 doi:10.20944/preprints202210.0025.v1

Article

Mitochondrial-derived Peptide Single-nucleotide Polymorphisms Associated with Cardiovascular Complications in Type 2 Diabetes

Enrique García Gaona [1*], Alhelí García Gregorio [2*], Camila García Jiménez [3*], Mildred Alejandra López-Olaiz [4*], Paola Mendoza-Ramírez [5*], Daniel Fernandez-Guzman [6*], Laura Yareni Zuñiga [7*], María Guadalupe Sánchez-Parada [8,9], Ana Elizabeth González Santiago [8,9], Luis Miguel Román Pintos [7,9], Rolando Castañeda Arellano [8,9], Luis Daniel Hernández-Ortega [8,9], Arieh Roldán Mercado-Sesma [7,9], Felipe de Jesús Orozco-Luna [10] and Raúl C. Baptista-Rosas [7,9,11,*]

[1] Facultad de Medicina, Benemérita Universidad Autónoma de Puebla, México, enrique.garciaga@alumno.buap.mx, ORCID 0000-0003-3572-7536 (E.G.-G.)
[2] Facultad de Enfermería, Universidad Veracruzana, México; zs18005332@estudiantes.uv.mx, ORCID 0000-0002-3517-3062 (A.G.-G.)
[3] Facultad de Ciencias Médicas y Biológicas "Dr. Ignacio Chávez", Universidad Michoacana de San Nicolás de Hidalgo, México; 1593027a@umich.mx, ORCID 0000-0003-4101-8630 (C.G.-J.)
[4] Escuela de Nutrición, Universidad del Valle de Atemajac, México; milyolaiz@gmail.com ORCID 0000-0002-1929-346X (M.A.L.-O.)
[5] Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla, México; paola.mendozaramirez1@gmail.com ORCID 0000-0001-9368-260X (P.M.-R.)
[6] Escuela Profesional de Medicina Humana, Universidad Nacional de San Antonio Abad del Cusco, Cusco, Perú; danferguz@gmail.com ORCID 0000-0002-9441-1067 (D.F.-G.)
[7] Departamento de Ciencias de la Salud-Enfermedad como Proceso Individual. Centro Universitario de Tonalá, Universidad de Guadalajara, México; lauray.zuniga@academicos.udg.mx, ORCID 0000-0001-9462-5746 (L.Y.Z.); luis.roman@acaemicos.udg.mx, ORCID 0000-0002-5180-3810, (L.M.R.-P.); arieh.mercado@academicos.udg.mx, ORCID 0000-0002-9025-9328 (A.R.M.-S.)
[8] Departamento de Ciencias Biomédicas, Centro Universitario de Tonalá, Universidad de Guadalajara, México. maria.sparada@academicos.udg.mx ORCID 0000-0002-2583-9625 (M.G.S.-P.); ana.gonzalez@academicos.udg.mx ORCID 0000-001-6923-9771 (A.E.G.-S.); rolando.castaneda@academicos.udg.mx ORCID 0000-0001-6940-5899 (R. C. -A.);
luis.hortega@academicos.udg.mx ORCID 0000-0003-2285-4376 (L. D.H.-O.).
[9] Centro de Investigación Multidisciplinaria en Salud, Universidad de Guadalajara, México.
[10] Centro de Análisis de Datos y Supercómputo, Universidad de Guadalajara, México, felipe.orozco@academicos.udg.mx; ORCID 0000-0002-3712-9997 (F.J.O.-L.)
[11] Hospital General de Occidente, Secretaría de Salud Jalisco, México, raul.baptista@academicos.udg.mx; ORCID 0000-0002-0273-4740 (R.C.B.-R.)

Abstract: Since the discovery of mitochondrial-derived peptides (*MDP*), their participation in cellular metabolism is no longer considered as the sole function of the mitochondria, but importance was also attached to its role as a source of protective factors of metabolic stress. These peptides are encoded in the mitochondrial genome and translated into the mitochondria or cytoplasm, to signal within the cell or be released and bind to membrane receptors. The objective of this work was explored and compare the frequency of *MT-RNR1* and *MT-RNR2* variants in sequences obtained from T2D individuals and control population. 213 different mitochondrial polymorphisms previously reported in the literature associated with T2D and cardiovascular diseases were analyzed. We can found three variants in the *MT-RNR1* not related with *MOTS-c* coding sequence: m.1189T>C (rs28358571), m.1420T>C (rs111033356), and m.1438A>G (rs2001030); and secondly, three polymorphisms associated to *MT-RNR2* m.2667T>C (rs878870626) related to *humanin,* m.1811A>G (rs28358576) in *SHPL3* and m.3027T>C (rs199838004) in *SHPL6* associated with statistical differences between the T2D and control group. All these findings were previously related to cardiovascular complications in literature and, as far as we know, relating for the first time in diabetic patients.

Keywords: mitochondria; Type 2 diabetes; *MDP; MOTS-c; Humanin; SHLP*

 doi:10.20944/preprints202210.0025.v1

1. Introduction

Mitochondria play a central role in cellular energy metabolism and have essential functions, including the regulation of intracellular calcium, production and removal of reactive oxygen species, and regulation of cell death by apoptosis [1].

Mitochondrial dysfunction and oxidative stress are largely involved in the cellular senescence process observed in malignant neoplasms, aging, and different neurodegenerative and cardiovascular diseases [2]. Although heart failure is multi-factorial, some studies have described an increased risk in diabetic patients, even after therapeutic control measures and prophylaxis for coronary artery disease and hypertension [3]. Multiple reports have implicated mitochondrial injury as a major player in the pathophysiology of diabetic heart disease [4–8].

Metabolic syndrome, beyond hyperglycemia related to peripheral insulin resistance in Type 2 diabetes (T2D), has also been associated with other metabolic abnormalities, which usually coexist and are often related, such as the re-distribution of body fat leading to a predominance of abdominal obesity and dyslipidemia. These conditions are usually associated with an increased risk of developing cardiovascular diseases [9], and have thus become a public health problem with a significant economic and social impacts in modern societies in the both developed and developing world [10–15].

Previous analyses have shown that the interaction of genetic variants and environmental factors contribute to the increasing status of metabolic syndromes [16,17]. Several lines of evidence have indicated the roles of oxidative stress and mitochondrial dysfunction in the pathogenesis of aging, including age-related metabolic and neurodegenerative diseases [1,3,9,18–21]. However, the basic mechanisms underlying the pathogenesis of metabolic syndromes and cardiovascular complications remain largely unknown.

Since the discovery of mitochondrial-derived peptides (*MDPs*), participation in the cellular metabolism is no longer considered the sole function of the mitochondria, but importance has also been attached to its role as a source of protective factors against metabolic stress, whether autocrine, paracrine, or endocrine. These peptides are encoded in the mitochondrial genome and translated into the mitochondria or cytoplasm, either for signaling within the cell or to be released and bind to membrane receptors. As *MDPs* can be detected in the blood, they have recently attracted attention as possible biomarkers of diverse pathologies, including cardiovascular diseases [22].

One of the first *MDPs* described was the mitochondrial open reading frame of 12s rRNA-c (*MOTS-c*), between positions 642 and 1888 of the sequence located within 51 bp of the region that codes for the mitochondrial ribosomal small sub-unit on the *MT-RNR1* gene. *MT-RNR1* transcribes for a peptide consisting of 16 amino acids with a structure similar to adiponectin, which has been implicated in the regulation of insulin sensitivity and metabolic homeostasis, with a special main action in muscle tissue [23].

MOTS-c promotes carbohydrate metabolism by glucose clearance and, when administered exogenously, improves carbohydrate tolerance, and decreases insulin resistance in obese mice. In humans, decreases in circulating levels of MOTS-c have been associated with obesity, insulin resistance, T2D, chronic kidney disease, and endothelial dysfunction [22].

Some polymorphisms in mitochondrial DNA could impact the function of *MDPs.* It has been described that the variant m.1382A>C (rs111033358) in the coding region of *MOTS-c* causes a replacement of amino acids K14Q (replacement of lysine to glutamine) in the peptide product of the expression of MOTS-c, a situation associated with an increased risk of T2D in male patients, dependent on the physical activity of the individual. Studies in mice have suggested that this mutation leads to inactivation of MOTS-c, contributing to the risk of T2D [22].

In previous cross-sectional clinical trials, it was shown that the expression of MOTS-c is down-regulated and decreased levels are presented in patients with T2D, correlated with plasma levels of HbA1c. This evidence supports the idea that mitochondrial dysfunction contributes to glycemic dysregulation and metabolic defects in diabetic individuals

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 October 2022 doi:10.20944/preprints202210.0025.v1

[24]. In addition to the polymorphisms found in the reading frame of MT-RNR1 for this metabolism-regulating peptide, other *MDPs* have been associated with atherogenesis and dilated cardiomyopathy [25–31]. These data open the possibility regarding the relationship between these previously un-noticed sequences, which would have a great impact on the related pathophysiology; in particular, on the chronic complications described in the diabetic patient.

The objective of this work was to compare and explore the frequency of mitochondrial variants in the MT-RNR1 and MOTS-c regions in sequences obtained from T2D individuals and control population, in order to identify their relationship with cardiovascular complications.

2. Results

We identified 2663 complete mtDNA sequences from patients in the *NCBI Nucleotide* database, using the search criteria specified in the *Material and Methods* section and in supplementary figure S1, of which 1261 were sequences of individuals diagnosed with T2D and 1105 sequences were from control individuals. Most of the information was derived from the research of Li et al. (2014) (n = 2000), while the rest were from the works of Achilli et al. (2011) (n = 7), Soini et al. (2012) (n = 64), Tanaka et al. (2004) (n = 129), Loo et al. (2014) (n = 149), Maksum et al. (2010) (n = 5), and Vijaya et al. (2010) (n = 19) (Table S1) [32–37].

To determine the population origin, haplotyping results demonstrated different clades and sub-clades, broadly distributed in 37 haplogroups (Table 1). The most frequent group dominating the population with T2D was haplogroup H, with 502 individuals representing 39.8% of the population (Table S2), followed by haplogroup U, representing 12.7% (n = 161) of the population and haplogroups T, J, and K, who together represented 21.3% (n = 269) of the population. Haplogroups A, B, and C—usually associated with East Asian and Native American populations—corresponded to only 3.1% (n = 40) of individuals in the group with T2D.

Regarding the population with haplogroup H, the general results of the haplotyping are shown in Table S2. Haplogroup H was found to have the greatest number and presented the greatest diversity of clades (47 in total), with the most common being H1 and H2 (with 194 and 63 individuals, representing 38.6% and 12.5%, respectively).

Haplogroup U, with a population of 161 individuals representing 12.7% of the total diabetic population, was the next most prevalent group, with a diversity consisting of 8 different clades, although 60% of its population corresponded to clade U5 (Table Spl. 3).

Regarding haplogroup T, consisting of 108 individuals representing 8.5% of the diabetic population studied (Table S4), was structured into 2 clades, with most distributed in T2 (78.7%, n = 103).

Uncommon exotic sub-clades were found in haplotypes X, Y, and Z, together representing 1.8% of the total (Table Spl. 5). Haplogroups L4, L3 associated with the Afro-descendant population [38] were only found in three individuals, contributing only 0.2%, making it the least-represented haplogroup in the sample.

As for the two populations studied, most of the *MT-RNR-1* polymorphisms were found in all analyzed sequences, between m.663 A>G and m.1438A>G.

Table 1. Frequency distribution of the different haplogroups in diabetic and control cases.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 October 2022 doi:10.20944/preprints202210.0025.v1

Macrohaplogroup	[ALL] N=2366 (100%)	Controls N=1105 (46.7%)	T2D N=1261 (53.3%)
Haplogroups L			
L4	1 (0.08)	0 (0.00)	1 (0.08)
L3	2 (0.16)	0 (0.00)	2 (0.16)
Haplogroups M			
M (includes Q)	10 (0.86)	6 (0.54)	4 (0.32)
M7	48 (4.02)	19 (1.72)	29 (2.30)
M8 (includes C, Z)	9 (0.71)	0 (0.00)	9 (0.71)
M9 (includes E)	22 (1.80)	5 (0.45)	17 (1.35)
G	10 (0.84)	4 (0.36)	6 (0.48)
D	64 (5.32)	22 (1.99)	42 (3.33)
Haplogroups N			
N1 (includes I)	65 (5.52)	33 (2.99)	32 (2.54)
N2 (includes W)	33 (2.82)	18 (1.63)	15 (1.19)
N9 (includes Y)	16 (1.36)	8 (0.72)	8 (0.63)
A	13 (1.06)	3 (0.27)	10 (0.79)
X	31 (2.57)	10 (0.90)	21 (1.67)
Haplogroups R			
R (includes P)	7 (0.57)	1 (0.09)	6 (0.48)
R0 (includes HV, H, V)	1070 (90.36)	492 (44.52)	578 (45.84)
JT (includes J, T)	411 (34.96)	211 (19.10)	200 (15.86)
R9 (includes F)	43 (3.62)	19 (1.72)	24 (1.90)
B	49 (4.13)	22 (1.99)	27 (2.14)
U (includes K)	462 (39.23)	232 (21.00)	230 (18.24)

T2D: Type 2 diabetes.

In *MOTS-c*, the most frequent variant in both populations was m.1382A>C. In our analysis, *Humanin* was the most frequent gene with variation in both populations (T2D and controls), in the position m.2706A>G. In *SHLP2*, the frequent variants between both groups were m.2141 T>C and m.2158T>C. Regarding *SHLP3*, the most significant variants were between the polymorphisms m.1719G>A and 1811A>G. In S*HLP4*, the variant with the highest frequency in the group of diabetic individuals was m.2442T>C, compared to the control group (in which no frequency was observed). In SHLP5, the most frequent variant was m.2850 T>C and, finally, in *SHLP6*, the variants m.3010G>A and m.3027T>C were the most frequent in both groups of populations. Regarding *SHLP1*, no frequent variants were found between both populations.

In the control group, a lower number of polymorphisms in mitochondrial genome sequences were found; meanwhile, in the group of diabetic individuals, they were found more frequently (Table S6).

The coordinates of the *MT-RNR1* gene, located between positions 648 and 1601 with a sequence length of 954 bp, and the position of *MOTS-c*, between positions 1343 and 1393 with a sequence length of 51 bp, were established (Figure 1). In the *MT-RNR1* region, we identified 142 variants, of which the most frequent were m.750A>G (rs2853518) and m.1438A>G (rs2001030) between the diabetic population and the control group (Table 1).

Six variants differed between the T2D and control groups in the *MT-RNR1* gene, which were not associated to *MOTS-c* variants (Table 2).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 October 2022 doi:10.20944/preprints202210.0025.v1

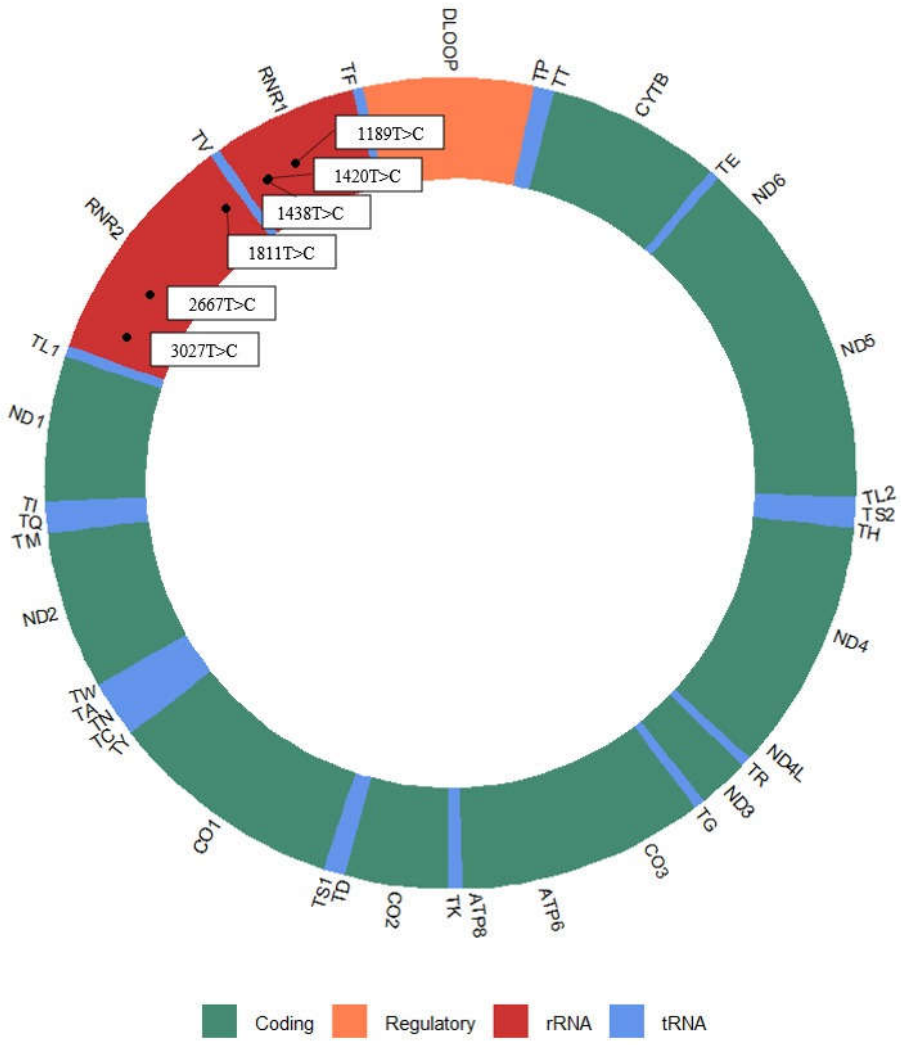

Figure 1. Position of polymorphisms associated with T2D in the human mitochondrial genome.

In the present investigation, 213 different mitochondrial polymorphisms previously reported in the literature as being associated with T2D [25–29,31] and cardiovascular diseases [26,30] were analyzed. In both groups, the most prevalent polymorphism was m.1438A>G (Table 2).

Regarding the identification of variants related to cardiovascular risk, three variants in the *MT-RNR1* not related to the *MOTS-c* coding sequence were identified: m.1189T>C (rs28358571), m.1420T>C (rs111033356), and m.1438A>G (rs2001030). These presented significant statistical differences between the T2D and control groups.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 October 2022 doi:10.20944/preprints202210.0025.v1

Table 2. Polymorphisms in *MDP* sequences related to T2D and controls.

mtDNA region Genes (positions)	Polymorphism	*NCBI dbSNP* ID	Type 2 diabetes n=1,261	Controls n=1,105	Odds ratio (CI95%)	*p*
MT-RNR1 (648-1,601)						
	m.1189T>C	rs28358571	52(4.12)	68(5.39)	0.66 (0.46-0.96)	0.024*
	m.1420T>C	rs111033356	11(0.87)	1(0.09)	9.72 (1.89-177.74)	0.007*
	m.1438A>G	rs2001030	1,142(90.56)	1,071(96.92)	0.41 (0.26-0.61)	0.000*
MT-RNR2 Humanin (2,633-2,707)	m.2667T>G#	rs878870626	9(0.71)	1(0.09)	7.94 (1.49-146.47)	0.019*
SHLP3 (1,703-1,819)	m.1811A>G	rs28358576	121(9.59)	151(11.97)	0.67 (0.52-0.87)	0.001*
SHLP6 (2,990-3,052)	m.3027T>C	rs199838004	18(1.42)	6(0.54)	2.66 (1.11-7.37)	0.03*

The results in type 2 diabetes and controls are n(%)
*X^2 test with one liberty degree, $p < 0.05$
#On the analysis only found *N* (any Nucleotide *A, C, G,* or *T* with non-gap).

3. Discussion

The heart is one of the most energy-consuming organs in the human body; therefore, a subtle energy deficit can rapidly induce contractile dysfunction. The uninterrupted generation of ATP depends on the continuous supply of oxygen and fuel substrates, as well as the integrity of oxidative phosphorylation, which produces virtually all of the ATP in the heart [39,40]. Although the heart can change its substrate preference, depending on the particular situation (i.e., related to the workload, oxygen supply, and hormones), its main energy substrate is fatty acids, estimated at between 60–70% of the total consumption. For this reason, due to their high energy demand, cardiomyocytes have a relatively larger number of mitochondria, compared to other cells [41,42].

Due to insulin resistance in T2D, the diabetic heart presents a higher rate of fatty acid oxidation. Previous studies have shown increased expression of the nuclear receptor transcription factors PPARα/δ/β. PPARs are important transcriptional regulators of fatty acid uptake and oxidation. In fact, PPARα regulates most of the enzymes involved in fatty acid oxidation. PPARα-knockout mice have been shown to have a reduced rate of fatty acid oxidation. Interestingly, over-expression of cardiac-specific PPARα significantly reduced enzymes involved in mitochondrial oxidative phosphorylation [43–45].

Experimental insights into genes for myocardial fatty acid utilization in both human and animal models, considering cardiac myofibers with progressive heart failure, described reduced fatty acid oxidation with a greater dependence on glucose metabolism in patients with compensated dilated cardiomyopathy [46]. As heart failure progresses, myocardial insulin resistance develops [47,48], further compromising the versatility of substrate use and increasing metabolic stress on the heart. Importantly, chronic heart failure patients with decreased systemic insulin sensitivity have a worse prognosis [49–52].

In particular, increased β-oxidation of fatty acids can be detrimental, as it requires more oxygen and generates a large amount of ROS. Interestingly, diabetic animals showed reduced cardiac efficiency, with increased myocardial oxygen consumption (VO_2) associated with increased fatty acid β-oxidation [53–59]. The increased demand for oxidant fatty acids and reduced cardiac efficiency may contribute to contractile dysfunction in the diabetic heart. Furthermore, altered substrate flexibility and change in oxygen consumption may potentially contribute to increased mortality after ischemic injury in diabetic patients [60,61].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 October 2022 doi:10.20944/preprints202210.0025.v1

Mitochondrial oxygen consumption is normally closely related to ATP synthesis (through the electron transport chain). The energy produced during electron transfer is used to create an electrochemical gradient by pumping protons from the mitochondrial matrix into the inter-membrane space. These protons generally re-enter the matrix by ATP synthase/complex V (proton pump) and generate ATP from ADP. However, it is sometimes possible for protons to bypass the ATPase system and re-enter the matrix through the use of uncoupling proteins, such as UCP-1, 2, 3, 4, and 5 [55,62,63].

This bypass system results in oxygen consumption that is not coupled to ATP production [64–66]. Mitochondrial uncoupling in experimental mouse heart models has previously been shown [67], with increased respiration in the presence of oligomycin (an ATP synthase inhibitor) and increased leakage of protons from isolated cardiac mitochondria. Adding guanosine diphosphate, an inhibitor of UCPs, resulted in restoration of proton leak, strongly suggesting that the increased uncoupling was UCP-mediated. A second potential mediator of mitochondrial uncoupling is the adenine nucleotide translocator [68–72]. Various studies in animal and human models have frequently demonstrated mitochondrial damage at different levels in the diabetic myocardium [1,19,73–80].

Dysfunctional mitochondria can cause increased ROS production and the release of factors that favor cell death, such as cytochrome C (an apoptosis-inducing factor) and Smac/DIABLO (the second activator of caspases) [21,74,81,82], and the induction of pyroptosis, characterized by activation of the inflammasome through the activation of caspase 1 and pro-inflammatory cytokines [83–85], although this sub-type of programmed cell death has previously been associated with infection by intracellular pathogens. Various ROS scavengers or antioxidants reduced cardiomyocyte death and attenuate diabetic heart injury in experimental animal models [81,86]. All of these cell destruction systems associated with mitochondrial dysfunction are apparently controlled by mechanisms related to non-coding RNA and epigenetic regulation [2,39,83,87,88].

Recent evidence has suggested that cardiac dysfunction in patients with T2D is related to metabolic abnormalities and more often associated with mitochondrial dysfunction. T2D and obesity—the major metabolic disorders—are characterized by elevated levels of circulating free fatty acids, resulting in increased cardiac fatty acid uptake, storage, and metabolism [44,89–95].

T2D has been reported to be heritable in 72% of individuals, with a higher incidence in women [96]. Notably, although there are forms of diabetes that are directly caused by mutations in mtDNA, these are extremely rare. Genetic analyses have revealed that mtDNA polymorphisms contribute to an increased risk of T2D in populations such as Asian and European populations, as these polymorphisms vary according to the population group analyzed [97].

Maternally inherited mtDNA consists of 16,569 base pairs in the average human and encodes 13 respiratory chain proteins, while the more than 70 remaining sub-units are encoded by the nuclear genome. In addition, mtDNA encodes 2 ribosomal RNAs and 22 tRNAs required for mitochondrial protein synthesis. mtDNA is more prone to mutation than nuclear DNA, resulting in variations that are used as a tool in population genetics, biological anthropology and forensic genetics. Certain polymorphisms mark branching points in the evolutionary phylogeny of the various human groups and define specific population haplogroups with common origins. Certain mtDNA haplogroups have been associated with susceptibility to various diseases, but also with beneficial traits such as longevity. Certain mtDNA polymorphisms have been postulated to decrease or increase the permeability of the mitochondrial respiratory chain and the production of harmful reactive oxygen species. In addition, some slightly harmful polymorphisms can produce subtle changes in the translation, replication, or production of mtDNA regulatory elements [33].

The segregation of mtDNA variants during cell division in individuals who inherit a low proportion from their mother and who—depending on epigenetic and environmental factors—favor their replication and the proportion within the heteroplasmic diversity of copies with polymorphisms associated with the disease, may cross a critical threshold that

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 October 2022 doi:10.20944/preprints202210.0025.v1

could be a risk factor for complex diseases such as T2D. It has been proposed that recent evolutionary "bottlenecks" may lead to an over-representation of minor mtDNA alleles in the population and the emergence of risk factors related to population-specific diabetes [98].

Preliminary studies using machine learning and artificial intelligence methodologies with various cardiac risk biomarkers in T2D patients have determined statistically significant relationships between single-nucleotide variants in mtDNA that were associated with diabetes [110], specifically the polymorphism m.73A>G (rs869183622) located in variable segment 2 of the control region (MT-HV2), as well as m.16126T>C (rs147029798) and m.16362T>C (rs62581341), both located in the variable segment of the control region (MT-HV1).

Other variants associated with coding regions of the mitochondrial genome have been described as important in various metabolic mechanisms, through the expression of small peptides of less than 100 amino acids, of which eight different variants have been identified so far, which are all related to each other. *MDP*s, as they have been called, are susceptible to metabolic stress and promote an adaptive response to it. They have been studied in the context of metabolic functions and in association with cardiovascular diseases [99–101]. These include *MOTS-c* (mitochondrial open reading frame of the twelve rRNA-c) and the currently re-classified *MT-RNR1* gene, the former of which transcribes for a peptide consisting of 16 amino acids, with a structure similar to adiponectin, transcribed from a functional open reading frame of 51 bp located in the region that codes for the mitochondrial ribosomal small sub-unit (between positions 642 and 1888 of the sequence). Among its multiple functions, it has been implicated in the regulation of insulin sensitivity and metabolic homeostasis, with a special main action in muscle tissue [101].

By inducing over-expression of *MOTS-c* in cell cultures, this peptide promotes carbohydrate metabolism by glucose clearance and, when administered exogenously, improves carbohydrate tolerance and decreases insulin resistance in obese mice. In humans, decreased circulating levels of MOTS-c have been associated with obesity, insulin resistance, T2D, chronic kidney disease, and endothelial dysfunction [22].

In the *MT-RNR1* gene, the m.1189T>C variant (with identification code rs28358571 in *NCBI dbSNP* database *https://www.ncbi.nlm.nih.gov/snp/*) have previously been classified as benign and not related to any disease [102–104]; furthermore, m.1420T>C (as rs111033356) has also been classified as benign [34,105–108], while the m.1438A>G variant (with identification code rs2001030) has been shown to produce an increased risk of T2D development in Japanese populations [109,110].

With respect to the variants found in *MT-RNR2*, m.1438A>G was found to have a statistical difference between group, indicated as the only polymorphism found in our research related to previous reports. Otherwise, m.2667T>G (as rs878870626), m.1811A>G (as rs28358576) and m.3027T>C (as rs199838004) found in *MT-RNR2* gene have not been reported previously in *ClinVar*, and they have not been related with any known human diseases.

4. Materials and Methods

4.1. Study design and Data processing

An analytical cross-sectional study was designed. Based on complete mitochondrial genome sequences available in the *NCBI Nucleotide* database (*https://www.ncbi.nlm.nih.gov/nuccore*), to identify sequences we used Booleans and keywords in the following search string: (*(015400[SLEN]:016700[SLEN]) AND Homo [Organism] OR Homo sapiens [organism]) AND mitochondrion [FILT] AND ("Type 2 diabetes" OR "non-insulin dependent diabetes" OR diabetes OR T2D*). In this way, we filtered and selected complete mitochondrial sequences. Once the sequences were identified in the database, the metadata associated with each sequence was explored, in order to validate the place of origin, which should correspond to the *Homo sapiens* species, having a length of 16569 ± 10 base pairs, and whether the sequences corresponded to patients with diabetes or to

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 October 2022 doi:10.20944/preprints202210.0025.v1

controls (i.e., patients without comorbidities who did not have diabetes), eliminating those that do not correspond to inclusion criteria. In case of doubts regarding any metadata, the authors contacted those responsible for obtaining the sequences.

4.2. Bioinformatic Analysis

For identification of haplogroups and polymorphisms of the selected sequences, we used the *Genebank* sequence number to determine the haplotype, while polymorphisms were identified using *MITOMASTER* (*http://www.mitomap.org/foswiki/bin/view/MITOMASTER*), from which a database was built with the identified polymorphisms. The criteria for the classification of the different haplogroups can be seen in more detail in the Phylotree database (*http://www.phylotree.org/*) [111]. In addition, for the construction of the database, the alignment of sequences was carried out in the UCSC Genome Browser (*https://genome.ucsc.edu*), in order to analyze the presence of the polymorphisms of interest in each of the sequences manually, recording deletions, insertions, and substitutions when compared to the *rCRS* reference sequence.

The methodology used to obtain and process the sequences is summarized in Supplementary Figure S1. To explore the coding regions of *MDP*, the positions in the genomic coordinates of the region of interest were established using the sequence information available in *GenBank* (*NCBI Reference Sequence*) with ID number NC_012920.1 for *MT-RNR1* and in the European Nucleotide Archive with ID number KP715230 for *MOTS-c*. The files were downloaded in *FASTA* format and aligned with the reference genome using the *BLAT* tool in the *UCSC Genome Browser*. All the genomic sequences obtained in the search were aligned and analyzed using the genomic browser at the positions of the *MT-RNR1* gene, which transcribes for 12sRNA and is located at coordinates 648–1601, where *MOTS-c* was explored at position 1343–1393; and, for the *MT-RNR2* gene, which transcribes for 16s RNA and is located at coordinates 1669–3231, *HUMANIN* was explored at position 2633–2707, *SHLP1* at 2485–2559, *SHLP2* at 2088–2168, *SHLP3* at 1703–1819, SHLP4 at 2442–2522, *SHLP5* at 2780–2854, and *SHLP6* at 2990–3052.

The variants associated with cardiovascular risk in diabetic patients described by Pinti et al. (2019) [110] and the obtained information were combined with the information on 52 different variants related to cardiovascular diseases published by Dabravolski et al. (2022) [28] and Volobuena et al. (2019) [26].

4.3. Statistical analysis

For the statistical analysis, we used *R Studio* https://www.rstudio.com/ and the *R* software version 4.2.1 https://cran.r-Project.org/. For the descriptive analysis, the polymorphisms were presented as absolute and relative frequencies. For bivariate analysis, we evaluated the association of polymorphisms with the presence or absence of T2D through the Chi-square test. To assess the strength of association, we used generalized linear models of the Poisson family with the log link function, and crude prevalence ratios and odds ratios with their respective 95% confidence intervals (95% CIs) were calculated. Values of $p < 0.05$ were considered statistically significant.

5. Conclusions

MDP variants in T2D patients were associated with statistical differences, when compared to non-diabetic individuals. In the *MT-RNR1* gene, we found three variants not associated with *MOTS-c*; while, in *MT-RNR2,* the polymorphism m.2667T>C related to *humanin* and m.3027T>C in *SHPL6* were associated with T2D. Of the 213 different mitochondrial polymorphisms previously reported in the literature to be associated with T2D and cardiovascular diseases, in our analysis, we found that most prevalent single-nucleotide variant was m.1438A>G; however, m.1420T>C had a higher association, based on the odds ratio estimation. We believe that, in the end, it is not possible to conclude on the existence of either a relationship or an association, due to the small sample (n) used in our study. Other main questions to explore include whether mitochondrial polymorphisms in

diabetic patients associated with these cardiovascular risk variants in T2D individuals are a cause or an effect.

Supplementary Materials: Figure S1. Methodology and bioinformatics pipeline; Table S1: Variants and related references; Table S2, Haplogroup H analysis; Table S3, Haplogroup U analysis; Table S4, Haplogroup T analysis; Table S5, Haplogroups X, Y, and Z analysis; Table S6, Polymorphisms comparative analysis.

Author Contributions: Researchers working at the Pacific Scientific and Technological Research Summer https://programadelfin.org.mx 2019 (M.A.L.-O. and P.M.-R.), 2020 (M.A.L.-O.), 2021 (P.M.-R, E.G.G., A.G.G., C.G.J., and D.F.-G.), and 2022 (E.G.G., A.G.G., C.G.J., and L.Y.Z.) should be considered the first authors of the article. Conceptualization, R.C.B.-R.; Data curation, A.G.G., C.G.J., and E.G.G.; Formal analysis, D.F.-G., E.G.G., and R.C.B.-R.; Investigation, E.G.G., A.G.G., C.G.J., M.A.L.-O., P.M.-R., and D.F.-G.; Resources, L. D.H.-O ; Methodology, E.G.G., A.G.G., C.G.J., M.A.L.-O., P.M.-R., and D.F.-G.; Project administration, A.R.M.-S. and R.C.B.-R.; Software, D.F.-G. and F.J.O.-L.; Supervision, A.R.M.S. and R.C.B.-R.; Validation, M.A.L.-O, P.M.-R. and R. C. -A.; writing—review and editing, L.Y.Z, M.G.S.-P., A.E.G.S., L.M.R.P., R.C.A., L.D.H.O., A.R.M.-S., and R.C.B.-R. All authors have read and agreed to the published version of the manuscript.

Funding: None.

Institutional Review Board Statement: Not applicable. This research does not involve humans or animals.

Informed Consent Statement: Not applicable.

Data Availability Statement: The identification for access to the sequences used for this work are fully referred to in Table supplementary S1. and are available from the *NCBI Nucleotide* database (*https://www.ncbi.nlm.nih.gov/nuccore*).

Acknowledgments: We appreciate and acknowledge the kind help of Anders Albretchsen at University of Copenhagen (Denmark), Rasmus Nielsen at University of California Berkeley (U.S.A), Mikkel Schierup Aarhus at University (Denmark), and Shengting Li at MGI-Tech Co (Shenzhen, China) for t sequences identification.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Verma, S.K.; Garikipati, V.N.S.; Kishore, R. Mitochondrial Dysfunction and Its Impact on Diabetic Heart. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **2017**, *1863*, 1098–1105, doi:10.1016/j.bbadis.2016.08.021.
2. Parker, A.M.; Tate, M.; Prakoso, D.; Deo, M.; Willis, A.M.; Nash, D.M.; Donner, D.G.; Crawford, S.; Kiriazis, H.; Granata, C.; et al. Characterisation of the Myocardial Mitochondria Structural and Functional Phenotype in a Murine Model of Diabetic Cardiomyopathy. *Front. Physiol.* **2021**, *12*, 672252, doi:10.3389/fphys.2021.672252.
3. Sharma, R.; Reinstadler, B.; Engelstad, K.; Skinner, O.S.; Stackowitz, E.; Haller, R.G.; Clish, C.B.; Pierce, K.; Walker, M.A.; Fryer, R.; et al. Circulating Markers of NADH-Reductive Stress Correlate with Mitochondrial Disease Severity. *Journal of Clinical Investigation* **2021**, *131*, e136055, doi:10.1172/JCI136055.

4. Kubli, D.A.; Gustafsson, Å.B. Unbreak My Heart: Targeting Mitochondrial Autophagy in Diabetic Cardiomyopathy. *Antioxidants & Redox Signaling* **2015**, *22*, 1527–1544, doi:10.1089/ars.2015.6322.
5. Sivitz, W.I.; Yorek, M.A. Mitochondrial Dysfunction in Diabetes: From Molecular Mechanisms to Functional Significance and Therapeutic Opportunities. *Antioxidants & Redox Signaling* **2010**, *12*, 537–577, doi:10.1089/ars.2009.2531.
6. Sung, H.J.; Ma, W.; Wang, P.; Hynes, J.; O'Riordan, T.C.; Combs, C.A.; McCoy, J.P.; Bunz, F.; Kang, J.-G.; Hwang, P.M. Mitochondrial Respiration Protects against Oxygen-Associated DNA Damage. *Nat Commun* **2010**, *1*, 5, doi:10.1038/ncomms1003.
7. Duncan, J.G. Mitochondrial Dysfunction in Diabetic Cardiomyopathy. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* **2011**, *1813*, 1351–1359, doi:10.1016/j.bbamcr.2011.01.014.
8. Galloway, C.A.; Yoon, Y. Mitochondrial Dynamics in Diabetic Cardiomyopathy. *Antioxidants & Redox Signaling* **2015**, *22*, 1545–1562, doi:10.1089/ars.2015.6293.
9. Bhatti, J.S.; Bhatti, G.K.; Reddy, P.H. Mitochondrial Dysfunction and Oxidative Stress in Metabolic Disorders — A Step towards Mitochondria Based Therapeutic Strategies. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **2017**, *1863*, 1066–1077, doi:10.1016/j.bbadis.2016.11.010.
10. Bello-Chavolla, O.Y.; Rojas-Martinez, R.; Aguilar-Salinas, C.A.; Hernández-Avila, M. Epidemiology of Diabetes Mellitus in Mexico. *Nutr Rev* **2017**, *75*, 4–12, doi:10.1093/nutrit/nuw030.
11. Mapa-Tassou, C.; Katte, J.-C.; Mba Maadjhou, C.; Mbanya, J.C. Economic Impact of Diabetes in Africa. *Curr Diab Rep* **2019**, *19*, 5, doi:10.1007/s11892-019-1124-7.
12. Urakami, T.; Kuwabara, R.; Yoshida, K. Economic Impact of Diabetes in Japan. *Curr Diab Rep* **2019**, *19*, 2, doi:10.1007/s11892-019-1122-9.
13. Khanijahani, A.; Akinci, N.; Iezadi, S.; Priore, D. Impacts of High-Deductible Health Plans on Patients with Diabetes: A Systematic Review of the Literature. *Primary Care Diabetes* **2021**, *15*, 948–957, doi:10.1016/j.pcd.2021.07.015.
14. Lago - Peñas, S.; Rivera, B.; Cantarero, D.; Casal, B.; Pascual, M.; Blázquez-Fernández, C.; Reyes, F. The Impact of Socioeconomic Position on Non-Communicable Diseases: What Do We Know about It? *Perspect Public Health* **2021**, *141*, 158–176, doi:10.1177/1757913920914952.
15. Tinajero, M.G.; Malik, V.S. An Update on the Epidemiology of Type 2 Diabetes. *Endocrinology and Metabolism Clinics of North America* **2021**, *50*, 337–355, doi:10.1016/j.ecl.2021.05.013.
16. Giglio, R.V.; Stoian, A.P.; Patti, A.M.; Rizvi, A.A.; Sukhorukov, V.; Ciaccio, M.; Orekhov, A.; Rizzo, M. Genetic and Epigenetic Biomarkers for Diagnosis, Prognosis and Treatment of Metabolic Syndrome. *CPD* **2021**, *27*, 3729–3740, doi:10.2174/1381612827666210412145915.
17. Wu, Q.; Li, J.; Sun, X.; He, D.; Cheng, Z.; Li, J.; Zhang, X.; Xie, Y.; Zhu, Y.; Lai, M. Multi-Stage Metabolomics and Genetic Analyses Identified Metabolite Biomarkers of Metabolic Syndrome and Their Genetic Determinants. *eBioMedicine* **2021**, *74*, 103707, doi:10.1016/j.ebiom.2021.103707.
18. Jha, S.K.; Jha, N.K.; Kumar, D.; Ambasta, R.K.; Kumar, P. Linking Mitochondrial Dysfunction, Metabolic Syndrome and Stress Signaling in Neurodegeneration. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **2017**, *1863*, 1132–1146, doi:10.1016/j.bbadis.2016.06.015.
19. Kruse, R.; Sahebekhtiari, N.; Højlund, K. The Mitochondrial Proteomic Signatures of Human Skeletal Muscle Linked to Insulin Resistance. *IJMS* **2020**, *21*, 5374, doi:10.3390/ijms21155374.
20. Chella Krishnan, K.; Vergnes, L.; Acín-Pérez, R.; Stiles, L.; Shum, M.; Ma, L.; Mouisel, E.; Pan, C.; Moore, T.M.; Péterfy, M.; et al. Sex-Specific Genetic Regulation of Adipose Mitochondria and Metabolic Syndrome by Ndufv2. *Nat Metab* **2021**, *3*, 1552–1568, doi:10.1038/s42255-021-00481-w.
21. Parker, A.M.; Tate, M.; Prakoso, D.; Deo, M.; Willis, A.M.; Nash, D.M.; Donner, D.G.; Crawford, S.; Kiriazis, H.; Granata, C.; et al. Characterisation of the Myocardial Mitochondria Structural and Functional Phenotype in a Murine Model of Diabetic

Cardiomyopathy. *Front. Physiol.* **2021**, *12*, 672252, doi:10.3389/fphys.2021.672252.

22. Merry, T.L.; Chan, A.; Woodhead, J.S.T.; Reynolds, J.C.; Kumagai, H.; Kim, S.-J.; Lee, C. Mitochondrial-Derived Peptides in Energy Metabolism. *American Journal of Physiology-Endocrinology and Metabolism* **2020**, *319*, E659–E666, doi:10.1152/ajpendo.00249.2020.
23. Wu, Y.; Sun, L.; Zhuang, Z.; Hu, X.; Dong, D. Mitochondrial-Derived Peptides in Diabetes and Its Complications. *Front. Endocrinol.* **2022**, *12*, 808120, doi:10.3389/fendo.2021.808120.
24. Ramanjaneya, M.; Bettahi, I.; Jerobin, J.; Chandra, P.; Abi Khalil, C.; Skarulis, M.; Atkin, S.L.; Abou-Samra, A.-B. Mitochondrial-Derived Peptides Are Down Regulated in Diabetes Subjects. *Front. Endocrinol.* **2019**, *10*, 331, doi:10.3389/fendo.2019.00331.
25. Sobenin, I.A.; Sazonova, M.A.; Postnov, A.Y.; Bobryshev, Y.V.; Orekhov, A.N. Changes of Mitochondria in Atherosclerosis: Possible Determinant in the Pathogenesis of the Disease. *Atherosclerosis* **2013**, *227*, 283–288, doi:10.1016/j.atherosclerosis.2013.01.006.
26. Volobueva, A.; Grechko, A.; Yet, S.-F.; Sobenin, I.; Orekhov, A. Changes in Mitochondrial Genome Associated with Predisposition to Atherosclerosis and Related Disease. *Biomolecules* **2019**, *9*, 377, doi:10.3390/biom9080377.
27. Miller, B.; Kim, S.-J.; Kumagai, H.; Mehta, H.H.; Xiang, W.; Liu, J.; Yen, K.; Cohen, P. Peptides Derived from Small Mitochondrial Open Reading Frames: Genomic, Biological, and Therapeutic Implications. *Experimental Cell Research* **2020**, *393*, 112056, doi:10.1016/j.yexcr.2020.112056.
28. Dabravolski, S.A.; Nikiforov, N.G.; Starodubova, A.V.; Popkova, T.V.; Orekhov, A.N. The Role of Mitochondria-Derived Peptides in Cardiovascular Diseases and Their Potential as Therapeutic Targets. *IJMS* **2021**, *22*, 8770, doi:10.3390/ijms22168770.
29. Schiano, C.; Franzese, M.; Geraci, F.; Zanfardino, M.; Maiello, C.; Palmieri, V.; Soricelli, A.; Grimaldi, V.; Coscioni, E.; Salvatore, M.; et al. Machine Learning and Bioinformatics Framework Integration to Potential Familial DCM-Related Markers Discovery. *Genes* **2021**, *12*, 1946, doi:10.3390/genes12121946.
30. Dabravolski, S.A.; Khotina, V.A.; Sukhorukov, V.N.; Kalmykov, V.A.; Mikhaleva, L.M.; Orekhov, A.N. The Role of Mitochondrial DNA Mutations in Cardiovascular Diseases. *IJMS* **2022**, *23*, 952, doi:10.3390/ijms23020952.
31. Rochette, L.; Rigal, E.; Dogon, G.; Malka, G.; Zeller, M.; Vergely, C.; Cottin, Y. Mitochondrial-Derived Peptides: New Markers for Cardiometabolic Dysfunction. *Archives of Cardiovascular Diseases* **2022**, *115*, 48–56, doi:10.1016/j.acvd.2021.10.013.
32. Achilli, A.; Olivieri, A.; Pala, M.; Hooshiar Kashani, B.; Carossa, V.; Perego, U.A.; Gandini, F.; Santoro, A.; Battaglia, V.; Grugni, V.; et al. Mitochondrial DNA Backgrounds Might Modulate Diabetes Complications Rather than T2DM as a Whole. *PLoS ONE* **2011**, *6*, e21029, doi:10.1371/journal.pone.0021029.
33. Soini, H.K.; Moilanen, J.S.; Finnila, S.; Majamaa, K. Mitochondrial DNA Sequence Variation in Finnish Patients with Matrilineal Diabetes Mellitus. *BMC Res Notes* **2012**, *5*, 350, doi:10.1186/1756-0500-5-350.
34. Tanaka, M.; Cabrera, V.M.; González, A.M.; Larruga, J.M.; Takeyasu, T.; Fuku, N.; Guo, L.-J.; Hirose, R.; Fujita, Y.; Kurata, M.; et al. Mitochondrial Genome Variation in Eastern Asia and the Peopling of Japan. *Genome Res.* **2004**, *14*, 1832–1850, doi:10.1101/gr.2286304.
35. Loo, J.-H.; Trejaut, J.A.; Yen, J.-C.; Chen, Z.-S.; Ng, W.-M.; Huang, C.-Y.; Hsu, K.-N.; Hung, K.-H.; Hsiao, Y.; Wei, Y.-H.; et al. Mitochondrial DNA Association Study of Type 2 Diabetes with or without Ischemic Stroke in Taiwan. *BMC Res Notes* **2014**, *7*, 223, doi:10.1186/1756-0500-7-223.
36. Li, S.; Besenbacher, S.; Li, Y.; Kristiansen, K.; Grarup, N.; Albrechtsen, A.; Sparsø, T.; Korneliussen, T.; Hansen, T.; Wang, J.; et al. Variation and Association to Diabetes in 2000 Full MtDNA Sequences Mined from an Exome Study in a Danish Population. *Eur J Hum Genet* **2014**, *22*, 1040–1045, doi:10.1038/ejhg.2013.282.
37. Vijaya Padma, V.; Anitha, S.; Santhini, E.; Pradeepa, D.; Tresa, D.; Ganesan, P.; Ishwarya, P.; Balakrishnan, R. Mitochondrial and Nuclear Gene Mutations in the Type 2 Diabetes Patients of Coimbatore Population. *Mol Cell Biochem* **2010**, *345*, 223–229, doi:10.1007/s11010-010-0576-5.

38. Brehm, A.; Rosa, A. African Human MtDNA Phylogeography At-a-Glance. *Journal of Anthropological Sciences* **2011**, 1–34, doi:10.4436/jass.89006.
39. Du, H.; Zhao, Y.; Li, H.; Wang, D.W.; Chen, C. Roles of MicroRNAs in Glucose and Lipid Metabolism in the Heart. *Front. Cardiovasc. Med.* **2021**, *8*, 716213, doi:10.3389/fcvm.2021.716213.
40. Wang, L.; Cai, Y.; Jian, L.; Cheung, C.W.; Zhang, L.; Xia, Z. Impact of Peroxisome Proliferator-Activated Receptor-α on Diabetic Cardiomyopathy. *Cardiovasc Diabetol* **2021**, *20*, 2, doi:10.1186/s12933-020-01188-0.
41. Fourny, N.; Beauloye, C.; Bernard, M.; Horman, S.; Desrois, M.; Bertrand, L. Sex Differences of the Diabetic Heart. *Front. Physiol.* **2021**, *12*, 661297, doi:10.3389/fphys.2021.661297.
42. Tomar, N.; Zhang, X.; Kandel, S.M.; Sadri, S.; Yang, C.; Liang, M.; Audi, S.H.; Cowley, A.W.; Dash, R.K. Substrate-Dependent Differential Regulation of Mitochondrial Bioenergetics in the Heart and Kidney Cortex and Outer Medulla. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **2022**, *1863*, 148518, doi:10.1016/j.bbabio.2021.148518.
43. Sack, M.N.; Rader, T.A.; Park, S.; Bastin, J.; McCune, S.A.; Kelly, D.P. Fatty Acid Oxidation Enzyme Gene Expression Is Downregulated in the Failing Heart. *Circulation* **1996**, *94*, 2837–2842, doi:10.1161/01.CIR.94.11.2837.
44. Spyropoulos, F.; Sorrentino, A.; van der Reest, J.; Yang, P.; Waldeck-Weiermair, M.; Steinhorn, B.; Eroglu, E.; Saeedi Saravi, S.S.; Yu, P.; Haigis, M.; et al. Metabolomic and Transcriptomic Signatures of Chemogenetic Heart Failure. *American Journal of Physiology-Heart and Circulatory Physiology* **2022**, *322*, H451–H465, doi:10.1152/ajpheart.00628.2021.
45. Yamamoto, T.; Sano, M. Deranged Myocardial Fatty Acid Metabolism in Heart Failure. *IJMS* **2022**, *23*, 996, doi:10.3390/ijms23020996.
46. Dávila-Román, V.G.; Vedala, G.; Herrero, P.; de las Fuentes, L.; Rogers, J.G.; Kelly, D.P.; Gropler, R.J. Altered Myocardial Fatty Acid and Glucose Metabolism in Idiopathic Dilated Cardiomyopathy. *Journal of the American College of Cardiology* **2002**, *40*, 271–277, doi:10.1016/S0735-1097(02)01967-8.
47. Nikolaidis, L. The Development of Myocardial Insulin Resistance in Conscious Dogs with Advanced Dilated Cardiomyopathy. *Cardiovascular Research* **2004**, *61*, 297–306, doi:10.1016/j.cardiores.2003.11.027.
48. Oleynikov, D.A. Metabolic Markers of Myocardium Insulin Resistance in Dogs with Heart Failure. *Open Vet J.* **2021**, *10*, 363–370, doi:10.4314/ovj.v10i4.2.
49. Doehner, W.; Rauchhaus, M.; Ponikowski, P.; Godsland, I.F.; von Haehling, S.; Okonko, D.O.; Leyva, F.; Proudler, A.J.; Coats, A.J.S.; Anker, S.D. Impaired Insulin Sensitivity as an Independent Risk Factor for Mortality in Patients With Stable Chronic Heart Failure. *Journal of the American College of Cardiology* **2005**, *46*, 1019–1026, doi:10.1016/j.jacc.2005.02.093.
50. Salvatore, T.; Pafundi, P.C.; Galiero, R.; Albanese, G.; Di Martino, A.; Caturano, A.; Vetrano, E.; Rinaldi, L.; Sasso, F.C. The Diabetic Cardiomyopathy: The Contributing Pathophysiological Mechanisms. *Front. Med.* **2021**, *8*, 695792, doi:10.3389/fmed.2021.695792.
51. Prandi, F.R.; Evangelista, I.; Sergi, D.; Palazzuoli, A.; Romeo, F. Mechanisms of Cardiac Dysfunction in Diabetic Cardiomyopathy: Molecular Abnormalities and Phenotypical Variants. *Heart Fail Rev* **2022**, doi:10.1007/s10741-021-10200-y.
52. Wu, H.; Norton, V.; Cui, K.; Zhu, B.; Bhattacharjee, S.; Lu, Y.W.; Wang, B.; Shan, D.; Wong, S.; Dong, Y.; et al. Diabetes and Its Cardiovascular Complications: Comprehensive Network and Systematic Analyses. *Front. Cardiovasc. Med.* **2022**, *9*, 841928, doi:10.3389/fcvm.2022.841928.
53. Buchanan, J.; Mazumder, P.K.; Hu, P.; Chakrabarti, G.; Roberts, M.W.; Yun, U.J.; Cooksey, R.C.; Litwin, S.E.; Abel, E.D. Reduced Cardiac Efficiency and Altered Substrate Metabolism Precedes the Onset of Hyperglycemia and Contractile Dysfunction in Two Mouse Models of Insulin Resistance and Obesity. *Endocrinology* **2005**, *146*, 5341–5349, doi:10.1210/en.2005-0938.
54. Boudina, S.; Sena, S.; O'Neill, B.T.; Tathireddy, P.; Young, M.E.; Abel, E.D. Reduced Mitochondrial Oxidative Capacity and Increased Mitochondrial Uncoupling Impair Myocardial Energetics in Obesity. *Circulation* **2005**, *112*, 2686–2695, doi:10.1161/CIRCULATIONAHA.105.554360.

55. Banke, N.H.; Lewandowski, E.D. Impaired Cytosolic NADH Shuttling and Elevated UCP3 Contribute to Inefficient Citric Acid Cycle Flux Support of Postischemic Cardiac Work in Diabetic Hearts. *Journal of Molecular and Cellular Cardiology* **2015**, *79*, 13–20, doi:10.1016/j.yjmcc.2014.10.015.
56. Mazumder, P.K.; O'Neill, B.T.; Roberts, M.W.; Buchanan, J.; Yun, U.J.; Cooksey, R.C.; Boudina, S.; Abel, E.D. Impaired Cardiac Efficiency and Increased Fatty Acid Oxidation in Insulin-Resistant *Ob*/*Ob* Mouse Hearts. *Diabetes* **2004**, *53*, 2366–2374, doi:10.2337/diabetes.53.9.2366.
57. Jankauskas, S.S.; Kansakar, U.; Varzideh, F.; Wilson, S.; Mone, P.; Lombardi, A.; Gambardella, J.; Santulli, G. Heart Failure in Diabetes. *Metabolism* **2021**, *125*, 154910, doi:10.1016/j.metabol.2021.154910.
58. Moriyama, H.; Endo, J.; Ikura, H.; Kitakata, H.; Momoi, M.; Shinya, Y.; Ko, S.; Ichihara, G.; Hiraide, T.; Shirakawa, K.; et al. Qualitative and Quantitative Effects of Fatty Acids Involved in Heart Diseases. *Metabolites* **2022**, *12*, 210, doi:10.3390/metabo12030210.
59. Karwi, Q.G.; Sun, Q.; Lopaschuk, G.D. The Contribution of Cardiac Fatty Acid Oxidation to Diabetic Cardiomyopathy Severity. *Cells* **2021**, *10*, 3259, doi:10.3390/cells10113259.
60. Giacco, F.; Brownlee, M. Oxidative Stress and Diabetic Complications. *Circ Res* **2010**, *107*, 1058–1070, doi:10.1161/CIRCRESAHA.110.223545.
61. Karwi, Q.G.; Sun, Q.; Lopaschuk, G.D. The Contribution of Cardiac Fatty Acid Oxidation to Diabetic Cardiomyopathy Severity. *Cells* **2021**, *10*, 3259, doi:10.3390/cells10113259.
62. Bai, J.; Liu, C.; Zhu, P.; Li, Y. Novel Insights Into Molecular Mechanism of Mitochondria in Diabetic Cardiomyopathy. *Front. Physiol.* **2021**, *11*, 609157, doi:10.3389/fphys.2020.609157.
63. Wang, L.; Zhang, Q.; Yuan, K.; Yuan, J. MtDNA in the Pathogenesis of Cardiovascular Diseases. *Disease Markers* **2021**, *2021*, 1–8, doi:10.1155/2021/7157109.
64. Skulachev, V.P. Uncoupling: New Approaches to an Old Problem of Bioenergetics. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1998**, *1363*, 100–124, doi:10.1016/S0005-2728(97)00091-1.
65. Napolitano, G.; Fasciolo, G.; Venditti, P. Mitochondrial Management of Reactive Oxygen Species. *Antioxidants* **2021**, *10*, 1824, doi:10.3390/antiox10111824.
66. Bertholet, A.M.; Kirichok, Y. Mitochondrial H^+ Leak and Thermogenesis. *Annu. Rev. Physiol.* **2022**, *84*, 381–407, doi:10.1146/annurev-physiol-021119-034405.
67. Boudina, S.; Sena, S.; Theobald, H.; Sheng, X.; Wright, J.J.; Hu, X.X.; Aziz, S.; Johnson, J.I.; Bugger, H.; Zaha, V.G.; et al. Mitochondrial Energetics in the Heart in Obesity-Related Diabetes. *Diabetes* **2007**, *56*, 2457–2466, doi:10.2337/db07-0481.
68. Schilling, J.D. The Mitochondria in Diabetic Heart Failure: From Pathogenesis to Therapeutic Promise. *Antioxidants & Redox Signaling* **2015**, *22*, 1515–1526, doi:10.1089/ars.2015.6294.
69. Sung, M.M.; Hamza, S.M.; Dyck, J.R.B. Myocardial Metabolism in Diabetic Cardiomyopathy: Potential Therapeutic Targets. *Antioxidants & Redox Signaling* **2015**, *22*, 1606–1630, doi:10.1089/ars.2015.6305.
70. Wai, T.; Langer, T. Mitochondrial Dynamics and Metabolic Regulation. *Trends in Endocrinology & Metabolism* **2016**, *27*, 105–117, doi:10.1016/j.tem.2015.12.001.
71. Abel, E.D. MITOCHONDRIAL DYNAMICS AND METABOLIC REGULATION IN CARDIAC AND SKELETAL MUSCLE. *Trans Am Clin Climatol Assoc* **2018**, *129*, 266–278.
72. Jubaidi, F.F.; Zainalabidin, S.; Mariappan, V.; Budin, S.B. Mitochondrial Dysfunction in Diabetic Cardiomyopathy: The Possible Therapeutic Roles of Phenolic Acids. *IJMS* **2020**, *21*, 6043, doi:10.3390/ijms21176043.
73. Tomita, M.; Mukae, S.; Geshi, E.; Umetsu, K.; Nakatani, M.; Katagiri, T. Mitochondrial Respiratory Impairment in Streptozotocin-Induced Diabetic Rat Heart. *Jpn Circ J* **1996**, *60*, 673–682, doi:10.1253/jcj.60.673.
74. Frustaci, A.; Kajstura, J.; Chimenti, C.; Jakoniuk, I.; Leri, A.; Maseri, A.; Nadal-Ginard, B.; Anversa, P. Myocardial Cell Death in Human Diabetes. *Circulation Research* **2000**, *87*, 1123–1132, doi:10.1161/01.RES.87.12.1123.

75. Kucharská, J.; Braunová, Z.; Ulicná, O.; Zlatos, L.; Gvozdjáková, A. Deficit of Coenzyme Q in Heart and Liver Mitochondria of Rats with Streptozotocin-Induced Diabetes. *Physiol Res* **2000**, *49*, 411–418.
76. Bugger, H.; Dale Abel, E. Molecular Mechanisms for Myocardial Mitochondrial Dysfunction in the Metabolic Syndrome. *Clinical Science* **2008**, *114*, 195–210, doi:10.1042/CS20070166.
77. Thapa, D.; Nichols, C.E.; Lewis, S.E.; Shepherd, D.L.; Jagannathan, R.; Croston, T.L.; Tveter, K.J.; Holden, A.A.; Baseler, W.A.; Hollander, J.M. Transgenic Overexpression of Mitofilin Attenuates Diabetes Mellitus-Associated Cardiac and Mitochondria Dysfunction. *Journal of Molecular and Cellular Cardiology* **2015**, *79*, 212–223, doi:10.1016/j.yjmcc.2014.11.008.
78. Bombicino, S.S.; Iglesias, D.E.; Rukavina-Mikusic, I.A.; Buchholz, B.; Gelpi, R.J.; Boveris, A.; Valdez, L.B. Hydrogen Peroxide, Nitric Oxide and ATP Are Molecules Involved in Cardiac Mitochondrial Biogenesis in Diabetes. *Free Radical Biology and Medicine* **2017**, *112*, 267–276, doi:10.1016/j.freeradbiomed.2017.07.027.
79. Kobayashi, S.; Zhao, F.; Zhang, Z.; Kobayashi, T.; Huang, Y.; Shi, B.; Wu, W.; Liang, Q. Mitochondrial Fission and Mitophagy Coordinately Restrict High Glucose Toxicity in Cardiomyocytes. *Front. Physiol.* **2020**, *11*, 604069, doi:10.3389/fphys.2020.604069.
80. Gomes, K.P.; Jadli, A.S.; de Almeida, L.G.N.; Ballasy, N.N.; Edalat, P.; Shandilya, R.; Young, D.; Belke, D.; Shearer, J.; Dufour, A.; et al. Proteomic Analysis Suggests Altered Mitochondrial Metabolic Profile Associated With Diabetic Cardiomyopathy. *Front. Cardiovasc. Med.* **2022**, *9*, 791700, doi:10.3389/fcvm.2022.791700.
81. Shen, X.; Zheng, S.; Metreveli, N.S.; Epstein, P.N. Protection of Cardiac Mitochondria by Overexpression of MnSOD Reduces Diabetic Cardiomyopathy. *Diabetes* **2006**, *55*, 798–805, doi:10.2337/diabetes.55.03.06.db05-1039.
82. Belali, O.M.; Ahmed, M.M.; Mohany, M.; Belali, T.M.; Alotaibi, M.M.; Al-Hoshani, A.; Al-Rejaie, S.S. LCZ696 Protects against Diabetic Cardiomyopathy-Induced Myocardial Inflammation, ER Stress, and Apoptosis through Inhibiting AGEs/NF-KB and PERK/CHOP Signaling Pathways. *IJMS* **2022**, *23*, 1288, doi:10.3390/ijms23031288.
83. Xu, Y.; Fang, H.; Xu, Q.; Xu, C.; Yang, L.; Huang, C. LncRNA GAS5 Inhibits NLRP3 Inflammasome Activation-Mediated Pyroptosis in Diabetic Cardiomyopathy by Targeting MiR-34b-3p/AHR. *Cell Cycle* **2020**, *19*, 3054–3065, doi:10.1080/15384101.2020.1831245.
84. Elmadbouh, I.; Singla, D.K. BMP-7 Attenuates Inflammation-Induced Pyroptosis and Improves Cardiac Repair in Diabetic Cardiomyopathy. *Cells* **2021**, *10*, 2640, doi:10.3390/cells10102640.
85. Cai, Z.; Yuan, S.; Luan, X.; Feng, J.; Deng, L.; Zuo, Y.; Li, J. Pyroptosis-Related Inflammasome Pathway: A New Therapeutic Target for Diabetic Cardiomyopathy. *Front. Pharmacol.* **2022**, *13*, 842313, doi:10.3389/fphar.2022.842313.
86. Fiordaliso, F.; Bianchi, R.; Staszewsky, L.; Cuccovillo, I.; Doni, M.; Laragione, T.; Salio, M.; Savino, C.; Melucci, S.; Santangelo, F. Antioxidant Treatment Attenuates Hyperglycemia-Induced Cardiomyocyte Death in Rats. *Journal of Molecular and Cellular Cardiology* **2004**, *37*, 959–968, doi:10.1016/j.yjmcc.2004.07.008.
87. Zhang, W.; Xu, W.; Feng, Y.; Zhou, X. Non-coding RNA Involvement in the Pathogenesis of Diabetic Cardiomyopathy. *J Cell Mol Med* **2019**, *23*, 5859–5867, doi:10.1111/jcmm.14510.
88. Mekala, N.; Kurdys, J.; Vicenzi, A.P.; Weiler, L.R.; Avramut, C.; Vazquez, E.J.; Ragina, N.; Rosca, M.G. MiR 208a Regulates Mitochondrial Biogenesis in Metabolically Challenged Cardiomyocytes. *Cells* **2021**, *10*, 3152, doi:10.3390/cells10113152.
89. Carley, A.N.; Severson, D.L. Fatty Acid Metabolism Is Enhanced in Type 2 Diabetic Hearts. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **2005**, *1734*, 112–126, doi:10.1016/j.bbalip.2005.03.005.
90. Hafstad, A.D.; Khalid, A.M.; Hagve, M.; Lund, T.; Larsen, T.S.; Severson, D.L.; Clarke, K.; Berge, R.K.; Aasum, E. Cardiac Peroxisome Proliferator-Activated Receptor-α Activation Causes Increased Fatty Acid Oxidation, Reducing Efficiency and Post-Ischaemic Functional Loss. *Cardiovascular Research* **2009**, *83*, 519–526, doi:10.1093/cvr/cvp132.
91. Goyal, B.; Mehta, A. Diabetic Cardiomyopathy: Pathophysiological Mechanisms and Cardiac Dysfuntion. *Hum Exp Toxicol* **2013**, *32*, 571–590, doi:10.1177/0960327112450885.
92. Malfitano, C.; de Souza Junior, A.L.; Carbonaro, M.; Bolsoni-Lopes, A.; Figueroa, D.; de Souza, L.E.; Silva, K.A.S.; Consolim-Colombo, F.; Curi, R.; Irigoyen, M.C. Glucose and Fatty Acid Metabolism in Infarcted Heart from Streptozotocin-Induced

Diabetic Rats after 2 Weeks of Tissue Remodeling. *Cardiovasc Diabetol* **2015**, *14*, 149, doi:10.1186/s12933-015-0308-y.

93. de las Heras, N.; Lahera, V. Relevance of Mitochondrial Dysfunction in Heart Disease Associated with Insulin Resistance Conditions. *Pflugers Arch - Eur J Physiol* **2022**, *474*, 21–31, doi:10.1007/s00424-021-02638-8.
94. Wang, L.-Y.; Chen, C. Energy Metabolism Homeostasis in Cardiovascular Diseases. *J Geriatr Cardiol* **2021**, *18*, 1044–1057, doi:10.11909/j.issn.1671-5411.2021.12.006.
95. Ma, X.M.; Geng, K.; Law, B.Y.-K.; Wang, P.; Pu, Y.L.; Chen, Q.; Xu, H.W.; Tan, X.Z.; Jiang, Z.Z.; Xu, Y. Lipotoxicity-Induced MtDNA Release Promotes Diabetic Cardiomyopathy by Activating the CGAS-STING Pathway in Obesity-Related Diabetes. *Cell Biol Toxicol* **2022**, doi:10.1007/s10565-021-09692-z.
96. Willemsen, G.; Ward, K.J.; Bell, C.G.; Christensen, K.; Bowden, J.; Dalgård, C.; Harris, J.R.; Kaprio, J.; Lyle, R.; Magnusson, P.K.E.; et al. The Concordance and Heritability of Type 2 Diabetes in 34,166 Twin Pairs From International Twin Registers: The Discordant Twin (DISCOTWIN) Consortium. *Twin Res Hum Genet* **2015**, *18*, 762–771, doi:10.1017/thg.2015.83.
97. Zempo, H.; Kim, S.-J.; Fuku, N.; Nishida, Y.; Higaki, Y.; Wan, J.; Yen, K.; Miller, B.; Vicinanza, R.; Miyamoto-Mikami, E.; et al. A Pro-Diabetogenic MtDNA Polymorphism in the Mitochondrial-Derived Peptide, MOTS-c. *Aging* **2021**, *13*, 1692–1717, doi:10.18632/aging.202529.
98. Benton, M.L.; Abraham, A.; LaBella, A.L.; Abbot, P.; Rokas, A.; Capra, J.A. The Influence of Evolutionary History on Human Health and Disease. *Nat Rev Genet* **2021**, *22*, 269–283, doi:10.1038/s41576-020-00305-9.
99. Charununtakorn, S.T.; Shinlapawittayatorn, K.; Chattipakorn, S.C.; Chattipakorn, N. Potential Roles of Humanin on Apoptosis in the Heart. *Cardiovasc Ther* **2016**, *34*, 107–114, doi:10.1111/1755-5922.12168.
100. Muzumdar, R.H.; Huffman, D.M.; Calvert, J.W.; Jha, S.; Weinberg, Y.; Cui, L.; Nemkal, A.; Atzmon, G.; Klein, L.; Gundewar, S.; et al. Acute Humanin Therapy Attenuates Myocardial Ischemia and Reperfusion Injury in Mice. *ATVB* **2010**, *30*, 1940–1948, doi:10.1161/ATVBAHA.110.205997.
101. Bachar, A.R.; Scheffer, L.; Schroeder, A.S.; Nakamura, H.K.; Cobb, L.J.; Oh, Y.K.; Lerman, L.O.; Pagano, R.E.; Cohen, P.; Lerman, A. Humanin Is Expressed in Human Vascular Walls and Has a Cytoprotective Effect against Oxidized LDL-Induced Oxidative Stress. *Cardiovascular Research* **2010**, *88*, 360–366, doi:10.1093/cvr/cvq191.
102. Duzkale, H.; Shen, J.; McLaughlin, H.; Alfares, A.; Kelly, M.; Pugh, T.; Funke, B.; Rehm, H.; Lebo, M. A Systematic Approach to Assessing the Clinical Significance of Genetic Variants: A Systematic Approach to Assessing Variant Clinical Significance. *Clin Genet* **2013**, *84*, 453–463, doi:10.1111/cge.12257.
103. Finnilä, S.; Hassinen, I.E.; Majamaa, K. Phylogenetic Analysis of Mitochondrial DNA in Patients with an Occipital Stroke. *Mutation Research/Mutation Research Genomics* **2001**, *458*, 31–39, doi:10.1016/S1383-5726(01)00012-7.
104. Rieder, M. Automating the Identification of DNA Variations Using Quality-Based Fluorescence Re-Sequencing: Analysis of the Human Mitochondrial Genome. *Nucleic Acids Research* **1998**, *26*, 967–973, doi:10.1093/nar/26.4.967.
105. Herrnstadt, C.; Elson, J.L.; Fahy, E.; Preston, G.; Turnbull, D.M.; Anderson, C.; Ghosh, S.S.; Olefsky, J.M.; Beal, M.F.; Davis, R.E.; et al. Reduced-Median-Network Analysis of Complete Mitochondrial DNA Coding-Region Sequences for the Major African, Asian, and European Haplogroups. *The American Journal of Human Genetics* **2002**, *70*, 1152–1171, doi:10.1086/339933.
106. Ingman, M.; Kaessmann, H.; Pääbo, S.; Gyllensten, U. Mitochondrial Genome Variation and the Origin of Modern Humans. *Nature* **2000**, *408*, 708–713, doi:10.1038/35047064.
107. Li, Z.; Li, R.; Chen, J.; Liao, Z.; Zhu, Y.; Qian, Y.; Xiong, S.; Heman-Ackah, S.; Wu, J.; Choo, D.I.; et al. Mutational Analysis of the Mitochondrial 12S RRNA Gene in Chinese Pediatric Subjects with Aminoglycoside-Induced and Non-Syndromic Hearing Loss. *Hum Genet* **2005**, *117*, 9–15, doi:10.1007/s00439-005-1276-1.
108. Matthijs, G.; Claes, S.; Longo-Mbenza, B.; Cassiman, J.-J. Non-Syndromic Deafness Associatedwith a Mutation and a Polymorphism Inthe Mitochondrial 12S Ribosomal RNAGene in a Large Zairean Pedigree. *Eur J Hum Genet* **1996**, *4*, 46–51, doi:10.1159/000472169.
109. Tawata, M.; Ohtaka, M.; Iwase, E.; Ikegishi, Y.; Aida, K.; Onaya, T. New Mitochondrial DNA Homoplasmic Mutations

Associated With Japanese Patients With Type 2 Diabetes. *Diabetes* **1998**, *47*, 276–277, doi:10.2337/diab.47.2.276.

110. Pinti, M.V.; Fink, G.K.; Hathaway, Q.A.; Durr, A.J.; Kunovac, A.; Hollander, J.M. Mitochondrial Dysfunction in Type 2 Diabetes Mellitus: An Organ-Based Analysis. *American Journal of Physiology-Endocrinology and Metabolism* **2019**, *316*, E268–E285, doi:10.1152/ajpendo.00314.2018.
111. van Oven, M.; Kayser, M. Updated Comprehensive Phylogenetic Tree of Global Human Mitochondrial DNA Variation. *Hum. Mutat.* **2009**, *30*, E386–E394, doi:10.1002/humu.20921.