Metabolic Biomarker Profiling to Understand Rheumatic Heart Disease Pathogenesis

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Background

Rheumatic heart disease (RHD) is leading cause of mortality among inflammatory heart diseases and acquired cardiovascular diseases globally [1].

RHD is a sequela of Group A *Streptococcus* infection and acute rheumatic fever (ARF) (Fig.1).

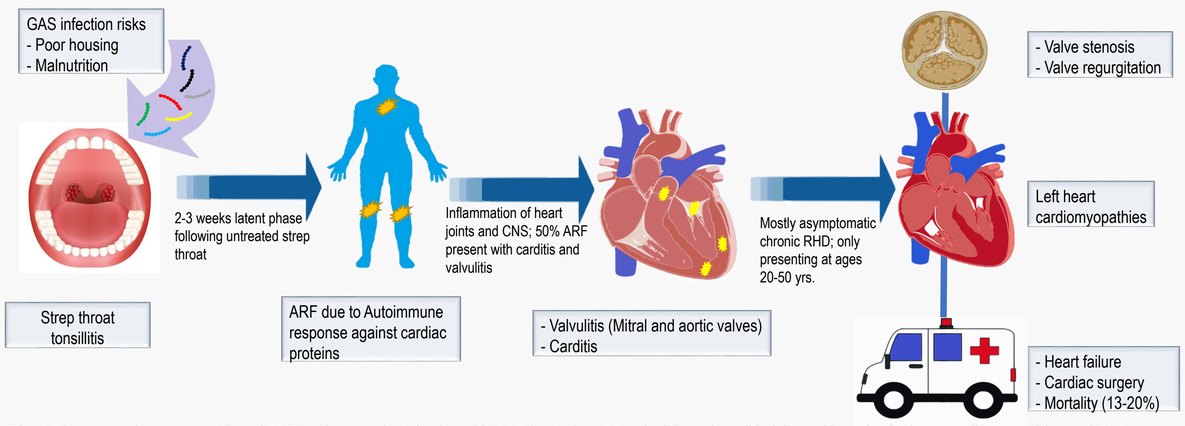

Fig. 1 *Diagrammatic representation of RHD pathogenesis. Infection with GAS bacteria, ARF Valvulitis and carditis followed by valve lesions, cardiomyopathies and RHD related mortalities*

RHD prevalence at 4.6-7.9/1000 in LMICs leading to 1,426 disability-adjusted life-years per 1000 individuals [1].

Cardiac surgery is the only proven intervention but not readily available in resource-limited regions especially in sub-Saharan Africa [2].

Biomarkers to be used for early detection and diagnosis of latent RHD [3].

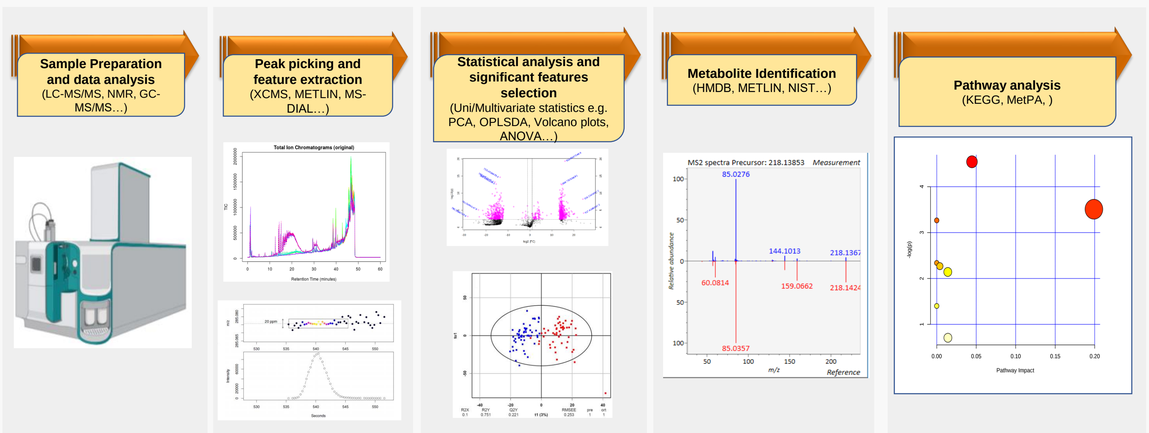

Fig. 2 *Schematic outlook of untargeted metabolomics workflow*

Objectives

The study aimed at applying metabolomics techniques to profile plasma/serum metabolic dysregulation biomarkers in patients diagnosed with RHD

Materials and Methods

Sampling
- Cases (RHD patients)
- Healthy Controls (age, sex & race)

Inclusion criteria
- RHD patients diagnosed at GSH (WHF 2014 criteria)
- Patient's age; 18-65 years old

Exclusion criteria
- Infective endocarditis
- Other inflammatory diseases
- Patients unable to consent
- HIV positive patients
- With other known cardiac diseases

Sample collection and processing
(Metabolites extraction)

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Histology
(H&E staining)

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LC-MS
Fresh frozen tissues and serum, plasma
(AB Sciex X500R Q-TOF-LCMS/MS)

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Metabolomics biomarker discovery
- MS spectra processing (XCMS Online 3.7.1)
- Statistical analysis for biomarker extraction (MetaboAnalyst 4.0)
- MS features annotation (CAMERA 1.34.0)
- Feature identification and matching (METLIN)

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Correlate molecular markers with macroscopic features

Results

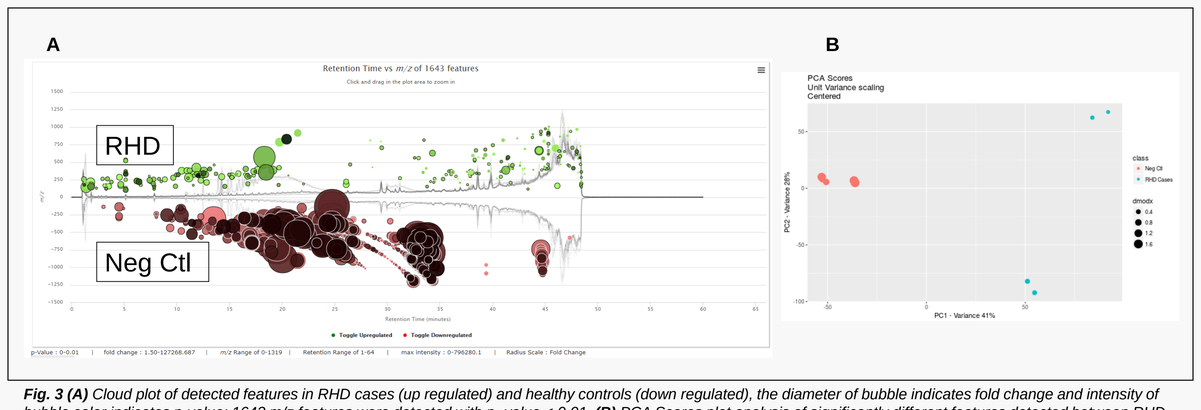

Fig. 3 (A) *Cloud plot of detected features in RHD cases (up regulated) and healthy controls (down regulated), the diameter of bubble indicates fold change and intensity of bubble color indicates p-value; 1643 m/z features were detected with p- value < 0.01.* ***(B)*** *PCA Scores plot analysis of significantly different features detected between RHD cases and healthy controls.*

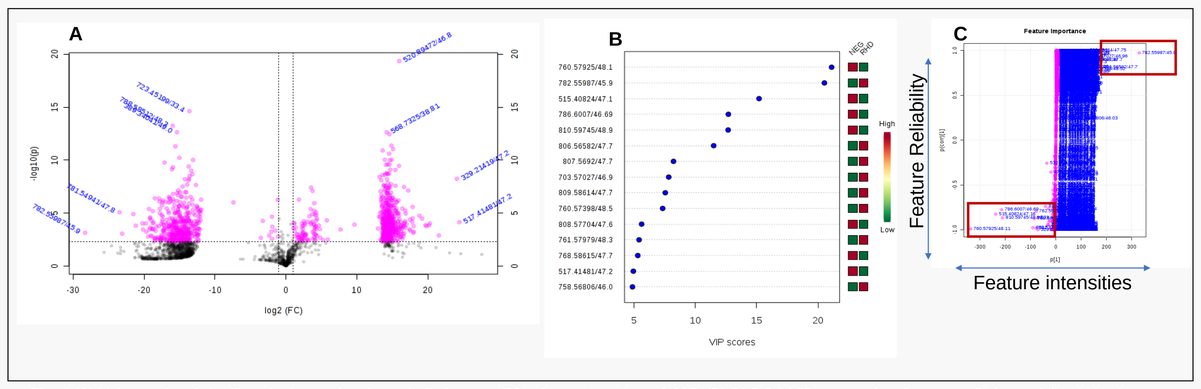

Fig. 4 (A) *Volcano plot of significantly dysregulated metabolic features FC > 2; t-test p value < 0.005.* ***(B)*** *PLS-DA VIP scores plot of most predictive and discriminative features.* ***(C)*** *Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) S-plot indicating important features with high reliability and intensities.*

Table. 1 *List of annotated and identified metabolic features indicating the rate of change (d.value), variability among replicates (stdev), p value and adjusted p value (qValue), name of metabolite, adducts ions, HMDB identifier and general role of the metabolites.*

Features m/z	d.value	stdev	rawp	q.value	Metabolite Name	Adducts	General Role	HMDB ID
809.58614	36.831	0.082005	1.20E-05	0.002551	Phosphatidylglycerol(18:0/18:1)	M+CH3OH+H	Cardiolipin biosynthesis. Heart failure, ischemia/reperfusion	HMDB10603
329.21419	-36.442	0.020788	2.39E-05	0.002551	Cortexolone	M+H-H2O	Androgen excess, virilization, and hypertension	HMDB0000015
781.54941	19.376	0.078066	5.98E-05	0.002614	Phosphatidylethanolamine(38:6)	M+NH4	Remodeling or regulation of cardiac muscle	HMDB08977
603.5298	16.628	0.024712	7.18E-05	0.002614	Triacylglycerol	M+H-2H2O	Atherosclerosis	HMDB11188
122.09597	15.193	0.080653	8.38E-05	0.002614	2,6-Xylidine	M+H	Lidocaine (local anesthetic) action pathway	HMDB0060677
928.61148	5.8237	0.028884	0.000371	0.002614	Lactosylceramide (d18:1/12:0)	M+K	Cell proliferation, adhesion, migration and angiogenesis, cardiovascular diseases	HMDB00173

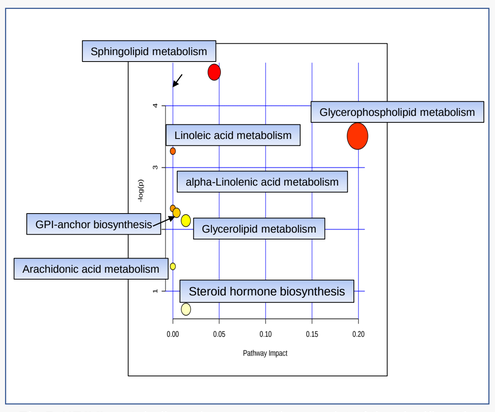

Fig 5. *KEGG metabolic pathways enrichment plot of the significantly identified metabolic biomarkers. Pathway impact determined by number of detected metabolites mapping onto the pathway*

Using statistical analysis, significant features were extracted (Fig. 3-4). The significant features were annotated for metabolite identification using HMDB library matching and the general role of metabolites evaluated (Table. 1). Seventy-nine significant metabolites were identified as potential biomarkers (p value<0.05). Phosphatidylglycerol, phosphatidylcholine and cortexolone were among the highly significant potential biomarkers (relative difference >30, p<0.001). Identified metabolites were mapped on to 8 super pathways using high quality KEGG metabolic pathways; sphingolipid, glycerophospholipid, linoleic, alpha-linoleic acid, arachidonic acid and glycerolipid metabolism (Fig.5).

Conclusions and recommendations

These preliminary results indicate application of metabolomics and bioinformatics in identification of potential biomarkers in RHD. The results suggest a strong association between lipid metabolism dysregulation and RHD pathophysiology. We recommend conducting further experiments with a bigger sample size.

References

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