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African-origin Mitochondrial DNA Variants as a Contributing Factor to Susceptibilities for Diabetes and Age-related Diseases

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Abstract

African-origin populations are more susceptible to diabetes and other age-related diseases compared to European-origin populations, but mechanisms for the differential susceptibility remain unknown. Human mitochondrial (mt) DNA haplogroups are maternally inherited ancient polymorphisms representing different geographic origins of populations. Haplogroups are defined by accumulations of single nucleotide polymorphisms (SNPs), some of which cause changes in amino acids and rates of mtDNA replication and transcription. Several studies have shown that age-related diseases and their complications can be linked to mtDNA haplogroup subsets. Previous studies report that transmitochondrial cybrids (cytoplasmic hybrids), which contain identical nuclei but either European-origin (H) or African-origin (L) haplogroup mtDNA, have significantly different bioenergetic profiles, production levels of reactive oxygen species, and expression levels for complement, inflammation and apoptosis genes, thus suggesting that major biological pathways can be modulated by mtDNA. Using GeneChip arrays and Q-PCR, we show that African-origin L cybrids show significantly different expression levels for three Wnt pathway genes (DKK3, SFRP1, and KREMEN1) and three diabetes-related genes (RPS6KA4, ADAMTS9, and VEGFA) compared to European-origin H cybrids. Our current findings, along with others, support the hypothesis that an individual's mtDNA can modulate the expression of important Wnt signaling and diabetes-related genes, which may contribute to the racial/ethnic disparities associated with diabetes and other age-related diseases.

Keywords

mtDNA, Wnt pathway/β-catenin, Disease Susceptibilities, Diabetes, African-origin population, European-origin population

Abbreviations

ABI: Applied Biosystems; ADAMTS9: ADAM metallopeptidase with thrombospondin type 1 motif, 9; ARPE-19: Retinal pigmented epithelium cell line; ATP: Adenosine triphosphate; CSNK1E: Casein kinase 1, epsilon; DKK3: Dickkopf 3 homolog; DMEM: Dulbecco's modified Eagle's medium; DNA: Deoxyribonucleic Acid; GSK3A: Glycogen synthase kinase 3 alpha; KREMEN1: Kringle containing transmembrane protein 1; µl: Microliter; ng: Nanogram; OXPHOS: Oxidative phosphorylation; Q-PCR: Quantitative polymerase chain reaction; PCR: Polymerase chain reaction; RARA1: Retinoic acid receptor, alpha; RPS6KA4: Ribosomal protein S6 kinase, 90kDa, polypeptide 4; SEM: Standard error of the mean; SNPs: Single nucleotide polymorphisms; SFRP1: Secreted frizzled-related protein 1; UCLA: University of California, Los Angeles; VEGFA: Vascular endothelial growth factor A.

Introduction

All mitochondria (mt) contain maternally inherited, circular, double-stranded DNA consisting of 16,569 nucleotide pairs. The coding region of mtDNA encodes for 37 genes including 13 protein subunits essential for OXPHOS, 2 ribosomal RNAs and 22 transfer RNAs [1-3]. The non-coding mtDNA D-loop region is important for replication and transcription. Recently evidence shows that mtDNA can also code for small, biologically active "Mitochondrial Derived Peptides" [4,5] supporting a more robust role for the mitochondrial genome in diseases.

Human mtDNA haplogroups are ancient polymorphisms inherited along maternal lineages and have been used for large ancestral population studies. These adaptive single nucleotide polymorphism (SNP) changes have occurred over thousands of years and define mtDNA backgrounds connected to geographic origins of

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human populations. There are eight different European haplogroups with the most common being the H haplogroup (approximately 30%, www.MitoMap.org). Individuals of maternal African-origin belong to the L haplogroups and being the oldest haplogroups, they contain the largest diversity of SNPs.

Some racial/ethnic populations are more prone to develop specific diseases [6-11]. The incidence of diabetes is highest in the non-Hispanic black population with the non-Hispanic white adults having the lowest risk (http://www.diabetes.niddk.nih.gov/dm/pubs/statistics). Alzheimer's disease (AD) is another example where the prevalence is associated with the racial/ethnic background of the individual. Compared to the non-Hispanic white population, the African-Americans are twice as likely to develop AD or dementia [12-14] and also have higher incidence of systemic lupus erythematosus (SLE) [10,11]. The causes for these racial/ethnic differences are not known but epigenetic changes may play a role [15]. However, there is mounting evidence that mtDNA haplogroups, which define different racial populations, may contribute to differences in disease susceptibilities [16].

The majority of studies related to genetics and diseases have focused on the contribution of nuclear genotypes with many fewer studies on the mtDNA genome. One reason has been the difficulty to assign functional, cellular changes to the mtDNA variants. However, with the development of the transmitochondrial cybrid (cytoplasmic hybrid) model, many questions related to the functional importance of mtDNA haplogroup variants and mitochondrial-nuclear interactions can be addressed. Cybrid studies comparing European-origin haplogroups (H and J) and African-origin haplogroups (L) showed differences in cell growth, oxygen consumption rates, expression of non-energy-related genes, responses to stressors, and rates of glycolysis [16-20]. Based upon many differences in the cellular behavior in the H and L cybrids, we hypothesized that the cybrids might also have different gene expression patterns for Wnt/β-catenin signaling genes, which are important for cell proliferation, cell migration, apoptosis, development and numerous diseases, including lipid metabolism, glucose homeostasis, diabetes, Alzheimer's disease and cancers [21-23].

Our findings demonstrate that cybrids containing African-origin mtDNA (L haplogroup) have significantly different expression levels of three Wnt pathway genes (DKK3, SFRP1, and KREMEN1) and three diabetes-related genes (RPS6KA4, ADAMTS9, and VEGFA) compared to the European-origin H haplogroup cybrids. Analyses of the Functional Pathways on the GeneChip array showed differences between L and H cybrids for development and cancer systems. Our current findings suggest that subjects with African-origin mtDNA versus European - origin mtDNA may have different expression levels of Wnt signaling and diabetes-related genes, which thereby may contribute to the racial/ethnic disparities associated with diabetes, cancers and Alzheimer's diseases.

Materials and Methods

Transmitochondrial cybrids and culture conditions

Institutional review board approval was obtained from the University of California, Irvine (#2003-3131). For DNA analyses, 10mls of peripheral blood were collected via venipuncture in tubes containing 10mM EDTA from normal young volunteers. DNA was isolated with a DNA extraction kit (PUREGENE, Qiagen, Valencia and CA). Platelets were isolated by a series of centrifugation steps and final pellets were suspended in Tris-buffered saline. The ARPE-19 cells deficient in mtDNA (Rho0) were created by serial passage in low dose ethidium bromide [24]. Cybrids were produced by polyethylene glycol fusion of platelets with Rho0 ARPE-19 cells according to modified procedures of Chomyn [25]. All experiments used passage 5 cybrid cells for the assays described below.

Identification of cybrid haplogroups

Verification of transfer of the mitochondria into the Rho0 ARPE-19 cells was accomplished by polymerase chain reaction (PCR), restriction enzyme digestion, or sequencing of the mtDNA to identify the mitochondrial haplogroup of each cybrid. Briefly, cybrid DNA was extracted from cell pellets using a spin column kit (DNeasy Blood and Tissue Kit, Qiagen) and quantified using the Nanodrop 1000 (Thermo Scientific, Wilmington, DE). PCR and restriction enzyme digests [26], allelic discrimination and sequencing were performed to determine mitochondrial haplogroups. The primers for allelic discrimination were synthesized by ABI Assay-by-Design. The samples were run at GenoSeq, the UCLA Genotyping and Sequencing Core, on an ABI 7900HT. Data were analyzed with Sequence Detection Systems software from ABI. The SNPs defining the H haplogroup were T7028C and G73A. Further subtyping showed the H cybrids were H5a (n = 1), H66a (n = 1), H11a (n =1), H4a (n = 1) and H (n = 4). The L cybrids were further subtyped to be L0a (n = 1), L1b (n = 1), L1c (n = 1), L2a (n = 2) and L2b (n = 1) defined by the SNP variants (Figure 1).

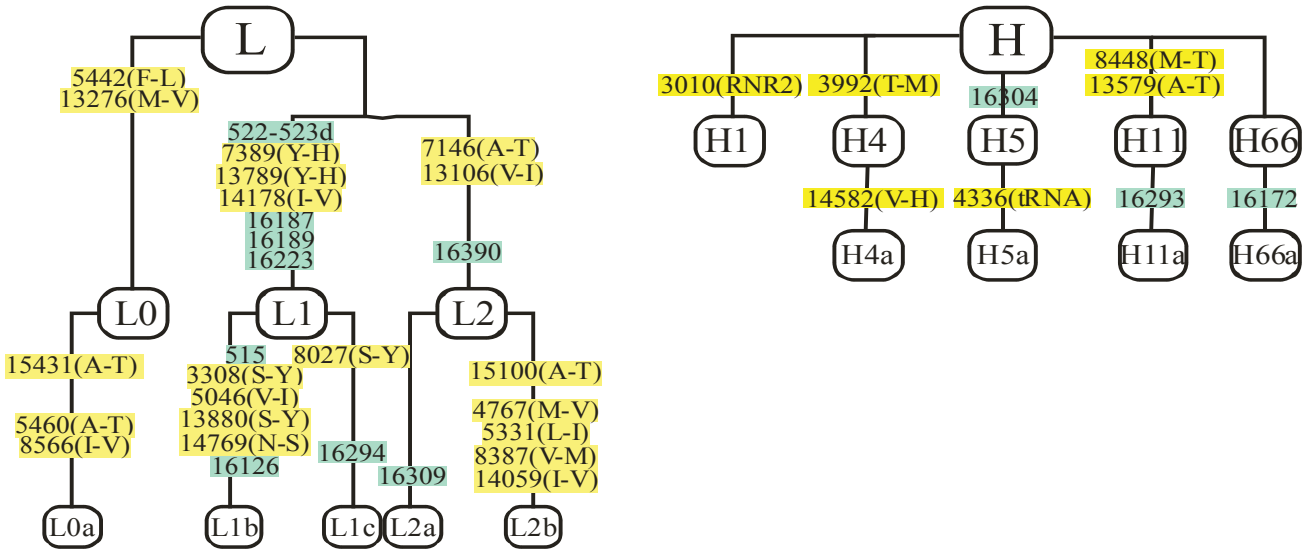

Figure 1: Branching diagram of L haplogroups subsets showing the different SNPs that define the specific haplogroup. The yellow highlighted SNPs are non-synonymous and cause amino acid changes in the coding regions of the mtDNA (5 amino acid changes for L0a; 7 amino acid changes for the L1b and L2b individuals, 2 amino acid changes for L2a and 4 amino acid changes for L1c, respectively) compared to the European H haplogroup (Cambridge reference). The green highlighted SNPs occur in the Control Region (non-coding MT-Dloop), which is critical for replication and transcription. The L haplogroups, which represent older geographic origins, contain higher numbers of non-synonymous SNPs than the European haplogroups and these differences may contribute to the racial/ethnic susceptibilities associated with diseases. MT, mitochondrial; SNP, single nucleotide polymorphism

Table 1: Genes expressed in WNT/β-catenin signaling pathways for L cybrids versus H cybrids as determined by IPA summary program analyses of Affymetrix Human U133 Plus 2.0 Array

Probe Set ID	Gene Symbol	Gene Title	Public ID	CYBD L Signal	CYBD H Signal	L vs H (fold)
202332_at	CSNK1E	casein kinase 1, epsilon	NM_001894	911.68	1188.09	-1.30
214247_s_at	DKK3	dickkopf homolog 3 (Xenopus laevis)	AU148057	19697.09	28552.76	-1.56
204602_at	DKK1	dickkopf homolog 1 (Xenopus laevis)	NM_012242	13771.8	19942.5	-1.45
202035_s_at	SFRP1	secreted frizzled-related protein 1	AI332407	89.16	241.93	-2.71
202036_s_at	SFRP1	secreted frizzled-related protein 1	AF017987	205.26	648.14	-3.16
202037_s_at	SFRP1	secreted frizzled-related protein 1	NM_003012	1571.58	9099.23	-5.79
243029_at	KREMEN1	kringle containing transmembrane protein 1	AL533967	23.95	4.04	5.92
227250_at	KREMEN1	kringle containing transmembrane protein 1	BF221745	353.32	165.83	2.13
230643_at	WNT9A	wingless-type MMTV integration site family, member 9A	BE220265	192.04	116.99	1.64
221029_s_at	WNT5B	wingless-type MMTV integration site family, member 5B	NM_030775	174.95	347.80	-1.99
202210_x_at	GSK3A	glycogen synthase kinase 3 alpha	NM_019884	150.06	175.70	-1.17
216300_x_at	RARA	retinoic acid receptor, alpha	BE383139	146.71	8.27	17.74
200785_s_at	LRP1	low density lipoprotein receptor-related protein 1	NM_002332	688.22	747.71	-1.09
205606_at	LRP6	low density lipoprotein receptor-related protein 6	NM_002336	83.00	97.29	-1.17
244616_x_at	MDM2	Mdm2 p53 binding protein homolog (mouse)	BE732830	383.73	430.03	-1.12
204632_at	RPS6KA4	ribosomal protein S6 kinase, 90kDa, polypeptide 4	NM_003942	238.23	126.32	1.89
1554697_at	ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9	AF488803	55.91	8.28	6.75
211527_x_at	VEGFA	vascular endothelial growth factor A	M27281	131.84	179.74	-1.36
210512_s_at	VEGFA	vascular endothelial growth factor A	AF022375	1806.69	2809.74	-1.56

Table 2: Description of Functional Pathways Regulated by Wnt/β-catenin Signaling Pathway as Determined by IPA Summary Program Analyses of Affymetrix Human U133 Plus 2.0 Array

L Cybrids versus H Cybrids	
Functional Pathways Possessing Wnt Pathway Molecules	
Category	**P-Value**
Cellular Movement	1.88E - 07 - 1.77E - 02
Cancer	4.34E - 07 - 1.74E - 02
Organismal Development	8.27E - 07 - 1.72E - 02
Visual System Development and Function	8.27E - 07 - 1.72E - 02
Tissue Development	4.53E - 06 - 1.77E -02
Cellular Development	7.33E - 05 - 1.66E - 02
Developmental Disorder	1.2E - 04 - 1.36E - 02
Cell Morphology	1.26E - 04 - 1.76E - 02
Connective Tissue Development and Function	5.04E - 04 - 1.51E - 02
Embryonic Development	5.04E - 04 - 1.72E - 02
Organ Development	5.04E - 04 - 1.72E - 02
Reproductive System Disease	7.14E - 04 - 1.11E - 02
Tissue Morphology	7.46E - 04 - 1.77E - 02

Analyses of GeneChip arrays and statistical analyses

For the GeneChip array analyses, equal amounts of RNAs (250 ng/μl per sample) from either H cybrids (n = 3) or L cybrids (n = 3) were combined into two separate samples. The two separate RNA samples were sent to the UCLA Clinical MicroArray Core Lab for analyses with the Affymetrix Human U133 Plus 2.0 Array data uploaded at Gene Expression Omnibus. Gene expression results were analyzed with IPA summary pathway analysis software (INGENUITY Systems, Redwood City, CA).

Quantitative polymerase chain reaction (Q-PCR) analyses

Gene expression changes identified by the GeneCHip array were verified by Q-PCR using 12 different primers (QuantiTect Primer Assay, Qiagen) for genes associated with the Wnt signaling or diabetes-related pathways. The Q-PCR analyses were performed on individual H or L cybrids and not on combined samples. Total RNA was isolated from individual pellets of cultured cells of haplogroup H cybrids (n = 8) and haplogroup L cybrids (n = 6) as described above. Q-PCR was performed using a QuantiFast SYBR Green PCR Kit (Qiagen) on a Bio-Rad iCycleiQ5 detection system. Analyses were performed in triplicate. Statistical analyses of gene expression levels were performed to measure differences between cybrids groups using Prism, version 5.0; (Graph Pad Software Inc.). Fold values were calculated using the standard 2^ddCt formula (ddCt = delta delta Ct). The H Cybrids were used as the relative control for L cybrids. The P-value < 0.05 was considered statistically significant.

Results

GeneChip array analyses

The pooled RNA isolated from H cybrids or L cybrids were analyzed using the GeneChip array that characterizes expression levels for over 40K genes. The IPA summary program of the GeneChip array showed that the pooled RNA from the L cybrids had differences in the Wnt/β-catenin signaling pathway compared to the pooled RNA from the H cybrids. More detailed analyses by the Ingenuity program shows that in the L cybrids, SFRP1 gene had at least 2-fold lower expression levels while the KREMEN1, RARA, and ADAMST9 genes showed greater than 2-fold increase when compared to the H cybrids (Table 1).

With the GeneChip array analyses, we were surprised by the differences in the Wnt/β-catenin signaling genes expression because all cybrids had the identical nuclei, cytoplasm and culture conditions but varied only in the mtDNA haplogroup of their mitochondria. This finding is important because there are many Functional Pathways that use the Wnt signaling pathway (Table 2). When the Functional Pathways were compared for the European-origin H cybrids versus the African-origin L cybrids, there were thirteen categories different between the two groups, including cancer, developmental, reproductive, cellular movement and morphology. These differences can be assigned to influences of the mtDNA variants since the nuclear genomes within all cybrids are identical.

Q-PCR analyses

To verify the GeneChip array results, we performed Q-PCR on individual RNA samples from the H (n = 8) or L (n = 6) cybrids for twelve nuclear genes associated with the Wnt/β-catenin signaling or diabetes-related pathways. We examined genes that showed greater than 2-fold differences in the H and L cybrids or genes that were critical for the Wnt pathway. Table 3 depicts GenBank Accession numbers and a brief description of the functions for nine genes associated with the Wnt/β-catenin signaling pathway (CSNK1E, DKK3, SFRP1, KREMEN1, WNT9, GSK3A, RARA1, LRP1 and MDM2), and three genes associated with diabetes (RPS6KA4, ADAMTS9 and VEGFA) [27].

Although all cybrids possess identical nuclei and culture conditions, the L cybrids had different expression levels for three Wnt-related genes compared to H cybrids (Table 4). Expression levels for DKK3 (0.06-fold, P = 0.03) and SFRP1 (0.5-fold, P = 0.003) were decreased while KREMEN1 levels were increased (1.5-fold, P = 0.005). The CSNK1E, WNT9A, GSK3A, RARA1, LRP1 and MDM2 gene expression levels in the L cybrids were similar to H cybrids

Table 3a: Description of Wnt/β-catenin Genes Analyzed by Q-PCR

Symbol	Gene Name	GenBank Accession No.	Function
CSNK1E	Casein kinase 1, epsilon	NM_001894 NM_152221	Serine/threonine protein kinase found in cytoplasm.
DKK3	Dickkopf 3 homolog	NM_013253 NM_015881 NM_001018057	Secreted protein involved in embryonic development via Wnt pathway.
SFRP1	Secreted frizzled-related protein 1	NM_003012	Soluble modulator of Wnt signaling. Maybe involved in polarity of photoreceptor cells.
KREMEN1	Kringle containing transmembrane protein 1	NM_032045 NM_153379 NM_001039570 NM_001039571	Involved in modulating Wnt signaling.
WNT9A	Wingless-type MMTV integration site family, member 9A	NM_003395	Member of WNT gene family that encodes secreted signaling proteins. Expressed in gastric cancer cell lines.
GSK3A	Glycogen synthase kinase 3 alpha	NM_019884	Multifunctional Ser/Thr protein kinase involved in WNT and PI3K signaling pathways.
RARA1	Retinoic acid receptor, alpha	NM_000964 NM_001033603 NM_001145301	Nuclear retinoic acid receptor. Regulates transcription. Involved with apoptosis, differentiation.
LRP1	Low density lipoprotein receptor -related protein 1	NM_002332	Endocytic receptor involved in intracellular signaling, lipid homeostasis and clearance of apoptotic cells.
MDM2	p53 E3 ubiquitin protein ligase homolog	NM_002392 NM_006878 NM_006880 NM_006881 NM_006882 NM_001145337 NM_001145339	Nuclear phosphoprotein that inhibits transactivation by p53, has E3 ubiquitin ligase activity, and affects cell cycle, apoptosis, and tumorigenesis.

Table 3b: Description of diabetes-related genes analyzed by Q-PCR

Symbol	Gene Name	GenBank Accession No.	Function
RPS6KA4	Ribosomal protein S6 kinase,90kDa, polypeptide 4	NM_001006944 NM_003942	Member of ribosomal S6 kinase family of Ser/Thr kinases.
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif, 9	NM_182920 NM_182921	Member of the ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) protein family. Implicated in the cleavage of proteoglycans and the inhibition of angiogenesis.
VEGFA	Vascular endothelial growth factor A	NM_001025366 NM_001025367 NM_001025368 NM_001025369 NM_001025370 NM_001033756 NM_001171622 NM_001171623 NM_001171624 NM_001171625 NM_001171626 NM_001171627 NM_001171628 NM_001171629 NM_001171630 NM_001204384 NM_001204385 NM_003376	Member of PDGF/VEGF growth factor family. Acts on endothelial cells. Causes increased vascular permeability, angiogenesis, vasculogenesis, endothelial cell growth, cell migration, and inhibits apoptosis. Mutations in this gene have been associated with proliferative and non-proliferative diabetic retinopathy.

(0.86-fold, P = 0.19; 1.32-fold, P = 0.13; 0.89-fold, P = 0.19, 0.95-fold, P = 0.6; 1.2-fold, P = 0.7; and 0.97-fold, P = 0.77, respectively).

The H and L cybrids also showed differential expression of diabetes-related genes. The diabetes-related gene, ADAMTS9, had

Table 4: Genes Expression Levels in L Cybrids versus H Cybrids Measured by Q-PCR

Gene	L Cybrids	H Cybrids
	Fold / P Value	
CSNK1E	0.86 / 0.19	1-fold
DKK3	0.06 / 0.03	1-fold
SFRP1	0.5 / 0.003	1-fold
KREMEN1	1.5 / 0.005	1-fold
WNT9A	1.32 / 0.13	1-fold
GSK3A	0.89 / 0.19	1-fold
RARA1	0.95 / 0.6	1-fold
LRP1	1.2 / 0.7	1-fold
MDM2	0.97 / 0.77	1-fold
RPS6KA4	0.5 / 0.003	1-fold
ADAMTS9	1.6 / 0.008	1-fold
VEGFA	0.79 / 0.02	1-fold

Positive values indicate up regulation of the gene. Negative values indicate down regulation of the gene.

The values for the H cybrids are equal to 1. Fold = $2^{\Delta\Delta CT}$;

There were N = 8 biologically different cybrids in the H cybrid group and N = 6 in the L cybrid groups,

with three replicate values for each sample.

Table 5: Systemic Diseases with Ethnic/Racial Disparities

African-origin Americans	European-origin Americans
Type 2 diabetes	Atrial fibrillation
Obesity	Autism
Hypertensive heart disease	Carotid artery disease
Lupus nephritis	Multiple sclerosis
Myeloma	Osteoporosis
Prostate cancer	Skin cancers
Stroke	
Systemic sclerosis	

Table 6: Eye Diseases with Ethnic/Racial Disparities

Glaucoma (African-origin)
Age-related macular degeneration (European-origin)
Polyploidal choroidal vasculopathy (Asian-origin)
Diabetic retinopathy (African-origin, Hispanic) • Arch Ophthalmol. 2004;122 : 477 - 485 • Arch Ophthalmol. 2008;126 (2) : 241- 245 • Arch Ophthalmol. 2011;129 (7) : 849 - 854

elevated expression levels (1.6-fold, P = 0.008) in the L cybrids. The VEGFA and RPS6KA4 gene expression levels were lower in the L cybrids compare to H cybrids (0.79-fold, P = 0.02 and 0.5-fold, P = 0.003, respectively).

Discussion

Different racial/ethnic populations have different susceptibilities to develop specific diseases (Table 5) [6,9]. For example, in populations twenty years or older, the age-adjusted percentage of individuals with diabetes varies: non-Hispanic blacks (13.2%), Hispanic/Latinos (12.8%), Asian-Americans (9.0%) and non-Hispanic whites (7.6%) (http://www.cdc.gov/diabetes/pubs/statsreport14/national-diabetes-report-web.pdf). Approximately, 11% of all African-Americans 20 years of age or older have diabetes and 25% of African-Americans have diabetes by ages 65-74 (http://www.diabetes.niddk.nih.gov/dm/pubs/statistics). African-Americans are twice more likely to have diabetes-related vision loss. In the Canary Islands, there is an ethnic susceptibility to diabetes due directly to maternal mtDNA [28]. In this population, the likelihood of inheritance is higher from an affected mother rather than an affected father [29]. Epidemiological studies illustrate that racial/ethnic disparities can also be found in eye diseases (Table 6) [30-32]. The causes for different susceptibilities to disease are not known, however, there is mounting evidence that mtDNA haplogroups, which define different racial populations, may be a contributing factor [16,33].

The DKK and SFRP families of molecules play critical roles in inhibition of the Wnt/β-catenin pathway. By Q-PCR analyses, the African-origin L cybrids showed decreased gene expression levels for both DKK3 and SFRP1, which could lead to increased transcription for downstream genes. DKK3, previously called RIG (Regulated in Glioma), has been associated with tumorigenesis [34-36] and its levels were decreased in malignant glioblastoma cells compared to low-grade astrocytoma and normal brain cells [37]. Low levels of DKK3 and SFRP1 have been reported in high-grade endometrial endometrioid adenocarcinoma and lower progression-free survival [38]. In addition, aberrant methylation status of DKK3 promoter has been associated with human breast cancer [39] and correlated with increasing age in male subjects [40]. DKK3 also plays a role in differentiation of pluripotent stem cells into smooth muscle cells and dopaminergic neurons [41,42] and co-localizes to amyloid-β proteins within senile plaques of Alzheimer's brains [43]. The differential expressions of DKK3 and SFRP1 in cybrids containing African-origin mtDNA need to be studied further because there is poorer prognosis of various cancers in this population [44-46].

The altered regulation of the Wnt/β-catenin pathway also plays a role in the development of diabetes. Genetic studies show that mutations in the LDL receptor proteins (e.g., LRP5 and LRP6) are associated with increased risks of type 1 diabetes, obesity, metabolic syndrome and coronary artery disease [47-50]. In addition, the polymorphisms within the TCF7L2 and WNT5B genes are associated with increased risk of type 2 diabetes [51-54]. These studies demonstrate the Wnt/β-catenin pathway is critical to pre-diabetic and diabetic conditions in different individuals. Therefore, the different expression of Wnt regulatory molecules in the African-origin L cybrids versus the European-origin H cybrids might alter significantly the Wnt/β-catenin signaling and metabolic conditions within the cells and could greatly affect the cellular homeostasis. In African-origin cybrids, the decreased gene expression for the inhibitors DKK3 and SFRP1 suggests that the Wnt/β-catenin signaling pathway would be in the "ON" state, allowing β-catenin to localize into the nucleus to drive transcription and leading to the downstream effects, compared to the European-origin cybrids that would have higher levels of these Wnt inhibitors.

In addition, there are Wnt/β-catenin independent pathways, the Wnt-planar-cell polarity (Wnt-PCP pathway) and the Wnt-calcium pathway (Wnt-Ca^{2+} pathway), both of which can be regulated by the levels of SFRP1. With decreased expression SFRP1 in cells with the African-origin mtDNA, these pathways would also be affected, possibly contributing to the development of diabetes, cancers and bone diseases [55-57]. The modulation of Wnt pathway expression may play a role in the significantly altered transcription for nuclear genes related to the complement, innate immunity, inflammation, and apoptosis pathways reported in L cybrids compared to H cybrids [16]. Further investigations are needed to understand the influence of the mtDNA on the Wnt/β-catenin independent pathways.

The DKK proteins interact with KREMEN transmembrane receptors to regulate Wnt signaling. By both the GeneChip array and Q-PCR, the African-origin L cybrids had increased expression levels for KREMEN1 compared to the European-origin H cybrids. While the DKK1 protein and lipoprotein receptor-related protein 6 (LRP6) have been studied extensively in other systems, in our GeneChip analyses, the African-origin L cybrids showed similar expression levels for DKK1 (-1.4-fold) and LRP6 (-1.2-fold) genes compared to the European-origin H cybrids, suggesting their transcription levels may not be influenced by the mtDNA variants.

The H and L cybrids also showed differential expression of diabetes-related genes. Our cybrid model shows that the mtDNA variants within the African-origin mtDNA can upregulate expression levels of ADAMTS9, which is associated with insulin resistance and Type 2 diabetes [58]. Studies of European-American and African-American populations show increased association of ADAMTS9 with diabetes and different risk allele burdens between the racial/ethnic groups [27]. The RPS6KA4 and VEGFA gene expression

levels were lower in the L cybrids compare to H cybrids. RPS6KA4, which represents the 90kDa polypeptide 4 of the ribosomal S6 kinases (RSK), a family of Ser/Thr kinases, is involved with cell proliferation, maturation, survival, migration, and invasion [59]. Expression levels of RSK can regulate insulin signaling and glucose metabolism [60], key events in maintaining metabolic balance within cells. With lower expression of RPS6KA4 in cells with the African-origin mtDNA, there may be diminished cell proliferation and survival, leading to cell death which is characteristics of diabetic tissues.

The differences between the European-origin cybrids and African-origin cybrids could be due to the numerous changes in amino acids that result from the non-synonymous SNPs found in L haplogroups versus H haplogroups (Figure 1). Comparisons of SNP changes that have occurred over time have shown that in mtDNA coding regions, the ratios of synonymous to non-synonymous SNPs are variable depending upon an African, Asian or European origin [61]. For example, the European JT cluster has a high degree of non-synonymous variability in the MT-ND2 gene compared to the European H or African L haplogroups, while the HV cluster has large non-synonymous variability in the MT-ND5 gene. The African mtDNA lineages are highly conserved in MT-ATP6 and MT-ATP8. These SNP patterns within the haplogroup clusters may play a role in either high risk or protection in some complex diseases [28, 62-65]. In any case, in the present study the African-origin L cybrids showed remarkably different expression levels in the DKK3, SFRP1 and KREMEN1 of the Wnt signaling and the diabetes-related RPS6KA4, ADAMTS9 and VEGFA genes compared to the European-origin H cybrids, which may contribute to racial differences in diseases and responses to medications.

Summary

Based upon our data, along with others, we hypothesize that mtDNA haplogroups representing populations of different geographic origins (African versus European) play a role in racial/ethnic susceptibilities to diseases. Our cybrid results show that a person's mtDNA haplogroup background can act as a "modifier" for the Wnt/β-catenin and diabetes-related genes, and this may influence the cellular homeostasis. Recognizing that the mtDNA haplogroups may contribute to the development and severity of diseases is an important step toward understanding complex diseases. Future work with the cybrid model is needed to identify the retrograde signaling between the mitochondria and nucleus mediating differential transcription of the important Wnt/β-catenin signaling pathway.

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Ethical Standards

All studies have been approved by the appropriate ethics committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All individuals involved in this study gave their informed consent prior to their inclusion in this study.

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