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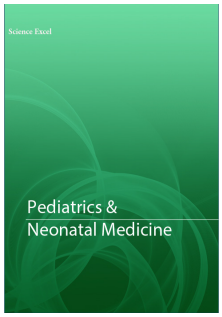

Variations Analysis of *Mitochondrial Cytochrome b (MT-CYB)* Gene in Malnourished Children from Senegal

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Abstract

Introduction: Child malnutrition is a major cause of infant morbidity and mortality worldwide and thus a major public health problem. The study of interactions between nutrition and genes— nutritional genomics—encompasses two domains: nutrigenetics and nutrigenomics. Malnutrition (deficiency or excess) can thus affect gene expression and genome stability. **Objective:** The objective of this work is to investigate mutations of interest of *Mitochondrial Cytochrome b* (*MT-CYB)* that may be related to child malnutrition and to study the genetic diversity of *MT-CYB*. **Materials and methods:** We analyzed the variability of *MT-CYB* in 23 malnourished children and six healthy children via PCR-sequencing. The search of mutations in the *MT-CYB* gene was conducted using Surveyor Mutation software. Nine prediction software programs (i.e., SIFT, PROVEAN, POLYPHEN-2, DEOGEN, SNPs & go, PREDICTSNP, MUTATIONTASTER, PANTHER, and FATHMM) were used to determine the functional impact of mutations. The various parameters of the genetic variety as well as the genetic differentiation of *MT-CYB* were obtained using DNAsp Version 5.1001, Harlequin Version 3.1 and Mega X. **Results:** A total of 24 mutations (Z-score ≥ 20) were identified in malnourished and healthy children. Among the non-synonym mutations present in malnourished children, mutations p.N206N/I, p.T336H, p.Y345A, p.T348T/N, and p.L357L/V were predicted pathogenic by at least five predictive software programs. The amino acids Ile, Lys, Arg and Asn demonstrate significant differences between normal and malnourished children. There is a predominance of T+A (53.72%) compared to C+G (46.28%). Our results show high haplotypic diversity (1.000+/-0.013) and low nucleotide diversity (0.10545+/- 0.00488). **Conclusion:** Our results allowed us to detect mutations in the MT-CYB gene that could be linked to childhood malnutrition. A decrease in isoleucine (Ile), asparagine (Asn), and arginine (Arg) may be correlated with the risk of malnutrition. This study will allow to readjust the strategies to fight against malnutrition.

Introduction

Nutrition plays an important role in health, particularly in children [1]. Nutrients and foods generally interact with genes, but this interaction may occasionally have fatal consequences [2]. Nutrition may contribute directly or indirectly to disease pathogenesis or onset. Malnutrition is thus defined as an imbalance between nutrient requirements and nutrient intake, which may result in cumulative deficits in energy, protein, or micronutrients that may affect growth and cognitive development [3] . Child malnutrition is a major public health problem worldwide, particularly in many low- and middle-income countries [4]. It is a leading cause of infant morbidity and mortality, accounting for 45% of deaths of children under five years of age [5,6]. The study of the interactions between genetics and nutrition is an old concern, which has gained new momentum with the completion of the decoding of the human genome. Indeed, sequencing has enabled the development of so-called "personalized" medicine, one of whose applications is called nutritional genomics [7]. Nutritional genomics is a relatively new field of study that investigates how diet may affect the expression of an individual's genetic information, how an individual's genetic make-

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up affects metabolism, and the response to nutrients and other bioactive components of food [8]. It encompasses two distinct fields: nutrigenomics and nutrigenomics [9]. Nutrigenomics studies the interaction between food components and the genome and regulatory changes in proteins and other metabolisms [10]. Nutrigenetics is the science that identifies and characterizes genetic variants associated with a differential response to nutrients and links this variation to variable disease states [2]. Malnutrition can thus affect gene expression and genome stability, leading to mutations in the genetic or chromosomal sequence. These mutations may result in abnormal dosage and gene expression, leading to undesirable phenotypes during different life stages [11].

Several studies have shown that mutations in *MT-CYB* caused an isolated Complex III deficiency, leading to various pathologies such as stunting, intolerance to exercise, cardiomyopathy, encephalommyopathy, and Leber hereditary optical neuropathy [12]. Cytochrome b (*Cyt b*) is the only Complex III polypeptide encoded by a mitochondrial gene [12]. It is involved in the transfer of transmembrane electrons by which the oxydo-reduction energy is converted into a driving force. Deleterious mutations in the *Cyt b* gene thus lead to the production of defective proteins, giving rise to diseases [13]. There is virtually no literature on the role of *Cyt b* in infant malnutrition. It is in this context that our subject is inscribed, which has the general objectives of researching *MT-CYB* mutations of interest involved in infant malnutrition and studying the genetic diversity of *MT-CYB* in malnourished children.

Materials and methods

Study population and sampling

The study involved 29 blood samples taken from healthy children (ES) and malnourished children (EM) in Senegal, aged 0 to 59 months. These children were recruited on the basis of informed consent and signed by a legal responsible. Our study was conducted at the Pediatric Social Institute of Guediawaye (IPS) and the National Hospital Center of Pikine (CHN-Pikine). For each child recruited, a blood sample was taken from a vacutainer tube with a 4-ml ethylenediaminetetraacetic (EDTA).

DNA extraction, PCR and sequencing

Genomic DNA was extracted using the Zymo research kit according to the manufacturer's instructions from a total of 200 µl of whole blood. From the total DNA, we amplified the *MT- CYB* gene by polymerase chain reaction (PCR). *MT-CYB* amplification was performed in a 50- µl reaction volume containing 2 µl genomic DNA, 25 µl Master Mix, 1 µl of each primer (forward and reverse), 1 µl MgCl 2, and 20 µl PCR water. PCR is performed in an Eppendorf thermocycler under the following conditions: initial denaturation at 94 °C for 3 min; 40 denaturation cycles at 94 °C for 45 s; annealing at 52 °C for 1 min; elongation at 72 °C for 1 min 30 s; and final elongation at 72 °C for 10 min; followed by extension at 72 °C for 10 min and holding at 10 °C. The primers H15915 (TCCCATTTCTGGTTTACAAGAC) and L14723 (ACCAATGACATGAAAAATCATGGTT) respectively correspond to the sense and antisense primers used in this study. All PCR products were purified and using the Sanger sequencing method.

Genetic analysis

MT-CYB sequences of healthy and malnourished children were thoroughly checked, corrected, and aligned with BioEdit software Version 7. 0. 8 [14]. To determine the presence of any mutation and its position relative to the *MT-CYB* gene, the raw gene sequencing data was submitted to Mutation Surveyor Software Version 5.1.2 (https://softgenetics.com/products/mutation-surveyor/) which compares the submitted chromatograms to the gene reference sequence. All chromatograms were compared to the reference sequence NC_012 920_14 247_F. Identification of the referenced pathogen mutations was performed using gnomAD software (https://gnomad.broadinstitute.org/). Nine predictive software packages were used to determine the functional impact of mutations (see Table 1). HOPE software (https://www3.cmbi.umcn.nl/hope/input/#) was used to analyze the structural effects of a point mutation in a protein sequence.

Basic parameters of genetic variability and indices of genetic diversity (i.e., nucleotide diversity [Pi] and haplotypic diversity [Hd]) were determined using DNAsp software Version 5.10.01 [15]. Nucleotide frequencies, molecular distances, and inter- and intra-population genetic distances are explained via Mega X software [16]. The degree of genetic differentiation (Fst), Tajima's D, Fu's Fs indices, and the demographic indices (i.e., raggedness and SSD) are calculated using Arlequin software Version 3.0 [17]. Mismatch distribution analysis was regenerated with DNAsp software Version 5.10.01 [15]. The significance threshold was set at 0.05.

Table 1. *Tools for predicting non-synonymous mutations*

Predictive tools	Link	Reference	Function
Polyphen-2	http://genetics.bwh.harvard.edu/pph2/	[18]	Structure and function of the protein
MutationTaster	http://www.mutationtaster.org/	[19]	
Provean	http://provean.jcvi.org/index.php	[20]	
Deogen2	http://deogen2.mutaframe.com/	[21]	Likelihood that mutations are the cause of the disease
SNPs&GO	http://snps.biofold.org/snps-and-go//snps-and-go.html	[22]	
PREDICTSNP	https://loschmidt.chemi.muni.cz/predictsnp/	[23]	
PANTHER	http://www.pantherdb.org/	[24]	Evolutionary conservation
SIFT	https://sift.bii.a-star.edu.sg/	[25]	
FATHMM	http://fathmm.biocompute.org.uk/	[26]	

Table 2. *Mutation in the MT-CYB gene*

Mutations	Score	dbSNP	Affected amino acid	Status	Nature of mutations
c.549C>T	25	New	p.F183F	Homozygous	Synonym
c.565A>G	21	rs35070048	p.I189V	Homozygous	Non-Synonymous
c.617A>AT	20	New	p.N206N/I	Heterozygous	Non-Synonymous
c.706C>T	22	rs193302994	p.L236F	Homozygous	Non-Synonymous
c.800A>AC	53	New	p.H267H/P	Heterozygous	Non-Synonymous
c.808C>CA	22	New	p.P270P/T	Heterozygous	Non-Synonymous
c.831C>CA	25	New	p.A277A/A	Heterozygous	Synonym
c.849C>CT	22	New	p.S283S/S	Heterozygous	Synonym
c.876T>TG	22	New	p.L292L/L	Heterozygous	Synonym
c.917T>TC	20	rs369851331	p.I306T	Heterozygous	Non-Synonymous
c.1008C>CA	28	New	p.T336T/T	Heterozygous	Synonym
c.1008C>A	20	New	p.T336H	Homozygous	Non-Synonymous
c.1008C>A	21	New	p.T336T	Homozygous	Synonym
c.1033T>TC	54	rs1603225480	p.Y345Y/H	Heterozygous	Non-Synonymous
c.1033T>TC	31	New	p.Y345A	Heterozygous	Non-Synonymous
c.1042A>T	22	New	p.T348L	Homozygous	Non-Synonymous
c.1043C>CA	24	New	p.T348T/T	Heterozygous	Synonym
c.1043C>CA	26	New	p.T348T/N	Heterozygous	Non-Synonymous
c.1044C>T	25	New	p.T348T	Homozygous	Synonym
c.1066G>GA	22	rs200336777	p.V356V/M	Heterozygous	Non-Synonymous
c.1069C>CG	34	New	p.L357L/V	Heterozygous	Non-Synonymous
c.1080A>AT	25	New	p.T360Q	Heterozygous	Non-Synonymous
c.1080A>AT	28	New	p.T360T/T	Heterozygous	Synonym
c.1083A>AG	26	New	p.T361T/T	Heterozygous	Synonym

Results

Profile of *MT-CYB* mutations

Table 2 presents the profiles of *MT-CYB* mutations in the two groups of children identified. The analysis of the chromatograms by Mutation Surveyor revealed the presence of 24 mutations (Z-score ≥ 20), of which 7 (29.17%) were homozygous and 17 heterozygous (70.83%). Fourteen mutations induce a changing of amino acid (i.e., non-synonymous mutations; 58.33%). Of all the non-synonymous mutations, nine (64.29%) are found in malnourished children (EM), three (21.43%) in healthy children (ES), and two (14.28%) are common to both malnourished and healthy children. Of these variants, only the c.565A>G, c.706C>T, c.917T>TC, c.1033T>TC, and c.1066G>GA variants were listed in the gnomAD database, and the remaining 18 are therefore considered new variants.

Prediction of the impact of non-synonymous mutations

The analysis of the pathogenicity of the non-synonymous mutations present in our cohort revealed that the prediction software predicted that the mutations p.N206N/I and p.T336H were deleterious and capable of altering the sequence, function, and structure of the protein, as well as inducing malnutrition. Mutations p.Y345A and p.L357L/V were predicted to be deleterious, thus inducing a modification of the protein function and structure. Nevertheless, disease prediction software predicted these variants to be benign. The p.T348T/N mutation was predicted to be pathogenic and to induce malnutrition by disease prediction software, but it was classified as neutral by function and structure prediction software. All non-synonymous mutations are not listed on the gnomAD. **Table 3** displays the results.

Table 4 shows the structure of each protein established using the HOPE software. According to the HOPE prediction, a mutant residue smaller than the wild-type residue was recorded at the mutations p.N206N/I and p.Y345A. This difference in size will result in a potential loss of external interactions. For these mutations, the mutant residue was more hydrophobic than the wild-type residue. Indeed, the mutation causes a loss of hydrogen bond at the heart of the protein and therefore disrupts the correct folding.

Mutant residues of the p.T336H and p.T348T/N mutations are larger and less hydrophobic than wild-type residues. Indeed, the wild residue of the T348N variant forms a hydrogen bond with serine in position 344 and tyrosine in position 345, while the wild residue of T336H forms a hydrogen bond with leucine in position 332. The residues of these variants are on the surface of the protein, so mutations in these residues can disrupt interactions with other molecules or other parts of the protein. Mutations could therefore lead to a loss of hydrophobic

Table 3. *Pathogenicity of non-synonymous mutations in the MT-CYB gene*

	Protein function and structure			Probability that mutations are the cause of the disease			Evolutionary conservation		
Mutations	Poly-phen-2	Provean	Mutation Taster	Deogen 2	PRE-DICTSNP	SNPs & GO	FATHMM	PANTHER	SIFT
c.565A>G p.I189V	Benign (0.12)	Neutral (-0.61)	Benign (0.99)	Benign (0.11)	Neutral (65%)	Neutral (0.408)	Deleterious (2.90)	Possibly damaging (0.5)	Tolerated (0.22)
c.617A>AT p.N206N/I	Probably damaging (1)	Deleterious (-6.88)	Deleterious (1)	Benign (0.15)	Deleterious (87%)	Disease (0.904	Deleterious (0.54)	Possibly damaging (0.85)	Deleterious (0)
c.706C>T p.L236F	Benign (0.0006)	Neutral (-2.24)	Benign (0.99)	Benign (0.11)	Neutral (71%)	Neutral (0.487)	Deleterious (3.01)	Possibly damaging (0.85)	Tolerated (0.10)
c.917T>TC p.I306T	Benign (0.002)	Neutral (-1.66)	Deleterious (1)	Benign (0.02)	Neutral (60%)	Neutral (0.250)	Deleterious (3.13)	Probably Benign (0.13)	Tolerated (0.06)
c.1008C>A p.T336H	Probably damaging (1)	Deleterious (-4.23)	Benign (0.79)	Benign (0.19)	Deleterious (87%)	Disease (0.827)	Deleterious (2.78)	Possibly damaging (0.85)	Deleterious (0)
c.1033T>TC p.Y345Y/H	Benign (0.137)	Neutral (1.74)	Deleterious (1)	Benign (0.01)	Neutral (74%)	Neutral (0.176)	Deleterious (2.95)	Probably Benign (0.13)	Tolerated (1)
c.1033T>TC p.Y345A	Probably damaging (0.994)	Neutral (-1.72)	Deleterious (1)	Benign (0.02)	Neutral (60%)	Neutral (0.328)	Deleterious (3.03)	Probably Benign (0.13)	Deleterious (0)
c.1042A>T p.T348L	Benign (0)	Neutral (0.92)	Benign (0.99)	Benign (0)	Deleterious (51%)	Neutral (0.490)	Deleterious (3.21)	Probably Benign (0.19)	Deleterious (0)
c.1043C>CA p.T348T/N	Benign (0.40)	Neutral (-1.44)	Deleterious (1)	Benign (0.02)	Deleterious (87%)	Disease (0.660)	Deleterious (3.09)	Probably Benign (0.19)	Deleterious (0.01)
c.1066G>GA p.V356V/M	Benign (0.002)	Neutral (-0.73)	Deleterious (1)	Benign (0.02)	Neutral (63%)	Neutral (0.376)	Deleterious (2.81)	Probably Benign (0.13)	Deleterious (0.04)
c.1069C>CG p.L357L/V	Probably damaging (0.993)	Neutral (-0.88)	Deleterious (1)	Benign (0.02)	Neutral (63%)	Disease (0.622)	Deleterious (2.88)	Probably Benign (0.19)	Deleterious (0)
c.1080A>AT p.T360Q	Benign (0.426)	Neutral (-1.25)	Deleterious (1)	Benign (0.08)	Deleterious (51%)	Neutral (0.493)	Deleterious (3.11)	Probably Benign (0.13)	Tolerated (0.07)

interactions with other molecules on the protein's surface. For the L357L/V mutation, it was noted that the mutant residue is smaller than the wild type residue. This mutation can disrupt protein function leading to possible loss of external interactions.

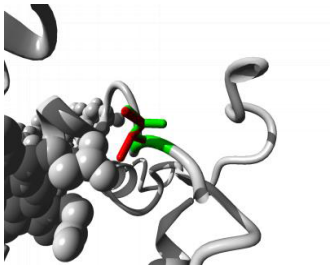
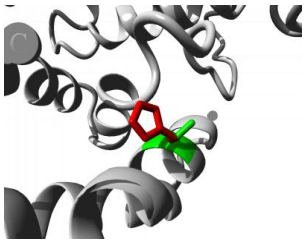
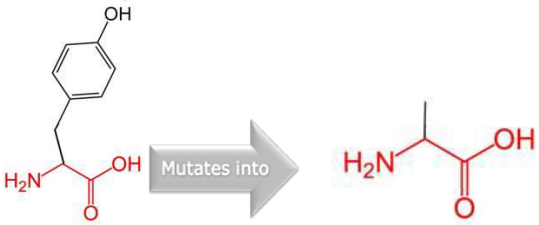
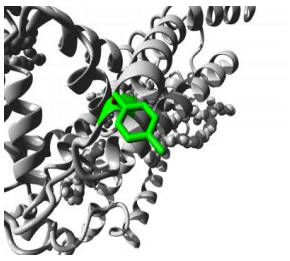
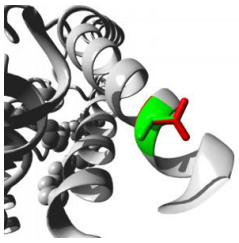
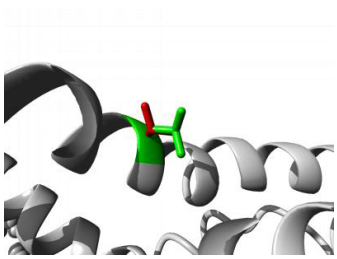
Variability of Cytb-encoded amino acids

Table 5 shows the amino acid frequencies of healthy and malnourished children. The amino acids Ile, Lys, Arg and Asn show significant differences (P-value ≤ 0.05) between healthy (ES) and malnourished children (EM). The frequency of Lys is slightly higher in malnourished children than healthy children, while the frequencies of Ile, Arg and Asn are slightly reduced at the level of malnourished children.

Genetic variability of MT-CYB in malnourished children

The *MT-CYB* gene has a high genetic diversity in malnourished children. **Table 6** shows a number of polymorphic sites equal to 158 (34.50%), of which 73.42% (116/158) are variable sites in parsimony. The total number of Eta mutations is 185, while the average number of nucleotide difference is 48.30. The percentages of transitions (57.98%) are more important than the percentages of transversions (42.02%). The transition/transverse ratio is estimated at 1.4. Our results showed a predominance of T+A (53.72%) compared to C+G (46.28%). The nucleotide base most represented is guanine (G), which is valid on all

***Table 4**. Amino acid structuring with HOPE software*

Mutation	Schematic structures of the original (left) and the mutant (right) amino acid.	Structure 3D
N206N/I		
T336H		
Y345A		
T348T/N		
L357L/V		

N.B : In the 3D structure, the protein is colored gray, the side chains of both the wild-type and the mutant residue are colored green and red, respectively

***Table 5**. Frequency of amino acids*

	ES (%)	EM (%)	P-value
Ala	2.32	2.86	0.269
Cys	3.91	3.71	0.280
Asp	0.98	0.85	0.104
Glu	6.11	5.59	0.215
Phe	3.79	3.90	0.829
Gly	20.63	21.58	0.090
His	0.98	0.97	0.342
Ile	2.56	2.14	0.052*
Lys	3.17	4.49	0.015*
Leu	17.09	16.18	0.224
Met	6.23	6.03	0.403
Asn	1.47	1.32	0.028*
Pro	1.59	2.29	0.224
Gln	2.20	1.95	0.214
Arg	4.27	3.52	0.058*
Ser	1.95	1.60	0.224
Thr	1.83	2.29	0.935
Val	10.87	9.90	0.375
Trp	7.20	7.73	0.666
Tyr	0.85	1.10	0.892

*Amino acids with significant P-value

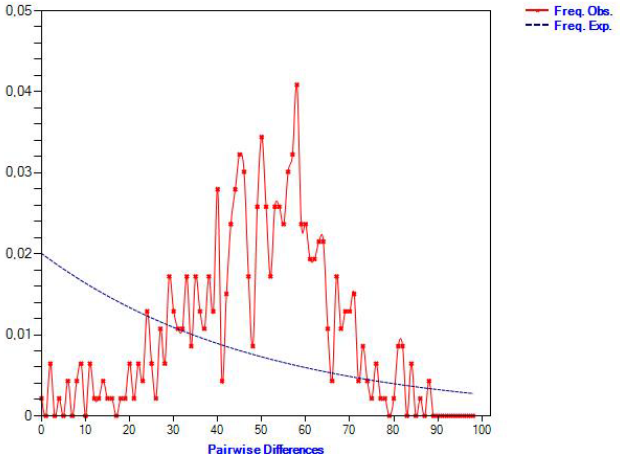


***Figure 1**. Mismatch distributions of malnourished children*

positions. Analysis of diversity and divergence indices revealed high haplotypic diversity (1000+/-0.013) and low nucleotide diversity (0.10545+/-0.00488).

Genetic differentiation between malnourished and healthy children

Table 7 presents the values of genetic distance and genetic differentiation. It appears that this distance is greater in malnourished children (d = 0.115) than in healthy children (d = 0.114). The distance between malnourished and healthy children is high (d = 0.118). The value of the degree of genetic differentiation (Fst) is significant.

Selection signature test

The selection signature test detection in malnourished children based on the allelic frequency spectrum indicates that Tajima's D demographic test is positive and non-significative (**Table 8**). The Fu's Fs test indicates a negative and significant value.

***Table 6**. Genetic diversity parameter of malnourished children*

Number of sites			**458**	
Monomorphic sites			300	
Polymorphic sites			158	
Singleton variable sites			42	
Parsimony informative sites			116	
Total number of mutations (Eta)			185	
Average number of nucleotide differences (k)			48.29644	
Rate of mutation (R)			1.36	
Haplotype diversity. Hd (Hd+/-SD)			1.000+/-0.013	
Nucleotide diversity (Pi+/-SD)			0.10545+/-0.00488	
Transition rate			57.98%	
Transverse rate			42.02%	
Tr/tv		1st position	2nd position	3rd position
	1.4	1	1.3	2.3
Nucleotide frequencies		1st position	2nd position	3rd position
T(U)	27.60	29.07	35.15	18.51
C	12.14	14.92	7.39	14.13
A	26.12	18.47	20.66	39.33
G	34.14	37.54	36.80	28.03

***Table 7**. Genetic distance (d) at intra and inter-population level and degree of differentiation (Fst)*

	Intra-population	**Inter-population**	**Fst (P-value)**
ES	0.114	0.118	0.0249 (0.0126)
EM	0.115		

***Table 8**. Neutrality tests for malnourished children*

	EM	P-value
Tajima's D	0.517	0.758
Fu's Fs	-4.563	0.029

***Table 9**. Values of SSDs and raggedness*

	EM	**P-value**
SSD	0.0069	0.35
Raggedness	0.0133	0.16

Distribution disparity analysis (mismatch distribution)

The analysis of the mismatch distribution curve under the assumption of a hypothesis population gives a multimodal aspect (**Figure 1**).

SSD and raggedness values under the population growth model for malnourished children are positive and not significant (see **Table 9**).

Discussion

The nucleotide variability analysis of mitochondrial cytochrome b (*MT-CYB*) was performed in malnourished Senegalese children aged 0–59 months to study the involvement of the *MT-CYB* gene in childhood malnutrition.

Genetic analysis of MutationSurveyor mutations revealed the presence of mutations in *MT-CYB* gene in malnourished Senegalese children (64.29%), as well as in healthy children (21.43%). Mutations in the *MT-CYB* gene are often sporadic and occur during embryogenesis, affecting a limited number of cells and resulting in tissue-specific phenotypes [27]. Mutations in *MT-CYB* in Complex III of the respiratory chain can lead to different pathologies such as stunting [12]. Of the variants obtained, 58.33% are non-synonymous mutations. It appears that over 100 false sense mutations have been reported in humans compared to the rCRS reference sequence [28]. All mutations listed on gnomAD were predicted to be benign compared to Leigh syndrome, with exception of the p.V356V/M mutation associated with Leber's hereditary optical neuropathy [29] .

Nine predictive tools that use various methods to predict pathogenic mutations evaluated synonymous polymorphisms located in the *MT-CYB* gene. Mutations p.N206N/I, p.T336H, p.Y345A, p.T348T/N, and p.L357L/V predicted pathogenic by at least five predictive software programs.

Mutations p.N206N/I and p.T336H were thus predicted to induce disease and affect the protein function and structure. Mutations p.Y345A and p.L357L/V have been predicted to cause deleterious changes in the function and structure of the protein but do not induce disease. The mutation p.T348T/N has been predicted pathogenic and induce malnutrition. These results demonstrate that the use of a combination of tools could adjust to program differences and improve the accuracy of the search for important polymorphisms, disease occurrence, or phenotypic variations [30].

The HOPE tool reinforces this hypothesis by demonstrating that the identified mutations can lead to a loss of hydrophobic interactions with other molecules on the surface of the protein or a loss of hydrogen bond at the core of the protein and will therefore disrupt the correct folding, thus affecting the protein function and structure. These pathogenic mutations are not referenced, and no literature exists on the function of these mutations in *MT-CYB*.

The transformation of nucleotide sequences into an amino acid sequence reported a change in frequency of a number of amino acids. In our study, a significant decrease in isoleucine (Ile), asparagine (Asn), and arginine (Arg) was observed in malnourished children. A study done in Malawi in 2016 showed a significant correlation between serum amino acid levels and stunting [31]. Indeed, Stunted children had a significant decrease in conditionally essential amino acids (arginine, glycine, glutamine), non-essential amino acids (asparagine, glutamate, serine) and 6 other sphingo lipids compared to children healthy [31]. Arginine is essential during growth and it can take on the characteristics of acid essential amine in certain situations [32]; while asparagine plays a role in protein biosynthesis [33].

A significant increase in lysine was observed in malnourished children. Indeed, lysine is an essential amino acid essential for growth and is necessary in the diet for the production of nitrogen [34].

Analysis of genetic diversity revealed that the genetic distance within the group of malnourished children was slightly higher than that of healthy children. However, the distance between malnourished and healthy children was elevated. Genetic variability of *MT-CYB* thus exists between malnourished and healthy children. Analysis of the genetic differentiation factor Fst revealed a significant difference in *MT-CYB* between malnourished and healthy children, which confirms the wide variation in inter-group distance.

Haplotypic diversity (Hd) represents the probability that two randomly sampled alleles are different, while nucleotide diversity (Pi) in malnourished children is defined as the average number of nucleotide differences per site in paired comparisons between DNA sequences [35]. The polymorphism of *MT-CYB* is highly marked in childhood malnutrition, particularly with a high haplotypic diversity (Hd) and a low nucleotide diversity (Pi), which implies that mutations of *MT-CYB* grow rapidly in malnutrition.

Neutrality tests of Tajima's D [36] and Fu's Fs [37] statistics for malnourished Senegalese children were performed. Those tests estimate the deviation from neutrality, which is based on the expectation of constant population size in the equilibrium mutation-derive [38]. Analysis of the molecular signature based on the neutrality test revealed a positive and non-significant Tajima's D. Indeed, a positive Tajima's D signifies a low level of polymorphism and a decrease in population size or balanced selection [38,39]. However, the statistically significant and negative Fs of Fu observed in malnourished children provides strong evidence of population expansion.

The mismatch distribution graph illustrated a multimodal shape showing a demographic equilibrium or a stable population. The raggedness SSD index under the population expansion model is not significant. This indicates that no difference exists between observed and simulated values and that the group of malnourished children is expanding demographically.

Conclusion

Malnutrition remains a public health problem with minimal genetic understanding. It is therefore necessary to know the genetic variability of certain genes likely to be affected by malnutrition. Our study used a population genetics approach to study the association of *MT-CYB* mutations with child malnutrition in Senegalese children under 5 years of age. Our results showed high variability in *MT-CYB* in malnourished children. Some of these mutations are silent, whereas others induce a change of amino acid. Of all the non-synonymous mutations, some were predicted by prediction software as pathogenic and therefore likely to induce malnutrition and affect protein function and structure in Senegalese children. The genetic distance between malnourished and healthy children appears to be high. Mutations in the *MT- CYB* gene are rapidly growing in malnutrition. A decrease in isoleucine (Ile), asparagine (Asn), and arginine (Arg) may be correlated with the risk of malnutrition. Neutrality tests have shown that the malnourished child group is expanding demographically. The results obtained should be evaluated in a wider sample. They pave the way for the study of the genetic diversity of child malnutrition, which until now has remained a scientific mystery due to focusing on other genes.

Conflicts of interest

The authors declare no conflict of interest.

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Ethics committee statement

Our study received approval from the UCAD Research Ethics Board under reference numbers: 0340/2018/CER/UCAD.

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