Rejection-associated Mitochondrial Impairment After Heart Transplantation

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Background. Mitochondrial dysfunction is associated with poor allograft prognosis. Mitochondrial-related gene expression (GE) in endomyocardial biopsies (EMBs) could be useful as a nonimmune functional marker of rejection. We hypothesize that acute cardiac allograft rejection is associated with decreased mitochondrial-related GE in EMBs. Methods. We collected 64 routines or clinically indicated EMB from 47 patients after heart transplant. The EMBs were subjected to mRNA sequencing. We conducted weighted gene coexpression network analysis to construct module-derived eigengenes. The modules were assessed by gene ontology enrichment and hub gene analysis. Modules were correlated with the EMBs following the International Society of Heart and Lung Transplantation histology-based criteria and a classification based on GE alone; we also correlated with clinical parameters. Results. The modules enriched with mitochondria-related and immune-response genes showed the strongest correlation to the clinical traits. Compared with the no-rejection samples, rejection samples had a decreased activity of mitochondrial-related genes and an increased activity of immune-response genes. Biologic processes and hub genes in the mitochondria-related modules were primarily involved with energy generation, substrate metabolism, and regulation of oxidative stress. Compared with International Society of Heart and Lung Transplantation criteria, GE-based classification had stronger correlation to the weighted gene coexpression network analysis–derived functional modules. The brain natriuretic peptide level, ImmuKnow, and Allomap scores had negative relationships with the expression of mitochondria-related modules and positive relationships with immune-response modules. Conclusions. During acute cardiac allograft rejection, there was a decreased activity of mitochondrial-related genes, related to an increased activity of immune-response genes, and depressed allograft function manifested by brain natriuretic peptide elevation. This suggests a rejection-associated mitochondrial impairment.

INTRODUCTION

Improvements in immunosuppressive therapies have increased survival rates after heart transplant (HTx).1 Nevertheless, acute allograft rejection remains a leading cause of allograft failure, death, and adverse long-term outcomes.1,2 Endomyocardial biopsy (EMB) is the gold standard method for surveillance in HTx rejection. The grading system is based on the International Society of Heart and Lung Transplantation (ISHLT) criteria.1,5 ISHLT grading is associated with a wide variation in the interpretation of EMB.3 This lack of precision has motivated the identification of additional biomarkers and methodologies to support accurate diagnostic evaluation. Gene expression (GE) profiling is a valuable tool for monitoring allograft rejection, and it has been incorporated into clinical practice in the United States.7-15 Gene-based classifications of EMBs have been suggested to improve accuracy in comparison to the ISHLT classification.16 Although alloimmune response is the central focus of evaluation in allograft rejection, the evaluation of nonimmune-related biomarkers can also provide valuable information about the graft function.

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The mitochondrion is a crucial organelle involved in biosynthetic reactions, such as ATP synthesis, and it also participates in proinflammatory molecular signaling. Additionally, GE signatures of mitochondrial impairment in allograft biopsies are associated with poor prognosis in kidney transplant recipients.

We sought to evaluate if mitochondrial-related GEIs in heart tissue specimens, obtained during rejection surveillance, provide useful information to improve the evaluation of cardiac transplant biopsies. The overarching hypothesis is that decreased GE of mitochondrial function–related genes in EMB correlates with cardiac allograft rejection.

**MATERIALS AND METHODS**

**Ethics Compliance**

The procedures in this study followed strict compliance with the ethical standards set forth by the World Medical Association. This study was approved by the University of California Los Angeles (UCLA) Office of Human Research Protection Program IRB (UCLA No. 12-001164) and the University of Alabama at Birmingham (UAB) (IRB protocol No. 080207014). All patients signed informed consent forms.

**Study Sample**

Cardiac allograft tissue samples were collected from 47 HTx patients at the time of EMB from 2 institutions. The tissue specimens (~1.5 mm in diameter) obtained during EMB were immersed in TRIzol, snap-frozen in liquid nitrogen immediately postprocedure, and stored in a −80°C freezer. Samples were subjected to next-generation mRNA transcriptome sequencing at the Universities’ genomic core facilities. Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) was used to test the quality of the total mRNA present. Illumina HSeq2000 TruSeq library generation platforms were used for whole genome next-generation mRNA sequencing (Illumina, San Diego, CA). The genome library consisted of random fragmentation of the poly(A) mRNA, followed by cDNA production by random polymers. The cDNA libraries were then subjected to quantification using qPC Clusters to yield approximately 725K–825K clusters/mm². After the first base addition, parameters were assessed and the cluster density and quality were determined. Single-end sequencing runs were conducted to align the cDNA sequences to the reference genome. FASTQ files obtained from mRNA sequencing were then imported into Avadis NGS 1.5 (Agilent, Palo Alto, CA, and Strand Scientific, Santa Barbara, CA) for alignment of the raw reads to the reference genome. All RNA-seq data were DESeq normalized and quality assessed using Avadis NGS v1.5.

**Clinical Information**

Hemodynamic information was available at the time of biopsy. For a subset of 30 samples, we also had the white blood cells (WBCs), brain natriuretic peptide (BNP), ImmuKnow, Allopasm (CareDx, Brisbane, CA), and left ventricular ejection fraction (LVEF).

**Coexpression Network Construction**

Weighted gene coexpression network analysis (WGCNA) was used to construct gene modules based on gene-gene interconnectivity. Gene network modules were constructed using biweight midcorrelation at a soft-thresholding power of 4. The weighted adjacency calculations were transformed into overlap dissimilarity measurements to minimize noise and provide more biologically meaningful clusters. WGCNA uses principal topological component analysis to compute a representative eigengene that summarizes the bulk expression of the entire module (see SDC, http://links.lww.com/TXD/A287, for further details; Figures S1 and S2, SDC, http://links.lww.com/TXD/A287). Cytoscape, plug-in ClueGO, was used to explore the gene ontology (GO) enrichment within each module using 2-sided hypergeometric test with Benjamini-Hochberg correction. Cytoscape, plug-in CytoHubba, was used to predict essential gene nodes within the modules by the Maximal Clique Centrality method.

**Unsupervised Classification of Cardiac Transplant Biopsies**

We analyzed the data using 2 different classifications as references. In addition to the ISHLT, we used a 3-class system we developed following an unsupervised analysis described elsewhere. Briefly, the output that the procedure provides is the matrix P0 (class, sample) containing the probability that each sample belongs to each class, and the subset of genes whose expression is most consistent with the classification found. To assign samples to classes, we evolve from a noninformative small random perturbation of the uniform assignment to its final value through a Bayesian procedure that uses the expression of each gene as new evidence to relax P0, thought of as a prior, toward the corresponding posterior. For each gene, we first used optimal transport to eliminate from its expression the effects of the outside confounding factors such as batch effect, age, gender, quilty lesion in EMB, and variations in prednisone dose. The algorithm finds the Wasserstein barycenter among the generalized batches—that is, the confounding factors—and maps each sample’s expression toward a convex combination of the barycenter, weighted by the corresponding values of P0 for that particular sample. Therefore, the unsupervised algorithm results in a class assignment of heart tissue samples, taking into account gene expression characteristics and filtering outside confounding factors.

**Statistical Analysis**

The gene modules were correlated to the clinical phenotypes; we calculated the correlation following ISHLT grades, unsupervised class, clinical variables, and module eigengenes. The distribution of clinical variables was compared using a general linear model. The statistical significance of each correlation was corrected using the Benjamini-Hochberg method.

**RESULTS**

**Patient Characteristics and Samples**

Tissue samples were collected at routine surveillance or clinically indicated cardiac biopsies from a total of 47 HTx patients; the mean age of the study population was 50.0 ± 14.7 y; 30% of the population were female, and 70% were of European ancestry (patient characteristics are provided in Table 1). Samples were categorized into groups based on the ISHLT grading system. The distribution of the groups within the sample population was 46.9% of the biopsies with grade 0R, 35.9% with grade 1R, 17.2% with grade 2R, and 6.3% antibody-mediated rejection (AMR) (Table 2).

**Gene Coexpression Network Analysis**

WGCNA resulted in groups of genes with related biological functions summarized into 16 modules. Modules had
Analysis Following the ISHLT Classification

The gene module-trait correlation showed divergent interaction between mitochondria and immune function modules. The rejection trait (ISHLT 1R n = 23/35.9% and 2R n = 11/17.2%) had a decreased activity of genes related to mitochondrial function (corr. −0.38, P = 0.002 and −0.22, P = 0.08) and enrichment for expressed genes related to immune function (corr. 0.51, P = 1.0 × 10−10) compared with the no-rejection trait (ISHLT 0R, n = 30/46.9%) (Figure 1).

Analysis Following the Unsupervised Classification

The class assignment using gene expression and optimal transport transformation resulted in 3 EMB classes: Unsupervised class (UC) UC1, UC2, and UC3. The UC1 class was closely related with the no-rejection group, and the UC2 class showed similarities to the rejection group. Compared with the ISHLT classification, gene-based classes had a stronger correlation with WGCNA-derived functional gene modules. For example, class 2 had a high activity of genes in the immune function module (corr. 0.78, P = 5.0 × 10−14) and a low activity of genes related to mitochondrial function 1 module (corr. −0.56, P = 1.0 × 10−6). UC3 had intermediate characteristics of classes 1 and 2 (Figure 1).

GO Enrichment and Hub Genes of the Mitochondrial Function Modules

Genes enriching the mitochondria function module I were predominantly involved in energy generation, regulation of oxidative stress, and substrate metabolism. The mitochondria function module II was mainly involved in the regulation of mitochondrial translation. The GO biologic processes of the mitochondrial-related modules are summarized in Table 3. The top hub genes in mitochondria module I included ACO2, AURKAIP1, ACOT2, and MAPKAPK3 among others. Table 4 summarizes the top Maximal Clique Centrality-based scored hub genes, and Figure 2 represents the role of the hub genes identified.

GO Enrichment and Hub Genes of the Immune Function Module

GO categories enriched by expressed genes in this module included T-cell costimulation, T-cell and B-cell proliferation, antigen processing and presentation, leukocyte migration, NK cell–mediated cytotoxicity, among others. Highest ranked Hub genes within this module included CD33, CTSS, HLA-DRA, IRF8, PTPRC, RAC2, IL10RA, HLA-DPB1, CD54, and MS4A6A. As we are focusing on nonimmune factors, the immune module is not discussed here.

Clinical Correlations

We found a negative correlation between expression of mitochondrial function genes and levels of BNP (corr. −0.35, P = 0.005 and −0.25, P = 0.05), and a positive correlation between activity of immune-response genes and levels of BNP (corr. 0.3, P = 0.02). Allomap scores presented a negative correlation to mitochondria function II and (−0.39, P = 0.01) and a positive correlation to immune module (corr. of 0.48, P = 5.0 × 10−3). ImmuKnow only presented significant negative correlation with the mitochondria function module II (−0.29, P = 0.02). The correlations between the gene modules and WBC, hemodynamic variables, and LVEF were weak and not statistically significant. Gene modules and clinical trait correlations are illustrated in Figure 1.

DISCUSSION

This study suggests that EMBs of patients with cardiac allograft rejection have decreased activity of mitochondrial-related genes and increased expression of genes involved in immune response. This molecular pattern is more evident by...
the molecular functional classification based on the GE (UC classification) in comparison to ISHLT, thus unveiling the underlying biology of the graft that is not evident under the microscope. Peripherally, elevated levels of BNP, ImmuneKnow, and Allomap scores are associated with decreased mitochondrial-related gene activity and increased activity of immune-response genes. This likely represents a rejection-associated mitochondrial impairment.

**Mitochondria Function and Transplantation**

Studies of mitochondrial function in humans following HTx are very limited. In murine models, disturbances were found in mitochondrial oxidative pathways during the acute rejection process, and there was evidence of decreased glycolytic enzymes.26-28

**Studies in humans showed that disturbances in intracardiac mitochondrial bioenergetics have a role in cardiac allograft rejection.**29,30 Recently, a study evaluated intracardiac mitochondrial function by high-resolution respirometry after HTx; the results showed oxidative capacity declination along with an increasing number of CD3+ lymphocytes. The impaired mitochondrial respiration improved after steroid pulse therapy.31 Another study in renal transplant recipients supports our findings, GE signatures of mitochondrial impairment were associated with allograft injury and worse long-term allograft survival.19

**Mitochondrial Function in Heart Disease**

The mitochondria module I was involved in energy generation, regulation of oxidative stress, and substrate metabolism.

**TABLE 3:** Relevant biologic processes in mitochondria modules

| Module | Term ID | Term name | Total genes | Genes | Enrichment P |
|--------|---------|-----------|-------------|-------|--------------|
| Mitochondrial function I: Energy generation and regulation of oxidative stress | GO:0019752 | Carboxylic acid metabolic process | 458 | 158 | 3.82 x 10^-13 |
| | GO:0006082 | Organic acid metabolic process | 530 | 170 | 5.85 x 10^-11 |
| | GO:004281 | Small molecule metabolic process | 1213 | 324 | 1.09 x 10^-10 |
| | GO:0055134 | Oxidation-reduction process | 517 | 165 | 2.59 x 10^-10 |
| | GO:0046395 | Carboxylic acid catabolic process | 109 | 54 | 1.85 x 10^-09 |
| | GO:0051186 | Cofactor metabolic process | 175 | 73 | 6.85 x 10^-09 |
| Mitochondrial function II: Regulation of mitochondrial translation | GO:0006413 | Translational initiation | 125 | 64 | 7.32 x 10^-38 |
| | GO:006613 | Cotranslational protein targeting to membrane | 45 | 35 | 1.43 x 10^-28 |
| | GO:000614 | SPP-dependent cotranslational protein targeting to membrane | 45 | 35 | 1.43 x 10^-28 |

Significant gene GO Biologic Processes within the mitochondria function modules. GO, gene ontology.
The mitochondria module II was mainly involved in regulation of mitochondrial translation (see Table 3).

**Energy Generation and Oxidative Stress**

Impaired mitochondrial function is a consistent feature in the pathophysiology of heart failure, and its role as one of the key contributors in heart failure is increasingly recognized. During oxidative stress, such an inflammatory state, reactive oxygen species (ROS) accumulate, which could potentially be a source of mitochondrial genomic instability. This leads to alterations of the mitochondrial bioenergetics and subsequently increases ROS. Excessive accumulation of ROS increases mitochondrial DNA damage, leading to altered mitochondrial function. Disturbances in intracardiac mitochondrial bioenergetics and responses to oxidative stress were also found in allograft rejection, suggesting that those features can play a role in disease development. Antioxidant therapy with coenzyme Q10 was proposed to prevent rejection. However, the concept is controversial.

**Substrate Metabolism**

Glucose, fatty acids, and amino acids (minor role) are key substrates of cardiac metabolism. Through various enzymatic pathways, these substrates generate high levels of ATP, which is essential to fuel continuous cardiac function. Alterations in substrate metabolism could consequently contribute to cardiac energetic inefficiency and disease progression.

**Mitochondrial Translation**

Nuclear encoded genes play an important role in mitochondrial maintenance, mitochondria translation, and transcription. Biogenesis of the mitochondrial machinery requires adequate translation and protein synthesis for the assembly and functioning of the oxidative phosphorylation complexes. Defects in these processes are associated with cardiomyopathies and cardiovascular diseases.

**Mitochondria and Inflammatory Response**

Mitochondrial dysfunction can contribute to the inflammatory response through both activation of the redox-sensitive inflammatory pathway and direct activation of the inflammasome. Activation of these 2 systems could lead to an overstimulation of the inflammatory response, which increases mitochondrial oxidative stress and promotes a vicious inflammatory cycle. The dysfunctional mitochondria need to be removed by mitophagy to keep cellular homeostasis. Deficient response to oxidative stress can lead to inadequate removal of dysfunctional mitochondria and mitochondrial DNA, contributing to the inflammatory process. Figure 2 represents the role of the hub genes identified and their possible contribution to inflammatory response.

**Molecular Classification of EMB**

Few studies have explored the potential of cardiac gene expression in the diagnosis of HTx rejection. In this study, we used an unsupervised classification based only

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**TABLE 4.** Top hub genes in mitochondria function modules (I and II)

| Module | Gene symbol | Gene name | Definition |
|--------|-------------|-----------|------------|
| Mitochondrial function I | ATP5O | ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit | ATP synthase, H+ transporting |
| | ALRKP1 | Aurora kinase A interacting protein 1 | Ribosomal subunit protein |
| | COX14 | Cytochrome C oxidase assembly factor | Role in coordinating cytochrome C oxidase |
| | COX41 | Cytochrome C oxidase subunit 4I1 | Terminal enzyme of the mitochondrial respiratory chain |
| | MYEOV2 | Myeloma-overexpressed gene 2 protein | Role in cell proliferation |
| | NDUF4A | NADH dehydrogenase (ubiquinone) 1 alpha, mitochondrial complex associated | NADH: transfers electrons from NADH to respiratory chain |
| | NDUF88 | NADH:ubiquinone oxidoreductase subunit B8 | NADH: transfers electrons from NADH to respiratory chain |
| | NDUFV2 | NADH:ubiquinone oxidoreductase core subunit V2 | NADH: transfers electrons from NADH to respiratory chain |
| | RPL23A | Ribosomal protein L23a | Involved in mediating growth inhibition by interferon |
| | UQCR11 | Ubiquinol-cytochrome C reductase, complex III subunit XI | Forms part of mitochondrial respiratory chain: Metabolism, electron transport, ATP synthesis, heat production by uncoupling proteins |
| Mitochondrial function II | ACO2 | Aconitase 2 | Enzyme involved in second step of TCA cycle |
| | ALDH2 | Aldehyde dehydrogenase 2 family (mitochondrial) | Oxidizes aldehydes to generate carboxylic acids for use in muscle and heart |
| | ATP5B | ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide | Subunit of mitochondrial ATP synthase (catalyzes ATP synthesis) |
| | FH | Fumarate hydratase | Component of TCA cycle |
| | MAPKAPK3 | Mitogen-activated protein kinase-activated protein kinase 3 | Induced by growth inducers and stress stimulation of cells: involved in cytokines production, endocytosis, cell migration, chromatin remodeling, and transcriptional regulation |
| | NDUFV1 | NADH:ubiquinone oxidoreductase core subunit V1 | NADH ubinit |
| | PDK2 | Pyruvate dehydrogenase kinase 2 | Regulates glucose/fatty acid metabolism through TCA cycle plays an important role in maintaining normal blood glucose levels. Plays a role in resistance to apoptosis under oxidative stress |
| | PEBP1 | Phosphatidylethanolamine binding protein 1 | Involved in modulating MAPK, NF-kappa B, GSK-3 signaling pathways |
| | SDHA | Succinate dehydrogenase complex flavoprotein subunit A | A complex of mitochondrial respiratory chain |
| | UQCR11 | Ubiquinol-cytochrome C reductase core protein I | A part of the mitochondrial respiratory chain |

Highest ranked Hub genes within mitochondrial modules ranked by MCC score.
MCC, Maximal Clique Centrality; NADH, nicotinamide adenine dinucleotide + hydrogen.
on GE to address the problem of wide variability in the classification of EMB and to improve evaluation accuracy. Genomic profile class 1 had characteristics of no-rejection, and class 2 had characteristics of rejection. The results showed a stronger gene module-trait correlation compared with the histology classification, thus, suggesting a possible improved representation of the underlying biologic process. Class 3 had intermediate characteristics of classes 1 and 2. The integrative analysis of transcriptomics and individual variability (confounding factors) show the potential of a precision medicine approach to refine diagnosis in HTx rejection.\textsuperscript{50,51}

Clinical Correlations

Although limited by sample size, we found that elevated levels of BNP, ImmuKnow, and Allomap scores are associated with decreased mitochondrial-related gene activity and increased activity of immune-response genes (Figure 1). This possibly represents immune activation and cardiac dysfunction, whereas the intragraft mitochondrial function decreases. Hemodynamics, WBC, and LVEF correlations were not significant, possibly because of the fact that most cases of acute rejection are diagnosed when the patient is asymptomatic. In a typical surveillance population, severe hemodynamic compromise is present in <5% of the patients and echocardiography parameters have limited diagnostic performance.\textsuperscript{52,53} If our findings are further confirmed, evaluation of mitochondrial function can potentially be used as a surrogate of allograft function in an early stage when no other diagnostic markers reveal abnormal allograft function. Taken together, the mitochondrial function could serve to explore potential diagnostic markers and therapeutic strategies that could provide further insights into allograft function. Additionally, our unsupervised method offers opportunities to improve evaluation accuracy of the EMB in HTx rejection.

Limitations

We acknowledge that there are limitations to this study. The sample size was small, but it is consistent with sample sizes in gene expression profile studies.\textsuperscript{13,48,49,54} Samples with well-defined AMR were limited, so reliable conclusions about AMR cannot be extracted. This study was not conducted longitudinally but highlights the importance of biobanking systematically to be able to study mitochondrial gene expression before moderate/severe rejection and after treatment. Tissue samples comprise several different cell types; bulk RNA-seq methods are not able to capture and define the cell type responsible for the gene expression; this is a universal problem of the RNA-seq methods. Further studies should be sought to confirm our findings and clarify the cell type responsible for the mitochondrial-related gene expression. The use of single-cell transcriptomic profiles and high-resolution respirometry could provide further insights about the cell type and mitochondrial function.\textsuperscript{31,55} Additionally, as we are moving away from EMB as a surveillance test, with both peripheral GE and cell-free DNA, further research should correlate mitochondrial function to cell-free DNA. Also, exploratory research would be of interest in circulating mitochondria DNA and intragraft mitochondrial function.\textsuperscript{56,57}

CONCLUSIONS

Our findings suggest that intragraft mitochondrial impairment is involved in acute cellular rejection. This highlights the role of mitochondrial function in cardiac allograft rejection and offers opportunities to explore diagnostic markers and therapeutic targets. The molecular classification of EMB based only on gene expression better represents the underlying biologic process in comparison to the ISHLT criteria. This illustrates the clinical potential of a precision medicine approach to refine evaluation of cardiac allograft rejection.
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REFERENCES

1. Lund LH, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: thirty-second official adult heart transplantation report–2015; focus theme: heart-lung transplantation. J Heart Lung Transplant. 2015;34:1244–1254.

2. Lund LH, Edwards LB, Kucheryavaya AY, et al; International Society for Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report–2014; focus theme: retransplantation. J Heart Lung Transplant. 2014;33:996–1010.

3. Billingham ME, Cary NR, Hammond ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. The International Society for Heart Transplantation. J Heart Transplant. 1990;9:587–593.

4. Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection. J Heart Lung Transplant. 2005;24:1710–1720.

5. Winters GL, Marboe CC, Billingham ME. The International Society for Heart and Lung Transplantation grading system for heart transplant biopsy specimens: clarification and commentary. J Heart Lung Transplant. 1998;17:754–760.

6. Crespo-Leiro MG, Zuckermann A, Bara C, et al. Concordance among pathologists in the second Cardiac Allograft Rejection Gene Expression Observational Study (CARGO II). Transplantation. 2012;94:1172–1177.

7. Pham MX, Teuteberg JJ, Kfoury AG, et al; IMAGE Study Group. Gene-expression profiling for rejection surveillance after cardiac transplantation. N Engl J Med. 2010;362:1890–1900.

8. Schoels M, Dengler TJ, Richter R, et al. Detection of cardiac allograft rejection by real-time PCR analysis of circulating mononuclear cells. Clin Transplant. 2004;18:513–517.

9. Starling RC, Pharm M, Valentine H, et al; Working Group on Molecular Testing in Cardiac Transplantation. Molecular testing in the management of clinical cardiac transplant recipients: initial clinical experience. J Heart Lung Transplant. 2006;25:1389–1395.

10. Bodez D, Hocini H, Tchitchek N, et al. Myocardial gene expression profiling to predict and identify cardiac allograft acute cellular rejection: The GET-Study. PLoS One. 2016;11:e0167213.

11. Crespo-Leiro MG, Stymmann J, Schulz U, et al. Clinical usefulness of gene-expression profile to rule out acute rejection after heart transplantation: CARGO II. Eur Heart J. 2016;37:2591–2601.

12. Deng MC, Eisen HJ, Mehra MR, et al; CARGO Investigators. Noninvasive discrimination of rejection in cardiac allograft recipients using gene expression profiling. Am J Transplant. 2006;6:150–160.

13. Horowitz PA, Tsai EJ, Pritt ME, et al. Detection of cardiac allograft rejection and response to immunosuppressive therapy with peripheral blood gene expression. Circulation. 2004;110:3815–3821.

14. Loupy A, Duong Van Huyen JP, Hidalgo L, et al. Gene expression profiling for the identification and classification of antibody-mediated heart rejection. Circulation. 2017;135:917–935.

15. Mehra MR. The emergence of genomic and proteomic biomarkers in heart transplantation. J Heart Lung Transplant. 2005;24(7):622–630.

16. Halloran PF, Potena L, Van Huyen JD, et al. Building a tissue-based molecular diagnostic system in heart transplant rejection: The Heart Molecular Microscope Diagnostic (MMDx) System. J Heart Lung Transplant. 2017;36:1192–1200.

17. Sandhir R, Halder A, Sunkaria A. Mitochondria as a centrally positioned hub in the innate immune response. Biochim Biophys Acta Mol Basis Dis. 2017;1863:1000–1007.

18. Angajala A, Lim S, Phillips JB, et al. Diverse roles of mitochondria in immune responses: novel insights into immune-metabolism. Front Immunol. 2018;9:1605.

19. Zepeda-Orozco D, Kong M, Scheuermann RH. Molecular profile of mitochondrial dysfunction in kidney transplant biopsies is associated with poor allograft outcome. Transplant Proc. 2015;47:1675–1682.

20. Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics. 2008;9:559.

21. Bindea G, Mlecnik B, Hackl H, et al. ClueGO: a cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. Bioinformatics. 2009;25:1091–1093.

22. Chin CH, Chen SH, Wu HH, et al. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014;8(Suppl 4):S11.

23. Tabak EG, Turner CV. A family of nonparametric density estimation algorithms. Commun Pure Appl Math. 2013;66:145–164.

24. Agnelli JP, Cadeiras M, Tabak EG, et al. Clustering and classification through normalizing flows in feature space. Multiscale Modeling Simulation. 2010;8:1758–1802.

25. Trigila G, Tabak EG. Data-driven optimal transport. Commun Pure Appl Math. 2016;69:613–648.

26. Duboc D, Abastado P, Muffalt-Joly M, et al. Evidence of mitochondrial impairment during cardiac allograft rejection. Transplantation. 1990;50:751–755.

27. Abastado P, Duboc D, Marsac C, et al. Demonstration of abnormalities of myocardial mitochondrial oxygenation in cardiac graft rejection. Transplant Proc. 2007;39:1–3.

28. Maneus EB, Pumar-Moya JL, Climent F, et al. Glycolytic enzyme activities are decreased during acute rejection in transplanted rat hearts. Transplant Proc. 2005;37:4122–4127.

29. Gvozdyakova A, Kucharska J, Mizerova S, et al. Coenzyme Q10 depletion and mitochondrial energy disturbances in rejection development in patients after heart transplantation. Biofactors. 1999;9:301–306.

30. Kucharska J, Gvozdyakova A, Mizerova S, et al. Participation of coenzyme Q10 in the rejection development of the heart transplanted: a clinical study. Physiol Res. 1998;47:399–404.

31. Scheider B, Jelenik T, Horn P, et al. P6033 Impaired myocardial mitochondrial function correlates with inflammatory cell burden in humans following heart transplantation. Eur Heart J. 2017;38(suppl_1):1268.

32. Brown DA, Perry JB, Allen ME, et al. Expert consensus document: Mitochondrial function as a therapeutic target in heart failure. Nat Rev Cardiol. 2017;14:238–250.

33. Murphy E, Ardehali H, Balaban RS, et al; American Heart Association Council on Basic Cardiovascular Sciences, Council on Clinical Cardiology, and Council on Functional Genomics and Translational Biology. Mitochondrial function, biology, and role in disease: a scientific statement from the American Heart Association. Circ Res. 2016;118:1960–1991.

34. Ayoub KE, Pothineni NVK, Rutland J, et al. Immunity, inflammation, and oxidative stress in heart failure: emerging molecular targets. Cardiovasc Drugs Ther. 2017;31:593–608.

35. Ristow M. Unraveling the truth about antioxidants: mitochondriess explains ROS-induced health benefits. Nat Med. 2014;20:709–711.

36. Noroadi H, Louden BL, Frenneaux MP, et al. Cardiac metabolism—a promising therapeutic target for heart failure. Pharmacol Ther. 2019;192:95–114.

37. Fukushima A, Milner K, Gupta A, et al. Myocardial energy substrate metabolism in heart failure: from pathways to therapeutic targets. Curr Pharm Des. 2015;21:3654–3664.

38. Bertero E, Maack C. Metabolic remodelling in heart failure. Nat Rev Cardiol. 2018;15:457–470.

39. D’Souza AR, Minczuk M. Mitochondrial transcription and translation: overview. Essays Biochem. 2019;59:299–300.

40. Bocconcazi V, Horvath R, Mitochondria: impaired mitochondrial translocation in human disease. Int J Biochem Cell Biol. 2014;48:77–84.

41. Siasos G, Tsigkou V, Kosmopoulos M, et al. Mitochondria and cardiovascular diseases-from pathophysiology to treatment. Ann Transl Med. 2018;6:256.

42. Garg NJ. Inflammamomes in cardiovascular diseases. Am J Cardiovasc Drugs. 2012;12(5 Pt 4):807–815.

43. Broz P, Dotti VM. Inflammamomes: mechanism of assembly, regulation and signalling. Nat Rev Immunol. 2016;16:407–420.

44. López-Armada MJ, Reviro-Navarre RA, Vaamonde-García C, et al. Mitochondrial dysfunction and the inflammatory response. Mitochondrion. 2013;13:106–118.

45. Tatt SW, Green DR. Mitochondria and cell signalling. J Cell Sci. 2013;126(Pt 4):807–815.

46. Oka T, Hikoso S, Yamaguchi O, et al. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. Nature. 2012;485:251–255.

47. Gottlieb PA, Thomas A. Mitophagy and mitochondrial quality control mechanisms in the heart. Curr Pathobiol Rep. 2017;5:161–169.

48. Bodez D, Hocini H, Tchitchek N, et al. Myocardial gene expression profiling to predict and identify cardiac allograft acute cellular rejection: the GET-study. PLoS One. 2016;11:e0167213.
49. Holweg CT, Potena L, Luikart H, et al. Identification and classification of acute cardiac rejection by intragraft transcriptional profiling. *Circulation*. 2011;123:2236–2243.

50. Sirota M, Sarwal MM. Transplantomics: toward precision medicine in transplantation research. *Transplantation*. 2017;101:1777–1782.

51. Jameson JL, Longo DL. Precision medicine—personalized, problematic, and promising. *N Engl J Med*. 2015;372:2229–2234.

52. Lu W, Zheng J, Pan X, et al. Diagnostic performance of echocardiography for the detection of acute cardiac allograft rejection: a systematic review and meta-analysis. *PLoS One*. 2015;10:e0121228.

53. Mills RM, Naftel DC, Kirklin JK, et al. Heart transplant rejection with hemodynamic compromise: a multiinstitutional study of the role of endomyocardial cellular infiltrate. Cardiac Transplant Research Database. *J Heart Lung Transplant*. 1997;16:813–821.

54. Hollander Z, Lin D, Chen V, et al; NCE CECR PROOF Centre of Excellence. Whole blood biomarkers of acute cardiac allograft rejection: double-crossing the biopsy. *Transplantation*. 2010;90:1389–1393.

55. Kalisky T, Oriel S, Bar-Lev TH, et al. A brief review of single-cell transcriptomic technologies. *Brief Funct Genomics*. 2018;17:64–76.

56. An Q, Hu Y, Li Q, et al. The size of cell-free mitochondrial DNA in blood is inversely correlated with tumor burden in cancer patients. *Precis Clin Med*. 2019;2:131–139.

57. Liu J, Cai X, Xie L, et al. Circulating cell free mitochondrial DNA is a biomarker in the development of coronary heart disease in the patients with type 2 diabetes. *Clin Lab*. 2015;61:661–667.