM; http://ncgm.sinica.edu.tw/ncgm_02/index.html), and Affymetrix, Inc. To compare with other ethnicities, we used the gnomAD structural variants (SVs) database to examine the *GSTM3* deletion frequency in major worldwide populations.

2.5. Basal expression of GSTM3 gene in human adult right ventricle

BrS is believed to be a right heart disease [22]. Although *GSTM3* is expressed in human heart muscle [23], whether *GSTM3* is specifically expressed in right ventricular cells has never been investigated before. We used a cDNA library from a healthy human adult right ventricle (Invitrogen) and diluted it to a working concentration of 100 ng/μL. Then, using primers for human β-actin (*ACTB* gene) and *GSTM3*, we performed PCR and separated the products on a 2% agarose gel to check the amplified band.

2.6. Western blot of GSTM3 proteins extracted from HEK 293 cells and HL-1 cells

HEK293 cells (derived from human embryonic kidney) were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine, and penicillin/streptomycin at 37 °C, 5% CO_2. HL-1 cardiac muscle cells, an immortalised mouse atrial cell line, were cultured on a plate coated with gelatin/fibronectin, maintained in Claycomb medium containing 10% FBS, penicillin/streptomycin, L-glutamine, and 0.1 mM norepinephrine. Protein lysates (50 µg) were extracted from HEK293 cells, HL-1 cells, and mouse testis tissue (use as positive control), then separated by SDS-PAGE, blotted with the *GSTM3* antibody (Cusabio Technology, LLC), and detected using an enhanced chemiluminescence western blotting system (Amersham Biosciences). *GSTM3* protein is endogenously expressed in HEK293 cells but not in HL-1 cells **(Fig. S1)**. Therefore, we used HEK293 cells stably expressing Nav1.5 channel in further studies.

2.7. HEK293 cell culture and transfection

HEK293 cells stably express *SCN5A* (encoding the Nav1.5 channel), hereafter referred to as HEK Nav1.5 cells. HEK Nav1.5 cells were cultured in a controlled environment (5% CO_2, 37 °C) and maintained in DMEM (Euroclone, Italy) supplemented with FBS (10%), L-glutamine (2 mM), penicillin/streptomycin (100 U/mL, 100 μg/mL), and zeocin (200 μg/mL). The transfection was carried out using jetPRIME reagent (PolyPlus transfection, Illkirch, France) according to the manufacturer's instructions. Cells were transfected with *GSTM3* Silencer Select Pre-designed siRNA (20 nM; Ambion, Thermo Fisher Scientific, Italy) or with Silencer Select Negative Control #1 Pre-designed siRNA, a non-targeting siRNA providing a negative control to compare siRNA-treated samples (20 nM; Ambion, Thermo Fisher Scientific, Italy). Twenty-four hours after transfection, cells were harvested either to analyze the knockdown of endogenous *GSTM3* protein levels by western blotting or for cytotoxicity assays, or re-seeded for electrophysiological recording.

2.8. Western blot of GSTM3 protein extracted from HEK NAV1.5 cells transfected with siRNA specific for GSTM3 or with a non-targeting negative control siRNA

Cells were lysed and cytoplasmic proteins were extracted using a NE-PER© Nuclear and Cytoplasmic Extraction Reagents kit (Thermo Fisher Scientific, Italy). Protein concentrations were determined using Bradford assay reagent (Pierce Coomassie Plus Protein Assay; Thermo Fisher Scientific, Italy) following the manufacturer's instructions. Twenty-five μg of cytoplasmic protein were separated on homemade gels (12% acrylamide) in a standard running buffer and then transferred onto nitrocellulose membranes (GE Healthcare, Euroclone, Italy). Membranes were blocked with 5% non-fat dry milk in 1 × Tris-buffered saline containing 0.1% Tween 20 (TTBS 0.1%) for 90 min at room temperature, washed 3 times in TTBS 0.1%, and then incubated overnight at 4 °C with anti-α-tubulin (1:500 in TTBS 0.1% plus milk 5%, rabbit monoclonal; Cell Signaling, Danvers, MA, USA) and anti-human glutathione S-transferase mu 3 (1:1000 in TTBS 0.1% plus milk 5%, rabbit polyclonal; Cusabio Biotech Co, College Park, MD, USA). After 3 TTBS 0.1% washings, membranes were incubated for 90 min with an IgG HRP-conjugated antibody (donkey anti-rabbit, 1:5000 in TTBS 0.1% plus milk 1%; Santa Cruz Biotechnology) and then washed again 3 times in TTBS 0.1%.

Proteins were detected using an enhanced chemiluminescence detection kit (SuperSignal West Pico Chemiluminescent Substrate; Thermo Fisher Scientific, Italy) in an ImageQuant LAS 4000 instrument (GE Healthcare Life Sciences, Italy). Blots were analysed and quantified with ImageJ software.

2.9. Cytotoxicity assays

To measure the mitochondrial activity and membrane damage in HEK293 cells, 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT) (MTT Formazan powder; Sigma-Aldrich, Italy) and lactate dehydrogenase (LDH) (LDH Cytotoxicity Detection KitPLUS; Sigma-Aldrich, Italy) assays were performed following the manufacturer's instructions as in our previous work [24]. To create conditions of oxidative stress, cells were incubated for 30 or 60 min either with medium or with medium plus 15 mM tert–butyl hydroperoxide (tBHP; Sigma-Aldrich, Italy). Absorbance and emission were measured with a multi-label spectrophotometer (VICTOR3, Perkin Elmer, USA) at 570 nm and 490 nm, respectively.

2.10. Electrophysiological experiments in HEK NAV1.5 cells

Patch clamp experiments were performed at room temperature in HEK Nav1.5 cells in whole cell configuration. The experimental details are described in the Supplementary Note. To induce conditions of oxidative stress, cells were incubated for 30 min at room temperature with an extracellular solution containing 95 mM N-methyl-D-glucamine, 20 mM NaCl, 5 mM CsCl, 2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 10 mM HEPES, 5 mM glucose, and 15 mM tBHP, and recordings were obtained within 60 min from the beginning of treatment. The standard voltage clamp protocols have been previously described [25,26]. Membrane voltages were not corrected for liquid junction potential. To measure the kinetics of the onset of inactivation, the declining phase of I_{Na} traces recorded between −30 mV and 20 mV were fitted with a bi-exponential function. The number of cells is indicated in Table 3, and the "current density" column is greater than the "activation" column because not all cells could complete the protocol for the study of the activation properties. We did not consider applying TTX in order to define I_{Na}, as HEK Nav1.5 cells stably express Nav1.5 channel, and the comparison with the un-transfected HEK293 cells showed the absence of inward currents **(Fig. S2).**

2.11. Generation and characterization of CRISPR/Cas9-mediated GSTM3 knockout male adult zebrafish

All experimental procedures on zebrafish were approved by the committee for use of laboratory animals at National Taiwan University, Taipei, Taiwan (IACUC Approval ID: 103; Animal Use document no. 102) and carried out in accordance with the guidelines of the Animal Welfare Act. The details of generation and characterization of *GSTM3* knockout adult male zebrafish using CRISPR/Cas9 are described in the Supplementary Note. Briefly, among the 3 guide RNAs (gRNAs) tested, only the exon 5-targeted gRNA effectively induced distinct changes in melting curves compared to the untreated groups **(Fig. S3A).** We thus raised only the exon 5 gRNA-treated F0 embryos to adulthood and then crossed them with WT fish to obtain F1 embryos. The F1 embryos were raised to 2 months old to collect genomic DNA from their tail fins. We screened for the *GSTM3* mutation carriers by high resolution melting analysis, raised them to adulthood, and cross-bred them to obtain the F2 generation. The zygosity of the F2 fish was determined by capillary electrophoresis. We performed PCR using F2 fish tail fin genomic DNA to obtain amplicons from 24 fish. As shown in **Fig. S3B**, the WT fish showed only one band of 250 base pairs. In contrast, the homozygous mutant fish also had one band with a reduced size, which indicated a potential deletion in the *GSTM3* gene. The heterozygous fish contained both bands with reduced

intensity. To confirm the deletion in *GSTM3*, we sequenced those amplicons and found that all *GSTM3*-/- fish had a 7 bp deletion (-TCCGCAA-) at the gRNA binding site compared to the sequence of WT fish **(Fig. S3C).**

The WT GSTM3 protein contains 219 amino acids. The deletion results in a premature stop codon in the middle. The mutant GSTM3 has the native sequence of 106 amino acids at its N-terminus, followed by 25 mutated amino acids due to the frameshift. The amino acid sequence alignment of WT and mutant GSTM3 is presented in **Fig. S4.** The identified *GSTM3*+/- and *GSTM3*-/- fish were separated and used for further experiments.

2.12. Expression of GSTM3 in adult male zebrafish heart by real-time ***quantitative*** PCR

To examine expression of *GSTM3*, we isolated 3 hearts of adult male zebrafish. The hearts were homogenised to extract total RNA using TRIzol reagent (Invitrogen) for cDNA synthesis using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) according to the manufacturer's instructions. The cDNA was then subjected to real-time quantitative PCR analysis (Applied Biosystems). Taqman reactions were performed using one-step RT-PCR Master Mix Reagents with the ABI PRISM 7700 Sequence Detection System and analysed using the Sequence Detection System software. Gene expression was normalised to a commonly used reference gene (*EF1A*, elongation factor 1α). Primers and probes were based on GenBank sequences (*GSTM3*, NM_001162851.1; and *EF1A*, NM131263.1). The data were analysed using the relative gene expression (i.e., $\Delta\Delta$CT) method, as described in Applied Biosystems User Bulletin No. 2 and explained further by Livak and Schmittgen [27]. Briefly, the data were presented as the fold change in gene expression normalised to *EF1A* and relative to a calibrator. The results are presented in **Fig. S5**.

2.13. Arrhythmogenicity assessment and drug administration in zebrafish

Because BrS is a young adult male-dominant disease, we used adult male zebrafish (F2) for further experiments instead of larva. All experiments were performed at room temperature. The PR interval, RR interval, QT interval, and QRS duration were recorded. The PR interval was measured from the start of the P wave to the start of QRS. The QT interval was defined as the time from the start of the Q wave to the end of the T wave. QRS duration was defined from the start of the Q wave to the end of the S wave. The heart rate was measured from the RR interval, defined as the time interval between the peaks of 2 consecutive QRS complexes. Recordings were acceptable if the T-wave amplitude was $\geq$ 25 μV and did not deteriorate by $\geq$50% during the recording. Bazett's formula was used to correct the observed QT interval for variations in heart rate.

Since sodium channel blockers are known to unmask Brugada ECG in patients and induce arrhythmia [4], we performed a flecainide challenge in the *GSTM3*-/- adult zebrafish [28]. Because quinidine is used clinically to suppress the electrocardiographic and arrhythmic manifestations of BrS [4], we tested its ability to suppress arrhythmias in a zebrafish model of BrS. Drugs were diluted from DMSO stock solutions to final concentrations of 0.1 µM, 1 µM, or 10 µM in E3 solution. All concentrations were safe for adult zebrafish [29,30].

Wild-type (WT) and mutant 9–12 month-old adult male zebrafish (F2) were anesthetised by titration with tricaine solution (MS-222, Sigma) for 2 min. We administered flecainide and quinidine. Arrhythmias, PR interval, RR interval, QRS duration, QT interval, and QTc values were obtained at baseline and after 10 min of exposure to drug (**Fig. S6**). All parameters were recorded for 10 min after reaching a stable steady state. We performed programmed extra-systolic stimulation (PES) before and after drug administration as a previously study [31]. Two independent and blinded investigators confirmed all measurements. The Inter-observer agreement was determined by overall proportion of agreements and by using the Kappa statistic. The overall proportion of agreement in the ECG measurements among the two interpreters was 99.7% with a Kappa score of 0.95. If the ECG data had a discrepancy, we discarded the data. The details of the procedure and the PES protocol are described in the Supplemental Note.

2.14. Statistical analyses

Continuous variables were compared using the Student's *t*-test. Categorical variables and CNV proportions were analysed using the Fisher's exact test. The analysis of variance (ANOVA) method was performed for multiple group comparisons, followed by a modified *t*-test with Fisher LSD correction (ORIGIN 10). *P* values $<$0.05 were considered as statistically significant.

3. Results

3.1. Patient characteristics

The demographic characteristics of the study cohorts are shown in Table 1. The gender of all cohorts was male-predominant, and 63.1% of BrS patients overall were symptomatic. As expected, the percentage of the symptomatic BrS patients was higher in cohort 1 than in cohort 2 (95.5% vs. 54%), whereas the percentage of asymptomatic BrS patients was higher in cohort 2 than in cohort 1 (4.5% vs. 46%) because of our study design. There were no differences in gender, family history of SCD, and spontaneous type 1 Brugada ECG among the 2 cohorts.

3.2. Discovery of the CNV regions

The genomic landscape of CNV regions in BrS patients is illustrated in **Fig. S7.** A total of 502 aberrant regions were observed, including 447 deletions and 55 amplifications. Importantly, no CNVs were detected in the *SCN5A* gene. The Refseq database revealed 91 deleted and 11 amplified genes in total (**Table S2**). Among these 102 CNV genes, Ingenuity Pathway Analysis revealed 5 significantly enriched canonical pathways ($P<10^{-5}$, **Table S3,** Hypergeometric test). Notably, although the 5 pathways had different cellular functions, they were primarily identified on the basis of the same genes, particularly the GST mu family. Unexpectedly, all 5 genes in the GST mu family, including GSTM1-5, showed high frequencies of deletion, suggesting they make up a gene cluster deleted in BrS. Among the 5 genes, *GSTM3* is the gene nearest to the breakpoint of the CNV region spanning across the GST mu family. Therefore, *GSTM3* was selected for further investigations. Because the candidate CNV region of GSTM3 (chr1:109,733,932-109,739,407) was too long ($>$5 K bp) to use traditional Sanger sequencing to identify the breakpoints of this CNV region, we used WES technology to fine-tune this candidate CNV region in the unrelated 66 BrS patients instead. Fifteen of the 66 BrS patients (30%) had this *GSTM3* deletion, and WES showed the length of the *GSTM3* deletion (chr1:109,737,076-109,737,247, hg38) (**Fig. 2A**). Except for common variants (e.g., SNPs), no radical or missense mutations were identified in the *GSTM3* gene.

3.3. Validation and replication

In cohort 2, the *GSTM3* deletion was present in 23.4% of the 235 BrS patients using a PCR-based genotyping assay and direct sequencing (Fig. 2B and 2C). The length of the *GSTM3* deletion

Table 1
Clinical characteristics of BrS patients in 2 independent cohorts.

	Cohort 1 (N = 66)	Cohort 2 (N = 235)	All (N = 301)
Age at diagnosis (years)	42.0 ± 12.9	45.0 ± 15.8	44.3 ± 15.2
Gender (male)	63 (95.4%)	209 (88.9%)	272 (90.4%)
Presentations			
SCA	36 (54.5%)	58 (24.7%)	94 (31.2%)
Syncope	27 (40.9%)	69 (29.4%)	96 (31.9%)
No symptoms	3 (4.5%)	108 (46.0%)	111 (36.9%)
Family history of SCD	13 (19.7%)	49 (20.9%)	62 (20.6%)
Spontaneous type 1 Brugada ECG	59 (89.4%)	176 (74.9%)	235 (78.1%)

BrS, Brugada syndrome; ECG: electrocardiogram; SCD: sudden cardiac arrest.

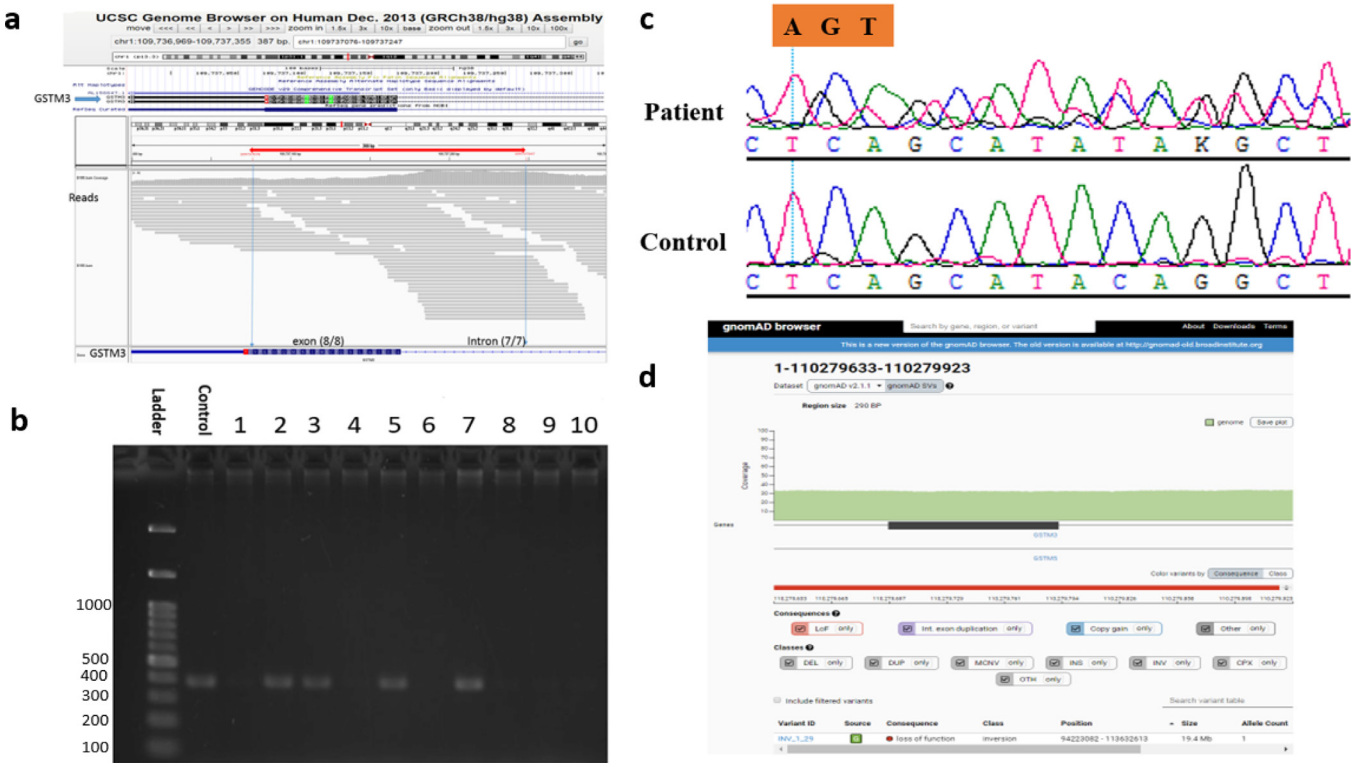

Fig. 2. Validation of the *GSTM3* deletion. (A) An example of a plot of *GSTM3* deletion detected by WES in a BrS patient. Red labels indicate the chromosomal position (chr1:109,737,076-109,737,247, hg38), containing part of intron 7, part of exon 8, and the transcription stop site (CCDS 812.1, hg38). **(B)** An example of a polyacrylamide gel of *GSTM3*. A PCR band indicated no deletion or heterozygous deletion of *GSTM3* (copy number=1 or 2; patients 2, 3, 5 7 and control); no PCR band indicated homozygous deletion of *GSTM3* (copy number=0; patients 1, 4, 6, 8, 9 and 10). **(C)** An example of the sequencing map of part of the deletion in exon 8 of *GSTM3* containing the transcription stop codon by a reverse primer in a BrS patient with heterozygous deletion and a control without deletion. **(D)** No *GSTM3* deletion (chr1:110,279,633-110,279,923, hg37) is reported in the gnomAD structural variants database (N = 10,738 unrelated individuals, (https://gnomad.broadinstitute.org/region/1-110279562-110279935?dataset=gnomad_sv_r2)).

is 291 base-pairs (chr1:109,737,011-109,737,301, hg38). Importantly, this deletion region in *GSTM3* contains exon 8 and the transcription stop codon (CCDS 812.1, hg38). In total, 23.9% of the BrS patients carried the *GSTM3* deletion and 94.4% of the BrS patients with the *GSTM3* deletion (68/72) were heterozygous carriers. Interestingly, when we screened this CNV in the 34 BrS patients excluded for having *SCN5A* mutations, this CNV was not detected. Co-segregation analysis in 10 families showed that the co-segregation rate was 20% (2 of 10 families; **Fig. S8, Supplementary Note**).

3.4. Evaluation of the GSTM3 deletion in the healthy controls

We evaluated the frequency of the *GSTM* deletion in 3 healthy controls (2 ancestral-matched local controls and 1 public CNV database). In the 2 local controls, the deletion was in 0.1% of 997 in-house unrelated controls whereas CNV analysis showed that 0.8% of the 15,829 ancestral-matched unrelated samples which passed the quality assessment have the *GSTM3* deletion in the TWB, which were significantly lower than that in BrS patients (both P<0.001, Proportional test). In the public CNV database, the WGS data from the gnomAD SVs database, which is 9% East Asian, showed that no such deletion in *GSTM3* was reported in any samples out of 10,738 unrelated individuals (P < 0.0001, Fig. 2D, Proportional test). These results support the deletion of *GSTM3* being significantly associated with Taiwanese BrS patients without *SCN5A* mutations.

3.5. Comparison of clinical demographics and severity of clinical presentation of the BrS patients with and without the deletion of GSTM3

The comparison of clinical characteristics between BrS patients with or without this deletion is shown in Table 2. In cohort 1 and cohort 2, there were no significant differences in age at diagnosis, gender, family history of SCD, or spontaneous type 1 Brugada ECG between the two cohorts. In cohort 1 and cohort 2, a

Table 2
Comparisons of clinical characteristics between BrS patients with or without deletion of GSTM3.

	Cohort 1 (discovery cohort)			Cohort 2 (replication cohort)			All	
CNV deletion of GSTM3	Without the CNV deletion of *GSTM3* (*N* = 41)	OR (95% CI)	*P* value	With the CNV deletion of *GSTM3* (*N* = 47)	Without the CNV deletion of *GSTM3* (*N* = 188)	OR (95% CI)	*P* value	With the CNV deletion of *GSTM3* (*N* = 72)	Without the CNV deletion of *GSTM3* (*N* = 229)
	41.5 ± 14		0.67	45.5 ± 15	44.9 ± 16		0.81	44.6 ± 13.7	44.3 ± 15.7
	39 (95.1%)		1	43 (91.5%)	166 (88.3%)		0.79	67 (93.1%)	205 (89.5%)
	18 (43.9%)	3.23 (1.01–11.27)	0.04*	19 (40.4%)	39 (20.7%)	2.58 (1.23–5.37)	0.007*	37 (51.4%)	57 (24.9%)
	20 (48.8%)	0.41 (0.12–1.32)	0.12	23 (48.9%)	46 (24.5%)	2.94 (1.44–6.03)	0.002*	30 (41.7%)	66 (28.8%)
	3 (7.3%)	0 (0–3.95)	0.28	5 (10.6%)	103 (54.8%)	0.10 (0.03–0.27)	<0.001*	5 (6.9%)	106 (46.3%)
	6 (14.6%)	2.24 (0.55–9.42)	0.21	11 (23.4%)	38 (20.2%)	1.21 (0.51–2.70)	0.68	18 (25.0%)	44 (19.2%)
	36 (87.8%)	1.59 (0.23–17.97)	0.7	39 (83.0%)	137 (72.9%)	1.81 (0.77–4.79)	0.19	62 (86.1%)	174 (76.0%)
	2	1.68 (0.11–24.67)	0.63	3	8	1.53 (0.25–6.72)	0.46	5	10
	167±15		0.83	173 ± 30	170 ± 31		0.55	171±28	169±29
	102±15		0.08	109±21	106±17		0.09	109.3 ± 21.2	105.3 ±16.7
	407±26		0.31	438±51	430±38		0.23	430±50	426±37
	82±13		0.01	88±17	83±12		0.02	89.4 ± 17.3	82.8 ±12.1
	3.7 ± 0.8		0.40	4 ± 0.9	3.8 ± 0.7		0.10	3.97±0.98	3.78±0.92

BrS: Brugada syndrome; ECG: electrocardiogram; SCA: sudden cardiac arrest; SCD: sudden cardiac death; *$P < 0.05$ by Fisher's exact test or Student's *t*- test;.

higher percentage of BrS patients with deletion of *GSTM3* experienced SCA compared to those without ($P = 0.003$, 0.007, respectively, Fisher's exact test). In total cohort, more BrS patients with deletion of *GSTM3* experienced SCA and syncope than those without ($P = 0.002$, 0.04, respectively, Fisher's exact test).

3.6. Establishment and validation of the cellular model

After confirming that *GSTM3* is expressed in human adult right ventricle (**Fig. S9**), we established a cellular model consisting of HEK293 cells stably expressing the *SCN5A* (HEK Nav1.5). To mimic the *GSTM3* deletion observed in BrS patients, we induced the down-regulation of *GSTM3* expression by transfecting the cells with siRNA targeting *GSTM3*. Western blotting showed that siRNA transfection reduced *GSTM3* expression by almost 50% (Fig. 3A).

To test the effects of *GSTM3* silencing on cell vitality, we used tBHP (15 mM), an organic peroxide, to create conditions of oxidative stress [32,33]. MTT assays showed that mitochondrial activity was reduced equally after 60 min of tBHP exposure in the negative control (84.4 ± 11.5% vitality, $P = 0.015$, ANOVA, followed by a modified t-test with Fisher LSD correction) and in the *GSTM3*-silenced cells (87.5 ± 8.7% vitality, $P = 0.028$, ANOVA, followed by a modified t-test with Fisher LSD correction) (Fig. 3B). LDH activity in the culture medium was also measured as an index of membrane damage after tBHP exposure. Again, the effect of tBHP on cell vitality was indistinguishable in control and *GSTM3* siRNA-transfected cells (81.8 ± 1.8% vitality, $P = 1.04\times10^{-4}$; and 83.3 ± 1.8% vitality, $P = 9.6 \times 10^{-6}$, respectively, ANOVA, followed by a modified t-test with Fisher LSD correction) (Fig. 3C). These data suggested that tBHP-induced cytotoxicity under these conditions was not critically relevant, at least from the metabolic point of view.

3.7. Effect of the loss of GSTM3 on the cardiac sodium current under conditions of oxidative stress

Whole-cell patch-clamp data showed that Nav1.5 current density measured at −10 mV was not affected by the siRNA transfections (Fig. 4A-B; Table 3). As expected from the literature [32,33], a condition of oxidative stress, created by the application of 15 mM of tBHP, triggered a decrease in I_{Na} of about 60% both in control cells and in cells transfected with the siRNA negative control. However, when *GSTM3* protein expression was silenced, tBHP reduced the I_{Na} density by approximately 75% (Fig. 4A-B; Table 3) and the onset of fast inactivation was accelerated (Fig. 3C).

Consistent with previous studies [34,35], no significant differences were found in activation after tBHP application (Fig. 5; Table 3), but tBHP exposure induced a significant negative shift in the availability of sodium channels (Fig. 5). When HEK Nav1.5 cells were treated with tBHP, $V_{1/2}$ significantly shifted approximately 8 mV in the negative direction, and a similar shift was observed in cells transfected with the non-targeting siRNA (Table 3). This hyperpolarizing shift was even more dramatic (approx. –18 mV) when *GSTM3* expression was reduced, after induction of oxidative stress (Fig. 5; Table 3). These data support the hypothesis that reduced expression of *GSTM3* amplifies the response of $Na_V1.5$ channels to oxidative stress, causing a more dramatic reduction in I_{Na}.

3.8. Comparisons of baseline ECG parameters and ventricular arrhythmic events before and after flecainide administration

Table S4 shows the ECG parameters and the number of WT, *GSTM3*+/- and *GSTM3*-/- zebrafish with ventricular arrhythmia (VA) before and after flecainide administration. The baseline PR interval, QRS duration, RR interval, and QTc interval were not significantly different among the 3 groups. After flecainide treatment

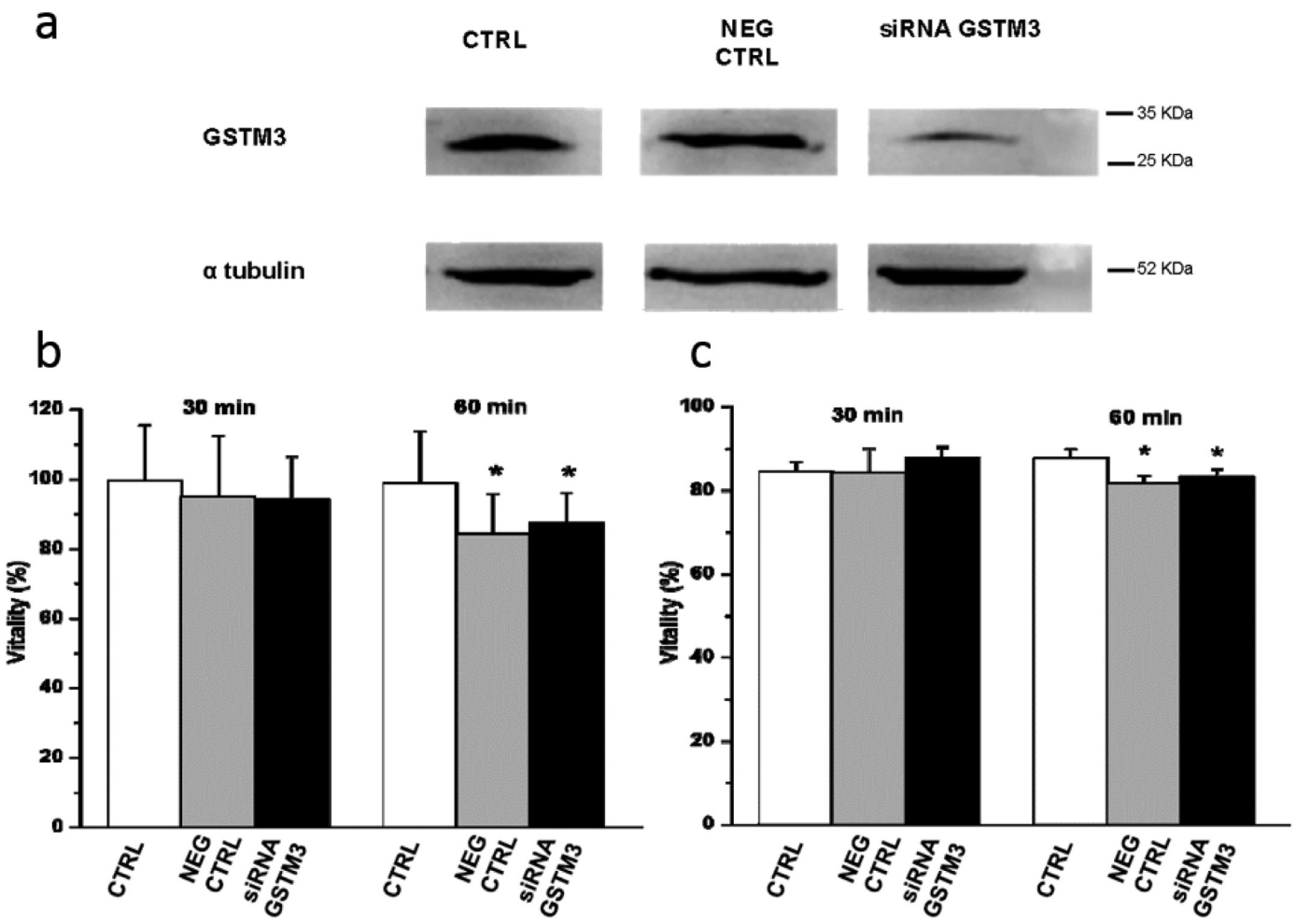

Fig. 3. ***GSTM3*** **knockdown and its effects on cell cytotoxicity. (A)** Results obtained from HEK Nav1.5 cells (CTRL), and cells transfected with a non-targeting siRNA (NEG CTRL) or siRNA specific for *GSTM3* (siRNA *GSTM3*), showing the GSTM3 protein is down-regulated by about 50% (normalised to α-tubulin protein expression). Three different transfections and at least 3 different western blots were performed. **(B and C)** Bar graphs showing the results of the cytotoxicity tests (MTT and LDH, respectively). Y-axis represents the vitality of the cells incubated for 30 or 60 min with 15 mM tBHP. Data are presented as mean values ± *S.E.* and were derived from at least 2 different experiments, each consisting of 6 different wells. $^{*}P<0.05$. (ANOVA, followed by a modified t-test with Fisher LSD correction).

Table 3
Properties of I_{Na} current in control and GSTM3-silenced cells in the presence or absence of 15 mM tBHP.

	Current density(pA/pF)	*Activation Curve* $V_{1/2}$ *(mV)*	*k*	*Availability Curve* $V_{1/2}{}^{(a)}$ *(mV)*	*k*
CTRL	−145.9 ± 24.3 (n = 44)	−24.3 ± 1.2 (n = 13)	7.5 ± 0.8	−64.0 ± 2.2 (n = 28)	8.4 ± 0.4)
CTRL + tBHP	−55.3 ± 16.1 (n = 13)*	−21.6 ± 2.2 (n = 13)	9.1 ± 1.5	−72.5 ± 0.9 (n = 6)*	8.2 ± 0.7
NEG CTRL	−146.9 ± 21.9 (n = 23)	−24.1 ± 2.8 (n = 6)	6.8 ± 0.7	−67.0 ± 1.8 (n = 10)	7.8 ± 0.3
NEG CTRL+ tBHP	−64.4 ± 21.9 (n = 18)*	−19.3 ± 2.8 (n = 6)	9.02±1.1	−75.8 ± 2.8 (n = 15)*	7.4 ± 0.6
GSTM3 siRNA	−164.4 ± 32.0 (n = 20)	−20.8 ± 4.7 (n = 4)	8.06±1.2	−64.4 ± 1.6 (n = 19)	8.2 ± 0.4
GSTM3 siRNA+ tBHP	−41.4 ± 8.5 (n = 23)*,#	−22.8 ± 5.3 (n = 12)	8.07±1.02	−82.8 ± 2.6 (n = 7)*,#,§	7.02±0.2

$V_{1/2}$, voltage of half-maximal activation; $V_{1/2}{}^{(a)}$, prepulse voltage where half-maximal inactivation occurred; k, slope factor. CTRL, untransfected cells; NEG CTRL, cells transfected with non-targeting siRNA. Values are means ± SE. n is the number of cells patched from at least three different experiments. $^{*}P<0.005$ vs. the respective tBHP untreated condition; $\#P<0.005$ vs CTRL + tBHP; $\S P<0.005$ vs NEG CTRL + tBHP. (ANOVA, followed by a modified t-test with Fisher LSD correction).

(1 μM and 10 μM flecainide), there was a significant increase of PR interval and QRS duration in *GSTM3*+/- and *GSTM3*-/- zebrafish compared with WT. At baseline, the number of WT, *GSTM3*+/-, and *GSTM3*-/- zebrafish with VA was not different but the number of *GSTM3*+/- and *GSTM3*-/- zebrafish with VA significantly increased after flecainide administration (Fig. 6A-C). Interestingly, the number of *GSTM3*+/- and *GSTM3*-/- zebrafish with VA decreased after quinidine infusion (Fig. 6D-F, **Table S5**). The pharmacologic responses observed in *GSTM3*-/- and *GSTM3*+/- fish are congruent with those encountered in clinical cases of BrS.

3.9. Comparison of arrhythmogenicity after programmed extra-systolic stimulation

Table S5 shows the number of WT, *GSTM3*+/- and *GSTM3*-/- zebrafish with inducible ventricular tachycardia (VT) or ventricular fibrillation (VF) after PES. After PES in the absence of drug treatment, the number of *GSTM3*+/- and *GSTM3*-/- zebrafish with inducible VT or VF was significantly higher than WT fish. With flecainide administration, the number of *GSTM3*+/- and *GSTM3*-/- zebrafish with inducible VT or VF increased significantly. In contrast,

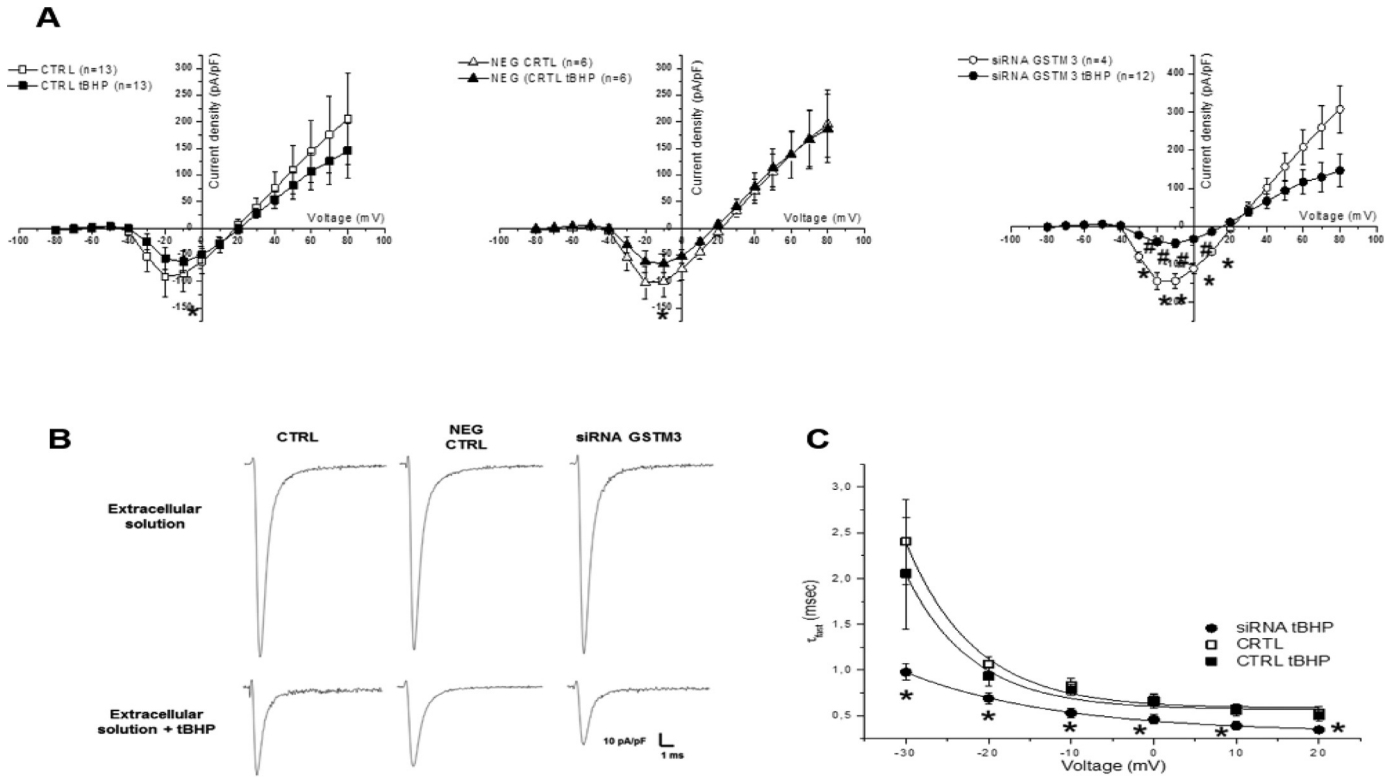

Fig. 4. Effect of tBHP on the Nav1.5 peak current density and kinetics of inactivation. (A) Current-voltage relationship of peak inward currents in HEK Nav1.5 cells (CTRL), in HEK Nav1.5 cells transfected with a non-targeting siRNA (NEG CTRL) or siRNA specific for GSTM3 (siRNA GSTM3) in the absence (empty symbols) or in presence (filled symbols) of tBHP. After performed multiple testing correction, $^{*}P<0.005$ vs the respective untreated condition; $\#P<0,005$ vs CTRL tBHP. In parentheses is the number of cells. **(B)** Peak currents evoked by a voltage step at −10 mV (holding potential −100 mV) normalised by cell capacitance, in the absence (upper traces) or presence (lower traces) of tBHP. **(C)** Kinetics of the onset of fast inactivation. $^{*}P<0.005$ after performed multiple testing correction. (ANOVA, followed by a modified t-test with Fisher LSD correction).
In Panel A and C, data are presented as mean values ± S.E.

the number of zebrafish with inducible VT or VF among the 3 groups was not different after quinidine infusion except for 0.1 μM quinidine.

4. Discussion

Previous CNV studies in BrS focused on the *SCN5A* gene alone [36-40]. This is the first whole-genome CNV study to investigate the role of genomic CNVs in influencing susceptibility to BrS and to examine the role of CNV in risk stratification of BrS patients. We identified a diallelic CNV deletion of *GSTM3* in 23.9% of Taiwanese BrS patients without *SCN5A* mutations. In contrast, the deletion of *GSTM3* was rarely observed in 0.1% of in-house controls ($N = 997$) and 0.8% in TWB samples ($N = 15,829$). Intriguingly, the *GSTM3* deletion was not reported in the large dataset based on whole-genome sequences (>10,000 individuals), suggesting that it is closely associated with BrS.

The value of genetic variants as a tool to evaluate recurrent arrhythmic risk in BrS is still undetermined [1]. In this study, although not all BrS families showed complete co-segregation of the *GSTM3* deletion with BrS, the frequency of SCA in BrS patients with deletion of *GSTM3* was significantly higher than in BrS patients without the deletion, suggesting that the clinical presentation of BrS patients with the deletion of *GSTM3* may be more severe. We also observed that the frequency of VA was significantly higher in *GSTM3* knockout zebrafish than in WT zebrafish. In other words, our findings suggest that deletion of *GSTM3* may exert modulatory effect on arrhythmia risk in Taiwanese BrS patients, and provide a reference for risk stratification of BrS patients.

GSTM3 encodes a glutathione S-transferase involved in antioxidant defense, protecting the cells from oxidative stress [41]. The deleted region of *GSTM3* contains the 8th exon and the transcription stop site. Thus, this deletion may result in failed transcription termination, leading to nonsense-mediated degradation of *GSTM3* mRNA [42]. Cardiac oxidative stress caused by reactive oxygen species (ROS) has been demonstrated to play an important role in the mechanism of cardiac arrhythmia and SCD [43,44]. In excitable cardiac cells, ROS regulate both cellular metabolism and ion homeostasis. Increasing evidence suggests that elevated cellular ROS can cause alterations of membrane current in isolated cardiac myocytes [45] and abnormal Ca^{2+} handling, leading to arrhythmogenesis [43]. In particular, ROS induce a reduction in the total cardiac sodium current and a leftward shift in the availability curve [34,45]. The pathogenic mechanism underlying BrS may involve an outward shift in the balance of current in the early phases of the action potential in the epicardium of the right ventricular outflow tract, secondary to a decrease in inward current (e.g., I_{Na}) or an increase in outward current (e.g., $I_{K\text{-}ATP}$). Oxidative stress, among other effects, produces both a decrease in I_{Na} and an increase in $I_{K\text{-}ATP}$. Our data from HEK293 Nav1.5 cells showed that, upon treatment with the direct-acting oxidative agent tBHP, the reduction in I_{Na} is amplified when *GSTM3* is reduced. A loss of I_{Na} has been shown to leave the transient outward current (I_{to}) less opposed, thus accentuating the epicardial action potential notch in the outflow tract of the right ventricle, leading to loss of the action potential dome and the development of phase 2 reentrant extrasystoles capable of precipitating VT/VF [46]. Reduced I_{Na} has also been proposed to contribute to the manifestation of BrS by slowing impulse conduction into the right ventricular outflow tract (RVOT)

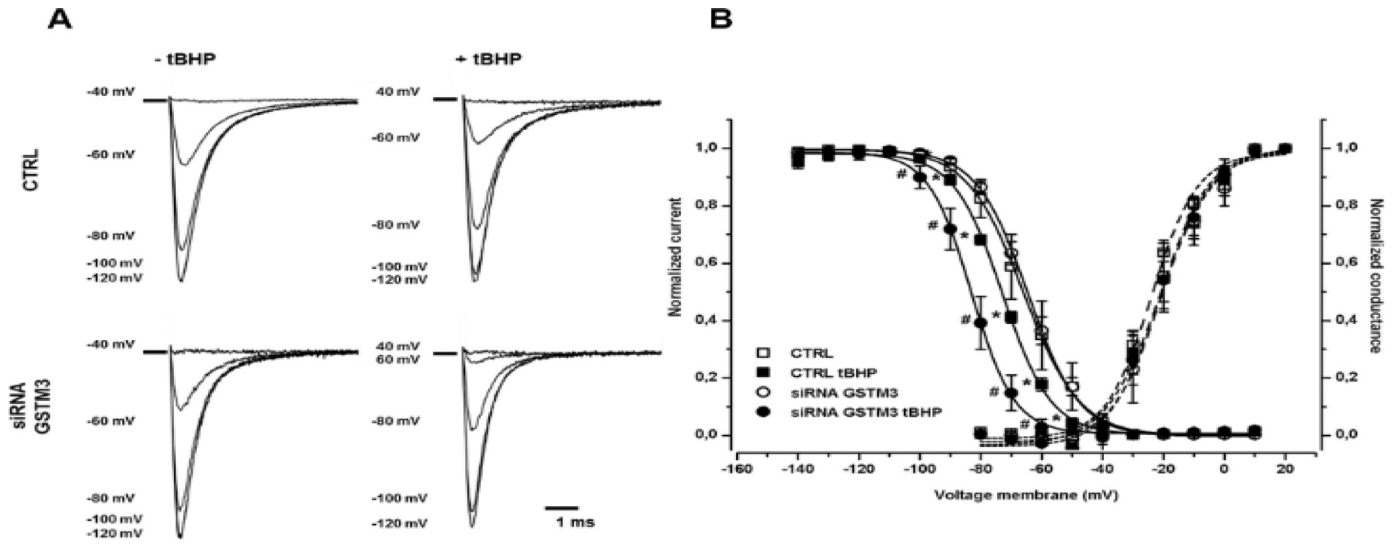

Fig. 5. Effect of tBHP on Nav1.5 activation curve and channel availability. (A) Families of current traces recorded in HEK Nav1.5 cells (CTRL) and in HEK Nav1.5 cells with *GSTM3* silenced (siRNA GSTM3), at selected voltages in the absence (-tBHP) or presence (+tBHP) of tBHP. **(B)** The silencing of *GSTM3* had no effect on the availability curves (solid lines) (CRTL vs siRNA *GSTM3*, open squares vs open circles). tBHP (15 mM) induced a hyperpolarizing shift in the $V_{1/2}$ of −8.5 mV in HEK Nav1.5 cells (CRTL vs CTRL tBHP; open squares vs filled squares) and of −18.4 mV when *GSTM3* was silenced (siRNA *GSTM3* vs siRNA *GSTM3* tBHP; open circles vs filled circles). No significant differences were observed in the activation properties (dashed lines). Results from the NEG CTRL cell line were omitted for the sake of clarity.

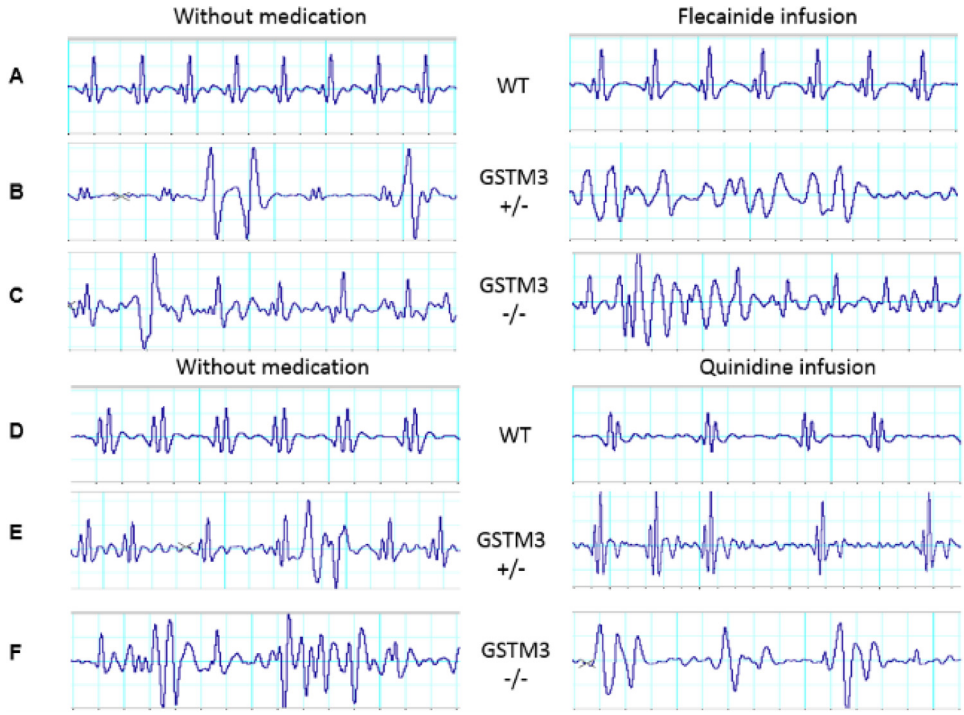

Fig. 6. An example of ventricular arrhythmia in WT, *GSTM3*+/-, and *GSTM3*-/- adult zebrafish recorded by a surface ECG system. Arrhythmic activities shown here were selected from experiments with 10 μM flecainide **(A-C)** and 0.1 μM quinidine **(D-F).** These representative ECG traces illustrate the ventricular arrhythmia of WT and *GSTM3* knockout fish before and after administrating flecainide and quinidine. .

[47]. Our findings suggest that a decrease in *GSTM3* copy number can amplify the effect of ROS to reduce I_{Na}, thus predisposing patients to the development of the BrS (Fig. 7). In the other hand, we found that Tpeak-Tend interval in *GSTM3* deletion group was statistically longer and had more episodes of SCA or syncope than that in no deletion group. Prolonged Tpeak-Tend interval, representing the dispersion of repolarization, has been reported as a risk factor for BrS [48-50]. Our findings were consistent with previous studies.

There are some limitations in this study. First, because this is not a prospective cohort study, we could not use the deletion of *GSTM3* to predict future ventricular events for asymptomatic BrS patients. Second, although our cellular and animal studies showed that deletion of *GSTM3* could alter electrophysiological stability and increase the frequency of VA, we cannot claim that this CNV is causal for BrS, because it exists in approximately 0.1–0.8% of healthy controls. Third, we do not have other ethnic BrS DNA samples to test our findings, and furthe studies in different ethnicities are warranted to validate this *GSTM3* deletion. Lastly, we utilized two different experimental approaches to evaluate the segment of the *GSTM3* deletion in this study. Because both microarray and ex-

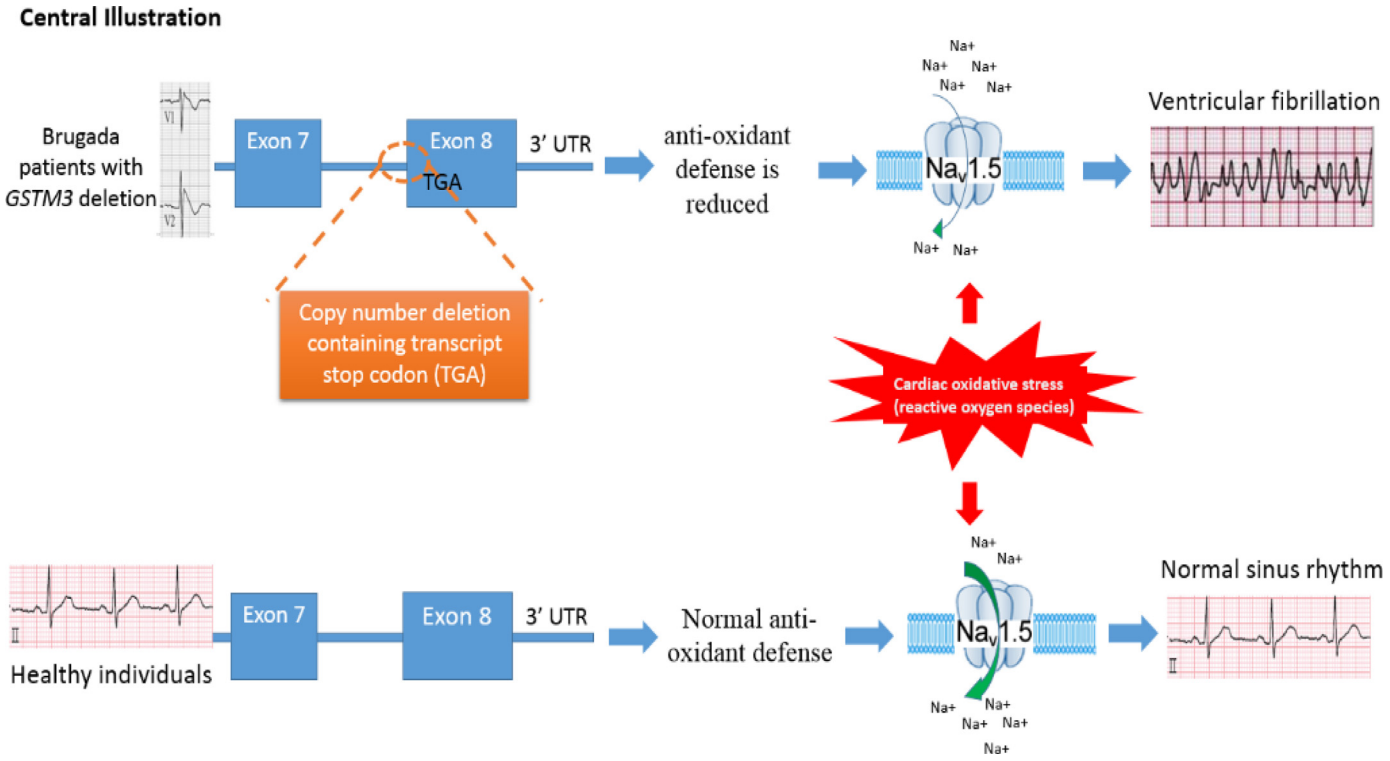

Fig. 7. ***GSTM3*** **deletion reduces antioxidant defense, thus increasing vulnerability of Brugada syndrome patients to development of life-threatening ventricular arrhythmias under conditions of oxidative stress**. Failed transcription termination is thought to lead to nonsense-mediated degradation of *GSTM3* mRNA, resulting in reduced levels of glutathione S-transferase and an inability to protect against the effects of reactive oxygen species, namely the reduction of cardiac sodium channel current density and precipitation of VT/VF.

ome sequencing belong to high-throughput genomic technologies, their advantage is to perform a genome-wide screening of possible segments with CNV. Therefore, these two high-throughput methods may have relatively higher false negative rates due to the absence of specific probes or sequencing reads in the corresponding regions. Alternatively, Sanger sequencing uses primers designed specifically for the region of interest. Thus, Sanger sequencing may have relatively lower false negative rates.

In terms of clinical implications, our study identified a deletion of *GSTM3* in BrS patients, which is associated with reduced I_{Na}, suggesting that the deletion could be a genetic modifier of the BrS phenotype. In addition, it could be used as a risk predictor in patients with BrS.

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Declarations of Competing Interest

The authors declared that they have no conflict of interests.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.102843.

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Erratum

Erratum regarding previously published research papers

The following Author Contribution statements were not included in the published versions of the Research Papers that appeared in previous volumes of *EBioMedicine*. The appropriate Author Contribution statements are included below.

Celastrol-induced degradation of FANCD2 sensitises pediatric high-grade gliomas to the DNA-crosslinking agent carboplatin. (*EBioMedicine* **50:** 81–92)

Author contributions: D.S.M. and E.H. conceived and designed the project. D.S.M., M.H.M., P.W. developed and validated the *in vitro* and *in vivo* models used in the study. D.S.M., B.B., M.H.M., and P.W. performed the functional *in vitro* experiments. D.S.M., P.W., and H.M. performed the functional *in vivo* experiments. J.K. provided bioinformatical expertise and support. B.B. and M.H.M., provided material and logistical support and advised on the project. G.J.K. and E.H. acquired funding and supervised the study. All authors contributed to writing the manuscript.

Epigenetically upregulated GEFT-derived invasion and metastasis of rhabdomyosarcoma via epithelial mesenchymal transition promoted by the Rac1/Cdc42-PAK signaling pathway. (*EBioMedicine* **50:** 122–134)

Author contributions: CL and FL designed the whole study and wrote the manuscript. LZ, WC, YP, JD, ZL, QL, HS, LM, WL, YW, YL, PW, YX, YW, LS, JH, and WZ contributed to experimental design and data collection. All authors have agreed with the manuscript and provide their consent for publication.

Combined identification of three miRNAs in serum as effective diagnostic biomarkers for HNSCC. (*EBioMedicine* **50:** 135–143)

Author contributions: CL and QZ conceived the study. ZYY, SYH, and DSZ participated in the study design. QZ and YYJ conducted the study, including acquisition, analysis, and interpretation of data. CL, ZZY, and ZWS drafted the manuscript. All authors critically reviewed, edited, and approved the manuscript and made the decision to submit for publication. All authors assume responsibility for the accuracy and completeness of the data and for the fidelity of the study to the protocol.

Phosphorylated Rasal2 facilitates breast cancer progression. (*EBioMedicine* **50:** 144–55)

Author Contributions: X.W., Y.K. and Z.M.Q. conceived, organized and supervised the study; X.W., M.Y.L. and Y.L.Y.performed the experiments and data collection; Y.L.Y., X.W., C.Q.and K.Y. contributed to the analysis of data and double checking. X.W., C.Q., Y.K., and Z.M.Q. prepared, wrote and revised the manuscript.

Sprouty4 correlates with favorable prognosis in perihilar cholangiocarcinoma by blocking the FGFR-ERK signaling pathway and arresting the cell cycle. (*EBioMedicine* **50:** 166–177)

Author contributions: Q.B, C. TL, S. RQ, L. ZL, Z. XM,and L. ZP carried out experiments. Z.ZL collected the samples. X. YF analysed data. X. YF conceived experiments and wrote the paper. All authors had final approval of the submitted and published versions.

Analysis of gene expression signatures identifies prognostic and functionally distinct ovarian clear cell carcinoma subtypes. (*EBioMedicine* **50:** 203–210)

Author contributions: RYH, TZT, and DSPT, designed and conceptualised the study. DL processed and reviewed OCCC samples. JY performed sample collection and experiments. NYLN curated and reviewed the clinical data of NUH cohort. TZT performed bioinformatics analyses. RYH, TZT, CVY, NYLN and DSPT analysed the data, interpret the results, and wrote the manuscript.

Pro-inflammatory monocyte profile in patients with Major Depressive Disorder and suicide behavior and how ketamine induces anti-inflammatory M2 macrophages by NMDAR and mTOR. (*EBioMedicine* **50:** 290–305)

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Author contributions: W.N. designed and performed *in vitro* experiments, analysed and discussed results, and critically revised the manuscript. L.N.G. and D.E.R. recruited and followed up patients with MDD and performed sample collection. I.G.E. and N.E. performed *in vitro* experiments. A.R.A. processed samples of patients with MDD. M.P.A. and L.M.S. designed and performed *in vivo* murine experiments, analysed and discussed results, and critically revised the manuscript. F.M.D. conceived and designed the study, recruited and followed up patients with MDD, discussed results, and wrote the manuscript. E.A.C.S. and A.E.E. conceived and designed the study, designed and performed experiments, analysed and discussed results, and wrote the manuscript.

Radiomics analysis of placenta on T2WI facilitates prediction of postpartum hemorrhage: A multicentre study. (*EBioMedicine* **50:** 355−365)

Author Contributions: Conception and design: Xiaoan Zhang, Jie Tian, Meiyun Wang. Collection and assembly of data: Qingxia Wu, Kuan Yao, Zhenyu Liu, Longfei Li, Xin Zhao, Shuo Wang, Honglei Shang, Yusong Lin, Zejun Wen. Development of methodology: Kuan Yao, Zhenyu Liu, Longfei Li, Shuo Wang, Yusong Lin, Jie Tian. Data analysis and interpretation: All authors. Manuscript writing: All authors. Final approval of manuscript: All authors.

TP63 Isoform Expression is Linked with Distinct Clinical Outcomes in Cancer. (*EBioMedicine* **51:** 102,561)

Author contributions: A.B. designed experiments, analyzed data and wrote the manuscript. T.M. performed PCR and RT-PCR experiments. Y.W. performed western blot validation experiments. P.B. contributed to statistical design and analysis of data. P.P. supervised experimental design, analyzed data and prepared the manuscript. All authors read and approved of final manuscript.

Serum IGFBP-1 as a potential biomarker for diagnosis of early-stage upper gastrointestinal tumor. (*EBioMedicine* **51:** 102,566)

Author contributions: Y-WX designed the study, searched the literature, performed the experiments, analysed and interpreted the data, did the statistical analysis, and wrote the manuscript. HC designed the study, collected patient samples, performed the experiments, analysed, and interpreted the data. C-QH designed the study, collected patient samples, searched the literature, did the statistical analysis, analysed, and interpreted the data. L-YC collected patient samples, performed the experiments, analysed and interpreted the data. S-HY analysed and interpreted the data. L-SH, and HG collected patient samples and clinical data. L-YC, C-TL, X-YH L-HL and S-LC collected patient samples and clinical data. Z-YW, Y-HP, L-YX, and E-ML conceptualized and designed the study, supervised the project, and revised the paper. All authors vouch for the respective data and analysis, and agreed to publish the manuscript.

Diagnostic accuracy and easy applicability of intestinal auto-antibodies in the wide clinical spectrum of coeliac disease. (*EBioMedicine* **51:** 102,567)

Author contributions: Study concept and design: Luigina De Leo, Tarcisio Not. Acquisition of data: Luigina De Leo, Stefano Martelossi, Grazie Di Leo, Matteo Bramuzzo. Analysis and interpretation of data: Luigina De Leo, Tarcisio Not, Stefano Martelossi, Grazia Di Leo, Matteo Bramuzzo, Vincenzo Villanacci, Chiara Zanchi. Drafting of the manuscript: Tarcisio Not, Luigina De Leo. Critical revision of the manuscript: Alessandro Ventura, Vincenzo Villanacci, Matteo Bramuzzo, Chiara Zanchi. Clinical decisions: Stefano Martelossi, Grazie Di Leo, Matteo Bramuzzo. Histological evaluation of biopsy samples: Vincenzo Villanacci. Intestinal antibodies immunoassays: Luigina De Leo, Michela Pandullo, Petra Riznik

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MEF2C Repressor Variant Deregulation Leads To Cell Cycle Re-Entry and Development of Heart Failure. (*EBioMedicine* **51:** 102,571)

Author contributions: AHMP, ACC designed and performed experiments, analyzed data, and wrote the manuscript. SRC, RRO, AS an MLBV designed and performed experiments. JRMS performed the echocardiography in animals. MFC analyzed data. AG, JLF, GCAR and MML provided human samples. JDM discussed the manuscript. KGF designed experiments, analyzed data, and wrote the manuscript. All authors reviewed and commented on the manuscript.

Developments in Zebrafish Avatars as radiotherapy sensitivity reporters – towards personalized medicine. (*EBioMedicine* **51:** 102,578)

Author contributions: R.F. and M.G.F. conceptualized the research; R.F. and B.C. supervised the research; S.F., B.C., V.P and R.F. performed research, acquisition, analysis and interpretation of data; P.F., R.R-T., N.F. provided primary tumor samples; M.J.C, S.V., J.S., performed calculations and set-up the accelerator, O.P., J.S. for fruitful discussions; R.F. and B.C. wrote the manuscript. S.F., C.G., O.P. and M. G.F. did critical reading and editing of the manuscript.

Multi-cancer V-ATPase molecular signatures: A distinctive balance of subunit *C* isoforms in esophageal carcinoma. (*EBioMedicine* **51:** 102,581)

Author contributions: JCVCS performed most of the experiments and analysis. PNN participated in the analysis and acquisition of data. EPC performed the in silico structural models. ARF and LFRP coordinated the project. JCVCS and ARF wrote the manuscript. JCVCS, PNN, TAS, ARF and LFRP performed study design. TAS and PNN participated in the collection of samples. ALOF and FFF provided specialized scientific and technical support. All authors discussed the results and manuscript text. All authors read and approved the final manuscript.

Heterogeneous nuclear ribonucleoprotein A2/B1 is a negative regulator of human breast cancer metastasis by maintaining the balance of multiple genes and pathways. (*EBioMedicine* **51:** 102,583)

Author Contributions: The authors' work in this study is listed as follows: *In vitro* and *in vivo* assays (YL, HL, FL, LBG, RH, CC and XD); RNA immunoprecipitation (YL); dual-luciferase reporter assay (YL and SL), signal pathways analysis (HL), proteomic analysis (YL), EMT markers test (HL, LBG and RH); real-time PCR (YL, SL, KL, LY, HMT, BBC and XL); and tissue microarray analysis (YL, DHX and XLD). SLS designed and supervised the study. YL and SLS analysed data and wrote manuscripts.

Genetic Risk for Dengue Hemorrhagic Fever and Dengue Fever in Multiple Ancestries. (*EBioMedicine* **51:** 102,584)

Author contributions: GP, ML, KH, IL contributed to the design; ML, SE, LG, GK, AB, IL, LP, CP, IF, RS, ED, FB, YR, PB, JN, LW, DS, SP, GP, AW, CR, LP acquisition of data; GP, ML, AB, LG, GK Interpretation of data; GP, ML, PS, IL drafted the manuscript; IF, LW, DS, SP, GP, AW, AB, ED, LG, GK, ML, RS, KH revised it for critical intellectual content; ML, SE, LG, GK, AB, IL, LP, CP, IF, RS, ED, FB, YR, PB, JN, LW, DS, SP, GP, AW, PS, GK, KH approved the final manuscript; PG, ML, PS, SE, IF, LW, DS, SP, GP, AW, JN, AB, ED, LG, GK, RS, KH agree to be accountable for all aspects of the work.

Cortical haemodynamic response measured by functional near infrared spectroscopy during a verbal fluency task in patients with major depression and borderline personality disorder. (*EBioMedicine* **51:** 102,586)

Author contributions: Syeda F. Husain: Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing-review & editing. Tong-Boon Tang: Supervision, Writing - review & editing. Rongjun Yu: Supervision, Writing - review & editing. Wilson W. Tam: Supervision, Methodology, Writing - review & editing. Bach Tran: Supervision, Writing - review & editing. Travis T. Quek: Participant recruitment, Writing – review & editing. Shi-Hui Hwang: Participant recruitment, Writing – review & editing. Cheryl W. Chang: Participant recruitment, Writing – review & editing. Cyrus S. Ho: Supervision, Writing - review & editing. Roger C. Ho: Conceptualisation, Participant Recruitment, Methodology, Writing – review & editing.

Impact of sitagliptin on endometrial mesenchymal stem-like progenitor cells: A randomised, double-blind placebo-controlled feasibility trial. (*EBioMedicine* **51:** 102,597)

Author contributions: Study concept, design, and overall supervision: J.J.B, S.Q. Prepared manuscript: J.J.B., S.Te., E.S.L., S.Q. Edited manuscript: L.L., L.J.E., M.J.M.D.C., K.J.F., J.M., P.J.B., A.P., P.K.K., R.F. Obtained funding: S.Q., J.J.B, S.Ta. Regulatory approvals: S.Q., S.Te. Patient enrolment, consenting, ultrasound and clinical assessments: S.Q., S.Te., A.P., L.J.E., L.L. CFU assays and analysis: E.S.L., P.J.B. Exploratory investigations: E.S.L., R.F., P.J.B, J.M., K.J.F., M.J.M.D.C., J.J.B. Data analysis: P.K.K., E.S.L., S.Te., J.J.B., S.Q.

The CD24+ Cell Subset Promotes Invasion and Metastasis in Human Osteosarcoma. (*EBioMedicine* **51:** 102,598)

Author contributions: Zhenhua Zhou wrote the manuscript. Zhenhua Zhou, Yan Li and Muyu Kuang performed cell culture, real-time PCR, flow cytometry and animal experiments. Xudong Wang carried out cell migration, invasion, proliferation assays, Western blot and protein mass spectrometry. Jingjing Hu and Jiashi Cao carried out the histological analysis and scores evaluation. Qi Jia and Sujia Wu carried out prognosis statistical analysis of clinical cases. Zhiwei Wang and Jianru Xiao conceived of the study and participated in its designation and helped to draft the manuscript. All authors read and approved the final manuscript.

The Transferability and Evolution of NDM-1 and KPC-2 co-producing Klebsiella pneumoniae from Clinical Settings. (*EBioMedicine* **51:** 102,599)

Author contributions: HW conceived the project and designed the experiments. QW collected samples and performed microbial identification. YL collected the medical records. RW, YL and LJ performed the microbiological experiments. HG performed the computational analyses. YL, HG, RW and HW wrote the manuscript. All authors read and commented on successive drafts and all approved the content of the final version. tumor immune cell infiltration and survival after platinum-based chemotherapy in high-grade serous ovarian cancer subtypes: A gene expression-based computational study. (*EBioMedicine* **51:** 102,602)

Author contributions: RL, WZ and HHZ contributed to the study design. YZ and RH contributed to data collection. RL performed statistical analysis, interpretation and drafted the manuscript. All authors contributed to critical revision of the final manuscript. RL approved the final version of the manuscript.

Mucosal microbial load in Crohn's disease: a potential predictor of response to fecal microbiota transplantation. (*EBioMedicine* **51:** 102,611)

Author contributions: C.M. and G.S. conceived and supervised the study. G.S., E.V., D.C., A.S, J.W. performed the experiments and data analysis. M.P. and C.M. performed the 16S rRNA data analysis and interpretation. S.L., M.M. and E.E. provided the explant tissues and reviewed the manuscript. C.E. provided the patients' clinical data. K.M. and S.V. provided the mucosal biopsies from CD patients and reviewed the manuscript. G.S. and C.M. wrote and reviewed the manuscript. A.C. revised the manuscript. All authors read and approved the final version of the manuscript.

Mesenchymal stem cells ameliorate β cell dysfunction of human type 2 diabetic islets by reversing β cell dedifferentiation. (*EBioMedicine* **51:** 102,615)

Author contributions: Conceptualization, Z.S., S.W.; Funding acquisition, Z.S., S.W.; Study design, L.W., T.L., R.L.; Investigation, L. W., T.L., R.L.; Data analysis, L.W., T.L., R.L.; Methodology, L.W., T.L., G. W., R.L., N.L., B.Z., Y.J.L., X.D., X.C., Y.L.; Data interpretation, S.W., Z.S., Z.W., X.X.; Supervision, S.W., Z.S., C.R.; Writing – original draft, R.L., L. W; Writing – review & editing, Z.S., S.W., X.X., C.R..

A practical model for the identification of congenital cataracts using machine learning. (*EBioMedicine* **51:** 102,621)

Author Contributions: HL, DL, WC, and YL contributed to the concept of the study and critically reviewed the manuscript. HL, DL, JC, ZL, YX, and XL designed the study and performed the literature search. HL, DL, JC, ZL, XL, XW, ZL, and WC collected the data. KZ, JH, LZ, and CG contributed to the design of the statistical analysis plan. DL, KZ, and JLH performed the data analysis and data interpretation. DL and HL drafted the manuscript. HL, DL, CC, YX, LW, and YZ critically revised the manuscript. HL, DL, WC, and YL provided research funding, coordinated the research and oversaw the project. All authors reviewed the manuscript for important intellectual content and approved the final manuscript.

MiR-765 functions as a tumor suppressor and eliminates lipids in clear cell renal cell carcinoma by downregulating PLP2. (*EBioMedicine* **51:** 102,622)

Author contributions: WX, CW and XPZ designed and performed the experiments. WX, JCX and CW wrote the manuscript. WX, KC and TW analyzed and performed the experiments. XGW and XPZ directed the experiments and analyzed and assembled the data. All authors read and approved the submitted manuscript.

Breast cancer induces systemic immune changes on cytokine signaling in peripheral blood monocytes and lymphocytes. (*EBioMedicine* **51:** 102,631)

Author contributions: LW and PPL designed experiments; LW, DLS, TYT and CA conducted experiments; LW and XL analyzed experimental data; AYC, FMD, JY, JW identified and recruited patients into this study; LW and PPL wrote manuscript. All authors read and approved the manuscript.

Near Infrared Photoimmunotherapy Targeting DLL3 For Small Cell Lung Cancer. (*EBioMedicine* **51:** 102,632)

Author contributions: The all authors checked and approved the final version of the manuscript. Y.I. and K.S. mainly conducted all experiments, performed analysis and wrote the manuscript; K.T., S.T., H.Y., Y.N., R.E., M.S., C.K., N.K., H.Y., Y.B., and Y.H. conducted analysis; S.N., T.F., K.K. and T.F.C.Y. conducted surgical operation to gather the specimens; K.S. supervised and conducted the project.

Gut microbiota composition during infancy and subsequent behavioural outcomes. (*EBioMedicine* **51:** 102,640)

Author contributions: AL and PV proposed the analysis. AL, MOH, ALP, and FC contributed to the statistical analysis. AL, PV, ALP, MOH, CS, FC, and MT contributed to data interpretation. FC contributed to biobanking. AL, PV, and MOH drafted the manuscript. All authors provided feedback and edits to the manuscript. Relevant grant funding applications were prepared by and awarded to: PV, ALP, JC, CS, FC, MT, SR, KA, RS, LH, PS, and the BIS Investigator Group.

Intracavernous injection of size-specific stem cell spheroids for neurogenic erectile dysfunction: efficacy and risk versus single cells. (*EBioMedicine* **52:** 102,656)

Author contributions: ZQL and YT designed the whole experiments and guided the entire experiments, and are responsible for the integrity of the data and the accuracy of the data analysis; YDX and ZQL contributed to performance the animal experiments, data analysis and manuscript drafting. HZ, CH, XMZ, YCZ, and RLG contributed to the performance of the experiments. ZCX and ZQL analyzed and interpreted the data, and critically revised the manuscript for important intellectual content. All authors approved the final version of the manuscript.

Identification and external validation of IgA nephropathy patients benefiting from immunosuppression therapy. (*EBioMedicine* **52:** 102,657)

Authors contributions: Research idea and study design: Z-HL, G-TX, C-HZ, T-YC, E-YX, T-GC; data acquisition: Z-HL, C-HZ, T-YC, E-YX, T-GC, XL; data analysis/interpretation: Z-HL, C-HZ, T-YC, E-YX, T-GC, XL, YZ; statistical analysis: T-YC, E-YX,T-GC,YZ; supervision or mentorship: Z-HL, C-HZ, YQ, S-SL, FX, D-DL. All authors read and approved the final version of the manuscript.

Classification of Primary Liver Cancer with Immunosuppression Mechanisms and Correlation with Genomic Alterations. .(*EBioMedicine* **52:** 102,659)

Authors contributions: H.N. conceived the study. M.F., R.Y., T.H, S.H., and K.K. performed the analysis. K.M., K.N., A.F., M.U., S.H., H.A., H.Y., K.C., and S.I. contributed materials and data. K.A. performed immunohistochemical analysis. S.S. and S.T. performed cell line experiments and expression analysis. H.T. and S.M. contributed to the supercomputer environment. M.F. and H.N. wrote the manuscript.

Silencing of circular RNA HIPK2 in neural stem cells enhances functional recovery following ischaemic stroke. (*EBioMedicine* **52:** 102,660)

Author contributions: H.Y. conceived and supervised this project. H.Y and G.W. designed the experiments. G.W., B.H., L.S., S.W., L.Y., J.L., F.W., M.L., S.L., F.Z., Y.Z., Y.B., Y.M. and B.C. conducted experiments and acquired, analysed and interpreted the data. H.Y. and G.W. wrote the manuscript. All authors read and approved the final version of the manuscript.

Genome-wide identification of FHL1 as a powerful prognostic candidate and potential therapeutic target in acute myeloid leukaemia. (*EBioMedicine* **52:** 102,664)

Author contributions: CC and YF designed the study. YF, MX, ZC performed the experiments. YF, ZY, ZZ, XY and XH analyzed the data. CC, MZ and XW obtained the funding. YF, MX, MZ and XW prepared the figures. YF, MX, ZC and CC wrote the manuscript. CC supervised the study. All authors read and approved the final manuscript.

Longitudinal Serum Autoantibody Repertoire Profiling Identifies Surgery-Associated Biomarkers in Lung Adenocarcinoma. (*EBioMedicine* **52:** 102,674)

Author contributions: S-C. T. and H-C. L. developed the conceptual ideas and designed the study. Y. L, S-J. G., H-W. J. performed the experiments, C-Q. L. and W. G. collected the sera samples. S-C.T., H-C. L., Y. L. and C-Q. L. wrote the manuscript with suggestions from the other authors.

A comprehensive analysis of candidate genes in familial pancreatic cancer families reveals a high frequency of potentially pathogenic germline variants. (*EBioMedicine* **53:** 102,675)

Author contributions: Study design: JE, NM and AC. Data collection: JE, MEC, VP, RF, MRG, TRA, LRD, ICG, MR, EMC and MM. Experimental work: JE, CG, JE2, EB, SGM, DG, GM. Data Analysis: JE, JE2, EB, DG, GM and JR. Interpretation of the data: JE, VP, RF, TRA, LRD, ICG, MR, EMC, NM and AC. Preparation of the manuscript: all authors

CircRNA-CIDN mitigated compression loading-induced damage in human nucleus pulposus cells via miR-34a-5p/SIRT1 axis. (*EBioMedicine* **53:** 102,679)

Author Contributions: Q.X. and L.K. designed the study protocol and wrote the manuscript; Q.X., L.K. and J.W. conducted the experiments; Z.L. and Y.S. established the ex vivo IVD cultured model; K.Z. and K.W. collected and analysed data; C.Y. collected the NP tissues and supervised the study; Y.Z. supported and supervised the study.

FGFR1 and FGFR4 oncogenicity depends on N-Cadherin and their co-expression may predict FGFR-targeted therapy efficacy. (*EBioMedicine* **53:** 102,683)

Author contributions: Conceptualization: A.Q., I.F., S.M.P., A.C., and L.P.A.; Methodology: A.Q., A.C., S.V.C, I.F., and S.M.P.; Investigation: A.Q., A.C., I.F., S.V.C., L.P.A. and S.M.P.; Validation: A.Q.,A.M., L.O., E.G., S.V.C, S.M.G, L.M, S.G. and F.L.R.; Formal Analysis: A.Q., I.F., J.Z., S. M.P; Writing – Original Draft: A.Q., I.F., A.C., S.M.P. and L.P.A., Writing – Review & Editing: A.Q., I.F., S.V.C, A.C., S.M.P. and L.P.A., Supervision: A.C., I.F., S.M.P., and L.P.A.; Funding Acquisition: S.M.P., I.F. and L.P.A. All authors read and approved the final version of the manuscript.

BAP18 is involved in upregulation of CCND1/2 transcription to promote cell growth in oral squamous cell carcinoma. (*EBioMedicine* **53:** 102,685)

Author contributions: Xue Wang, Chunyu Wang, and Guangqi Yan designed the study and wrote the manuscript, Xue Wang, Ge Sun, Yuanyuan Kang, Shengli Wang, Renlong Zou, Hongmiao Sun and Kai Zeng performed experiments and analyzed the data, Huijuan Song, Wei Liu, Ning Sun, and Wensu Liu conducted bioinformatic analyses and statistical analyses, Yue Zhao wrote and revised manuscript. All authors read the approved the final manuscript.

Systematic identification of CDC34 that functions to stabilize EGFR and promote lung carcinogenesis. (*EBioMedicine* **53:** 102,689)

Author Contributions: The project was conceived and designed by G.B.Z. The experiments were conducted by X.C.Z, G.Z.W., Q.H., L. W.Q., S.H.G., J.L., L.M., Y.F.Z., C.Z., H.Y., D.L.Z., and M.W.. Biospecimens were harvested/provided by Z.S.W., Y.C.Z., Y.C.H., B.Z., C.L.W., and Z.L.. The EGFR transgenic mice were provided by L.C.. Data were analyzed by G.B.Z., Y.Z., Z.L., L.C., and X.C.Z.. The manuscript was written by G. B.Z.. The study sponsor had no role in the design of the study; the data collection, analysis, or interpretation; the writing of the article; or the decision to submit for publication.

CBX4 transcriptionally suppresses KLF6 via interaction with HDAC1 to exert oncogenic activities in clear cell renal cell carcinoma. (*EBioMedicine* **53:** 102,692)

Author contributions: Conception and design of the study: Jiang N, Zhang CZ, Shen HM; Generation, collection, assembly, analysis of data: Jiang N, Niu G, Pan YH, Pan WW, Zhang MF; Drafting and revision of the manuscript: Jiang N, Zhang CZ, Shen HM; Approval of the final version of the manuscript: all authors.

Enhanced O-linked GlcNAcylation in Crohn's disease promotes intestinal inflammation. (*EBioMedicine* **53:** 102,693)

Author contributions: Q.H.S. wrote the manuscript. Z.X.X. contributed to the conception and writing. W.Y.S., Y.L.L., and Z.X.X. designed research; Q.H.S., Y.P.J., M.D.L., D.Z., R.X.Z., J.C., and Y.L., performed research; C.S.Q., Y.S.W., G.L., H.L.Z., Q.D., J.L., Y.L.L., and Z.X.X. analyzed the data. Q.H.S., G.L., H.L.Z., Q.D., and Z.X.X. revised the manuscript. All authors read and approved the final manuscript.

Elevated myocardial SORBS2 and the underlying implications in left ventricular noncompaction cardiomyopathy. (*EBioMedicine* **53:** 102,695)

Author contributions: Yingjie Wei. supervised the work; Yingjie Wei, Chunyan Li. designed the experiments with help from Fan Liu, Shenghua Liu, Haizhou Pan, Haiwei Du, Jian Huang, Yuanyuan Xie, Yanfen Li and Ranxu Zhao. Yingjie Wei, Chunyan Li and Fan Liu analyzed the data; Chunyan Li and Yingjie Wei cowrote the manuscript. All authors discussed the results and commented on the manuscript.

Artificial intelligence-assisted prediction of preeclampsia: development and external validation of a nationwide health insurance dataset of the BPJS Kesehatan in Indonesia. (*EBioMedicine* **54:** 102,710)

Author contributions: HS and ECYS developed the concept and design of this study. Dataset access was requested by HS. This author and YWW, and ECYS had full access to all data in the study. HS extracted and processed the data, performed training and validation of machine learning algorithms, conducted the literature search and wrote the draft of the manuscript. HS, YWW, and ECYS independently assessed the eligibility criteria of reviewed studies. YWW and ECYS critically revised the drafted manuscript. HS and ECYS take responsibility for data integrity and the accuracy of the analysis. All authors reviewed the final manuscript.

Plantar temperatures in stance position: A comparative study with healthy volunteers and diabetes patients diagnosed with sensoric neuropathy. (*EBioMedicine* **54:** 102,712)

Author Contributions: UN, MS, JM, AM and PRM contributed equally to this study. PRM and SK conceived and designed the study. ED, JK, SK, JM, AM, and IW recruited participants and performed the experiments. UN, MS, JM, AM and PRM analyzed the data. UN, MS, JM, and PRM drafted the manuscript. TS and PRM were responsible for the design and performance of the sensor-equipped insoles and for data retrieval.

TRAF4 acts as a fate checkpoint to regulate the adipogenic differentiation of MSCs by activating PKM2. (*EBioMedicine* **54:** 102,722)

Author contributions: SC, JL, ZC and YP designed the study and performed the experiments. ZS, ZL and GY performed the statistical analyses. GZ, ML, WL, WY and SW contributed study material and reagents. SC, ZX, PW and HS wrote the manuscript. ZX, PW and HS are the corresponding authors. All authors read and approved the final manuscript.

Identification, clinical manifestation and structural mechanisms of mutations in AMPK associated cardiac glycogen storage disease. (*EBioMedicine* **54:** 102,723)

Author Contributions: Dan.H, and Dong.H. designed the study. Dong.H., H.B.M, L.W.L., N.B.S., Y.L., B.W., F.Z., B.L.S., A.A., L.M., Y.X., S. W., C.A., M.H.G., P.M.E., Dan.H performed clinical and pathological phenotyping of study subjects. Dan.H, H.B.M, and Dong.H. coordinated the clinical evaluations. Dan.H, H.B.M, M.H.G., P.M.E., and Dong. H. supervised and coordinated the genetic laboratory work. Y.L., Y.X., S.W., Dan.H, and D.B., performed history analysis. H.M., K.M., K.L., Dan.H, and D.B., performed computational modeling calculations and transfer entropy analysis. Dan.H, H.B.M, and Dong.H. organized and summarized the database. Dan.H, H.B.M, L.W.L., D,B. and Dong.H. analyzed the data. Dan.H, D,B. C.A., M.H.G., P.M.E., and Dong.H. developed the conceptual approaches to data analysis. Dan.H, Dong.H. D,B. and H.B.M, wrote the manuscript. All co-authors contributed to critical editing of manuscript.

Precise pulmonary scanning and reducing medical radiation exposure by developing a clinically applicable intelligent CT system: Toward improving patient care. (*EBioMedicine* **54:** 102,724)

Author contributions: Conceptualization: Yang Wang and Bing Zhang; Experimental and data studies: Yang Wang, Xiaofan Lu, Yingwei Zhang, Xin Zhang, Kun Wang, Jiani Liu, and Xin Li; Technical Support: Renfang Hu, Xiaolin Meng, Shidan Dou, Huayin Hao, Xiaofen Zhao, Wei Hu, Cheng Li, and Yaozong Gao; Statistical analysis: Xiaofan Lu and Fangrong Yan; Construction of artificial intelligence network: Renfang Hu, Xiaolin Meng, Shidan Dou, Huayin Hao, Xiaofen Zhao, Wei Hu, Cheng Li, and Yaozong Gao; Manuscript editing: Yang Wang, Xiaofan Lu, Zhishun Wang, Guangming Lu, Fangrong Yan, and Bing Zhang; Funding acquistion: Fangrong Yan and Bing Zhang; Resources: Fangrong Yan and Bing Zhang; Supervision: Fangrong Yan and Bing Zhang. All authors read and approved the final version of the manuscript.

Clinical and genomic insights into circulating tumor DNA-based alterations across the spectrum of metastatic hormone-sensitive and castrate-resistant prostate cancer. (*EBioMedicine* **54:** 102,728)

Author Contributions: Conception of idea, MK; Acquisition of data, MK, WT, LH, KM, HF, EK, AA, SY; Data generation, AW, CM, CW; Analysis and interpretation of data, TZ, JY, MK, AW, CM, CW, PD, HF, EK, AA; Drafting of the manuscript, MK, AA, TZ, JY, WT; Critical revision of the manuscript for important intellectual data, WT, LH, SJ, KM, JY, TZ, SJ, HF, SY, EK, AA; Obtaining funding, MK, LH, AA, EK, KM.

Lifetime risk of autosomal recessive mitochondrial disorders calculated from genetic databases. (*EBioMedicine* **54:** 102,730)

Author contributions: MW and TK conceived the study. JT and MW defined a comprehensive list of mitochondrial disease genes and set up a list of pathogenic variants in these genes, supported by SLS, TMS, and SBW. JT and MW queried two databases (gnomAD and in house) to assess the allele frequencies of disease-causing variants in the general population and calculated the lifetime risks, supported by HP, TM, KO and TK. JT and MW drafted the manuscript which was then refined by all other authors and finalized by MW and TK.

Transcriptional and clonal characterization of B cell plasmablast diversity following primary and secondary natural DENV infection. (*EBioMedicine* **54:** 102,733)

Author contributions: A.T.W conceived of the project, designed and executed experiments, analyzed data, and wrote the paper. G.G. and W.R. designed and executed experiments, analyzed data, and provided subject matter expertise. M.K.M. and B.G. analyzed data. T.L., H.S., K.V., C.K., A.G., M.E.F., and J.L. generated data. A.M, A.S., E.D., S.F. provided subject matter expertise and supervised data generation. B.J.D. secured funding. T.E., S.T., and A.L.R. secured funding and provided subject matter expertise. R.G.J. provided project oversight, secured funding, and provided subject matter expertise. D.E. provided project oversight and subject matter expertise. J.R.C and H.F. conceived of the project, designed and executed experiments and analyzed data.

Zika Virus Envelope Nanoparticle Antibodies Protect Mice without Risk of Disease Enhancement. (*EBioMedicine* **54:** 102,738)

Author contributions: Literature search: SS; Figures: RS, RKS, SS, NK; Study design: SS, NK, JKL, FK; Data collection: RS, RKS, VR, UA, GB, JAA; Data analysis and interpretation: SS, NK, JKL, FK; Writing: SS and NK; Approval of final manuscript: all authors.

Bio responsive self-assembly of Au-miRNAs for targeted cancer theranostics. (*EBioMedicine* **54:** 102,740)

Author contributions: The authors' responsibilities were as follows: WC, LY, YW and XW devised the experiments and wrote the manuscript. WC conducted the synthesis of materials, purification, and materials/biological characterizations etc. HF contributed to the mouse model experiment. All other authors contributed to materials synthesis, purification/characterization, and/or discussion of the results.

Large-scale network dysfunction in the acute state compared to the remitted state of bipolar disorder: A meta-analysis of resting-state functional connectivity. (*EBioMedicine* **54:** 102,742)

Author Contributions: Yanlin Wang and Xiaoqi Huang designed the study, Yanlin Wang and Shi Tang collected data and performed analyses; Lu Lu, Lianqing Zhang, Xinyu Hu, Xuan Bu, Hailong Li, Xiaoxiao Hu, Xinyu Hu, Ping Jiang, and Zhiyun Jia provided helpful suggestions; Yanlin Wang, Yingxue Gao and Shi Tang drafted the main article; John A. Sweeney, Qiyong Gong and Xiaoqi Huang critically reviewed the manuscript.

Dynamics of within-host Mycobacterium tuberculosis diversity and heteroresistance during treatment. (*EBioMedicine* **55:** 102,747)

Author contributions: Study design: CN, JB, FB; Data collection: CN, KB, JM, AG, NP, MO; Data analysis: CN, FB; Data interpretation: CN, JM, MO, FB; Writing: CN, FB; Review and approval of manuscript: CN, KB, JM, AG, NP, MO, JB, FB; All authors have read and approved the final version of this manuscript.

Host transcriptomic signature as alternative test-of-cure in visceral leishmaniasis patients coinfected with HIV. (*EBioMedicine* **55:** 102,748)

Author contributions: All authors read and approved the final version of the manuscript. Wim Adriaensen: Conceptualization, data curation, formal analysis, investigation, visualization, writing & editing Bart Cuypers: Formal analysis, methodology, writing, review & editing Carlota F. Cordero: Formal analysis Bewketu Mengasha: Data collection and curation Séverine Blesson: Data curation, project coordination Lieselotte Cnops: Formal analysis, writing, review & editing Paul M. Kaye: Methodology, supervision, review & editing Fabiana Alves: Data curation, funding acquisition, project administration, review & editing Ermias Diro: Data curation, project coordination, funding acquisition, review & editing Johan van Griensven: Conceptualization, methodology, funding acquisition, project administration, supervision, review & editing

Motor transmission defects with sex differences in a new mouse model of mild spinal muscular atrophy. (*EBioMedicine* **55:** 102,750)

Author Contributions: Marc-Olivier Deguise: Generated the mouse model, designed study, produced and analyzed data for all figures, and wrote the manuscript. Yves De Repentigny: Data acquisition, data analysis and method description. Alexandra Tierney: Data acquisition and data analysis

Ariane Beauvais: Assistance with experiments. Jean Michaud: Assessment of histology of the skeletal muscle. Lucia Chehade: Data acquisition and data analysis. Mohamed Thabet: Assistance with electrophysiology. Brittany Paul: Data acquisition and data analysis. Aoife

Reilly: Assistance with experiments. Sabrina Gagnon: Maintenance of mouse models and genotyping. Jean-Marc Renaud: Electrophysiology and data analysis. Rashmi Kothary: Designed study and wrote manuscript.

Ileo-colonic delivery of conjugated bile acids improves glucose homeostasis via colonic GLP-1-producing enteroendocrine cells in human obesity and diabetes. (*EBioMedicine* **55:** 102,759)

Author Contributions: Conceptualization, AA, MC, FMG, and AV; Methodology, AM, AA, JR, BG, MC, FMG, and AV; Formal Analysis, GC, AM, JR, AA, FMG, and AV; Investigation, GC, AM, AA, JR, JD, IZ, GF, DB, GR, BG, SN, AA. Resources, FR, BG, AV, NFL, FMG, MC, AA. Writing − Original Draft: GC, AM, JR. Writing − Review & Editing, GC, AM, AA, JR, JD, GF, DB, GR, FR, BG, AV, NFL, FMG, MC, AA. Visualization, GC, AM, JR, Supervision FR, BG, AV, NFL, FMG, MC, AA Funding Acquisition, FMG, AA.

Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. (*EBioMedicine* **55:** 102,763)

Authors contributions: Conceptualization: JL, SML, JL, YH, DLY, XZ. Acquisition of data: BYL, XBW, HW, WL, QXT, JHY, LZ, LJX, CXG, JT, JZL, JHY, RP, HS, CP, TL, QZ, JW, LX, SHL, BJW, ZHW, CRH, HBZ, RZ, HLZ, XC, PY, BZ, LW,WQZ SSH,YWH, SHJ, PW, JAZ, YPL, WXW, LZ, LL, FQZ. Analysis and interpretation of data: JL, SML, JW. Writing-original draft Preparation: JL. Writing-review and editing: UD, MJL, JL, DLY, XZ. All authors reviewed and approved the final version of the manuscript.

A dysregulated bile acid-gut microbiota axis contributes to obesity susceptibility. (*EBioMedicine* **55:** 102,766)

Author contributions: Wei Jia was principal investigator of this study. Zhaoxiang Bian provided valuable support for C. scindens gavage animal experiment. Wei Jia, Aaihua Zhao, Xiaojiao Zheng, and Guoxiang Xie designed the study. Meilin Wei conducted key experiments of the study and perform the data analysis and drafted the manuscript. Fengjie Huang, Yunjing Zhang, Wei Yang, and Ling Zhao conducted the animal experiments. Kun Ge, Chun Qu, Mengci Li, Shouli Wang, and Xiaolong Han helped to perform the experiments and collected the data. Wei Jia and Cynthia Rajani revised the manuscript.

Prognostic and predictive value of a five-molecule panel in resected pancreatic ductal adenocarcinoma: A multicentre study. (*EBioMedicine* **55:** 102,767)

Author Contributions: Conception and design: JCG, SL, TPZ. Provision of study material and patients: JCG, SL, TPZ, ZGZ, BS, QL, MHD. Financial and administrative support: JCG, SL. Data analysis and interpretation: PZ, LZ, LY, QFL, ZYL, JL, DY, ADT, JS. Experimental support: PZ, LZ, LY, GGX. Manuscript writing: PZ, LZ, QFL. Final approval of the manuscript: All the authors.

CD24-targeted intraoperative fluorescence image-guided surgery leads to improved cytoreduction of ovarian cancer in a preclinical orthotopic surgical model. (*EBioMedicine* **56:** 102,783)

Author contributions: Literature search: E. McCormack, L. Bjørge, K. Kleinmanns, V. Fosse; Study design: E. McCormack, L. Bjørge, K. Kleinmanns, V. Fosse;

Development of methodology: E. McCormack, L. Bjørge, K. Kleinmanns, V. Fosse; Data collection (*in vitro* data, animal experiments, patient data): K. Kleinmanns, V. Fosse, B. Davidson, O. Tenstad, E. García de Jalón; Data analysis and interpretation of data (statistical analysis): K. Kleinmanns, V. Fosse, B. Davidson, O. Tenstad, E. García de Jalón; Writing, review and/or revision of the manuscript: K. Kleinmanns, V. Fosse, E. McCormack, L. Bjørge; Study supervision: E. McCormack, L. Bjørge. All authors read and approved the final version of the manuscript.

Low oxygen saturation during sleep reduces CD1D and RAB20 expressions that are reversed by CPAP therapy. (*EBioMedicine* **56:** 102,803)

Author contributions: TS, DJG, and SAG conceptualized the association study. TS, RL, RJ, HL, ACG, NK, BEC, JL, and SW performed statistical analysis and data harmonization. All authors critically reviewed the manuscript. YL, JR, and SR collected data and designed components of MESA and its gene expression study. DL collected data and designed components of FOS and the SABRe CVD initiative which collected genes expression data for FOS. RM, SRP, SFQ, SR, and DJG designed and executed the HeartBEAT study, and DJG and AS designed its gene expression study.

Clinical implications of serum neurofilament in newly diagnosed MS patients: A longitudinal multicentre cohort study. (*EBioMedicine* **56:** 102,807)

Author Contributions: FS, VF, TU, M Muthuraman, SGM, SG: Analysis and interpretation of data and drafting the manuscript. AS, RG: Study protocol, design and ethics implementation of the KKNMS cohort study. CL, AS, FL, TK, M Mühlau, LK, TR, A Bayas, A Berthele, FP, HPH, RL, CH, MS, BW, FTB, BT, TK, FW, UZ, UZ, HT, BH, HW, RG: Contributing data and revising the manuscript. SB, FZ: Design and conceptualisation of the study, analysis and interpretation of data, drafting the manuscript.

Molecular analysis of Chinese oesophageal squamous cell carcinoma identifies novel subtypes associated with distinct clinical outcomes. (*EBioMedicine* **57:** 102,831)

Author contributions: Lin Feng and Xiyan Wang designed the study. Meng Liu performed the data collection and data analysis. Wei Sun and Yuan Zhang collected Chinese ESCC samples. Haiyin An and Meng Liu extracted and quantified RNA and DNA. Shujun Cheng provided constructive feedback. Lin Feng and Ruozheng Wang supervised research and provided data interpretation. Meng Liu wrote and reviewed the manuscript.

Using Recombination-Dependent Lethal Mutations to Stabilize Reporter Flaviviruses for Rapid Serodiagnosis and Drug Discovery. (*EBioMedicine* **57:** 102,838)

Author contributions: C.B., X.X, and A.M. performed experiments. K.F. provided critical reagents. C.B., X.X., J.Z., and A.M. analyzed the data. C.B., X.X., J.Z., K.F., and P-Y.S. interpreted results. C.B., X.X., and P-Y.S. wrote the manuscript.

Broadly neutralizing antibodies potently inhibit cell-to-cell transmission of semen leukocyte-derived SHIV162P3. (*EBioMedicine* **57:** 102,842)

Author contributions: Study conception and design: RLG and MC. Acquisition of data: KS, MT, and SH. Management of animals: DD. VL, HM and GS contributed with key reagents and expertise. Analysis and interpretation of the data: KS, NDB, and MC. Draft of the manuscript: KS and MC. Critical revisions: HM, GS, RLG, and MC. All authors read and approved the final version of the manuscript.

GSTM3 variant is a novel genetic modifier in Brugada syndrome, a disease with risk of sudden cardiac death. (*EBioMedicine* **57:** 102,843)

Author Contributions: JMJJ, TPL, and CA performed literature search, conceived and designed the study and the experiments. JMJJ, TPL, AB, IR, SJL, CYJC, LCL, SFSY, EYC, and LPL conducted experiments and analysed the data. JMJJ, JJH, WCC, YBL, LYL, CCY, LTH, and HCH enrolled patients, collected and interpreted data. JMJJ, AB, IR, TPL, and CA wrote the paper.

Tumor budding, poorly differentiated clusters, and T-cell response in colorectal cancer. (*EBioMedicine* **57:** 102,860)

Author contributions: All authors contributed to review and revision. M.G., J.A.N., and S.O.: developed the main concept and designed the study. A.T.C., C.S.F., M.G., and S.O.: wrote grant applications. K.F., J.P.V., J.B., D.J.P., J.A.M., A.T.C., C.S.F., J.K.L., J.A.N., and S.O.: were responsible for collection of tumor tissue, and acquisition of epidemiologic, clinical and tumor tissue data, including histopathological, immunohistochemical, and immunofluorescent characteristics. K.F., J.P.V., J.B., D.J.P., K.H., J.A.M., C.S.F., J.A.N., and S.O.: performed data analysis and interpretation. K.F., J.P.V., J.B., D.J.P., and S.O.:

drafted the manuscript. K.A., K.H., J.K., N.A., T.U., M.C.L., S.G., S.S., M.Z., A.F.L.D.S., T.S.T, H.N., J.A.M., X.Z., K.W., M.G., J.A.N., and S.O.: contributed to editing and critical revision for important intellectual contents.

A surrogate of Roux-en-Y gastric bypass (the enterogastro anastomosis surgery) regulates multiple beta-cell pathways during resolution of diabetes in ob/ob mice. (*EBioMedicine* **58:** 102,895)

Author contributions: F.A., C.A. and C.M. designed the experiments. C.A.; J.C.; C.G.; A.L.; F.M., C.R., J.D.; E.G.; S.M.L., O.T. conducted the experiments. C.A.; F.A.; C.M.; O.T.; C.G. G.R. and R.R. analyzed data. K.C. contributed to patient recruitment and coordinated clinical investigation, patient phenotyping, and sample collection. F.A. and C. A. wrote the manuscript and C.A.; F.A.; C.M.; O.T.; T.S.; C.G.; R.R.; S.L.; R.S.; H.L.S.; E.G. and G.R. contributed to data presentation and the manuscript. All authors reviewed the manuscript. F.A. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Protection Against Mycobacterial Infection: a case-control study of mycobacterial immune responses in pairs of Gambian children with discordant infection status despite matched TB exposure. (*EBioMedicine* **59:** 102,891)

Author contributions: RB and BK conceived and designed the work. RB, MS, AS and UE conducted the clinical recruitment. RB and BS conducted and interpreted the BCG-GFP-LuxFO whole blood assays. BS and MG conducted the in-house interferon gamma release assays. BH conducted and interpreted the cytokine multiplex assays. RB and AK conducted the statistical analyses. RB and BK drafted the work. All authors revised the work for important intellectual content.

Brain Delivery of Supplemental Docosahexaenoic Acid (DHA): A Randomized Placebo-Controlled Clinical Trial. (*EBioMedicine* **59:** 102,883)

Author contributions: IC, NC, BK, DB participated in recruitment and study visits. HNY and MGH did lumbar punctures. XH, NK, and WJM conducted data analysis. NH, NK and MNB did imaging analysis. LD, CM, and HCC planned cognitive testing. AM, AS, BZ assisted with biomarkers. IC, VS, HH, MH, HCC, WJM, MNB, LSS and HNY wrote the manuscript. HNY and LSS designed the study.

Obesity-related hypoxia via miR-128 decreases insulin-receptor expression in human and mouse adipose tissue promoting systemic insulin resistance. (*EBioMedicine* **59:** 102,912)

Author contributions: B.A. and F.L.A. performed experiments *in vitro* and *in vivo*, in mouse systems; B.A. performed human tissue culture studies and analyzed data with the contribution of E.C., M.M., D. M.C., D.P.F and A.B; G.C. and G.N. provided tissues from surgery and clinical information; D.B., V.M. and UK contributed to the analysis of data from mouse experiments; B.A. and E.C. contributed to manuscript draft; F.S.B. helped collecting clinical data and drafted figures; I. D.G. edited the final version of the manuscript and contributed to data interpretation; A.B. conceived and supervised the study and wrote the manuscript.

All authors read and approved the final manuscript.