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Involvement of Cytochrome B in Acute Rheumatic Fever and Rheumatic Heart Disease

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Abstract: *Rheumatic heart disease (RHD), the only long-term consequence of acute rheumatic fever, is the deterioration of heart valves as immunological sequelae of group A streptococcal infection. Several studies have indicated that mutations in mitochondrial Desoxyribonucleic acid (DNA), including MT-CYB coding for cytochrome B in complex III (CIII), were associated with cardiological disorders. We therefore hypothesized that mutations in this gene could play an important role of causality or modification in RHD. In this study, we had 99 individuals divided into two populations: one composed by 23 controls, and the other grouping 76 patients, with various RHD, followed at the Clinic of Thoracic and Cardiovascular Surgery of the National University Hospital Center of Fann (CHNUF), between 2014 and 2017. The DNA was extracted from whole blood and MT-CYB amplified and sequenced. Our results highlighted 113 new mutations, transversions, but mostly transitions that would have appeared in patients following the pathology. Among these mutations, four were the most common: L320L (6.58%), and L281F (6.58%), L299F (3.95%), T368I (13.16%). 24 of our mutations were found in breast cancer either with the same basic substitution or with different base substitutions. Our results also showed that there was some genetic variability among patients. From the results obtained, we could conclude that mutations of MT-CYB are implicated in HVD, and that these mutations are rather sporadic than diagnostic.*

Keywords: *Acute rheumatic fever, rheumatic heart disease, MT-CYB*

1. Introduction

Rheumatic heart disease (RHD) remains the most common cardiovascular disease among people under 25 developing countries. A meta-analysis of studies published between January 1993 and June 2014 reported the prevalence of RHD among children and adolescents in 37 populations, including six from Africa [1]. The incidence and exact prevalence of RHD in Africa are poorly specified [1, 2].

Several studies have shown that 3 to 6% of people infected with group A streptococcus develop an acute rheumatic fever [1-3]; this indicates the existence of genetic susceptibility in some hosts and therefore the role of certain genetic variants. Khatami and his group have shown that mutations in the cytochrome B gene (*MT-CYB*), which is the only component of the complex III of the respiratory chain coded by a mitochondrial gene, were associated with cardiological disorders, especially in congenital heart defects [4]. The involvement of *MT-CYB* in coronary heart disease and hypertrophic cardiomyopathy has also been highlighted [5].

The objective of our study was to show that there are mutations of *MT-CYB* characteristics of rheumatic heart disease.

2. Materials and Methods

2.1. Study Population

Our study population consisted of ninety-nine individuals aged from 4 to 66, including seventy-one women and twenty-eight men. This population was split into two groups of individuals: a group of twenty-three healthy individuals (control group), and a group of seventy-six patients with various rheumatic valvular pathologies and monitored between 2014 and 2017 at the Clinical of Thoracic and Cardiovascular Surgery of Fann National University Hospital Centre.

2.2. Sample Collection

The study was approved by the Ethics Committee of Cheikh Anta Diop University of Dakar (Reference N°:Protocol0086/2015/CER/UCA D). Whole blood samples were taken in EDTA tubes after a written informs consent was provided by patients.

2.3. Genetic Study

2.3.1. DNA Extraction, Amplification and Sequencing

DNA extraction was performed from whole blood using the Qiagen DNeasy kit. Quality of the extracted DNA was verified by electro phoretic migration in 1,5% of agarose gel; DNA was then stored at a temperature of 20°C.PCR amplification of *MT-CYB* was carried out at a reaction volume of 50 µL containing 2 µL of concentrated DNA and 48 µL of the PCR mix comprising 29.8 µL of MilliQ water, 5 µL of buffer, 1 µL of supplementary MgCl2, 2 µL of dATP, dCTP, dGTP, and dTTP, 5 µL of H15915, 5 µL of L14723, and 0.2 µL of Taq polymerase. L14723 (5'ACCAATGACATGAAAAATCATG GTT-3') and H15915 (5'-TCTCC ATTTCT GGTTTA CAAGAC-3') were the forward and reverse primers, respectively.

The PCR program included the following conditions: 94°C for 3 min; 40 cycles (94°C for 45 s; 52°C for 1 min; 72°C 1 min for 30 s); 72°C for 10 min. PCR products were purified and sequenced.

Sequencing reactions were performed using an MJ Research PTC-225 Peltier thermocycler with the ABI PRISM kit and electrophoresed in an ABI 3730 XL sequencer.

2.3.2. Molecular Analysis

Sequences obtained were submitted to the Bio Edit software version 7.1.9 [6], which uses the clustal W algorithm to perform the correction and alignment of the sequences. We then realized blastx 2.8.0. [7] to compare our sequences with NCBI-based reference protein sequences (refseq_protein).

Changes in nucleotides (transitions or transversions) found in both controls and patients were considered as polymorphisms, whereas those only found in patients were considered as mutations. To determine the exact position of our mutations in the mitochondrial genome, we performed a realignment of our sequences using the Cambridge reference sequence (rCRS) as a control sequence. These latter were then compared to those listed as somatic mutations in mitomap (www.mitom ap.org).

Exact position of each mutation as well as the corresponding amino acid (AA) were obtained using the Alamut Visual 2.12 software which, at the same time, determines pathogenicity of some of these mutations through Polyphen-2 prediction test [8], and Sift [9].

The first test ranks the results according to three criteria: probably harmful (p less than 5%), potentially harmful (p between 5 and 10) and benign (p higher than 10%), and the second depending on whether they are tolerated or affected the protein function. Then, we used Mega 7.0.2.6 software [10] to translate the nucleotide sequences into amino acid (AA) sequences, and determined the synonyms (S) and non-synonymous (NS) substitutions using the second reading frame which had no stop codon. In addition of Polyphen-2, two others predictive tests were used to determine the pathogenicity of non-synonymous mutations: Mutation assessor [8] which classifies the mutations according to the pathogenicity in neutral, low, medium (median) or high, and Provean test [11] which distinguishes two types of mutations: deleterious mutations and neutral mutations.

A non-synonymous mutation was considered pathogenic if it was not listed as a polymorphism and if at least two of the above programs defined it as such [12].

With the DNAsp software version 5.10.01 [13], we estimated the parameters of variability and genetic divergence, the size of the population, the total number of sites (N), the monomorphic sites, the polymorphic information sites (s) and non-informative (Pi). We could also bring out with the same software the number of mutations (Eta), the number of haplotypes, the haplotypic (Hd) and nucleotide (Pi) diversity, and the average number of nucleotides differences (k).

With Mega 7.0.2.6 software [10], we determined the frequency for each of the four nucleotides in controls (HC) and patients as well as their frequencies at each codon's position.

Then, we determined mutation rates, nucleotide frequency, AA frequency, and synonymous (Ks) and non-synonymous substitutions frequencies (Kns). The same is true for genetic distance within and between populations (controls, patients). For this analysis, we worked with the Tamura Nei model, which corresponds to the best model. The determination of the genetic

structuring and molecular variance analysis (AMOVA) was performed by Harlequin software [14]. A comparison of genetic structures was made first within and between population, and then considering one of the criteria of gender, ethnicity, area of residence and finally the socio-economic category.

We then carried out the Z-test or selection test in order to determine the type of evolution of MT-CYB mutations, by assuming that Ho: dN = dS (dN = non-synonymous substitutions, dS = synonyms substitutions), as hypothesis H1: dN>dS for positive selection and H1: dN<dS for negative selection; all these hypotheses have been verified using the modified Nei-gojobori method (proportion) model [15].

3. Results

3.1.1. Polymorphisms of MT-CYB

3.1.2. Characterization of MT-CYB Mutations

After correcting and aligning, we ended up with 99 sequences; nucleotide variations were declined as polymorphisms when they were present at same time in controls and patients, and as mutations when they were present only in patients. Thus, number of mutations was 113 with 63 mutational points. 24 mutations detected in our dataset were referenced in mitomap (breast cancer), and 9 of them exhibited the same nucleotide base substitutions.

These substitutions accounted for 39.68% for transversions and 60.32% for transitions; of these, 63.16% concerned substitution of C for T. Four mutations were present at high frequencies in patients: the sense mutation L320L (6.58%) and the missense mutations L281F (6.58%), L299F (3.95%), and T368I (13.16%). All these results are shown in Table 1.

Table1. *Characteristics of MT-CYB mutations*

Mutations	Nature	p.rCRS	p.AA	Proportions (%)	Sift	Polyphen-2	References
C>T	T	15 371	L209L	1,31	Tolerated	Benign	
C>T	T	15 381	T212I	1,31	Tolerated	Benign	
C>T	T	15 382	T212T	2,63			
C>T	T	15 386	H214T	1,31	Affect	Benign	
C>T	T	15 433	A229A	1,31		Benign	
C>T	T	15 434	L230F	1,31	Tolerated	Benign	
C>T	T	15 436	L230L	1,31			
C>T	T	15 476	L244L	1,31			
C>T	T	15 494	L250L	1,31			
T>A	t	15 495	L250P	1,31	Affect	PD	
C>G	t	15 496	L250L	1,31			
G>C	t	15 498	G251D	1,31	Tolerated	PD	
C>G	t	15 499	G251G	1,31			
G>A	T	15 500	D252N	1,31	Affect	PD	
C>T	T	15 569	L275L	1,31			+
C>G	t	15 577	A277A	1,31			+(C>A)
C>T	T	15 587	L281F	6,58	Tolerated	PD	
C>T	T	15 589	L281L	1,31	Affect	PD	+(C>A)
C>T	T	15 595	S283S	1,31			+
T>C	T	15 601	P285P	1,31			+(T>A)
T>A	t	15 608	L288L	1,31			+(C>A),(C>G)
C>T	T	15 626	L294L	2,63			
G>T	t	15 628	L294L	1,31			+(A>C)
C>G	t	15 632	L296L	1,31			
T>G	t	15 633	L296P	1,31	Tolerated	PD	
T>C	T	15 639	I298T	1,31	Affect	PD	
C>T	T	15 641	L299F	3,95	Affect	PD	+
T>G	t	15 642	L299P	1,31	Affect	PD	
A>G	T	15 644	I300V	2,63	Tolerated	PD	
C>A	t	15 646	I300I	1,31			+(C>T)
C>T	T	15 647	L301	2,63			+(C>A)
T>C	T	15 648	L301P	2,63			
A>G	T	15 649	L301L	2,63			
C>T	T	15 651	A302V	2,63	Tolerated	Benign	

Table1. *MT-CYB mutations Characterization*

Mutations	Nature	p.rCRS	p.AA	Proportions (%)	Sift	Polyphen-2	References
A>C	t	15 652	A302A	1,31			
A>G	T	15 653	M303V	2,63	Tolerated	Benign	+
T>A	t	15 654	M303T	1,31			+(T>C)
A>G	T	15 655	M303M	2,63			+(A>C)
A>G	T	15 656	I304V	1,31	Tolerated	Benign	
A>T	t		I304F	1,31			
T>C	T	15 657	I304I	2,63			
C>T	T	15 664	I306T	1,31			+
C>T	T	15 665	L307F	1,31	Tolerated	PD	
T>C	T	15 670	H308H	2,63			+(T>G)
C>T	T	15 672	M309T	1,31	Tolerated	Benign	+(T>C)
A>C	t	15 682	Q312Q	1,31			+(A>G)
C>T	T	15 698	R318C	1,31	Affect	PD	+(C>G)
C-A	t	15 704	L320L	6,57			+(C>T)
C>G	t	15 728	L328V	1,31			
C>G	t	15 743	L333F	2,63			+(C>T)
C>G	t	15 749	L335L	1,31	Tolerated	Benign	+(C>T)
A>G	T	15 758	I338V	2,63	Tolerated		
A>G	T	15 766	G340G	1,31			+
G>C	t	15 777	S344N	1,31			+(G-GG)
C>T	T	15 790	T348T	2,63			+
A>G	T	15 799	G351G	1,31			
C>G	t	15 811	S355S	1,31			+; +(C>T)
C>G	t	15 820	T358T	1,31			+(C>A)
C>A	t	15 828	T361M	2,63			+(C>T)
A>G	T	15 842	M366V	1,31	Tolerated	Benign	+(A>T)
C>T	T	15 849	T368I	13,16	Tolerated	Benign	+;+(C>A)
A>G	T	15 851	I369V	1,31	Tolerated	Benign	
T>A	t	15 852	I369T	1,31			
C>T	T	15 853	I369I	1,31			

p.rCRS: position relative to the Cambridge reference sequence; p: position of the amino acid relative to the reference protein sequence (Uniprot accession number: P00156); T: transition; t: transversion. +: referenced in mitomap; the letters in parentheses correspond to the interchanged bases. PD: probably damaging.

3.2. Correlation between Amino Acid Substitution (AA) and Cytochrome B Protein Functionality

There was a rate of 56.82% of non-synonymous mutations in patient’s group; among them, 9 were moderately pathogenic and 19, highly pathogenic according to at least two of the predictive tests used.These results are shown in Table 2.

Table2. *Functional impact of amino acid (AA) substitutions in Cytochrome b protein*

Mutations	P00156 position	Polyphen-2	Mutation assessor	Provean	Protein binding site	Changed Codons	Trs/Trv	Patients
T6I	212	Benign	Neu	Neu	1	ACC->ATC	1trs	M-Sg154
H8D	214	Benign	Neu	Neu	1	CAT->GAT	1trv	M-Sg160
A23T(Polym)	229	Benign	Med	Neu	1	GCC->ACC	1trs	TS-Sg99
L24F	230	PD	Low	Del	1	CTC->TTC	1trs	M-Sg161
L38V	244	PoD	Med	Neu	1	CTA->GTA	1trv	M-Sg163
L44Q	250	PD	High	Del	1	CTG->CAG	1trv	M-Sg154
G45A	251	PoD	Med	Del	1	GGC->GCG	2trv	M-Sg154
D46N	252	PD	High	Del	1	GAC->AAC	1trs	M-Sg154
L75F	281	PD	High	Del	1	CTC->TTC	1trs	M-Sg9, M-Sg141, M-Sg148, M-Sg152, M-Sg163

L82M	288	PD		Neu	1	CTA->ATA	1trv	M-Sg163
L90R	296	PD	High	Neu	1	CTA->CGA	1trv	M-Sg56
L90V	296	PoD	Med	Neu	1	CTA->GTA	1trv	M-Sg80
I92T	298	PD	High	Del	1	ATC->ACC	1trs	M-Sg163
L93V	299	PoD	Med	Neu	1	CTC->GTC	1trv	M-Sg17

Table2. *Functional impact of amino acid (AA) substitutions in Cytochrome b protein*

Mutations	P00156 position	Polyphen-2	Mutation assessor	Provean	Protein binding site	Changed Codons	Trs/Trv	Patients
L93F	299	PD	Med	Del	1	CTC->TC	1trs	M-Sg80,MSg163
L93G	299	PD		Del	1	CTC->GGC	2trv	M-Sg34
I94V	300	PoD	Neu	Neu	1	ATC->GTG	1trs, 1trv	M-Sg34, M-Sg48
L95R	301	PD	High	Del	1	CTA->CGG	1trv, 1trs	M-Sg47
L95G	301	PD		Del	1	CTA->GGA	2trv	M-Sg48
L95V	301	PD	High	Del	1	CTA->GTA	1trv	M-Sg66
L95K	301	PD		Del	1	CTA->AAG	2trv, 1trs	M-Sg34
A96V	302	Benign	Neu	Neu	1	GCA->GTA	1trs	M-Sg48
M97E	303	Benign		Del	1	ATA->GAG	2Trs,1Tv	M-Sg78
M9A	303	Benign		Neu	1	ATA->GCG	3Trs	M-Sg34
I98N	304	PD	High	Del	1	ATC->AAC	1trv	MSg3,MSg78
I98F	304	PoD	Low	Neu	1	ATC->TTC	1trv	M-Sg59
I98V	304	Benign	Neu	Neu	1	ATC->GTC	1trs	M-Sg34
P99A	305	PD	High	Del	1	CCC->GCC	1trv	M-Sg78
I100F	306	Benign	Neu	Neu	1	ATC->TTC	1trv	M-Sg53
L101F	307	PD	Med	Del	1	CTC->TTC	1trs	M-Sg163
H102Q	308	PD	Med	Del	1	CAT->CAA	1trv	M-Sg37
M103I	309	Benign	Low	Neu	1	ATA->ATT	1trv	M-Sg37
M103T	309	Benign	Neu	Neu	1	ATA->ACA	1trs	M-Sg163
Q106H	312	PD	Low	Del	1	CAA->CAC	1trv	M-Sg163

PD: probably damaging; PoD: potentially damaging; Del: deleterious; Med: medieum; Neu: neutral; trs: transition; trv: transversion.

3.3. Amino Acids Variability

Aspartic acid and histidine were absent in both controls and patients; Pro and Thr absent in controls were present in patients; but in both cases, none of corresponding p-values was significant.However, Leu level increased in patients with a significant p-value of 0.003941. These results are shown in Table 3.

Table3. *Amino acids frequency by population*

Amino acids	Controls	Patients	P-values
Ala	0,667	0,685	0,08668
Cys	4,698	4,692	0,5987
Asp	0	0	0,5847
Glu	3,944	3,953	0,3428
Gly	21,374	21,140	0,362
His	0	0	0,8102
Asn	2,668	2,671	0,1699
Pro	0	0,053	1
Gln	1,334	1,371	0,4578
Arg	7,337	7,504	0,4578
Ser	3,335	3, 356	0,7589
Tyr	2,001	1,986	1
Ile	2,668	2,679	0,9018
Leu	20,679	20,727	0,003941
Lys	1,972	1,986	0,2691
Met	5,974	6,019	0,6521
Phe	2,668	2,697	0,4422
Thr	0	0,017	0,1411
Trp	8,643	8,576	0,7589
Val	10,035	9,885	0,0556

3.4. Determination of the genetic diversity of *MT-CYB*

Genetic diversity analysis carried out for two populations gave the following results: control population consisted of 23 individuals, and patient population (P) of 76 individuals. All sequences had the same number of sites (492 bp). The degree of polymorphism was lower in controls (98.17% of invariable sites compared to 83.74% in patients); number of variable sites was greater in patients (80) with a number of singletons sites greater than the number of polymorphic sites in both controls and patients. The total number of mutations followed the same logic with a value of 9 in controls and 94 in patients. The patients group had the highest number of haplotypes like the haplotype diversity (0.890 ±0.00097 in patients and 0.581 ±0.01446 for controls); in the same logic, the nucleotide difference Pi and the average number of nucleotide differences were much greater in patients.

Non-synonymous (Kns) and synonymous (Ks) substitutions were of equal value in controls; these rates were much higher in patients (Ks = 0.009 +/- 0.004, Kns = 0.006 +/- 0.001). All these results are shown in Table 4.

Table4. *Population genetic diversity parameters*

Populations parameters		Controls				Patients			
Population size (N)		23				76			
Number of sites (n)		492				492			
Invariables sites		483				412			
Polymorphic Sites		Total	Pi	S		Total	Pi	S	
		9	2	7		80	23	57	
Number of mutations		9				94			
Number of haplotypes		8				39			
Haplotype diversity	Hd	0,581				0,890			
	Std Dev.	0,01446				0,00097			
Nucleotide diversity	Pi	0,00206				0,00794			
	Std Dev	0,00496				0,03898			
Average number of nucleotides differences (K)		1,012				3,908			
Ks		0,002±0,001				0,009±0,003			
Kns		0,002±0,001				0,006±0,001			
Nucleotide frequencies		T	C	A	G	T	C	A	G
General		29,5	9,3	25,2	36	29,5	9,5	25,2	35,8
Position 1		21	9,8	42,1	27,4	21	9,8	42,1	27,4
Position 2		29	17,1	20,7	32,9	29	17,4	20,9	32,6
Position 3		38	1,2	12,7	47,6	38	1,3	12,8	47,6
Nature of mutations	Transitions	99,66				88,62			
	Transversions	0,34				11,38			
Binding type	C+G	54,7				45,3			
	A+T	45,3				54,7			

Std Dev: *standard deviation*

3.5. Assessment of differentiation and genetic structuring by population

Analysis of genetic distances revealed a smaller intra-population distance in controls (0.002±0.001) than in patients (0.008±0.001). Genetic distance between controls and patients was 0.005±0.001. These results are shown in Table 5.

The determination of genetic differentiation (Fst) revealed that there was differentiation between controls and patients (p = 0.018417); this result is shown in Table 6.

Table5. *genetic distances*

Populations	Within population	Between populations
Controls	0,002±0,001	0,005±0,001
Patients	0,008±0,001	

D. *genetic distance; SD: standard deviation*

Table6. *Genetic Differentiation Factor*

Populations	Genetic Variation	Fst	P-Value
Controls	100,40%	0,01574	
Patients	100,40%	0,01003	
Controls -Patients	0,40%	0,59922	0,01847

3.6. Correlation between the degree of differentiation and clinical parameters

Values of the degree of genetic differentiation (Fst) were not significant between individuals belonging to different sexes, (Men/Women), to different ethnic groups and to different areas of residence. These results are shown in Table 7.

Table7. *Correlation Fst and social criteria*

Populations	Genetic Variation	Fst	P-Values
SEX			
Women(W)	99,70%	0,00306	
Men(M)		0,00289	
Women-Men(W-H)	0,30%	0,00301	0,273
Ethnic group			
E3	99,04	0,01321	
E6		0,00643	
E7		0,00153	
E8		0	
AE		0,04542	
E3-E6-E7-E8-AE	0,96	0,00963	0,1505
Area of residence			
ZR1	100,25	0	
ZR3		0,00353	
ZR4		0	
AZR		0	
ZR1-ZR3-ZR4-AZR	0,25	0	0,5464

E. *letter represents the ethnic groups, the letters ZR the zones of residence; AE: other ethnicities represent the ethnic groups very poorly represented in our study population; AZR: other areas of residence, corresponds to areas of residence with a small number of individuals; E3: lebou; E6: Wolof; E7: Fulani; AE: Bambara, Diola, Mandingo, Mankagne, Socé, toucouleur, Punu, Ndiago; E8: serere; ZR1: Dakar; ZR3: Pikine; ZR4: Rufisque; AZR: Guediawaye, Thiés, Mbour, Tivaouane, Mbacké, Fatick, Gossas, Ziguinchor.*

Difference in Fst studied between populations following clinical signs developed by different individuals constituting our two populations was not significant between controls and patients: for SC1 (Fst = 0, p = 0.588); for SC2 ((Fst = 0.1145 p = 0.1613), SC3 (Fst = 0 p = 0.651), SC4 (Fst = 0.00404 p = 0.3275).These results are shown in Table 8.

Table8. *Fst Correlation and Clinical Parameters*

Populations	Genetic Variation	FST	P-Value
SC1			
HC	100,44	0,01426	
Patients		0	
HC-Patients	0,44	0	0, 588
SC2			
HC	98,85	0,02947	
Patients		0	
HC-Patients	1,15	0,1145	0,16129
SC3			
HC	100,53	0,1739	
Patients		0	
HC-Patients	0,53	0	0,651
SC4			
HC	99,60	0,02581	
Patients			
HC-Patients	0,44	0,00404	0,3275

SC: clinical sign; SC1: repeated tonsillitis; SC2: rheumatic fever; SC3: orthopnea; SC4: anasarque

3.7. Evolution of *MT-CYB*: Z-Test Mutations

In the HC ", dN / dS = 0.338 P = 0.368 null hypothesis Ho: dN = dS is accepted: selection is neutral.

In patients, the p-value was not significant for the hypothesis of neutral selection. Results are shown in Table 9.

Table9. *Results of the Z-test selection*

Status	Controls	Patients
R	0,002±0,001	0,008±0,008
dN/Ds	0,338	0,683
Probability	0,368	1

4. DISCUSSION

Our work focused on the genetic characterization of *MT-CYB* in ninety-nine patients, twenty-three of whom are healthy controls and seventy-six of patients with various valvular heart diseases.

Our results showed existence of 9 types of mutations that could be considered as neutral polymorphisms because they are present in both controls and patients, and could be considered as having no functional relevance [16-19]. Existence of this polymorphism on *MT-CYB*, despite being a coding sequence, could be explained by its high variability due to a replication that is not very faithful to the image of the entire mitochondrial genome [20]. Another reason is that mitochondrial polymerase is less faithful than that of the nucleus and the rate of renewal of the mitochondrial DNA and therefore of replication is greater than that of the nuclear DNA [21]. In addition, there is not only a deficiency or absence of correction and repair systems in the mitochondrial genome, but also an apparent lack of recombination.

Among mutations observed in patients, transversions (T) were less frequent than transitions (t) (60.32% versus 39.68%), and these were dominated at 63.16% by a mutation of C towards T. Our results agree with [22], that the general characteristics of mtDNA mutations are CT and AG transitions. However, frequency of transversions was greater in patients than in HC (11.38 against 0.34); in addition, although Ks was superior to Kns in patients, the latter was quite important (Ks = 0.009 +/- 0.003, Kns = 0.006 +/- 0.001) These results show that new mutations of *MT-CYB* (both transitions transversions) would have appeared in patients, demonstrating involvement of Cyt b nucleotide variations of in RHD. These results are in line with the conclusions of [23, 24] on superiority. Numerical studies have also demonstrated in their studies that, as in RHD, transitions are superior in number to transversions in breast cancer.

Other mutations were found in both breast cancer and RHD, among which four in high proportions in patients and therefore are characteristic of RHD: it is nonsense mutations L281F, L299F, T368I and, L320L sense mutation. The latter, although not inducing a change in AA, the C15704A position to which it corresponds could serve as a molecular marker, which had a proportion of 6.58% could and nonsense mutations L281F (6.58%), L299F (3.95%), T368I (13.16%). In the case of the L281F and L299F mutations, substitution of a leucine which is an aliphatic AA with an aromatic phenylalanine has certain consequences for the functioning of the protein. In addition, these mutations were not only predicted pathogenic by the prediction tests tested on our dataset, but are also located at the C-terminal domain containing ubiquinone / ubiquinol binding sites [25,26]. In addition, these mutations are also part of the 9 have also been listed in mitomap in breast cancer, with the same base substitutions. Although the T368I mutation corresponds to the replacement of polar threonine by apolar isoleucine, and is located on a conserved domain in mammals, it is located outside the c-terminal domain, which could explain why was predicted benign by "Sift" and "polyphen-2". This same mutation was also found on mitomap in the context of cancer but with a different nucleotide base substitution. The numerical superiority of the transitions with respect to the transitions of *MT-CYB*, the similarity of the mutations observed in RHD and in cancer advocate in favor of the postulate according to which these pathologies are both due to an autoimmune reaction of the organism involving the T lymphocytes [27,28]

Of all the NS mutations recorded, 14.71% are moderately, while 50% are strongly; these mutations could be at the origin of a poor quality of life that could lead to a reduction in life expectancy of some "operated on" [29,30]. M-Sg163, M-Sg78, M-Sg34, M-Sg139, M-Sg154 had the highest number of mutations. The frequency of mutations in these individuals should be explained by the socio-economic criteria that characterize them; however, by making a correlation between them (age, sex, ZR, ethnicity) and the nature or frequency of the mutations, no explanation could be found. This lack of results could be explained by our sampling which was not balanced with respect to the social criteria.

Studying functions of the variability of AA in population had shown that proline (Pro) and

Threonine (Thr) that were absent in TS were present in patients, which would probably be due to the observed nonsense mutations. Leucine (Leu) increased significantly in patients (p-value = 0.0039). This result would allow us to conclude that leucine (Leu), essential AA not supplied by the diet, plays a certain role in the genesis of RHD, and that in the image of cancer [31] variations in some AAs play a major role in occurrence of this pathology. However, our study would highlight an increase in leucine (Leu) in patients with RHD, while those of [32] reported an increase in glutamine (Gln) in cancer patients. Several other mutations have been found in other cardiovascular pathologies. Indeed, the R318C mutation was found in L318P form responsible for severe reduction of complex I and III activities in skeletal muscle and is believed to be responsible for isolated muscle impairment and a clinical picture involving intolerance to exercise [33]. This region therefore seems to play a vital role in muscle activity, which could explain the dyspnea of effort that is the subject of many patients. Nonsense mutation G251A has been found in children with hystiocytoid cardiomyopathy [34], confirming the results of [35] who concluded that DNA mutations mitochondrial, including *MT-CYB* are involved in cardiovascular disease. [28] found in the *MT-CYB* gene of five patients with a sporadic form of mitochondrial myopathy in which exercise intolerance is the predominant symptom, a total of three different non-sensory mutations (G15084A, G15168A and G15723A), a missense mutation (G14846A) and a deletion of 24bp (nucleotides 15498 to 15521). Other authors have highlighted the involvement of other areas of *MT-CYB* in heart diseases: [31], which implicated the 16189T>C mutation in myocardial dysfunction, [36] demonstrated the involvement of 14927A>G, *MT-CYB* 15236A>G, *MT-CYB* 15452A>G mutations in cardiomyopathies. It appears that other mutations located in other regions of the *MT-CYB* are involved in mitochondrial and especially cardiac pathologies, but our study concerns a small portion of 492 base pairs. A complete study of all *MT-CYB* would allow us to detect more mutation and better understand rheumatic heart disease.

The haplotypic diversity (hd) was 0.581 +/- 0.01446 in the HC with a Pi nucleotide diversity of 0.00206 +/- 0.00496, which would show that there is significant variability among "patients". These results were corroborated by the value of intra-population genetic distance (d) which was 0.002 in HC and 0.008 in "patients"; which would confirm that patients "" are much more distant from one another than HC; Intra-population genetic distances are more important than inter-population genetic distances (distance between HC and "sick"): genetic diversity would therefore be due to the difference between individuals within the same populations. The index of genetic variability (Fst) between HC and "patients" (Fst = 0.599, p-value = 0.018) would go in the same direction since it does not allow to invalidate our hypothesis, the p-value not being significant. These results build on the fact that the difference in genetic structuring between HC and "patients" would only be due to specific or diagnostic mutations and not to a different rate of mutations between the two populations. In summary, there would be no genetic difference between populations, or significant genetic distance, which could be explained in part by the fact that the target DNA of "patients" comes from blood and not from valvular tissue.

5. Conclusion

The main objective of our study was to demonstrate that mutations of *MT-CYB* play an important role in the occurrence of rheumatic heart disease and therefore of RAA. Given the importance of the mutations observed in the majority of individuals that make up our two populations (healthy and sick controls), we can conclude that our hypothesis is true: mutations of *MT-CYB* are involved in RHD.

If our research allowed us to support our thesis, it allowed us to understand that the rheumatic valvulopathies are the fruit of a positive selection with change of amino acids with appearance of new mutations resulting in a constraint on the protein.

The individual variability detected in the patients would allow us to conclude that this pathology would not be due to diagnostic mutations, but rather to sporadic mutations.

However, our study knows some limitations, namely the partiality of sequencing (a small portion of *MT-CYB* studied), as well as a sampling that is not sufficiently representative, hence the interest of carrying out a multicentric study. Indeed, this type of study would allow us to have a sample with an equal number of individuals with all types of rheumatic valvulopathies (mitral, aortic, mitral and

tricuspid, mitral and aortic and finally, mitral, aortic and tricuspid) with socio-economic characteristics A study more oriented towards a balance between the numbers of patients with (origin, socio-economic level) more varied.

The possible consequences of the pathogenicity of postoperative mutations make it necessary to search for the DNA of the causal agent of this pathology, haemolytic streptococcus, in the valve tissues in order to explain the occurrence and the consequences of these disorders. Postoperative mutations.

Our study also allowed us to highlight the significant rise in leucine; a quantification of this AA could help in the diagnosis of these rheumatic valve diseases.

A study of the mutations of *MT-CYB* in three populations: a population of HC, a population of non-operated RHD patients and a population of patients operated after a RHD would allow us to specify what are the mutations born of the pathology and what are mutations caused by surgery.

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