ASSOCIATION BETWEEN A SIRTUIN 5 SNP (rs SIRT5 SNP, rs9382222) AND THREE FUNCTIONAL MARKERS OF BRAIN HEALTH

by

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Medical Doctor, Universidad Autonoma de Nuevo Leon,

Mexico, 2005

Submitted to the Graduate Faculty of

Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2011

UNIVERSITY OF PITTSBURGH

GRADUATE SCHOOL OF PUBLIC HEALTH

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It was defended on

July 22nd, 2011

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ASSOCIATION BETWEEN A SIRTUIN 5 SNP (rs SIRT5 SNP, rs9382222) AND THREE FUNCTIONAL MARKERS OF BRAIN HEALTH

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This study is based on an a priori hypothesis for a particular SNP in the SIRT5 gene (rs9382222; C→T) for which we have evidence that the common C-allele is associated with an older biological age of the brain. Digit Symbol Substitution Test (DSST), 20 meters timed-walk (Gait Test) and Epidemiological Studies Depression Scale (CES-D) are functional markers of brain health and applicable tests to measure cognitive function, motor function and depressed mood. HYPOTHESIS: At baseline, subjects carrying the common C/C risk genotype at the SIRT5 SNP will display poorer function on cognitive function tests (lower DSST score) and motor function tests (longer time to walk 20 meters), and have increased self-reported symptoms of a depressed mood (higher CES-D score), as compared to all other subjects. METHODS: The linear model type, one-way analysis of covariance (ANCOVA), was fitted using SAS GLM procedure to test for between-group differences in functional outcomes. Concordance in SNP effects were investigated for the three interrelated functional markers in subjects carrying the specific genotype (C/C, C/T, T/T). RESULTS: We detected a borderline significant association between DSST and SNP in the black population (p=0.051, mean diff.=-0.05, SD=0.95) with C/C subjects displaying lower DSST scores vs. C/T (almost 2 units lower than heterozygotes). There is a trend for an association between CES-D and SNP in the white population (p=0.08, mean diff.=-1.85, SD=0.03) with the C/C risk group reporting higher depression-like symptoms vs. C/T. Gait Test were no statistical significant associated to the SNP. CONCLUSIONS: The C/C previously

linked with older biological brain age was associated with (1) lower DSST scores in the black population and (2) displayed trend-level higher CES-D depressive-like scores in the white population, hence suggesting the SIRT5 C/C genotype as a probable risk factor for both biological brain age and related functional outcomes. PUBLIC HEALTH SIGNIFICANCE: Emotional and cognitive fitness is rapidly becoming a major determinant to the quality of life during old age. Study the genetic component of the brain aging as this SNP would help to 1) identify people at risk, and 2) address public health programs to achieve a successful aging.

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PREFACE

Etienne Sibille provided support for the overall analysis and biological aspect of the project, Caterina Rosano assisted in the data acquisition and statistical analysis, Candace Kammerer assisted in the data acquisition and genetic analysis and Christopher Walsh assisted in the data request process.

1.0 INTRODUCTION

Worldwide, the proportion of people over 60 years age is significantly increasing compared to other age groups [1]. Since 2002 it has been calculated that the number of old people has tripled over the last half century; it has been estimated it will more than triple in the next half century [2]. Pooled factors such as drop in fertility and increase in average life span have made this epidemiologic transition possible [3]. This global phenomenon is occurring at different rates among countries [4].

According to the U.S. Bureau of the Census, the World Health Organization, and the United Nations on U.S. and global trends in aging, the worldwide population of persons aged >65 years was projected to 420 million in the year 2000, increasing 9.5 million just in the year 1999 [5]. It is estimated that from 2000 to 2030, the worldwide population aged >65 years will augment by approximately 550 million to 973 million [6]. Proportionally it will grow from 6.9% to 12.0% worldwide (15.5% to 24.3% in Europe, from 12.6% to 20.3% in North America, from 6.0% to 12.0% in Asia, from 5.5% to 11.6% in Latin America and the Caribbean [5], and from 2.9% in to 3.7% in Sub-Saharan Africa) with a remarkable increase in developing countries where it is estimated to increase almost three-fold (249 million in 2000 to an estimated 690 million in 2030) [5]. In the developing countries the world's population aged >65 years is estimated to increase from 59% to 71%. The increase of persons aged >65 worldwide is already challenging the public health system. Medical attention and social services are the principal

demands by this population; they are the most likely to develop chronic illnesses which produce disability, decrease the quality of life, and increase medical care costs and attention.

In the US, people over 65 years old are expected to grow from 12.4% in 2000 to 19.6% in 2030 [6]. It means in 2030 there will be 71 million people in this age group[6]. Although Florida has the largest population in people over 65 years old, according to the U.S. Bureau of Census, Pennsylvania was one of the nine states in the country that had more than one million persons over 65 years old fifteen years ago [7] and it is expected to grow more than 15% by 2025 [8].

The expected increase of older adults is already challenging public health system. Chronic diseases which decrease the quality of life and increase the medical care cost have already required special public health interventions; however, there is a growing concern about the future [3].

Because of this epidemiology transition, quality of life has become an important issue in older people. It is well know that improvements in health services and living conditions have considerably contributed to the increased average human lifespan over the last century. As a result, emotional and cognitive fitness is rapidly becoming a major determinant and unmet challenge to the quality of life during old age. Specifically, while successful aging is achievable, for numerous individuals, low mood is too often an early symptom and significant contributor to the downward spiral of aging, which includes further cognitive and motor decline.

1.1 HAP-MAP PROJECT

This project is a partnership of scientists and funding agencies from Canada, China, Japan, Nigeria, the United Kingdom and the United States to develop a public resource to help researchers find genes affecting health, disease, and responses to drugs and environmental factors [9]. The international Hap-Map Project describes the common patterns of human DNA sequence variation and was built with the goal to develop a haplotype map of the human genome. The information produced by the Project is freely available.

1.2 HARDY-WEINBERG EQUILIBRIUM

The Hardy-Weinberg Equilibrium (HWE) law is the cornerstone of diploid population genetics [10]. It states that both allele and genotype frequencies in a population are in equilibrium from generation to generation unless specific disturbing influences (including non-random mating, mutations, selection, limited population size, "overlapping generations", random genetic drift, gene flow and meiotic drive) break this balance. Prior to performing association analyses between genetic maters and traits, genotype frequencies are tested for HWE [10].

As a quick review, sites in the genome where the DNA sequences of many individuals differ by a single base are called single nucleotide polymorphisms (SNPs). For example, some people may have a chromosome with a C at a particular site where others have a chromosome with a T; each different letter (nitrogenous base) is called an allele.

1.3 SIRTUINS

Sirtuins are proteins that have been reported to influence aging, stress and metabolism [11]. These proteins are localized in the mitochondria; the reason of the sub cellular localization is because these proteins in the human genome shared both a non-variable Sirt2 catalytic core domain and a variable amino- and carboxyl-terminal extensions [11].

Since mitochondrial contain some sirtuins and are the principal organ in charge of the energy output[11], scientific research has been involved in the process of illnesses such as diabetes, aging, neurodegenerative disorders and cancer with mitochondrial dysfunctions [12-14]. The suggestion to address a relation between sirtuins and mitochondrial dysfunction is due to the acetylating process factor. It has been observed that during the well nutrition state the mitochondrial proteins are mostly acetylated [15, 16], but in the diet with considerable depletion of calories the acetylated-protein levels vary [15, 17]; thus, it is suggested that multiple enzymes depend on the removal of the acetyl groups from sirtuins to work properly. Also it is inferred that because of their role to preserve the proper function of the mitochondria, sirtuins are related in different disease processes (i.e. chronic and degenerative diseases).

Their catalytic activity may be controlled by their chemical structure [11]. New research has revealed that sirtuins regulate the metabolism through the modulation of metabolic enzymes via protein deacetylation or mono-ADP-ribosylation, acting as clout in the metabolic efficiency [11]. The specific enzymes that catalyze the removal of acetyl groups from the (epsilon)-amino group of lysine residues are the Histone/Protein deacetylases [11]. Histone deacetylases are classified in three classes (I-III), and sirtuins belong to class III because of a distinctive chemical reaction which consumes nicotinamide adenine dinucleotide (NAD+) and generates nicotinamide, O-acetyl-ADP-ribose (OAADRr), and deacetylated substrate [18-20]. Sirtuins do

not belong to class I and II because proteins in those classes only catalyze simple hydrolysis of acetyllysine [21, 22].

Currently, there are seven sirtuins known to be present in the human genome [23, 24]. SIRT1, SIRT2, SIRT6, SIRT7, SIRT3, SIRT4 and SIRT5 have different sub cellular locations and targets: SIRT1 is the nucleus and acts as a transcriptional repressor via histone deacetylation, regulating the transcription factors such as, MyoD, FOXO, p53, and NF-(KAPPA)B [25-31]. SIRT2 is in the cytoplasm and is related with the microtubules and deacetylates lysine of (alpha) tubulin [32]. SIRT6 is also in the cytoplasm and acts as histone H3K9 deacetylase to regulate telomeric chromatine [33]. SIRT7 is found in the nucleolus and regulates the RNA polymerase I transcription [34]. SIRT3, SIRT4 and SIRT5—all from the mitochondrial matrix—have controversial functions; SIRT 3 and 5 are NAD+ dependent deacetylases, which means they change the acetyl lysine proteins by taking off their acetyl groups to compose the 2'-O-acetyl-ADP-ribose and nicotinamide. One of the functions of SIRT4 is to remove the ADP-ribose group [11].

1.4 SIRTUIN 5

SIRT5 is an endogenous protein localized in the matrix of the mitochondria [11, 29]. It is located specifically in the mitochondria intermembrane space [35, 36]. Molecularly, it has 36 amino acid mitochondrial targeting signals in its N terminal which is removed once in the mitochondria [37, 38]. SIRT5 is expressed in multiple tissues: brain, muscle, heart, liver and kidney [37, 39].

Recent findings show that a polymorphism in SIRT5 (rs9382222) is associated with molecular aging. This polymorphism has been located it in a mouse/human conserved region

using two separate programs to contain a promoter [40], such as TSSG CGG Nucleotide Sequence Analysis and Promoter 2.0.

SIRT5 has a deacetylase function. SIRT5 acts on acetylated histones or BSA28 against acetylated histone H4 peptide, showing its deacetylase activity, and against acetylated cytochrome C30, intermembrane mitochondrial space protein [41].

Biologically, carbamoil phosphate synthase (CPS1) –a mitochondrial matrix enzyme– has been identified as a substrate for SIRT5. CPS1 plays an important role in urea synthesis in the urea cycle. In fact, this enzyme acts as a rate-limiting enzyme modulating the urea synthesis. Specifically, CPS1 removes the ammonia generated by amino acid catabolism [42, 43]. SIRT5 increases the CPS1 activity because SIRT5 stimulates the deacetylation function of CPS1 with NAD+ in vitro[38]. Thus, SIRT5 increase the urea formation in conditions when the nutrient intakes are low, the ammonia generation is high, and the amino acid catabolism is also high [11]. A loss of ammonia is seen in metabolism with low calorie intake and high-protein diet (HPD), and this is when SIRT 5 regulates CPS1 [11].

A reduced calorie condition is a circumstance that regulates the SIRT5 expression. Once the calorie restriction begins the SIRT 5 start to deacetylate CPS1 triggering the activity of CPS1 enzyme; this activation causes the exchange of ammonia in carbamoyl phosphate. This exchange consequently causes the excretion of carbamoyl phosphate as urea in the urea cycle [11].

New findings have shown a controversy about whether or not SIRT5 increase acetylation and/or hyperacetylation of CPS1 during diets with calorie restrictions [17]. Researchers based this theory on an experiment where, under a low calorie intake diet, they study the acetylation of the CPS1; the results show that 24 sites were acetylated but seven sites were hyperacetylated. In that study no site was found as deacetylated [17].

Other studies has shown nine acetylating sites in CPS1, but in contrast with other experiments it shows that 4 sites were acetylated during feeding and fasting, another 4 sites were acetylated upon fasting, and one site was deacetylated [15].

SIRT5 activity remains under study due to these controversial results from different biased experiments. These studies generally suggest that SIRT5 could be related to other mitochondrial substrates [38], although new studies and concordant results are needed to understand in depth the activity of SIRT5 and clarify its blurred functions. The SIRT5 gene has also been correlated with malignancies [39].

1.4.1 SIRT5 and age-of-onset for neurological diseases

The biological mechanism or pathways by which characteristics of age-of-onset for neurological diseases are expressed are mostly unknown, but the connection between transcriptome changes ("molecular aging") and normal brain aging has been reported [40].

A cross-cohort microarray analysis found that many neurological disease pathways are associated with molecular aging [40]. From five candidates of longevity gene polymorphism, the SIRT5 gene (longevity gene) was used in this study. In fact, this research associated the low-expressing polymorphism of this specific gene with older brain molecular ages [40]. Moreover, SIRT5 was suggested as a risk factor since it influences positively the proper function of the mitochondria.

Many genes working as transcription regulators have been identified. Previous research about the relation between genes and neurological diseases has suggested that their expression promotes positively the progression of the disease. Some studies have been focused on finding a

relation between the sirtuin mechanism and different pathways to address preventive methods and treatments for neurological diseases.

Based on previous post-mortem studies, subjects carrying the SIRT5 risk allele may be at increased risk for mitochondrial function- and age-related early declines [40]. These studies suggest that SIRT5 is associated with a downward spiral of aging and low expression of structural and functional markers of brain health. Thus, the purpose of the current study is to investigate the associations between the risk allele SIRT5 and three functional markers of brain health in the Health ABC epidemiological cohort. These associations may clarify the role of the risk allele SIRT 5 and the low expressions of functional markers of brain health and assist in early protection from the detrimental age-dependent effects.

2.0 HYPOTHESIS

At baseline, subjects carrying the common C/C "risk" genotype at the SIRT5 SNP (rs9382222), which has been associated with older biological age of the brain, will display poorer function on cognitive function tests (lower DSST score) and motor function tests (longer time to walk 20 meters), and have increased self-reported symptoms of a depressed mood (higher CES-D score), as compared to all other subjects.

3.0 METHODS

3.1 POPULATION

The Health, Aging and Body Composition (Health ABC) database [44] was chosen due to its large scale prospective investigation of multiple factors in subjects 65 years of age and older, consistent domain monitoring across studies and extensive expertise in the analysis of those data.

The Health ABC is a longitudinal cohort study which from 1997 to 1998 enrolled 3,075 Medicare affiliated eligible healthy individuals aged 70-79 years from Pittsburgh, Pennsylvania and Memphis, Tennessee. The cohort consisted of 52% women and 42% blacks with a mean age of 73.6 years. The Process of recruitment consisted of contacting Medicare affiliated eligible people; the information was obtained through the Centers for Medicare & Medicaid Services (once called the Health Care Financing Administration). Samples of the white and black populations were taken randomly through predesigned zip code areas near the Health ABC designed centers. Also eligible people from household members were also included in the population samples [45]. Since well-functioning individuals were included in this study the exclusion criteria were difficulty to perform basic daily activities such as difficulty walking. People who reported trouble walking at least one quarter of a mile and climbing ten steps without resting were also excluded [45]. Depressive symptoms were not recorded as part of the exclusion criteria. Less than 3% of the eligible people reported the use of anti-depressive medications [46].

Based in the use of three continuous outcome measurements (functional markers of brain health) and a categorical exposure variable (the genotype: C/C, C/T, T/T). The estimation of the sample size was based in the assumption that we will do 3 T-test (using the formula for a T-test and Bonferroni-corrected alpha – i.e., 0.5 / 3 = 0.17). Our sample calculation in the design indicated us that with our sample size of 2768 we have enough power (80%) to detect as specific standard deviation as the 0.08 (Figure 1).

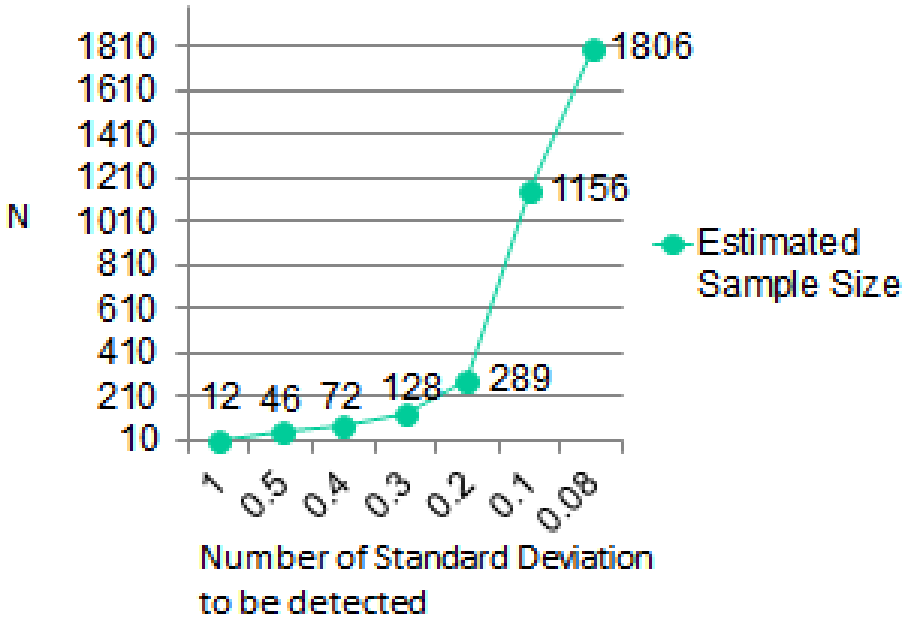


Figure 1. Estimated sample sizes assuming a power of 80%, and alpha = 0.17 (0.5 / 3). It is observed that with the sample size of 1806 individuals we can detect a specific standard deviation as the 0.08.

3.2 CLINICAL/DEMOGRAPHIC DATA

The data from the Health ABC were used to find associations between a SIRT5 SNP and three functional markers of brain health. These three markers or variables of interest were based on in

the results of three standardized tests: the Cognitive test, Digit Symbol Substitution Test (DSST); Motor function test, Gait speed measure; and the Mood or depressive symptoms test, Center for Epidemiology Studies Depression Scale (CES-D). While these measures may not be as refined or sensitive as other approaches, there are highly appropriate for epidemiologic settings, and also these measures are useful in studying older populations [44, 47, 48].

3.3 BRAIN FUNCTION TESTS

3.3.1 Cognitive test

The Digit symbol substitution test (DSST) is a pencil and paper test of psychomotor performance, which requires incidental memory, perceptual organization, visuomotor coordination, selective attention and the ability to filter out irrelevant information (e.g., symbols that may look alike)[49]. This test is associated with mood [50], mobility [51] and physical disability [10]. In this test people are provided with a key grid of numbers and matching symbols together with a test section with numbers and empty boxes. The process of this test consists of filling out as many empty boxes as possible with a symbol matching each number in 90 seconds; in other words, it consists of encoding and retrieving numbers and matching symbols. Basically, in order to solve the DSST, people must memorize the encoded number in the test section which is temporarily stored, and then visually scan the key grid to search for the number-symbol match. Once the number is recognized they must match the symbols in the test section and copy those below each matched number. The score is given by correct number-symbol matches. The reliability of this test is high [52].

3.3.2 Motor function test

Gait Test is a reliable and valid measure of motor performance. This test measures the time people take to walk 20 meters from a stand-still position to a straight course setup along a hallway [53]. Using a stopwatch, the time was recorded from the first step people take until the last step at the end of the 20 meters. The detailed process consisted in asking participants to stand at a starting line marked with tape and, after the staff's indication, start to walk normally until the finish line.

3.3.3 Mood or depressive symptoms test

The Center for Epidemiologic Studies Depression scale (CES-D) is a short self-report scale designed to measure depressive symptomatology in the general population. It has been widely used in studies of late-life depression [51]. CES-D was initially developed to measure depression symptoms in community samples [54]. Today it is considered a sensitive measure for general emotional distress [55, 56]. This scale has good psychometric properties [51]. The CES-D has demonstrated good test-retest reliability, validity in older adults [54] across different ethnic/racial populations [57], as well as high correlations with significant life events and clinical diagnosis of depression [54, 58, 59]. The scores obtained from the questionnaire responses range from 0 to 60, with higher scores indicating more symptoms of depression [54]. CES-D scores of 16 to 26 are considered indicative of mild depression and scores of 27 or more indicative of major depression [60]. We chose to use the CES-D since elevated scores are associated with current and future cognitive and motor impairment in the Health ABC [44, 47, 48], thus allowing our

hypothesis tests of low mood as a putative indicator for overall age-related decline in subjects at elevated biological risk.

3.4 HAP-MAP PROJECT

The International Hap-Map Project was used to compare the allele frequency of SIRT5 SNP versus the genotypes obtained in the Health ABC. White and black populations from the Health ABC database genotype frequencies were compared to the Hap-Map project. In the Hap-Map project the Yoruba in Ibadan Nigeria (YRI) were used as the black population and Utah residents with ancestry from northern and western Europe (CEU) as the white population. In addition, through the SIRT5 SNP information from the Hap-map project, we identified the respective genotype (C/C, C/T, T/T) for the Health ABC database genotype coded in numbers (0,1,2).

3.5 HARDY-WEINBERG EQUILIBRIUM

We listed the counts of the genotype distributions because the database with the rs9382222 SNP genotypes was already tested for HWE. This test can indicate if there are data-acquisition flaws or violations of assumptions of no mutation, selection, population substructure, etc.

3.6 ANCESTRY GENETIC POPULATION ASSUMPTION

Because of the assumption that members of a preconceived “race” share common ancestry that may include genetic risk factors [61] and also because the Health ABC was divided into white and black populations, we separated the database by both races; in other words, all analyses were performed on each of the function measures separately by race, (European American–white population and African American–black population). Once the databases were stratified by race, contingency tables and chi-square tests were used to assess associations between frequencies of SIRT5 SNP (rs9382222) and distributions by race.

3.7 BRAIN FUNCTION MARKERS: NORMAL DISTRIBUTION/TRANSFORMATION

The distributions of the three brain function markers were examined: Cognition test, Motor function test and Mood or depressive symptoms test. We also transformed the distributions as necessary until the three brain function markers were normally distributed.

3.8 STATISTICAL ANALYSIS

3.8.1 Exploratory analysis

The following variables from the Health ABC database were used: for phenotype, age (CVAGE), sex (GENDER), race (RACE), cognition test (Y1DSS), motor function test

(Y1MTR20SD) and mood or depressive symptoms test (Y1CES_D); for genotype, SIRT5 SNP (rs9382222).

We tested for significant association and correlation between the three functional outcomes (CES-D, DSST and Gait speed test) and two independent variables (sex and age) using chi-square and Pearson correlation statistics. These analyses were guided by previous analyses of these traits in the Health ABC cohort, e.g., Rosano et al., 2005.

3.8.2 Main Analysis

One way analysis of covariance (ANCOVA) was performed using SAS GLM procedure to test for between-group differences in functional outcomes (CES-D score, DSST score and Gait Test score) and two covariates (sex and age). The statistically significant models were selected and subsequently, we tested for association the three possible genotypes of SIRT 5 SNP (C/C, C/T, T/T). Finally, all the selected models with statistically significant differences among the three possible SIRT5 genotypes were plotted using box plots to show the differences among the three brain functional markers (test scores) in people carrying any of the three possible genotypes of SIRT5 SNP.

4.0 RESULTS

4.1 HEALTH ABC DATABASE AND HAP-MAP PROJECT GENOTYPE COMPARISON

The genotype frequencies in the Health ABC database compared to the Hap-Map project respectively, were as follows: in the white population, the common homozygote (CC) was 47.02% vs. 43.3%, heterozygote (CT) was 42.57% vs. 46.7% and uncommon homozygote (TT) was 10.41% vs. 10%; in black population the common homozygote (CC) was 67.85% vs. 78.9%, heterozygote (CT) was 28.15% vs. 19.3% and uncommon homozygote (TT) was 4% vs. 1.8% (Figure 2). In addition, from the SNP information of the Hap-Map project, we identified the respective allele pair of bases (letters) to the registered Health ABC allele codes (numbers): C/C= 0, C/T=1, T/T=2.

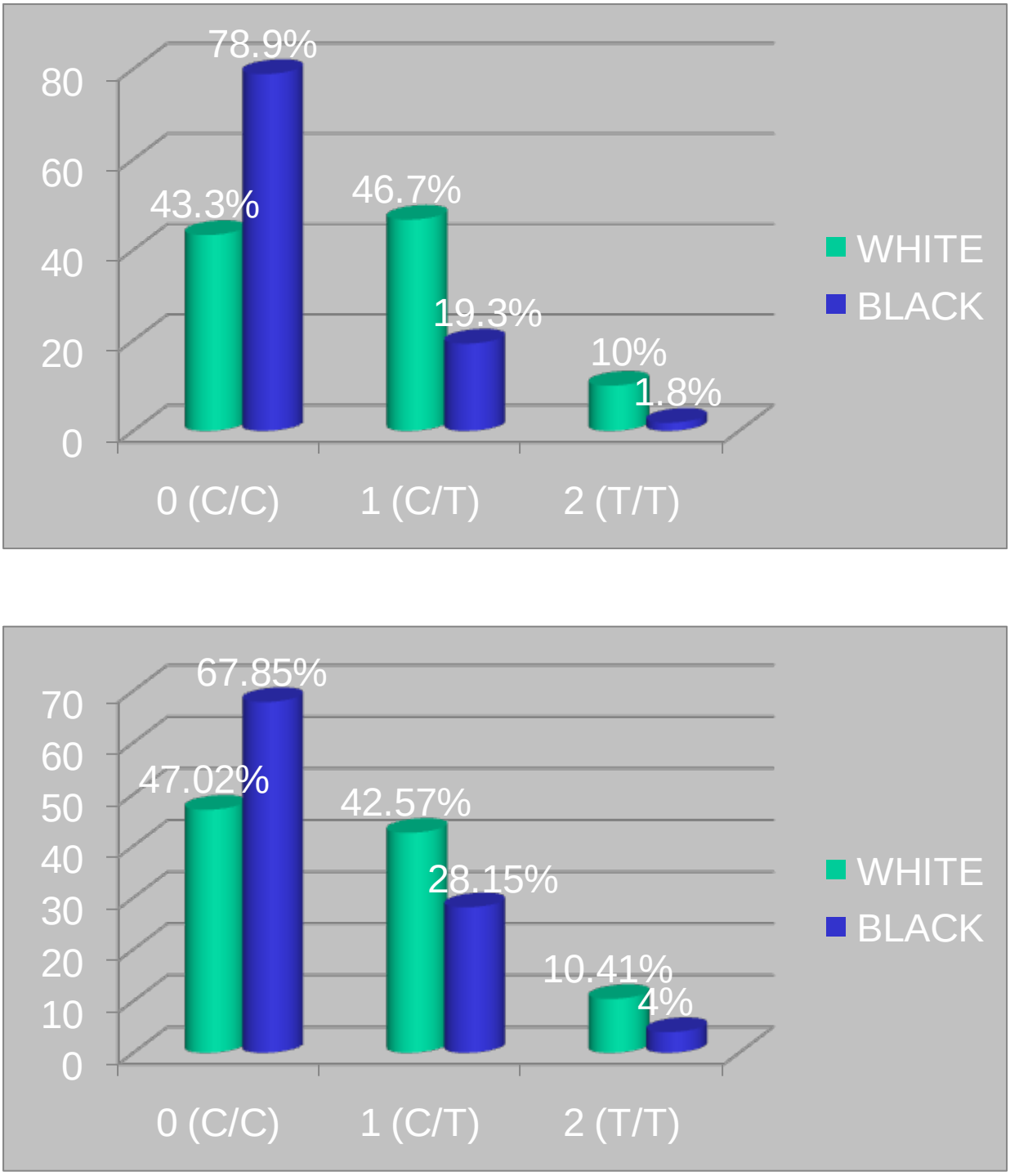


Figure 2. Genotype frequencies of the HAP-MAP project (above) and the Health ABC database.

4.2 HARDY-WEINBERG EQUILIBRIUM

The counts of the genotype distributions are listed in Table 1.

Table 1. Genotype and Allele Frequencies in the Health ABC database.

	Genotype Frequencies			Allele Frequencies		
Population	Genotype	Frequency	Count	Allele	Frequency	Count
Health ABC Database	C/C	55.49	1536	C	73.84	2044
	C/T	36.71	1016	T	26.51	724
	T/T	7.08	216			
Total		100	2768		100	2768

4.3 SNP DISTRIBUTION BY RACE

After the Health ABC data base was stratified by race, the following results were obtained in the association in the SNP distribution by race (χ^2 125.3323, P <.0001): from the 2768 total population, 1642 (59.32%) were white and 1126 (40.68%) were black. Regarding the genotype distribution by race, among the white people, 772 (47.02%) were homozygote for the common allele, 699 (42.57%) were heterozygote and 171 (10.41%) were homozygotes with uncommon allele; among the black people, 764 (67.85%) were homozygotes for the common allele, 317 (28.15%) were heterozygotes and 45 (4%) were uncommon allele homozygotes (Table 2)

Table 2. Distribution of the SIRT 5 SNP in white and black population.

SNP/ RACE	C/C	C/T	T/T	TOTAL
WHITE	47.02%	42.57%	10.41%	100%
BLACK	67.85%	28.15%	4%	100%

4.4 BRAIN FUNCTION MARKERS: NORMAL DISTRIBUTION / TRANSFORMATION

DSST (Skewness: -0.2416207, Kurtosis: -0.0529059) and Gait (Skewness: 0.2172326, Kurtosis: 0.31890667) showed a normal distribution. CES-D (Skewness: 2.06812055, Kurtosis: 6.14335799) did not show a normal distribution and was transformed by logarithms (base 10) to reduce non-normality (Skewness: -0.0467108, Kurtosis: -0.7317499) (Figure 3). As additional information the Skewness and Kurtosis for a normal distribution is zero, and any symmetric data should have these values near zero.

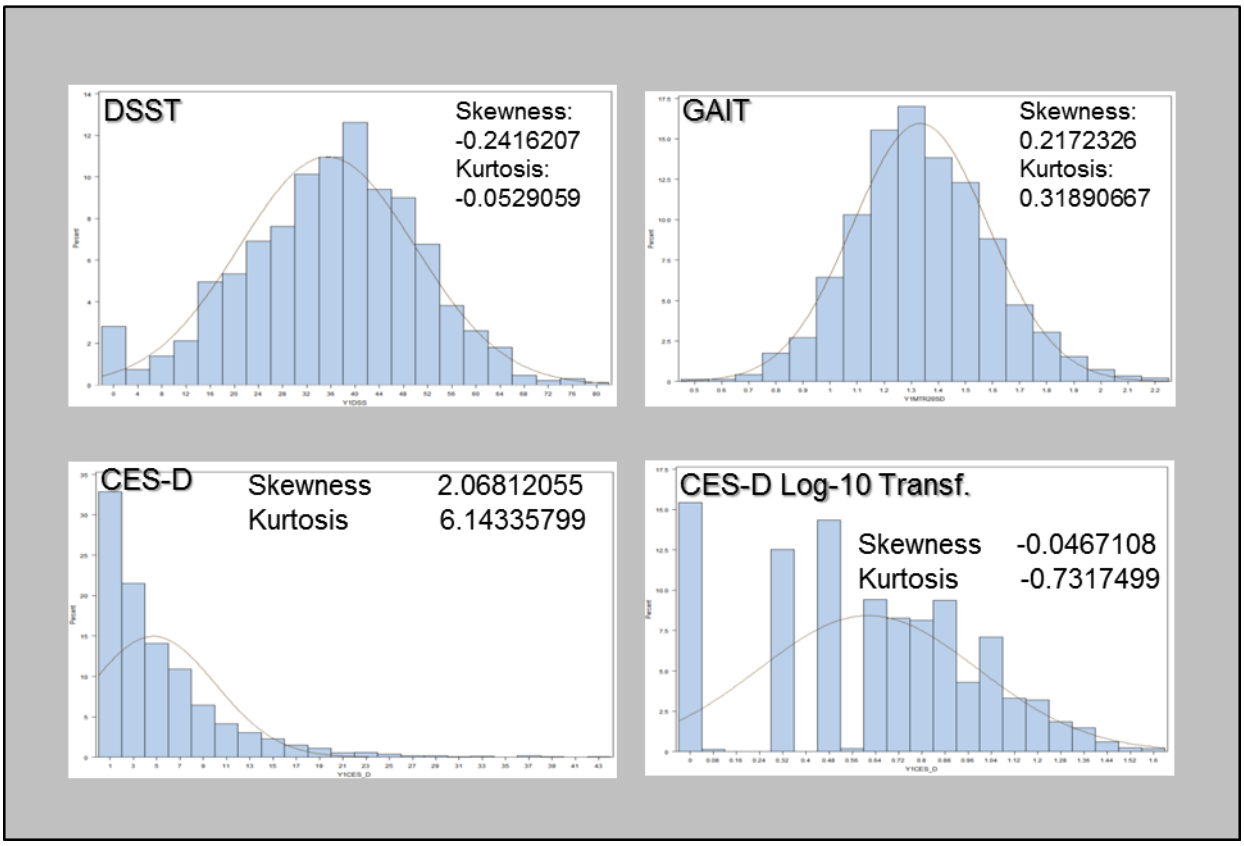


Figure 3. Normal distribution graphs of the three brain functional markers. DSST (mean=35.39, SD=14.55), Gait Test (mean=1.33, SD=0.25) and CES-D Log 10 transformed (mean=0.61, SD=0.37) resulted with a normal distribution transformation. CES-D (without transformation) was not normally distributed.

4.5 STATISTICAL ANALYSIS

4.5.1 Analyses of Covariate effects on DSST, Gait Test and CES-D.

The exploratory analysis resulted in the following significant associations: the associations between DSST score and sex in the white population showed a higher mean in females (43.0530

vs. 39.0279; t= -6.88, p <.0001) than males (Figure 4). The association between DSST score and sex in the black population showed a higher mean in females (29.6582 vs. 23.7655; t= -6.97, p <.0001) than males (Figure 4). The association between Gait Test and sex in the white population showed a higher mean in males (1.4607 vs. 1.3385; t= 10.15, p <.0001) than females (Figure 5). The association between Gait Test and sex in the black population showed a higher mean in males (1.2964 vs. 1.1707; t= 8.63, p <.0001) than females (Figure 5). The association between CES-D score and sex in the white population showed a higher mean in females (5.3912 vs. 3.9606; t= -4.60, p <.0001) than males (Figure 6Figure 7). The association between CES-D score and sex in the black population resulted in no significant association although there was a higher mean in females (5.021 vs. 4.560; t= -0.37, p <0.7094) than males (Figure 6 Figure 7). In addition, the exploratory analysis results in the following significant correlations: DSST score and age in white population showed a significant negative correlation - white subjects with older ages tend to have lower DSST scores (Pearson correlation: -0.17968, p <0.0001) (Figure 8). DSST scores and age in black population showed a significant negative correlation: black subjects with older ages tend to have lower DSST scores (Pearson correlation: -0.20233, p <0.0001) (Figure 8). Gait test and age in white population showed a significant negative correlation: subjects with older ages tend to have lower Gait Test scores (Pearson correlation -0.1562, p <0.0001) (Figure 9). Gait Test and age in black population showed a significant negative correlation: subjects with older ages tend to have lower Gait Test scores (Pearson correlation: -0.19153, p <0.0001) (Figure 9). CES-D and age resulted in no significant correlation in either white (Pearson correlation:-0.00267, p =0.9233) or black (Pearson correlation: 0.02962, p =0.3791) populations (Figure 10 &Figure 11). These results are summarized in Table 3.

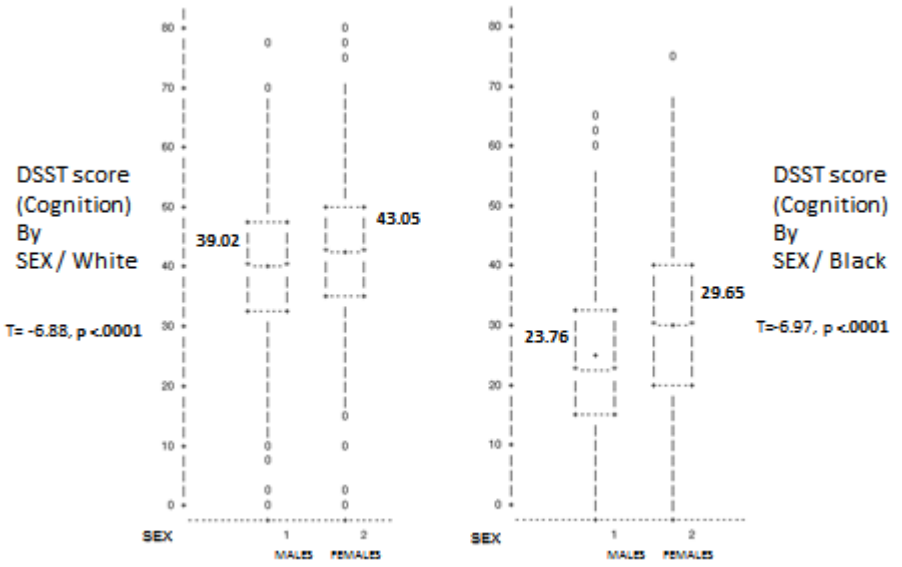


Figure 4. Graphs of DSST score by sex and stratified by race.

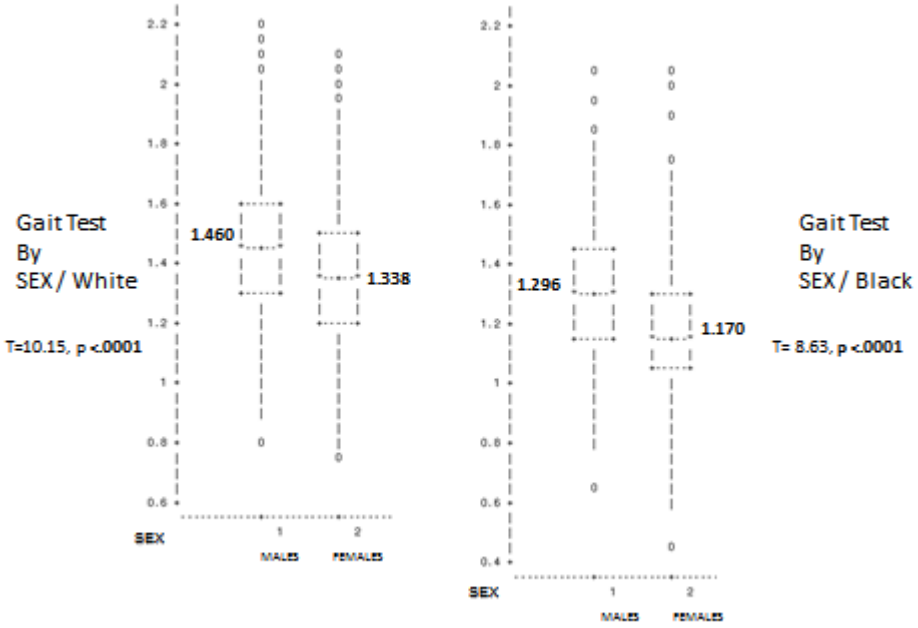


Figure 5. Figure Graphs of Gait Test score by sex and stratified by race.

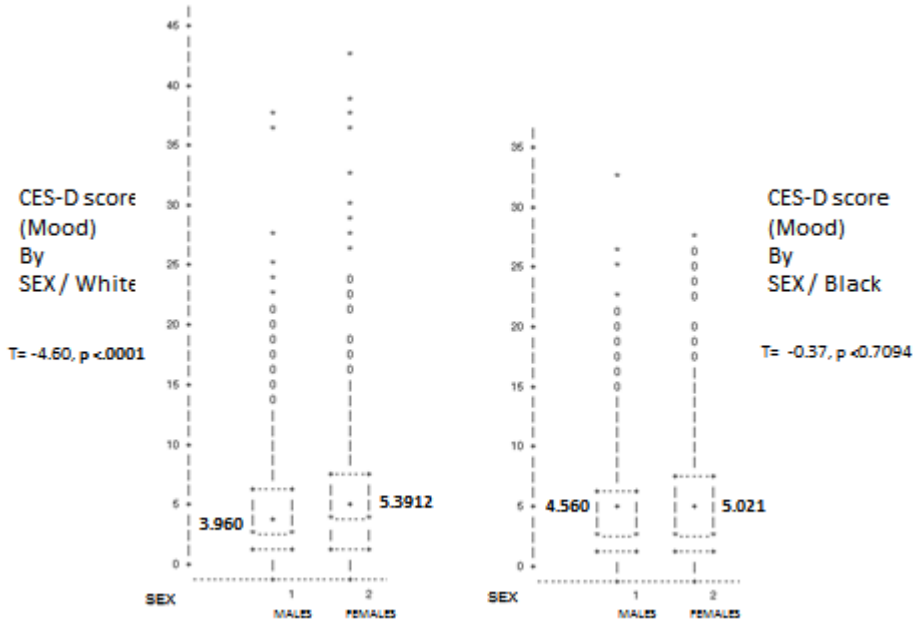


Figure 6. Graphs of CES-D (without transformation) score by sex and stratified by race.

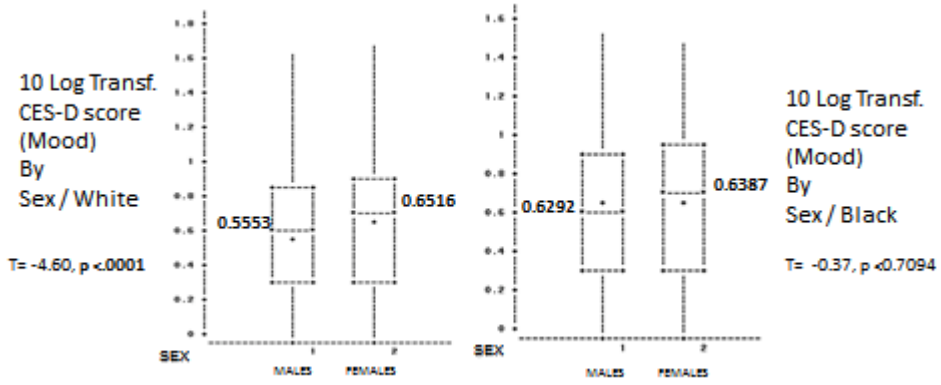
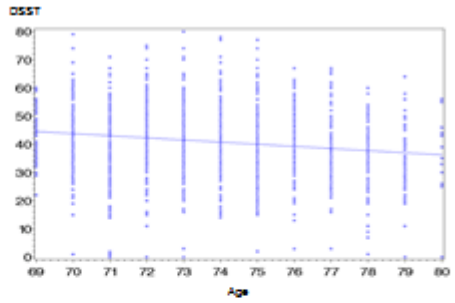


Figure 7. Graphs of CES-D (Log 10 scale transformed) by sex and stratified by race.

DSST score
By Age/ White
Pearson Correlation:
-0.17968, P <0.0001



DSST score
By Age/ Black
Pearson Correlation:
-0.20233, P <0.0001

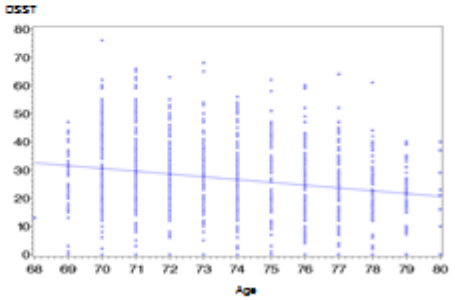
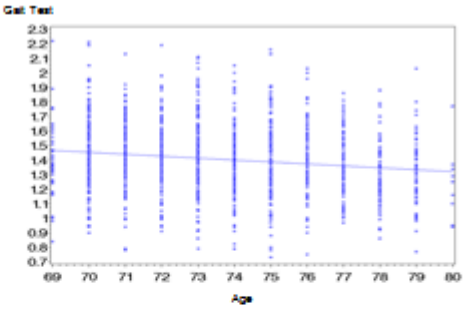


Figure 8. Graphs of DSST score by age and stratified by race.

Gait Test score
By Age/ White
Pearson Correlation:
-0.1562, P <0.0001



Gait Test score
By Age/ Black
Pearson Correlation:
-0.19153, P <0.0001

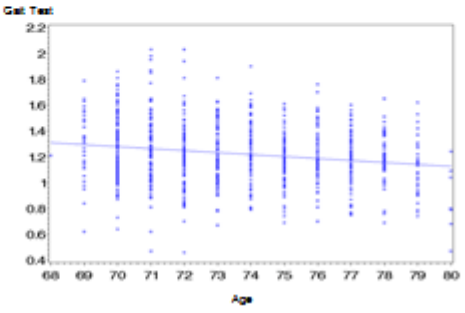
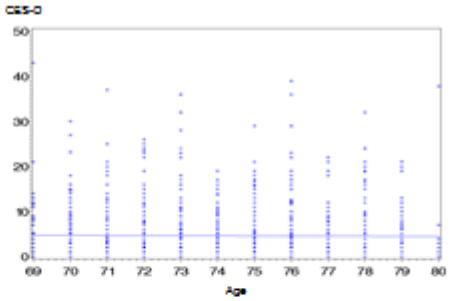


Figure 9. Graphs of Gait Test score by age and stratified by race.

CES-D score
By Age/ White
Pearson Correlation:
-0.00267, P 0.9233



CES-D score
By Age/ Black
Pearson Correlation:
0.02962, P 0.3791

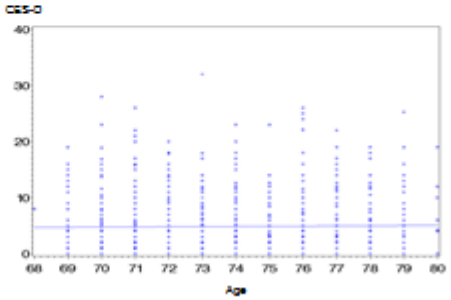
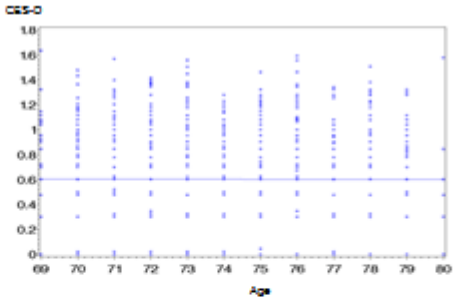


Figure 10. Graphs of CES-D (without transformation) score by age and stratified by race.

Log 10 Transf.
CES-D (Mood)
By Age/ White
Pearson Correlation:
-0.00267, P 0.9233



Log 10 Transf.
CES-D (Mood)
By Age/ Black
Pearson Correlation:
0.02962, P 0.3791

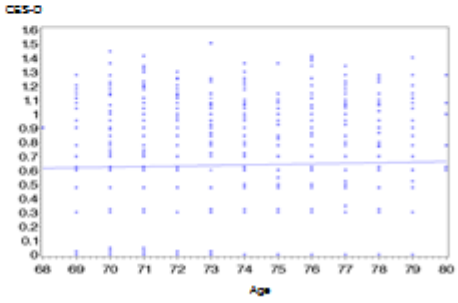


Figure 11. Graphs of CES-D (Log 10 scale transformed) by age and stratified by race.

Table 3. Summary of results from the analyses of Covariate effects on DSST, Gait Test and CES-D.

	Variable	DSST	Gait Test	CES-D
White Population	AGE	**YES Correlation (Pearson= -0.17968, p <0.0001)**	**YES Correlation (Pearson= -0.1562, p <0.0001)**	NO Correlation (Pearson= -0.00267, p 0.9233)
	SEX	**YES Association (t= -6.88, p <0.0001)**	**YES Association (t= 10.15, p <0.0001)**	**YES Association (t= -4.60, p <0.0001)**
Black Population	AGE	**YES Correlation (Pearson: -0.20233, p <0.0001)**	**YES Correlation (Pearson: -0.19153, p <0.0001)**	NO Correlation (Pearson:0.02962, p 0.3791)
	SEX	**YES Association (t= -6.97, p <0.0001)**	**YES Association (t= 8.63, p <0.0001)**	NO Association (t= -0.37, p <0.7094)

4.5.2 Association with SIRT5 SNP

Based on the associations and correlations among the three functional outcomes of brain health (DSST, Gait Test and CES-D) and the independent variables (age and sex), the following models were run (Table 4) and graphed (Figure 12). The results of each of the designed models are summarized in Table 5; the first five models were statistically significant: Model 1 ($F= 33.36$, $p <0.001$), Model 2 ($F= 32.89$, $p <0.001$), Model 3 ($F= 50.32$, $p<0.001$), Model 4 ($F= 41.25$, $p <0.001$), Model 5 ($F= 12.61$, $p <0.001$). Model 6 was not statistically significant ($F= 0.64$, $p <0.526$).

From the overall models (Table 5) only models 2 and 5 were significant for the SIRT5 SNP: in Model 2, people lost 0.94 units for every year of age and the emphasis on sex in the

model made people increase 5.65 units, both changes in the DSST; in Model 5, the SIRT 5 SNP effect of sex in the model made people lose 0.09 units in the CES-D. The results from the rest of the Models (not statistically significant) were as follows: in model 1, people lost 0.72 units for every year of age and the emphasis on sex in the model made people increase 3.83 units in the DSST; in Model 3, people lost 0.01 units for every year of age and the emphasis of sex in the model made people lose 0.12 units in Gait test; and in Model 4, people lost by 0.01 units for every year of age and the emphasis of sex in this Model made people lose 0.13 units.

Table 4. Models designed for the association with SIRT5 SNP.

No.	Outcome	Model	F-test	Significant
	DSST			
1	WHITE	DSST= $\beta_0 + \beta_1$ x GENOTYPE + β_2 x AGE + β_3 x SEX	33.36, p <0.001	Yes
2	BLACK	DSST= $\beta_0 + \beta_1$ x GENOTYPE + β_2 x AGE + β_3 x SEX	32.89, p <0.001	Yes
	Gait Test			
3	WHITE	Gait T= $\beta_0 + \beta_1$ x GENOTYPE + β_2 x AGE + β_3 x SEX	50.32, p <0.001	Yes
4	BLACK	Gait T= $\beta_0 + \beta_1$ x GENOTYPE + β_2 x AGE + β_3 x SEX	41.25, p <0.001	Yes
	CES-D			
5	WHITE	CES_D= $\beta_0 + \beta_1$ x GENOTYPE + β_2 x SEX	12.61, p <0.001	Yes
6	BLACK (ANOVA)	CES_D= $\beta_0 + \beta_1$ x GENOTYPE	0.64, p <0.526	No

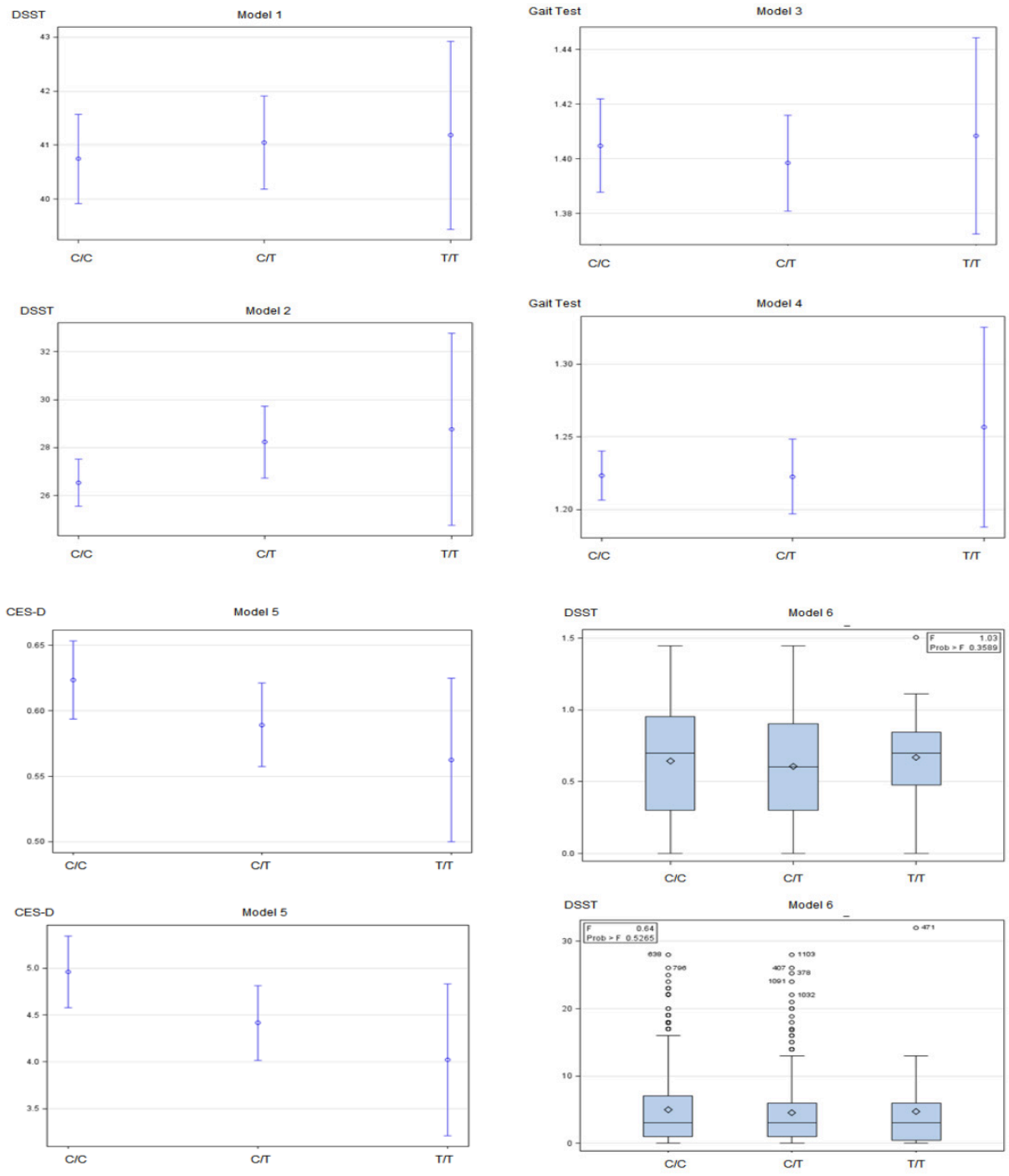


Figure 12. **Graphs of each Model designed for the association with SIRT5 SNP.** Model 1: DSST in white population= $\beta_0 + \beta_1$ GENOTYPE + β_2 x AGE + β_3 x SEX (F 33.36, p<0.001); Model 2: DSST in black population= $\beta_0 + \beta_1$ x GENOTYPE + β_2 x AGE + β_3 x SEX (F 32.89, p<0.001); Model 3: Gait Test in white population = $\beta_0 + \beta_1$ x GENOTYPE + β_2 x AGE + β_3 x SEX (F 50.32, p<0.001); Model 4: Gait Test in black population= $\beta_0 + \beta_1$ x

GENOTYPE + β_2 x AGE + β_3 x SEX (F 41.25, p<0.001); Model 5: CES-D in white population = β_0 + β_1 x GENOTYPE + β_2 x SEX (F 50.32, p<0.001) (Log 10 scale transformed); Model 6: CES-D in black population = β_0 + β_1 x GENOTYPE (F 1.03, p<0.358) (Log 10 scale transformed); Model 5: CES-D in white population = β_0 + β_1 x GENOTYPE + β_2 x SEX (without transformation); Model 6: CES-D in black population = β_0 + β_1 x GENOTYPE (without transformation).

*Last two graphs about model 5 and 6 using untransformed data were showed as a reference to appreciate the changes in the relation compared to the non-log 10 transformed CES-D scores.

Table 5. Summary of results from the association with SIRT5 SNP.

Model Number	**Outcome/Race**	**Model's Variables**	**Parameter Estimate**	**t Value**	**Pr > |t|**
1	DSST/White	Intercept	88.6300	11.69	<.0001
		RS938222	0.2495	0.58	0.5651
		AGE	**-0.7255**	**-7.15**	**<.0001**
		SEX	**3.8386**	**6.65**	**<.0001**
2	DSST/Black	Intercept	87.35	8.24	<.0001
		RS938222	**1.45**	**1.97**	**0.0486**
		AGE	**-0.9487**	**-6.66**	**<.0001**
		SEX	**5.6574**	**6.82**	**<.0001**
3	Gait Test/White	Intercept	2.6148	16.77	<.0001
		RS938222	-0.0011	-0.12	0.9017
		AGE	**-0.0139**	**-6.67**	**<.0001**
		SEX	**-0.1250**	**-10.55**	**<.0001**
4	Gait Test/Black	Intercept	2.6118	14.28	<.0001

Table 5 continued

		RS938222	0.0065	0.52	0.6024
		AGE	**-0.0161**	**-6.56**	**<.0001**
		SEX	**-0.1307**	**-9.16**	**<.0001**
5	CES-D/White	Intercept	0.45136	1.62	0.1052
		RS938222	**-0.03185**	**-2.04**	**0.0419**
		AGE	0.00035	0.10	0.9239
		SEX	**0.09711**	**4.63**	**<.0001**
6	CES-D/Black	Intercept	0.3333	1.02	0.3064
		RS938222	-0.0165	-0.72	0.4702
		AGE	0.0039	0.90	0.3668
		SEX	0.0099	0.39	0.6977

From the Models 2 and 5 (Table 6) which were selected to be tested for their association among each of the three possible genotypes of SIRT5 SNP (C/C, C/T & TT), only Model 2 had found a borderline significant association in the genotype C/C vs. C/T (common homozygote vs. heterozygote) with a statistically significant difference of p=0.051, mean difference=-0.05, Standard Deviation=0.95, (Table 7)(Figure 13); in other words, in the black population, the recessive allele “T” causes the DSST score to increase, or vice-versa. The dominant allele “C” makes the DSST score decrease. Model 5 had an association between the genotype C/C vs. C/T (common homozygotes vs. heterozygotes); however, the statistical significance was p=0.08, mean difference=-1.85, Standard deviation=0.03 (Table 8).

4.5.2.1 DSST

In this test, which were involved models 1 and 2, just model 2 had found a borderline significant association in the genotype C/C vs. C/T (common homozygote vs. heterozygote) with a statistically significant difference of p=0.051, mean difference=-0.05, Standard Deviation=0.95 (Table 7)(Figure 13).

4.5.2.2 Gait Test

Models 3 and 4 were involved in this specific test and neither of these models was statistically significant associated to the genotype.

4.5.2.3 CES-D

In this test, which were involved in models 5 and 6, just model 5 had an association between the genotype C/C vs. C/T (common homozygotes vs. heterozygotes); however, the statistical significance was p=0.08, mean difference=-1.85, Standard deviation=0.03 (Table 8).

Table 6. Models 2 and 5 divided by their three different genotype components (C/C, C/T, T/T).

Model No.	OUTCOME	MODEL
	DSST	
2	BLACK Population	$DSST= \beta_0 + \beta_1 \times GENOTYPE0 + \beta_2 \times AGE + \beta_3 \times SEX$ $DSST= \beta_0 + \beta_1 \times GENOTYPE1 + \beta_2 \times AGE + \beta_3 \times SEX$ $DSST= \beta_0 + \beta_1 \times GENOTYPE2 + \beta_2 \times AGE + \beta_3 \times SEX$
	CES-D	
5	WHITE Population	$CES\_D= \beta_0 + \beta_1 \times GENOTYPE0 + \beta_2 \times SEX$ $CES\_D= \beta_0 + \beta_1 \times GENOTYPE1 + \beta_2 \times SEX$ $CES\_D= \beta_0 + \beta_1 \times GENOTYPE2 + \beta_2 \times SEX$

Table 7. Comparison (T-test) among common homozygote C/C vs. heterozygote C/T from Model 2.

DSST in black population= $\beta_0 + \beta_1$ x GENOTYPE (C/C,C/T) + β_2 x AGE + β_3 x SEX.

Contrast	SD	t-value	P-value
Common Homozygote vs. Heterozygote	0.956	1.950	0.051

Table 8. Comparison (T-test) among common homozygote C/C vs. heterozygote C/T from Model 5.

CES_D= $\beta_0 + \beta_1$ x GENOTYPE (C/C,C/T) + β_2 x SEX.

Contrast	SD	t-value	P-value
Common Homozygote vs. Heterozygote	0.0352	1.73	0.0830

Figure 13. Graph of Model 2 comparing two genotypes -C/C & C/T-. Model: DSST in back population= $\beta_0 + \beta_1$ x GENOTYPE (C/C, C/T) + β_2 x AGE + β_3 x SEX.

5.0 DISCUSSIONS

In overall, the results confirmed our hypothesis because we expected that at baseline, subjects carrying the common C/C risk genotype, which has been associated with older biological age of brain, would display poorer brain functions (i.e. lower DSST score, higher reported depressive-like symptoms) and we found that the C/C SIRT5 genotype was associated here with (1) lower DSST scores in the black population (almost 2 units lower than heterozygotes) and (2) displayed trend-level higher CES-D depressive-like scores in the white population, hence supporting the SIRT5 C/C genotype as a risk factor for both biological brain age and related functional outcomes.

Since Health ABC is a sample of two populations in the United States (US) and Hap Map is a sample of international populations, the comparison was useful to describe the expected allele frequencies in the international population versus those reported in the Health ABC.

If a particular genetic variant is in HWE then we can assume that there are unlikely to be data errors and that the assumptions of the association analyses are met, thus the analyses can proceed. If can also provide a baseline against with to measure change.

The reason we stratified the Health ABC database in black and white population is because we expected variations in allele frequencies between the populations [61]. By analyzing the populations separately, we prevented confounding due to allele frequency differences.

As expected, the analysis of covariates showed that females tend to have higher DSST scores than males; while in Gait scores the tendency is the opposite. Also according to our expectations, this analysis shows that older populations have the propensity for lower scores in DSST and higher Gait scores. As for CES-D scores, the results showed that white females tend to report more depressive symptoms than males. In DSST heterozygotes, members of the black population are more likely to have almost 2 units more than common homozygotes.

The Differences in CES-D among the white population are so small and the p-values are so far beyond the conventional 0.05 that it is not recommended to be considered. In fact, regarding our hypothesis, we expect people carrying the common genotype C/C report increasing depressive symptoms than people who do not carry these genotype; thus, although CES-D in white population was not statistically significant, the association between CES-D and the genotype was as expected. This finding is actually encouraging and should be followed up in other cohorts.

The results from the association with SIRT5 SNP analyses indicated an association between DSST and SIRT 5 SNP in the black population. These results suggest that future studies need to confirm our hypothesis because we expected that at baseline, subjects carrying the uncommon genotype T/T would display poorer function on the cognitive function test (lower DSST score) and we observed that the common genotype C/C is a risk factor for lower DSST scores, however this difference was modest; thus, we can suppose that the uncommon genotype is a protective factor that prevents from people obtaining lower DSST scores, in relation those with the common genotype in the black population.

Looking at these results from the allele perspective, we can say that the more SIRT5 “C” alleles people carry, the greater the risk for a lower DSST score. Alternatively, we could say that

people who carry the allele “T” have a protective factor to avoid lower DSST scores; for example, heterozygotes people C/T showed higher DSST scores than the most common homozygote individuals regarding their genotype SIRT5 SNP; thus, this assumption could mean that the allele “T” in heterozygotes is providing some kind of protection against the allele “C” in the DSST score.

Height is an important factor in the gait test score; usually it is expected than taller people have lower gait test scores than shorter people; the reason for this anthropometric aspect is that taller people usually have longer lower limbs; thus, they need less time to walk 20 meters than people with shorter legs who need more steps to complete the test. This factor could affect our results and interpretation in terms of motor function. In order to avoid this possible distortion of to our findings, in future studies it is important to adjust this factor (group people with the same height in the motor test scores) in the main analysis.

Because Health ABC selected healthy people, we can only make predictions between the SIRT 5 SNP and the three functional brain markers in the healthy older population, instead of an entire population (healthy and non-healthy individuals). In addition, we do not know if the association will show with prevalent, morbid cases. However, the focus of health ABC is for example to focus on why healthy people become demented, not why sick people become demented.

Cross-sectional studies usually involve observations of the entire population. However because Health ABC selected healthy people, we can only make predictions between the SIRT 5 SNP and the three functional brain markers in the healthy older population, instead of an entire population.

Basically, because we are studying healthy people, there would be no advantage between the prospective cohort studies (help to determine risk factors with longitudinal observations over time) over the Cross-sectional studies (follow representative subset of the population, at a defined time) to find an association (SNP-functional markers of brain health). Although this can be easily tested by comparing the cross-sectional with prospective in future analyses

Sample size is always an important factor in any research; the more individuals enrolled, the more accurate results will be obtained. Thus, the future use of different databases could affect the way these results are presented.

Several factors may increase a woman's risk of depression that about twice as many women as men experience depression. Probably because this depression gender gap lasts until after menopause, women are still more sensitive to detect and report depressive symptoms than men. This gap could influence the CES-D scores among both sexes.

Additional analyses could help our understanding of the association of SIRT5 alleles with brain function. For example, we could combine the three measures of brain function scores into one score, using principal components methods. This would enable a test of association between SIRT5 SNP and one multifunctional marker of brain health. Such a result could clarify the inherited interrelation of the three functional brain markers as a whole brain function associated with the SIRT5 SNP.

5.1 LIMITATIONS

However the Health ABC cohort is one of the most representative studies that have been done in the older population area, there still could be a collection bias. One major bias might be

individuals who are intellectually or physically disabled. Also those having low vision or reading difficulties could be a problem. This systematic favoritism present in data collection could affect positively or negatively the results of the study. For example if they select people based in the zip codes near to the Health ABC centers, the educational level, amenities to facilitate sport activities from selected population could affect the scores of the three functional marker of brain health. In addition, there is no perfect way to test this. However, one could look at the characteristics of those who provide complete data over the years of the study and compared to those who do not. Also, one could do test retest reliably.

5.2 CONCLUSIONS

The genotype frequency in the total population (Health ABC) tends to be the common homozygous C/C rather than heterozygous C/T and uncommon homozygous T/T.

Mean DSST heterozygotes C/T among black population, are more likely to be 2 units higher than mean DSST for the common homozygotes C/C, indicating that heterozygotes have a better performance in the DSST score than those who carry the C/C genotype.

Members of the black population who carry the common homozygote genotype C/C show a poorer performance in the DSST score than those who carry the heterozygote genotype C/T.

We can also conclude that SIRT5 SNP is a risk or protective factor to the DSST score depending on its allele frequency expressed in the black population. Allele “C” is a risk factor for a poor DSST score and allele “T” is a protective factor for this same test.

Besides the functional markers of brain health we used, probably the finding for other tests could change the direction of our results. For example, DSST scores could be no as accurate as other cognitive tests for older population. More over the health status could be related with the success in the DSST. We could expect that disable individuals would obtain lower DSST scores than healthy individuals, but probably this expectation change using another cognitive test. Same could happen with the CES-D and Gait Test.

The educational level also could be relevant to our results; it would be expected that people with higher education would obtain higher DSST scores than those who lack of this educational level; thus, if we do not aware the educational level of our selected population, we could overestimate our results by observing a stronger association between the SNP and the DSST.

Differences in CES-D among the white population were not significant. Given the lack of statistically significant findings, differences in CES-D scores among the white population were not conclusive in this study; however, this finding is auspicious and should be followed up in other cohorts and studies.

The lack of community health programs is a factor that also could influence the results of this study. The absence of recreational activities and psychological support from public health programs, at the time people were performing the CES-D and Gait Test, could underestimate the association of the SNP with both CES-D and Gait Test, since this activities enhance healthy cardiovascular condition and motivate people in their daily activities.

Finally, regarding the null statistical significant association of Gait Test and the SNP, probably we could consider that this particular SNP is not relevant to this specific test and/or

there could be others SNPs which could show a stronger association with the motor function using the Gait Test or other kind of tests.

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