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Public Interest in Carrier Screening in the Brazilian Population

Author
Dutra-Clarke, Marina

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Public Interest in Carrier Screening in the Brazilian Population

THESIS

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for the degree of

MASTER OF SCIENCE

in Genetic Counseling

by

Marina Dutra-Clarke

Thesis Committee:
Professor Maureen Bocian, MD, MS, Chair
Professor Kathryn Steinhaus French, MS
Professor Kathryn Osann, PhD, MPH

2018

DEDICATION

To

My dearest parents,

in recognition of their love and support

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ABSTRACT OF THE THESIS

Public Interest in Carrier Screening in the Brazilian Population

By

Marina Dutra-Clarke

Master of Science in Genetic Counseling

University of California, Irvine, 2018

Professor Maureen Bocian, MD, MS, Chair

Brazil has a heterogeneous population comprising indigenous, European, and African ancestral roots that have contributed to carrier risks for certain autosomal recessive disorders, such as sickle-cell disease, thalassemias, cystic fibrosis, and (in the Ashkenazi Jewish Brazilian population) Tay-Sachs disease.

An anonymous online survey was distributed to Brazilians using Facebook and Reddit. A total of 353 eligible participants responded. This study explored knowledge of these disorders, knowledge of autosomal recessive inheritance, perception of carrier risk, and interest in carrier screening. The mean knowledge score was 53% (range: 15% to 100%) and was not significantly associated with level of education. Physicians had significantly higher knowledge scores than all other professions. Non-physician healthcare professionals, however, did not have higher knowledge scores when compared to non-physician professionals. Overall, perception of carrier risk was low. Participants expressed high interest in carrier screening, regardless of demographic background. Seventy-eight percent of participants expressed high interest in carrier screening, and 91% expressed high interest specifically in carrier screening for life-threatening disorders if treatment were available. Participants preferred having carrier screening prior to pregnancy

compared to during pregnancy or waiting for newborn screening ($p<0.001$). Additionally, 86% of participants were interested in carrier screening for disorders that are not typically included in newborn screening.

Challenges to implementing a screening program in Brazil include the shortage of genetics-trained professionals and lack of infrastructure. The carrier risks for these disorders, and the interest presented here, justify a need for expansion of Brazil's genetic services to include population-wide preconception and prenatal carrier screening.

1. INTRODUCTION

1.1 Population genetics of Brazil

Genetic differences among populations can explain why certain autosomal recessive disorders are more common in one population than in another. Autosomal recessive disorders are a group of genetic disorders that occur only when both copies of the same gene contain a mutation, which subsequently leads to little-to-no function of the gene. These disorders are usually inherited from two parents who are "carriers" of the disorder. In a carrier, one copy of a gene contains a mutation while the other copy has no mutation. When two individuals are carriers of mutations in the same gene and have a child together, there is a 25% chance in each pregnancy that the child will inherit the mutated copy from each parent and be affected with an autosomal recessive disorder. This phenomenon is more likely to occur in individuals of a particular ancestry when there is a relatively higher frequency of carriers in that particular population. The prevalence of increased carrier frequencies in a population is influenced by various processes of evolution, including natural selection, new mutations, migration, and genetic drift (Rotter and Diamond 1987). For example, high prevalence of some single-gene disorders are consistently observed in small populations, either when a population is sharply reduced in size, such as a bottleneck effect, or when a small group separates from the main population to found a colony, known as a founder effect. To determine the carrier frequency of a disease-causing allele in a particular population, it is critical to understand the geographical origins of the population.

Brazil, which has a particularly heterogeneous population, is a classic model for population genetics studies on admixture populations (Giolo et al., 2012). The majority of the population is composed of either bi- or tri-hybrids of Caucasians, African descendants, and

Amerindians. Brazil comprised a native population until the Portuguese colonization took place in the year 1500. Not long after, Brazil became a primary destination of the African diaspora. Approximately four million Africans were taken as slaves to Brazil during and after the year 1550. Another European migratory wave followed, mainly from Italy, Germany, and Spain. The interbreeding among the indigenous peoples, Europeans, and Africans contributed to the heterogeneity in the present day population (Salzano and Bortolini, 2002).

Many studies have used genotyping of the DNA of Brazilian individuals to quantify African ancestry. For example, one study showed that in the present-day Black Brazilian representative population, 48% of the Y chromosome and 85% of the mitochondrial DNA haplogroups are of Sub-Saharan African origin (Gonçalves et al., 2008). In another study, mitochondrial DNA from a present-day White Brazilian population had as high as 33% Amerindian and 28% African contribution (Alves-Silva, et al., 2000). When using molecular markers, it has been shown that skin color is a poor predictor of genomic African ancestry on an individual level in the Brazilian population (Parra et al., 2002; Pena et al., 2011). Thus, most Brazilians, even those who self-classify as Caucasian, have some degree of African contribution to their genetic makeup.

Due to this African contribution, it is important to be aware of the increased incidence of hemoglobinopathies in individuals of African ancestry, especially sickle cell anemia and beta thalassemia. Hemoglobinopathies are a heterogeneous group of genetic blood disorders caused by defects in the globin-chain genes. Imbalances in these genes can lead to hemolysis and impaired erythropoiesis. The estimated carrier frequencies for sickle cell trait are 1 in 25 in the general Brazilian population and 1 in 10 in the Brazilian Afro-descendants (Horovitz et al.,

2013). For comparison, the carrier frequency for sickle cell anemia in Caucasians in the United States has been reported to be 1 in 330 to 1 in 500 (Ojodu et al., 2014).

It is thought that the migration of people from sub-Saharan Africa, Italy, and Portugal— the latter two having associated Mediterranean ancestry—introduced thalassemias into the present-day Brazilian populations (Zago et al., 1995). For example, the most common beta thalassemia mutation in southeast Brazil is the CD39 variant, which is also the predominant mutation in Mediterranean populations, including the Italian, Spanish, and Portuguese. In individuals of Mediterranean descent, the overall frequency of carriers for beta thalassemia is estimated to be between 1 in 20 and 1 in 30, and as high as 1 in 6 in Cyprus (Weatherall & Clegg, 2001). The estimated carrier frequency for beta thalassemia varies by region in Brazil; however, one study identified 1 in 76 beta thalassemia carriers in a random sample of over 13,000 individuals, primarily blood donors, in São Paulo, Brazil (Ramalho et al., 1999), which is similar to the 1 in 71 weighted-average in Brazil from Livingstone's study (1985). Alpha thalassemia is observed in higher frequency in those of Mediterranean descent and in those of African descent, both of which are populations that share common ancestry with present-day Brazilians. The frequency of carriers of alpha thalassemia in individuals of African descent is estimated to be between 1 in 20 and 1 in 33. The frequency of carriers of alpha thalassemia in individuals of Mediterranean descent is estimated to be between 1 in 30 and 1 in 50, and as high as 1 in 7 in Cyprus (Weatherall & Clegg, 2001). A recent study of the Brazilian population observed a carrier frequency of the alpha$^{3.7}$ mutation of 1 in 14 in a healthy admixed cohort of Northern Brazilian individuals in whom Amerindian admixture is higher than African admixture (Souza et al., 2009). Also, Sonati et al. (1991) observed a carrier frequency of 1 in 5 for the alpha$^{3.7}$ mutation in a healthy Black Brazilian population.

Another settlement in Brazil includes that of the Jewish population. Jews have inhabited Brazil since the Inquisition in Portugal in the 1540's. Now, Brazil has the ninth largest Jewish population in the world. The present-day Jewish community in Brazil comprises 75% Ashkenazi Jews of Polish and German descent and 25% Sephardic Jews of Spanish, Portuguese, and North African descent. A variety of genetic conditions occur with higher frequency among the Ashkenazi Jews, including Tay-Sachs disease, Canavan disease, cystic fibrosis, Gaucher disease (type I), and familial dysautonomia. Probable explanations of these increased frequencies in the worldwide Ashkenazi Jewish population range from a founder effect to reproductive compensation, which refers to the tendency of parents to replace offspring who are lost to genetic disorders in order to attain a given family size (Frisch et al., 2014; Koeslag and Schach, 1984). There is a theory of heterozygote advantage, particularly in Tay-Sachs disease, that postulates that mutation carriers have resistance to tuberculosis (Chakravarti and Chakraborty, 1978). In one study, the carrier frequency of Tay-Sachs disease in a Brazilian Ashkenazi Jewish population was observed to be 1 in 33 (Rozenberg et al., 2001), similar to that observed in the North American Ashkenazi Jewish population, where the carrier frequency is 1 in 29 (Peterson et al., 1983). For comparison, the carrier rate in Sephardic Jews and most non-Jews is between 1 in 250 and 1 in 300 (Kaback et al., 1999).

The carrier frequency of cystic fibrosis is higher in individuals of European descent, approximately 1 in 25. One Brazilian study observed a carrier frequency for cystic fibrosis estimated at 1 in 58 in Brazilian individuals selected from various regions and as high as 1 in 20 in Brazilians of European descent in the state of Rio Grande do Sul (Raskin et al., 2008).

1.2 Brazilian population statistics

The Brazilian population is nearly 210 million, which makes it the fifth largest population in the world (United Nations, 2017). In comparison, the United States population is roughly 327 million and ranks as the world's third largest population (United States Census Bureau, 2018). In Brazil, the average number of live births per woman is 1.8, and the infant mortality rate is 16 per 1,000 live births (United Nations 2010-2015). In the United States, the average number of live births per woman is 1.9, and the infant mortality rate is 6 per 1,000 live births, nearly three times lower than that of Brazil (United Nations 2010-2015). Brazil has a predominantly young population, with 31% of the population under 19 years old, greater than the United States at 26% (United Nations, 2017). Given its relatively young population with higher proportion of women of reproductive age, the availability of preconception genetic screening in Brazil is particularly relevant.

1.3 Prevalence of genetic disorders in Brazil

In recent decades, Brazil has shown substantial improvement in infection control and has reduced the incidence of diseases caused by poor nutrition in what is considered an "epidemiological transition." With reduced morbidity and mortality rates, now is an important time to address birth defects and genetic disorders, which are becoming relatively more significant causes of morbidity and mortality in Brazil's population (Horovitz et al., 2005).

The March of Dimes published data collected in 2001 on global rates of serious birth defects of genetic or partially genetic etiology (Christianson et al., 2006). Countries were ranked as follows: "highest" (greater than 69.9 per 1,000 live births), "high" (between 61-69.9 per 1,000 live births), "moderate" (between 52.1-60.9 per 1,000 live births), and "lowest" (less than 52.1 per 1,000 live births). The highest rates are observed in certain parts of Africa and the Middle

East, whereas the lowest rates are seen in the United States and certain parts of Europe. The rate in Brazil reported by the March of Dimes in 2001 was estimated as 57.2 per 1,000 live births, which is classified as a moderate rate. When classified by mode of inheritance, estimates of such disorders are 7 per 1,000 for autosomal dominant, 1.3 per 1,000 for X-linked, 3.9 per 1,000 for autosomal recessive, and 3.6 per 1,000 for chromosomal anomalies (Christianson et al., 2006). In the United States, the estimated rate of serious birth defects of genetic or partially genetic etiology is 47.8 per 1,000 live births, which is classified as one of the lowest rates of birth defects (Christianson et al., 2006).

Among the Brazilian population, the carrier frequencies for certain autosomal recessive conditions warrant population carrier screening. As described previously, the estimated carrier frequency for sickle cell trait in Brazil is 1 in 25 (40 per 1,000) in the general population and 1 in 10 (100 per 1,000) in the Afro-descendants (Horovitz et al., 2013). In the United States, the total incidence estimate for sickle cell trait is 15.5 cases per 1,000 births, ranging from 0.8 cases per 1,000 births in Montana to 34.1 cases per 1,000 births in Mississippi. The U.S. incidence estimate for sickle cell trait per ethnicity, based on information provided by 13 states, is 73.1 cases per 1,000 African American newborns and 3.0 cases per 1,000 White newborns (Ojodu et al., 2014).

Brazil has the 9th largest Ashkenazi Jewish population in the world. Ashkenazi Jewish individuals are at risk to be carriers for certain autosomal recessive condition, such as Tay-Sachs disease. In one study, the carrier frequency of Tay-Sachs disease in a Brazilian Ashkenazi Jewish population is 1 in 33, similar to the 1 in 30 frequency observed in the United States Ashkenazi Jewish population (Rozenberg et al., 2001). Additionally, the carrier frequency for cystic fibrosis, another autosomal recessive disorder, is 1 in 29 in Ashkenazi Jewish individuals.

The carrier frequency of cystic fibrosis is increased in individuals of European descent compared to other ancestral groups; in populations of European descent, the carrier frequency is 1 in 25. In the United States, the carrier rate in individuals of Northern European descent is 1 in 28. European origin makes up approximately 60% of the general Brazilian population and as high as 78% of the population in the Southern region, which means that many Brazilians are at increased risk for being carriers for cystic fibrosis (Pena et al., 2011; Rodrigues de Moura et al., 2015). One Brazilian study observed a carrier frequency for cystic fibrosis estimated at 1 in 58 Brazilian individuals selected from various regions, 1 in 44 in Brazilians of European descent, and as high as 1 in 20 specifically in Brazilians of European descent in the state of Rio Grande do Sul (Raskin et al., 2008) by screening for the common mutation, deltaF-508, in CFTR (the gene for cystic fibrosis). In their cohort of 500 Afro-Brazilians, the common delta-F508 mutation was observed in 1 in 60 individuals. This is similar to the observed carrier rate for cystic fibrosis in African-Americans in the United States, which is 1 in 61. However, it is important to note that delta-F508 is not the most common mutation that causes cystic fibrosis in individuals of African descent. For example, the 3120+1G>A mutation is a common mutation in African individuals and is observed in 1 in 91 South African Blacks (Padoa et al., 1999). Since the 3120+1G>A accounts for 15-65% of CFTR mutations in South African blacks, the corrected cystic fibrosis carrier rate was estimated as 1 in 14 to 1 in 59 in South African blacks. Therefore, the carrier rate for cystic fibrosis in Afro-Brazilians may be higher than the 1 in 60 observed in the Raskin study because they only screened for the delta-F508 mutation.

1.4 Barriers to genetic services in Brazil

Barriers to genetic services and testing in Brazil include lack of infrastructure for genetic testing facilities, a shortage of genetics professionals with expertise to conduct and interpret

tests, and inadequate coverage of costs for genetic testing by the current health system. In Brazil, genetic services are overcrowded, and when patients with genetic disorders are followed clinically on a regular basis, access to genetic services for new patients in need becomes even more difficult (Horovitz, 2013). It is estimated that only 25-30% of those in need of genetic services in Brazil receive them. One reason for the lack of genetic services is the shortage of qualified genetics specialists. A 2015 study estimated the total number of genetics specialists as 241 serving all of Brazil (Scheffer et al., 2015). Most of these individuals are located in the South-Southeast region, which poses an even greater barrier to the patient population outside of that region. One reason for a lack of genetics specialists is the inadequate genetics education provided to medical students; medical genetics is only an optional course in many medical schools in Brazil (Guimarães, 2010; Horovitz, 2013; Passos-Bueno et al., 2014). Genetic counseling in Brazil is typically done by medical geneticists and healthcare professionals from other specialties (Passos-Bueno et al., 2014). For example, many centers employ nurses to conduct cancer genetic counseling due to the lack of genetics-trained professionals. However, more genetic counseling training is underway for non-physician genetics specialists as part of a program to improve access to genetic services in Brazil (Passos-Bueno et al., 2014).

There is currently a lack of infrastructure for genetic services in Brazil. Hospitals that have medical genetics specialists and diagnostic laboratories with capabilities in biochemical testing, cytogenetic testing, and molecular testing are primarily available only in urban centers. Furthermore, genetic testing is expensive in Brazil, in part due to the lack of infrastructure (Schlatter et al., 2015).

Another barrier to genetic testing and services in Brazil is the current healthcare system, which comprises one public sector and two private sectors (Paim et al., 2011). The public sector

is called the Unified Health System (Sistema Único de Saúde) where health services are funded entirely by the government. One of the private sectors provides services funded by both private and public funds. The other private sector is the private health insurance sector, which offers various private health insurance plans. With very few public healthcare policies implemented for certain genetic tests, most genetic testing is not covered by the public sector (SUS) (Giugliani et al., 2016). Genetic testing is more readily available for Brazilians with private health insurance, but only 25% of Brazilians have some form of private health insurance. Preimplantation genetic diagnosis, non-invasive prenatal screening, expanded newborn screening, predictive testing, and pharmacogenetics are only available in the private sector though costs are not always covered by private health insurance (Horovitz et al., 2013).

The carrier frequencies for hemoglobinopathies and cystic fibrosis among Brazilians and for Tay-Sachs disease in the Brazilian Ashkenazi Jewish population argue in favor of a population carrier screening program in Brazil. However, the lack of genetic services, infrastructure, and inadequate funding are the likely reasons that a program has not yet been established.

1.5 Current state of the newborn screening programs in Brazil and in the United States

In both the United States and in Brazil, newborn screening is a public health program designed to identify newborns with specific disorders. This program involves taking a few drops of blood from the heel of a newborn to screen for certain disorders, the majority of which are genetic conditions. The significance of screening newborns is that the clinical features and symptoms of the disorders tested for are not always evident in the newborn period, and early detection, diagnosis, and intervention are often crucial in avoiding adverse sequelae. One

laboratory technique, which screens for various metabolic disorders simultaneously with a dried-blood spot, is called tandem mass spectrometry. Positive newborn screens detected using tandem mass spectrometry are then sent to a referral center for follow-up and confirmatory testing to rule out a false positive result. For hemoglobinopathy and sickle cell disease screening in newborns, the methodology is typically a combination of isoelectric focusing (IEF) and high performance liquid chromatography (HPLC). Other methodologies, such as DNA-based assays, may be used for confirmatory testing when there is an abnormal result (CDC, 2015).

In Brazil, newborn screening is commonly known as the "teste do pezinho," which translates to "little foot test." In Brazil, newborn screening was conducted through isolated initiatives beginning in 1976. It was not until 2001 that the Ministry of Health established newborn screening as a national public health program, called "Programa Nacional de Triagem Neonatal." The first phase of the national newborn screening program included congenital hypothyroidism and phenylketonuria, and these were universally tested for in all states in Brazil by 2006. Phase 2 added hemoglobinopathy testing and became available in 2013. Then phase 3, which was established later in 2013, added cystic fibrosis to the panel. Phase 4, established in 2014, included congenital adrenal hyperplasia and biotinidase deficiency. These six conditions are covered by the Brazilian government as part of the newborn screening program in all states of Brazil. In the United States, tests for 29 or more conditions (depending on the state) are offered as part of the newborn screen. In both the United States and Brazil, for an additional cost, parents have the option of a more expanded list of conditions than just those covered in the state newborn screening program. The DLE (Diagnosticos Laboratoriais Especializados) is a genetic testing laboratory that has pioneered newborn screening in Brazil. This laboratory offers expanded newborn screening options, all at an additional cost not covered by the Brazilian

government. DLE offers a Basic plan, which includes 17 disorders, the Amplified plan includes 20 disorders, the Plus plan includes 23 disorders, the Master plan includes 28 disorders, the Expanded plan includes 46 disorders, and the Complete plan includes 50 disorders. The additional cost poses a barrier to accessibility to those who are of lower economic status and who may not be able to afford the expanded panels.

In the United States, newborn screening is available in all states but is not standardized across the country. Every state and territorial jurisdiction varies widely with respect to the list of disorders included in their newborn screening tests—some states mandate as few as 29 disorders, whereas other states mandate screening for over 60 disorders. The Secretary of the Department of Health and Human Services published a recommended uniform screening panel, which consists of 34 core disorders and 26 secondary disorders as of November 2016. In certain states, the decision of which conditions to include in newborn screening is approved by the state legislature (Watson et al., 2006).

1.6 Current state of carrier screening for autosomal recessive disorders in the United States

Carrier screening for autosomal recessive disorders is a type of genetic testing that can determine if an individual has a mutation for a disorder that can be passed on to a child. Carrier screening provides prospective parents with meaningful information that they can use to guide pregnancy planning. If a couple finds that they are both carriers of the same autosomal recessive disorder, there would be a 25% chance in each pregnancy for the baby to be affected with the disorder. If this information is obtained in the preconception period, couples can be offered the option for preimplantation genetic diagnosis (PGD) after *in vitro* fertilization. This method involves analyzing one or two cells extracted from early embryos for mutations inherited from

the parents and then transferring an embryo that does not harbor the mutations into the prospective mother. In other cases, carrier screening is performed after a couple is already pregnant. In this case, if the parents were both found to be carriers of the same condition, prenatal testing of the fetus would be available through invasive procedures such as chorionic villus sampling or amniocentesis. Couples often use this information to guide pregnancy management decisions, such as pregnancy termination or having time to prepare for the condition in case of an abnormal result.

The United States is at the forefront of genetic testing services, which has greatly contributed to the population's accessibility to genetic testing. This, however, is not the case with genetics services in developing nations. In the United States, ethnic, pan-ethnic, or expanded carrier screening are all widely accepted testing options. The American College of Medical Genetics and Genomics (ACMG) has formulated position statements and protocols for prenatal and preconception population carrier screening that "have successfully guided reproductive decision-making for millions of families" (Grody et al., 2013). The ACMG emphasizes the importance of developing clear criteria for the selection of disorders to include in carrier screening panels, rather than screening for as many disorders as possible. Carrier screening recommendations by the ACMG include population carrier screening for certain autosomal recessive disorders, such as cystic fibrosis and spinal muscular atrophy, as well as a panel of conditions specifically for disorders more common in the Ashkenazi Jewish population. The American College of Obstetricians and Gynecologists (ACOG) published a recent committee opinion (number 690) titled "Carrier screening in the age of genomic medicine" (Rink et al., 2017). The recommendations proposed were that "all patients considering pregnancy or who are already pregnant, regardless of screening strategy and ethnicity, should be offered carrier

screening for cystic fibrosis and spinal muscular atrophy as well as a complete blood count and screening for thalassemias and hemoglobinopathies." The ACOG published another committee opinion in March 2017, number 691, titled "Carrier screening for genetic conditions." In this letter, the recommendations were for a complete blood count performed on all pregnant women to assess risk for hemoglobinopathies and that hemoglobin electrophoresis would be indicated if there were suspicion of a hemoglobinopathy, based on ethnicity or low red blood cell mean corpuscular volume. Screening for spinal muscular atrophy was recommended due to the relatively high carrier frequency observed in the general population (the carrier frequency for spinal muscular atrophy in most populations is between 1 in 40 to 1 in 60). Carrier frequencies for cystic fibrosis are higher in individuals of certain ancestries than in others (the carrier frequency for cystic fibrosis can be deconstructed based on various ethnicities as is 1 in 25 in Caucasians, 1 in 65 in Hispanics, 1 in 61 in African Americans, and 1 in 90 in Asians), but because it is challenging to assign a single ethnicity to each individual, it is part of standard population screening offered to everyone in the United States. In the Ashkenazi Jewish population, there are several autosomal recessive conditions with increased carrier frequency, including 1 in 30 for Tay-Sachs disease, 1 in 26 to 1 in 29 for cystic fibrosis, 1 in 40 for Canavan disease, 1 in 89 for Niemann-Pick A, 1 in 90 for Fanconi Anemia, 1 in 100 for Bloom syndrome, 1 in 18 for Gaucher disease type 1, and 1 in 30 for Familial Dysautonomia. The ACOG committee opinion proposed that the criteria for disorders to be in a carrier screening panel should include disorders with a carrier frequency of 1 in 100 or greater, a well-defined phenotype, and a detrimental effect on the quality of life.

Currently, there is no preconception or prenatal population carrier screening program in Brazil. The increased carrier frequency for several autosomal recessive disorders in the Brazilian

population calls for the implementation of routine population carrier screening. For example, the carrier frequency for cystic fibrosis in the Brazilian population is comparable to that of the United States and is even higher (as high as 1 in 20) in the southern regions of Brazil – yet no population screening is offered. The carrier frequencies in Brazil for cystic fibrosis, Tay-Sachs disease (among the Ashkenazi Jewish population), sickle cell disease, and thalassemias all meet the criteria and recommendations of the ACOG and the ACMG position statements on carrier screening. Thus, it would be justifiable to implement population carrier screening.

1.7 Implementation of genetic screening programs

Carrier screening programs have been widely successful and, at times, unsuccessful. The most notable failed carrier screening program was in the United States in the early 1970's, when a national screening program for sickle cell disease was implemented (Culliton 1972). Many African-Americans reported feeling forced to undergo testing and experienced employment, health insurance, life insurance, and marriage discrimination. The associated shame that came with this screening program reflected a lack of public understanding of sickle cell anemia and the benign nature of being a carrier for the disorder. In some cultures, sickle cell and other hemoglobinopathy screening has resulted in women feeling stigmatized and believing that their knowledge of their carrier status could affect their ability to marry (Giordano et al., 2009).

The inception of a voluntary carrier screening program for Tay-Sachs disease in the North American Ashkenazi Jewish population in the 1970's led to a dramatic 90% decrease in the incidence of Tay-Sachs disease (Kaback et al., 1993), and the incidence of Tay-Sachs disease in the American and Canadian Ashkenazi Jewish populations actually became lower than that in the non-Ashkenazi Jewish population. It was the first study of population-based screening, and its organization and strategy led to the success of the program. A pilot study for voluntary carrier

screening for Tay-Sachs disease was implemented in Brazil between the years 1998 and 2000 (Rozenberg et al., 2001). Senior students from Jewish high schools were recruited for the study. The voluntary participation rate was 70%, an indication of the desire for carrier screening. The carrier rate in this cohort was 1 in 33, similar to that of other countries with reported carrier rates for Tay-Sachs disease in the Ashkenazi Jewish population of 1 in 30. The lead author proposed that a Tay-Sachs screening program should be initiated in Brazil. Such screening programs have now been implemented, mainly in the research setting (Rozenberg et al., 2006). Some concerns have been raised about these programs, such as the importance of sharing test results with at-risk relatives and of offering cascade testing for those relatives. For example, Rozenberg mentioned that a family member of one of his patients with Tay-Sachs disease was found to be a carrier through a screening program, but the information was not shared with the family members (2006).

The same strategies used in the successful Tay-Sachs screening program and the advent of newer technologies have aided in the implementation of other genetic screening programs, including carrier detection and prenatal screening for cystic fibrosis (Kaback et al., 1993). Cystic fibrosis carrier detection has been widely used for decades in the United States, but it has limitations. Since the CFTR gene is large and over 1,900 mutations have been identified, it is not cost-effective to offer a full molecular analysis of the entire gene on a population basis. Thus, a common strategy for cystic fibrosis is ethnicity-based mutation panels, which include a certain number of the most common disease-causing *CFTR* gene mutations observed for specific ethnic groups. However, there are limitations of this strategy, in part due to the difficulty of assigning a single ancestry to an individual.

The inborn errors of metabolism (IEM) Brazil network has been a very successful comprehensive program in diagnosing individuals with suspected or confirmed diagnoses of metabolic disorders in all 27 states in Brazil since 2001 (Giugliani et al., 2016). This program was established to provide these patients with appropriate standard of care, including genetic testing and clinical care coordination.

The most recent implementation of a genetic screening program in Brazil is the Brazilian Hereditary Cancer Network, established to provide genetic screening for hereditary cancer predisposition syndromes. By 2009, a handbook was published to establish standardized guidelines for medical professionals. This program is of increased importance now given the recent discovery of a Brazilian founder mutation at codon 337 of the *TP53* gene (pArg337His, c.1010G>A), observed in 1 in 300 Southern Brazilian individuals. The *TP53* germline mutation is known to cause multiple, early cancers due to Li-Fraumeni syndrome, a cancer-predisposition syndrome (Paskulin et al., 2015). Currently, there are 10 centers in public hospitals, altogether providing approximately 7,000 outpatient consultations and risk assessments of hereditary cancer per year to the population assisted by the Public Health Care System (SUS) of Brazil (Ashton-Prolla et al., 2016). However, there are limitations to this program. Genetic testing for hereditary cancer syndromes is only covered by private health insurance, and only 25% of the Brazilian population has private healthcare coverage. Implementation of this program provides hope that in the future, Brazil will expand their genetic screening services for all patients.

2. MATERIALS AND METHODS

This research study was approved and registered under the category of 'exempt human subjects research' by the Institutional Review Board of the University of California, Irvine (HS#2017-3961) (APPENDIX E).

2.1 Participant eligibility

Individuals were eligible to participate in this study if they identified as Brazilian, either currently living in Brazil or living abroad, and were between 18-45 years of age. This age restriction was placed in an effort to target individuals of reproductive ages and/or considering family planning. There were 359 total respondents, but six were removed due to age ineligibility (one was less than 18 years of age and five were over 45 years of age). Both males and females were eligible to participate. The survey was only provided in Portuguese. As such, the participants were required to read and understand Portuguese. Internet access was required in order to participate in this study.

2.2 Recruitment methods

Participants were recruited through an advertisement posted solely online. With an online recruitment method, participants could have been currently living in Brazil or living abroad, but all self-identified as Brazilian by descent. The advertisement was posted to the lead researcher's Facebook page, several public Facebook groups specific to Brazilian individuals, including but not limited to, "Brasileiros nos Estados Unidos" and "You know you're Brazilian when…," and the "Brasil" subreddit group of Reddit (APPENDIX C-D).

2.3 Protection of participant privacy

Participants were asked to complete an anonymous web-based survey generated through SurveyMonkey, an online survey software. Participants accessed the online survey link in their own private settings. The privacy of participants was protected throughout the entirety of the data collection process. No personal identifiers were obtained in this study. This research study did not cause any harm to the participants. All research data was stored securely and confidentially.

2.4 Informed consent

Implied informed consent (unwritten consent) was obtained prior to partaking in the survey. On the first page of the online survey, participants were prompted to an IRB-approved study information sheet. This page included contact information for the lead researcher and faculty sponsor, the purpose of the study, the eligibility requirements, and the contact information of the UCI Institutional Review Board. By clicking 'OK,' the participants indicated that they consented to being a research participant. There was no compensation or reimbursement for participating in this study.

2.5 Survey

The survey was generated using SurveyMonkey and was accessed through the website link: https://pt.surveymonkey.com/r/brazilsurvey1. The survey consisted of a total of 47 questions, including an assortment of 33 multiple-choice questions, 13 Likert scale-based questions, and 1 multiple-answer question. The average completion time was 9 minutes. None of the respondents exited the survey early. The completion rate was high, with only 0.1% of the survey responses missing.

The survey questions included 10 demographic questions, 7 knowledge-based questions regarding the inheritance of autosomal recessive conditions, 11 perceived-knowledge questions regarding certain autosomal recessive conditions and associated carrier risk in the Brazilian population, and 19 questions exploring attitudes toward genetic testing.

The knowledge-based questions in this survey were adapted from a previously published study by Ferreira et al. (2012) entitled, "A model of genetic guidance for hemoglobinopathy patients and laboratory diagnosis of family members as educational and preventative measures." The Ferreira study surveyed 77 Brazilian individuals with hemoglobinopathies and found that the majority of the participants (68.6%) had a low level of knowledge of the inheritance, biology, and cause of sickle cell disease. This may have been due to the fact that the majority of the participants had considerably low education levels, reporting middle school as their highest level of education. The knowledge-based questions adapted from this study include: (1) "Which of the following describes how recessive genetic disorders are inherited?" (2) "When only one parent is a carrier of a genetic disorder, can the child have the disorder?" (3) "When both parents are carriers of a recessive genetic disorder, how often will their offspring have the disorder?" and (4) "Is there a difference between a person who is affected with a recessive genetic disorder and a person who is a carrier for the same genetic disorder?"

Several questions regarding attitudes toward genetic testing were adapted from unpublished studies by Shira Kohan et al. (2015) titled "Perceived Jewish Risk Perception of Genetic Disorders and Attitudes toward Genetic Testing and Screening" and Forghani et al. (2016) titled "Comparison of Attitudes Toward Genetic Testing in Ashkenazi, Persian, and Sephardic Jews in the Greater Los Angeles Area." These questions include: (1) "Would you prefer to have genetic testing before marriage or after marriage?" (2) "If you learned that you

were a carrier of a genetic disorder before marriage, do you think it would affect your ability to find someone to marry?" (3) "If you were pregnant and you and your partner found out you were both carriers of either sickle cell disease, Tay-Sachs disease, or cystic fibrosis, would you want to test your unborn baby?" (4) "How likely would you be to have genetic testing for disorders that could result in a miscarriage?"(5) "How likely would you be to have genetic testing for disorders that would result in severe physical and/or intellectual disability?" and (6)"How likely would you be to have genetic testing for disorders that would result in mild to moderate physical and/or intellectual disability?" The Kohan study used Pearson's correlation models to analyze the relationships between subgroups and how the participants responded to the questions pertaining to attitudes toward genetic testing.

The complete survey for this study is available in Appendix A. A translated version in English is available in Appendix B for reference. Only the Portuguese version was distributed to participants in this study.

2.5.1 Survey scoring

Certain demographic information was grouped due to small sample size. For age distribution, participants were grouped by ages 30 years of age or less and greater than 30.

Questions presented in Likert scale from 1-5: (1) Not at all, (2) A little bit, (3) Somewhat, (4) Quite a bit, (5) Very much.

For the knowledge-based questions, each participant received a total genetic knowledge score. This score is the percentage of total correct answers out of 13 for each participant. All data sets computing knowledge scores are based on a total of 350 participants due to the failure of three participants to complete the knowledge questions.

2.5.2 Data analysis

Survey analysis was conducted using the statistical software, Statistical Package for Social Sciences (SPSS).

This is a descriptive study comparing data on genetic knowledge, perceived genetic risk, and attitudes toward genetic testing among different subgroups (i.e., based on gender, age, considering planning a family within the next 5 years). The differences among subgroups were analyzed using chi-square tests or Fisher's exact tests for categorical variables. T-tests were used for continuous variables when there were two groups. For more than two groups, one-way analysis of variance (ANOVA) was used. Pairwise tests were conducted using the Tukey method with adjustment for multiple comparisons. One-sample Z-test was used to compare two groups within one population sample. P-values less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Demographic data

There were 353 eligible participants who responded to the survey (Table 1). Of these, 69% were female and 31% were male. Participants between ages 18-45 were eligible for this study. Twenty-four percent of participants were between ages 18-25, 53% were between ages 26-35, and 23% were between ages 36-45. Participants were asked their place of birth in Brazil; the majority were born in the South (43%) and Southeast (30%) regions of Brazil, followed by the Northeast (14.5%), the Central-West (7.4%) and the North (4.8%). In this cohort, 74.6% reported their ethnicity as White, and the second most commonly reported ethnicity was "Mestiço" (14%), which is a mix of European and Native Indian. Five percent of respondents reported Black or "Mulato", a mix of Black and White, four percent reported "other" and/or "other mixed ethnicity," 1% reported Indigenous ethnicity, 1% reported Asian ethnicity, and two respondents did not complete this question. The majority of respondents had completed a college or university education (43.5%). The second highest education level was completion of post-graduate studies (30%), followed by high school (26%), and, lastly, middle school (2%). One respondent did not complete this question. Professions were selected from a provided list of 18 profession categories; 3% (n=11) of respondents were physicians, and 10% (n=36) were non-physician healthcare professionals. There was an even split between Catholic and non-religious/atheist participants in this cohort—26% and 29%, respectively. Two percent of this cohort were Jewish (n=7). Marital/relationship status was evenly distributed between married/living with partner and single, 47% and 48%, respectively, and 5% of respondents reported being divorced/separated. One respondent did not complete this question.

Among the total participants, 28.9% reported that they had children, 36% were planning on having children within the next five years, 47% did not plan to have more children within the next 5 years, and 17% were undecided.

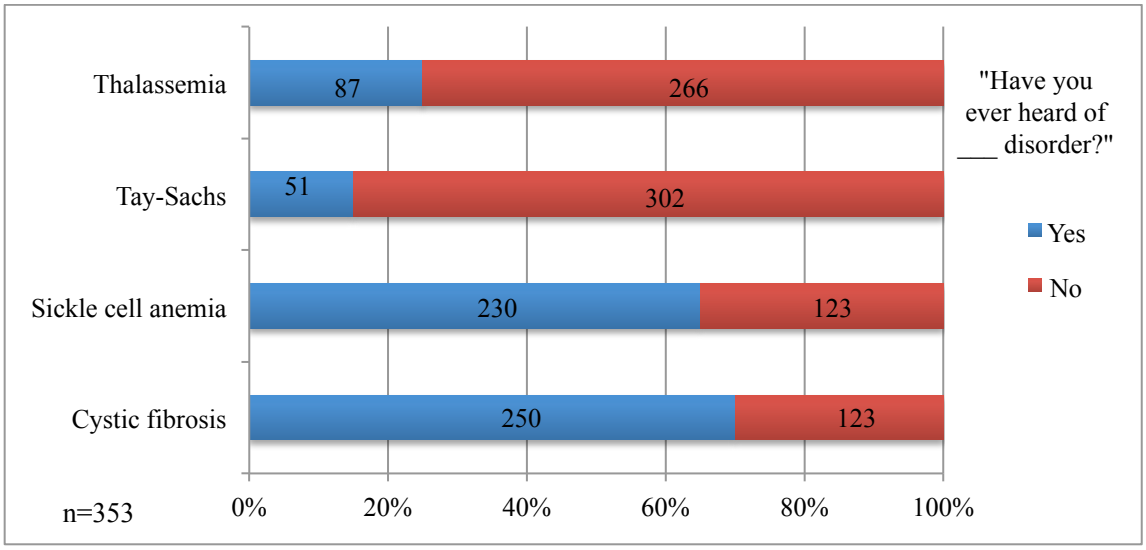
Table 1: Demographics of the participants	n	%
Age	**353**	**100%**
18-21	41	11.6%
22-25	44	12.5%
26-30	103	29.2%
31-35	83	23.5%
36-40	47	13.3%
41-45	35	9.9%
Gender	**353**	**100.0%**
Female	243	68.8%
Male	110	31.2%
Birthplace region	**353**	**100.0%**
North	17	4.8%
Northeast	51	14.5%
Central-West	26	7.4%
South	106	30.0%
South-East	153	43.3%
Ethnicity	**351**	**99.7%**
White	263	75.0%
Black/Mixed	17	4.8%
Mestico	50	14.0%
Indigenous	5	1.4%
Asian	4	1.1%
Other mixed	12	3.4%
Highest Level of Education	**352**	**100.0%**
Middle school	7	2.0%
High school degree	94	26.7%
College degree	153	43.5%
Post graduate degree	98	27.8%
No formal education	0	0.0%

Table 1: Demographics of the participants (continued)	n	%
Profession	**353**	**100.0%**
Physician	11	3.1%
Other healthcare professional	36	10.2%
Science/Technology/Engineering	42	11.9%
Lawyer	13	3.7%
Business/Finance	49	13.9%
Education	32	9.1%
Student	52	14.7%
Unemployed	16	4.5%
Other	102	28.9%
Relationship status	**352**	**100.0%**
Single	169	48.0%
Married/Living with partner	166	47.2%
Divorced/Separated	17	4.8%
Widowed	0	0.0%
Religion	**352**	**100.0%**
Buddhist	6	1.7%
Catholic	93	26.4%
Hindu	0	0.0%
Latter day Saints	0	0.0%
Jewish	7	2.0%
Muslim	0	0.0%
Protestant	49	13.9%
Not religious/atheist	102	29.0%
Other	78	22.2%
Prefer not to answer	17	4.8%
Do you have children?	**353**	**100.0%**
Yes	102	28.9%
No	251	71.1%
Do you plan on having children in the next 5 years?	**353**	**100%**
Yes	127	36%
No	165	46.7%
Undecided	61	17.3%

3.2 Awareness of certain genetic disorders

Participants were asked if they had ever heard of sickle-cell anemia, thalassemia, Tay-Sachs disease, and cystic fibrosis. The majority of participants had heard of cystic fibrosis (71%) followed by sickle-cell anemia (65%); smaller percentages had heard of thalassemia (25%) and Tay-Sachs disease (14.5%) (Figure 1).

Figure 1: Distribution of awareness of certain genetic disorders



Awareness of these disorders was not significantly associated with reported race (Table 2). There was a significant difference between males and females with respect to awareness of cystic fibrosis; 76% of females had heard of cystic fibrosis, whereas only 60% of males reported hearing of cystic fibrosis (p=0.003). There was a significantly increased association between awareness of cystic fibrosis and highest level of education (p=0.010). In addition, awareness of cystic fibrosis was significantly associated with participants over 30 years of age (p=0.001). None of the Jewish participants had heard of Tay-Sachs disease.

Table 2: Descriptive characteristics of respondents who were aware of certain genetic disorders

Demographic subgroups		Sickle-cell anemia		p-value	Thalassemia		p-value
Age	**Total**	**n**	**%**	**0.577**	**n**	**%**	**0.283**
≤ 30	188	120	64%		42	22%	
> 30	165	110	67%		45	27%	
Gender				**0.519**			**0.273**
Female	243	161	66%		64	26%	
Male	110	69	63%		23	21%	
Ethnicity				**0.623**			**0.879**
White	263	168	64%		63	24%	
Black/Mulato/Mestiço	67	47	71%		17	25%	
Other	21	14	67%		6	29%	
Highest level of education				**0.115**			**0.053**
High school or less	101	59	58%		21	21%	
College degree	153	100	65%		33	22%	
Post-graduate degree	98	71	72%		33	34%	
Religion				**0.704**			**0.177**
Catholic	93	63	68%		26	28%	
Non-religious/Atheist	102	69	68%		29	28%	
Jewish	7	4	57%		0	0%	
Other	150	93	62%		31	21%	

The total column represents the total number of participants of the survey cohort that correspond to the particular demographic subgroup labeled. The n column represents the total number of participants that correspond to the particular demographic subgroup who had heard of the disorder labeled. P-values were calculated using Pearson's Chi-Square Tests and Fisher's Exact Tests.

Table 2: Descriptive characteristics of respondents who were aware of certain genetic disorders (continued)

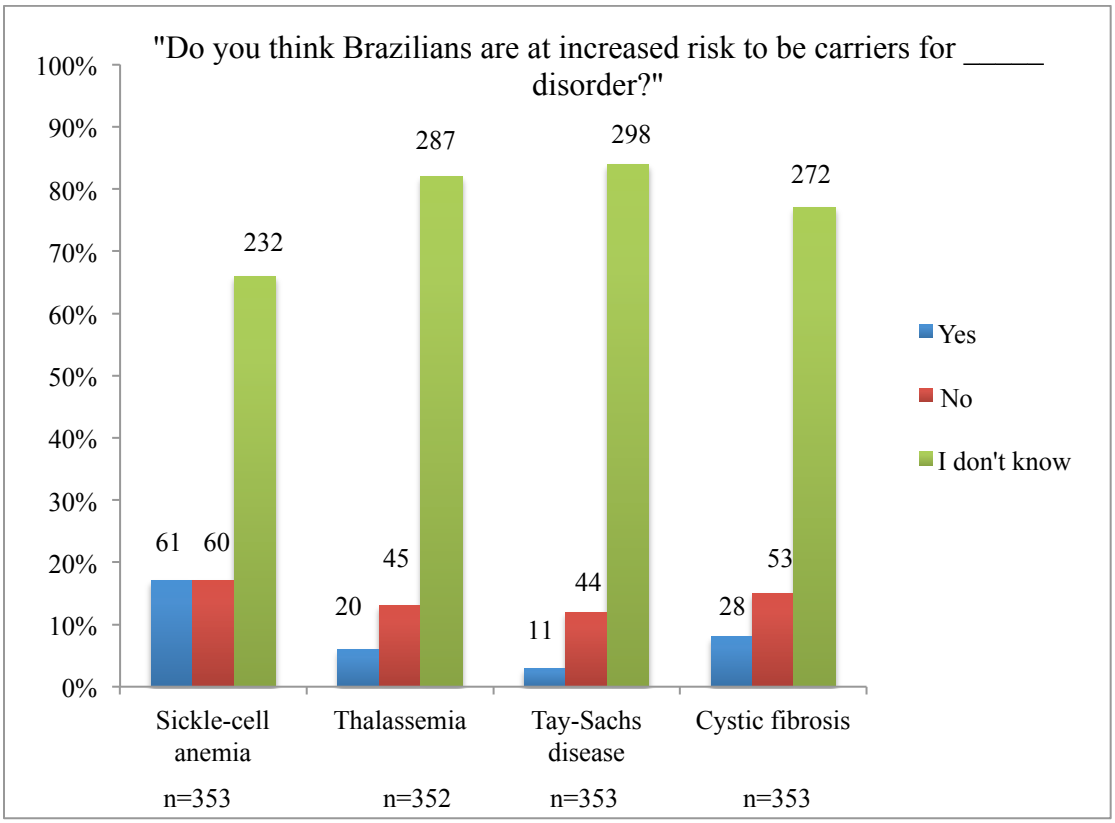
Demographic subgroups		Tay-Sachs disease		p-value	Cystic fibrosis		p-value
Age	**Total**	**n**	**%**	**0.389**	**n**	**%**	**0.001**
≤ 30	188	30	16%		119	63%	
> 30	165	21	13%		131	79%	
Gender				**0.491**			**0.003**
Female	243	33	14%		184	76%	
Male	110	18	16%		66	60%	
Ethnicity				**0.789**			**0.187**
White	263	39	15%		182	69%	
Black/Mulato/Mestiço	67	10	15%		53	79%	
Other	21	2	10%		13	62%	
Highest level of education				**0.831**			**0.010**
High school or less	101	14	14%		63	62%	
College degree	153	21	14%		107	70%	
Post-graduate degree	98	16	16%		80	82%	
Religion				**0.067**			**0.788**
Catholic	93	11	12%		68	73%	
Non-religious/Atheist	102	22	22%		73	72%	
Jewish	7	0	0%		4	57%	
Other	150	17	11%		104	69%	

The total column represents the total number of participants of the survey cohort that correspond to the particular demographic subgroup labeled. The n column represents the total number of participants that correspond to the particular demographic subgroup who had heard of the disorder labeled. P-values were calculated using Pearson Chi-Square Tests and Fisher's Exact Tests.

3.3 Perception of carrier risk of certain genetic disorders

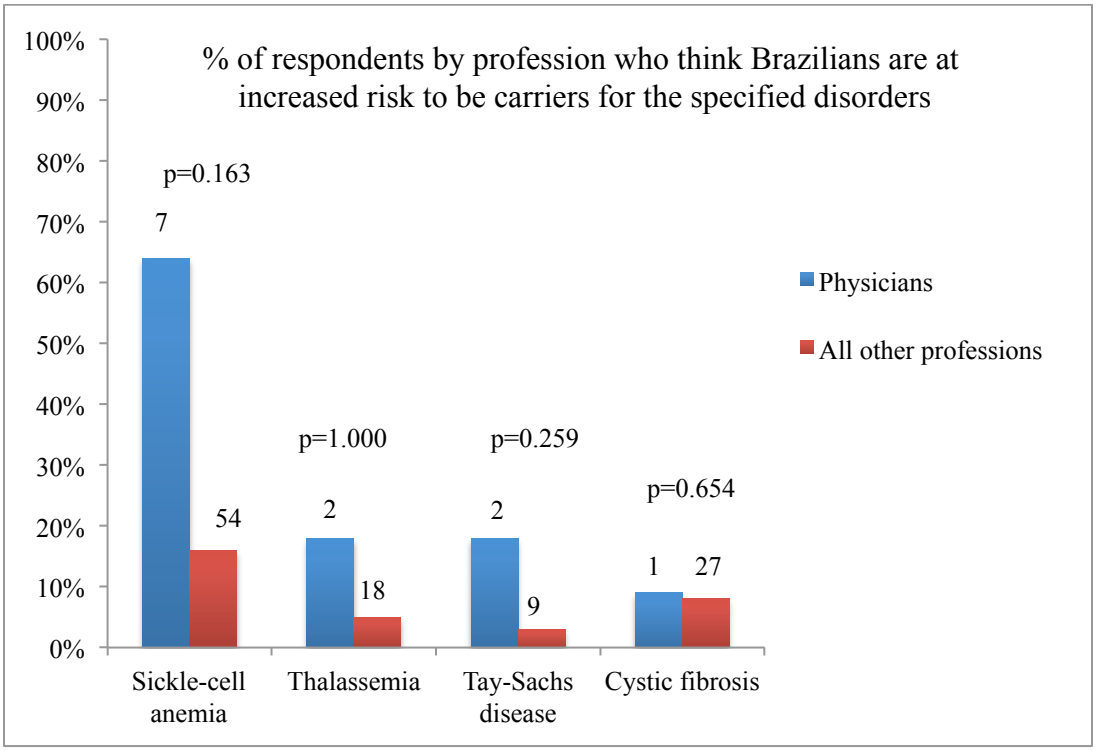
Participants were asked if they thought Brazilians were at increased risk to be carriers for sickle-cell anemia, thalassemia, Tay-Sachs disease, and cystic fibrosis. For each disorder, the majority of participants reported "I don't know;" 66% reported "I don't know" with respect to sickle-cell disease, 82% for thalassemia, 84% for Tay-Sachs disease, and 77% for cystic fibrosis (Figure 2). We refer to this as the perception of carrier risk.

Figure 2: Perception of carrier risk for certain genetic disorders



After removing the participants who responded, "I don't know" to the question about perception of carrier risk for these disorders, there was no statistically significant difference in the responses among the physicians compared to all other non-physician professions (Figure 3). Regarding perception of carrier risk for cystic fibrosis among the 11 physicians, six reported that they did not know if Brazilians had increased carrier risk for cystic fibrosis, one reported increased risk, and four reported no increased risk. This sample size is too small to draw definitive conclusions.

Figure 3: Differences in perception of carrier risk for certain disorders between physicians and non-physicians



P-values were calculated using Fisher's Exact Test between perceived increased carrier risk vs. no perceived increased carrier risk groups (those who answered, "I don't know" were removed from the analysis). Y-axis represents the percent of the physicians (n=11) and percent of non-physicians (n=342) who perceived increased carrier risk for the disorders labeled.

The perception of carrier risk for these disorders was assessed in the subset of participants who had previously heard of these disorders. For each disorder, participants were asked to respond "yes" or "no" for whether or not they thought there was increased carrier risk in the Brazilian population. Among the participants who had previously heard of sickle-cell anemia (n=230), 57% did not know if risk was or was not increased. Among those who answered yes or no (n=99), 53% reported yes, while 48% reported no. Using a one-sample z-test, these values were not significantly different from one another (p=0.551). This suggests little knowledge overall of carrier risk for sickle-cell anemia, since there was no statistically significant difference between the percentage who perceived increased carrier risk and the percentage who perceived

no increased carrier risk. Regarding the perception of carrier risk among the participants who had heard of cystic fibrosis (n=250), 72% did not know if risk was or was not increased. Among the participants who answered yes or no (n=69), 35% reported yes, while 65% reported no (p=0.013, z test). Among the participants who had heard of thalassemia (n=87), 63% reported that they did not know if risk was or was not increased. Among those who reported yes or no (n=32), 34% reported yes, while 66% reported no. This difference was not statistically significant (p=0.070, z test). Regarding perception of carrier risk for Tay-Sachs disease, none of the Jewish participants answered "yes," which is not surprising given that none of the Jewish participants in this cohort had ever heard of Tay-Sachs disease. Of the non-Jewish participants who had heard of Tay-Sachs disease (n=51), 53% did not know if there was increased carrier risk for this disorder. Of the participants who reported yes or no (n=24), 17% reported yes, and 83% reported no (p=0.001, z test). Among those who had heard of the disorders, there were no significant differences in perception of carrier risk for these disorders among participants of different ethnicities or places of birth (Table 3). Additionally, there was no significant difference in perception of carrier risks for these disorders between those who did or did not have children. There were significant differences in the perception of risk for thalassemia and Tay-Sachs disease among individuals planning on having children within the next five years compared to those who were not (p=0.007 and p=0.027, respectively). There was a significant increase in perception of carrier risk for cystic fibrosis in those 30 years of age or less compared to those over 30 (p=0.005), those with a high school education or less compared to those with a college or post-graduate education (p=0.021), and single individuals compared to those who are married/living with partner (p=0.032). Females had a significantly increased perception of carrier risk for sickle-cell anemia compared to males (p=0.004).

Table 3: Perception of carrier risk for genetic disorders by demographic characteristics

Demographic subgroups		Sickle-cell anemia		p-value		Thalassemia		p-value
Age	**Total (n=99)**	**n**	**%**	**0.208**	**Total (n=32)**	**n**	**%**	**1.000**
≤ 30	55	32	58%		23	8	35%	
> 30	44	20	46%		9	3	3%	
Gender				**0.004**				**0.216**
Female	61	39	64%		23	9	39%	
Male	38	13	34%		9	4	22%	
Ethnicity				**0.133**				**0.753**
White	74	43	58%		22	8	36%	
Black/Mulato/Mestiço	21	7	33%		9	3	33%	
Other	4	2	50%		1	0	0%	
Place of birth				**0.807**				**0.938**
North/Northeast	24	14	58%		9	3	33%	
Central-West	4	2	50%					
South/Southeast	71	36	51%		23	8	35%	
Highest level of education				**0.608**				**0.640**
High school or less	30	17	57%		9	2	22%	
College degree	40	22	55%		12	5	42%	
Post-graduate degree	29	13	45%		11	4	36%	
Marital status				**0.239**				**0.397**
Single	56	26	46%		24	7	29%	
Married/Living with partner	41	24	59%		8	4	50%	
Do you have children?				**0.513**				**1.000**
Yes	24	14	58%		5	2	40%	
No	75	38	51%		27	9	33%	
Planning on having children within the next five years				**0.302**				**0.007**
Yes	34	21	62%		9	6	67%	
No	49	22	45%		18	2	11%	
I don't know	26	9	53%		5	3	60%	

Data includes only those who had previously heard of the disorder, labeled as "total n". Respondents who answered, "I don't know" for carrier risk perception for these disorders were removed. The n values are the number of respondents who reported increased carrier risk for the disorder labeled. P-values were calculated with Pearson Chi-Square Tests and Fisher's Exact Tests. Under the thalassemia column, p=0.006 between those planning of having children within the next five years and those who were not, though p-value may not represent true difference due to the small sample size in one group.

Table 3: Perception of carrier risk for genetic disorders by demographic characteristics (continued)

Demographic subgroups		Tay-Sachs disease		p-value		Cystic fibrosis		p-value
Age	**Total (n=24)**	**n**	**%**	**1.000**	**Total (n=69)**	**n**	**%**	**0.005**
≤ 30	13	2	15%		33	17	52%	
> 30	11	2	18%		36	7	19%	
Gender				**1.000**				**0.206**
Female	17	3	18%		48	19	40%	
Male	7	1	14%		21	5	24%	
Ethnicity				**0.657**				**0.977**
White	20	3	15%		49	17	35%	
Black/Mulato/Mestiço	3	1	33%		16	6	38%	
Other	1	0	0%		3	1	33%	
Place of birth				**0.151**				**0.431**
North/Northeast	8	3	38%		17	6	35%	
Central-West	1	0	0%		3	0	0%	
South/Southeast	15	1	7%		49	18	37%	
Highest level of education				**0.301**				**0.021**
High school or less	6	0	0%		18	11	61%	
College degree	6	2	33%		23	5	22%	
Post-graduate degree	12	2	17%		28	8	29%	
Marital status				**0.300**				**0.032**
Single	13	1	8%		32	15	47%	
Married/Living with partner	11	3	27%		36	8	22%	
Do you have children?				**0.539**				**0.179**
Yes	6	0	0%		23	5	22%	
No	18	4	22%		46	19	41%	
Planning on having children within the next five years				**0.027**				**0.909**
Yes	4	2	50%		21	8	38%	
No	14	0	0%		31	10	32%	
I don't know	6	2	33%		17	6	35%	

Data includes only those who had previously heard of the disorder, labeled as "total n." Respondents who answered, "I don't know" for carrier risk perception for these disorders were removed. The n values are the number of respondents who reported increased carrier risk for the disorder labeled. P-values were calculated with Pearson's Chi-Square Tests and Fisher's Exact Tests.

3.4 Perception of knowledge of what it means to be a "carrier" of an autosomal recessive disorder and perception of knowledge of carrier screening

Participants were asked if they knew what it means for an individual to be a "carrier" of a genetic disorder. Ninety percent of participants responded "yes." When asked if they had ever heard of carrier screening, 43% had and 57% had not. There were no significant differences among demographic subgroups in responses to this question (Table 4).

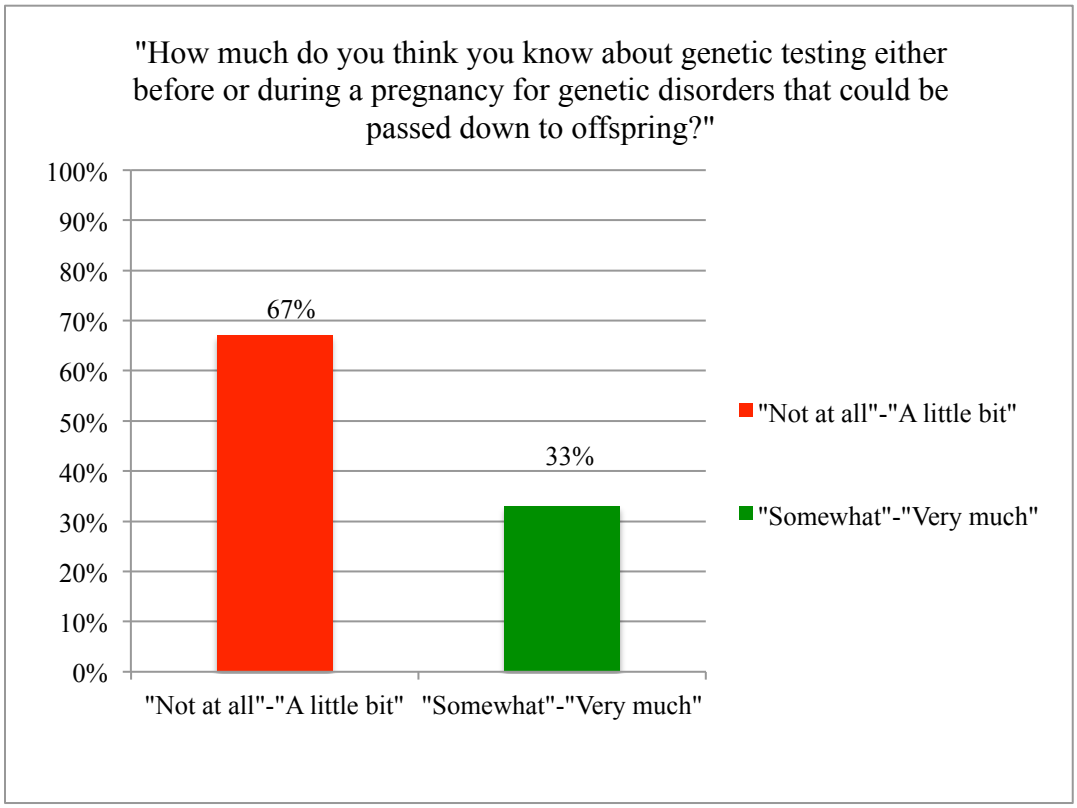
Table 4: Descriptive characteristics of those who had heard of carrier screening and those who had not

Demographic subgroups	Total n	Yes (n=152)	%	No (n=200)	%	p-value
Age						**0.258**
≤ 30	188	86	46%	101	54%	
> 30	165	66	40%	99	60%	
Gender						**0.828**
Female	243	104	43%	139	57%	
Male	110	48	44%	61	55%	
Ethnicity						**0.495**
White	263	108	41%	154	59%	
Black/Mulato/Mestiço	67	33	49%	34	51%	
Other	21	9	43%	12	57%	
Place of birth						**0.936**
North/Northeast	68	29	43%	39	57%	
Central-West	26	10	38%	15	58%	
South/Southeast	259	113	44%	146	56%	
Highest level of education						**0.812**
High school or less	101	41	41%	60	59%	
College degree	153	67	44%	86	56%	
Post-graduate degree	98	44	45%	54	55%	
Religion						**0.179**
Catholic	93	44	47%	48	52%	
Non-religious/Atheist	102	48	47%	54	53%	
Jewish	7	3	43%	4	57%	
Other	150	56	37%	94	63%	
Marital status						**0.016**
Married/Living with partner	166	80	48%	88	53%	
Single	169	70	41%	96	57%	
Divorced/Separated	17	2	12%	15	88%	
Do you have children?						**0.821**
Yes	102	45	44%	57	56%	
No	251	107	43%	143	57%	
Planning on having children within the next 5 years						**0.488**
Yes	127	52	41%	75	59%	
No	165	70	42%	95	58%	
I don't know	61	30	49%	30	49%	

P-values were calculated using Pearson's Chi-Square Test or Fisher's Exact Test. Under the marital status category, p-value may not represent true difference in marital status due to the small sample size in one group. When the divorced/separated group was removed, p=0.317.

Participants were specifically asked how much they thought they knew about genetic testing done, either before or during a pregnancy, for genetic disorders that can be passed on to their offspring, basing their responses on a Likert scale from 1-5: (1) “not at all” (2) “very little” (3) “somewhat” (4) “quite a bit” and (5) “very much.” The majority, 67%, reported a score of 1 or 2 (Figure 4).

Figure 4: Distribution of perception of knowledge about carrier screening



When categorized into two groups, low perception of knowledge about carrier screening (1-2) vs. high perception of knowledge about carrier screening (3-5), there were no significant differences among demographic subgroups in responses to this question (Table 5). The mean response from the physicians in this cohort was (3), which corresponds to “somewhat.”

Table 5: Perception of knowledge about carrier screening among different demographic subgroups

Demographic subgroups	Total n	Low perception (n=237)	%	High perception (n=115)	%	p-value
Age						**0.803**
≤ 30	188	127	68%	60	32%	
> 30	165	110	67%	55	33%	
Gender						**0.733**
Female	243	165	68%	78	32%	
Male	110	72	65%	37	34%	
Ethnicity						**0.715**
White	263	181	69%	82	31%	
Black/Mulato/Mestiço	67	43	64%	23	34%	
Other	21	13	62%	8	38%	
Place of birth						**0.185**
North/Northeast	68	52	76%	16	24%	
Central-West	26	16	62%	10	38%	
South/Southeast	259	169	65%	89	34%	
Highest level of education						**0.630**
High school or less	101	64	63%	36	36%	
College degree	153	103	67%	50	33%	
Post-graduate degree	98	69	70%	29	30%	
Religion						**0.245**
Catholic	93	59	63%	34	37%	
Non-religious/Atheist	102	64	63%	37	36%	
Jewish	7	4	57%	3	43%	
Other	150	110	73%	40	27%	

P-values were calculated using Pearson's Chi-Square tests and Fisher's Exact Tests.

Table 5: Perception of knowledge about carrier screening among different demographic subgroups (continued)

Demographic subgroups	Total n	Low perception (n=237)	%	High perception (n=115)	%	p-value
Profession						**0.248**
Physician	11	5	45%	6	55%	
Non-physician healthcare	36	23	64%	13	36%	
All other professions	306	209	68%	96	31%	
Marital status						**0.050**
Married/Living with partner	166	109	66%	59	36%	
Single	169	111	66%	55	33%	
Divorced/Separated	17	16	94%	1	6%	
Have children?						**0.503**
Yes	102	66	65%	36	35%	
No	251	171	68%	79	31%	
Planning on having children within the next 5 years						**0.449**
Yes	127	90	71%	37	29%	
No	165	105	64%	59	36%	
I don't know	61	42	69%	19	31%	

P-values were calculated using Pearson's Chi-Square Tests and Fisher's Exact Tests.

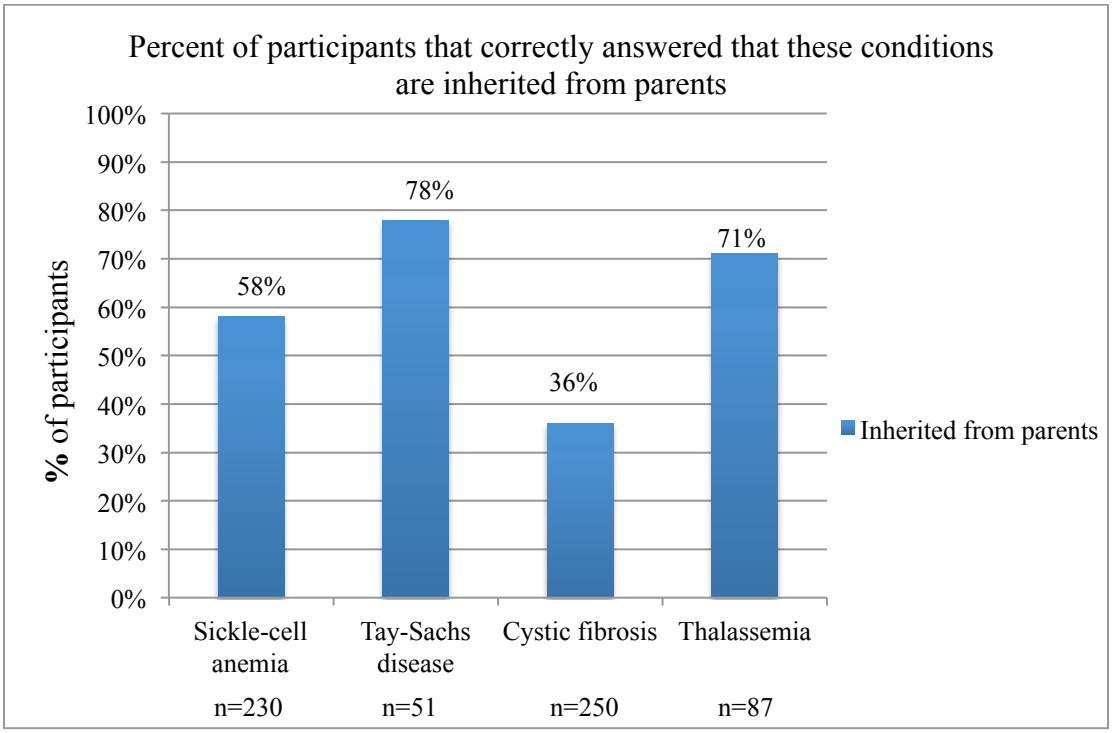
3.5 Genetic knowledge of hereditary disorders vs. nonhereditary disorders and knowledge of autosomal recessive inheritance among demographic groups

Genetic knowledge was ascertained by a combination of two knowledge-based question segments: (1) hereditary disorders vs. non-hereditary disorders (seven questions) and (2) the inheritance pattern of autosomal recessive disorders (six questions). A total genetic knowledge score, combining both segments, was calculated based on the number of correct answers out of 13 questions. The highest score in this cohort was 100% (13/13), and the lowest was 15% (2/13). The mean knowledge score was 53% (n=350) (standard deviation=21%). Participants scored higher in segment #1, which tested their ability to differentiate between disorders that are

inherited from parents and from disorders that are not, than in segment #2, which tested their knowledge of autosomal recessive inheritance.

For segment #1, participants were asked to "check all that apply" from a list of disorders if they thought the disorder was inherited from parents. Overall, 45% of participants checked sickle-cell anemia, 30% checked Tay-Sachs disease, 31% checked cystic fibrosis, 0.6% checked measles, 28% checked thalassemia, 0.9% checked tuberculosis, 3% checked Zika, 11% checked none of the above, and 37% checked that they did not know. Of the participants who had previously heard of sickle-cell anemia (n=229), 58% correctly indicated that it is a disorder inherited from parents. Of the participants who had heard of Tay-Sachs disease (n=50), 78% correctly identified it as a disorder inherited from parents. Of the participants who had heard of cystic fibrosis (n=248), 36% correctly checked that it is a disorder inherited from parents. Of the participants who had heard of thalassemia (n=86), 71% correctly indicated that it is a disorder inherited from parents. Of these four disorders, cystic fibrosis was the least recognized as a disorder inherited from parents (Figure 5).

Figure 5: Knowledge of heredity of certain genetic disorders by participants who had previously heard of the disorders



Segment #2 of the genetic knowledge questions comprised six questions that tested knowledge of autosomal recessive inheritance. The mean score in this segment of knowledge questions was 37% (2.2/6). There were significant differences among certain demographic groups in this segment (Table 6). Males had a significantly higher mean genetic knowledge score in this segment than females (p=0.010). Physicians had a significantly higher genetic knowledge score in this segment than those in the non-physician healthcare professions and all other professions (p=0.001). The mean genetic knowledge score was the same for non-physician healthcare professionals and those of all other non-physician professions; both groups had a mean score of 2.17 correct out of the six questions in this segment (36%). Participants 30 years of age or less had a significantly higher mean genetic knowledge score in this segment than participants over 30 years of age (p=0.022). Participants who did not have children had a

significantly higher mean score than those who had children (p=0.010). This is consistent with the findings that participants 30 years of age or less scored higher than those greater than 30 years of age, described above. Participants who were single had significantly higher mean knowledge scores in this segment than those who were married/living with partner (p=0.011). There was no significant difference between those planning on having children within the next five years and those who were not (p=0.487). There was no significant difference in mean knowledge score in this segment by level of education (p=0.356).

Table 6: Differences among demographic subgroups in mean genetic knowledge scores from segment #2 (genetic knowledge questions)

Demographic subgroups	mean score %	n	std. error	p-value
Age				**0.022**
≤ 30	40%	187	2.1	
> 30	33%	163	2.2	
Gender				**0.010**
Female	34%	242	1.8	
Male	43%	108	2.9	
Ethnicity				**0.866**
White	38%	260	1.8	
Black/Mulato/Mestiço	36%	67	3.5	
Other	38%	21	6	
Place of birth				**0.748**
North/Northeast	36%	68	3.7	
Central-West	41%	25	7.2	
South/Southeast	37%	257	1.7	
Highest level of education				**0.356**
High school or less	36%	101	3.0	
College degree	36%	151	2.2	
Post-graduate degree	41%	98	2.9	
Religion				**<0.001**
Catholic	41%	91	3.1	
Non-religious/Atheist	44%	102	3.0	
Jewish	31%	7	12.3	
Other	30%	149	2.0	

P-values were calculated using one-way analysis of variance.

Table 6: Differences among demographic subgroups in mean genetic knowledge scores from segment #2 (genetic knowledge questions) (continued)

Demographic subgroups	mean score %	n	std. error	p-value
Profession				**0.001**
Physician	68%	11	5.2	
Non-physician healthcare	36%	36	4.4	
All other professions	36%	303	1.7	
Marital status				**0.004**
Single	42%	168	2.4	
Married/Living with partner	33%	165	2.1	
Divorced/Separated	25%	16	5.0	
Have children?				**0.010**
Yes	31%	100	2.6	
No	40%	250	1.9	
Planning on having children within the next 5 years				**0.487**
Yes	35%	127	2.5	
No	39%	163	2.3	
I don't know	38%	60	3.8	

P-values were calculated using one-way analysis of variance. In the profession category, p=0.003 for physicians vs. non-physician healthcare professionals and p=0.001 for physicians vs. all other professions. In the marital status category, p=0.011 for single vs. married/living with partner. There was no statistical significance among participants who were single vs. divorced/separated (p=0.058) or married/living with partner vs. divorced/separated (p=0.528)

Each participant received a combined total genetic knowledge score out of the 13 genetic knowledge questions from both knowledge segments #1 and #2. Differences among demographic subgroups are depicted in Table 7. Mean total genetic knowledge scores were not significantly different between males and females, though a significant difference was observed in scores from segment #2 alone, described above.

Table 7: Mean genetic knowledge scores among demographic subgroups				
Demographics	**score %**	**n**	**std. error**	**p-value**
Age				**0.160**
≤ 30	54%	187	1.6	
> 30	51%	163	1.7	
Gender				**0.134**
Female	52%	242	1.33	
Male	55%	108	2.13	
Race				**0.678**
White	53%	260	1.3	
Black/Mulato/Mestico	52%	67	2.7	
Other	50%	21	4.4	
Birthplace				**0.910**
North	56%	17	5.1	
Northeast	51%	51	3.2	
Central-West	53%	25	5.3	
South	54%	106	1.9	
Southeast	52%	151	1.7	
Religion				**<0.001**
Catholic	56%	91	2.3	
Non-religious/Atheist	57%	102	2.1	
Other	48%	156	1.6	
Level of education				**0.294**
High school or less	52%	101	2.2	
College degree	52%	151	1.7	
Post-graduate degree	56%	98	2.2	
Profession				**<0.001**
Physician	82%	11	3.2	
Non-physician healthcare	53%	36	3.0	
All other professions	52%	303	1.2	

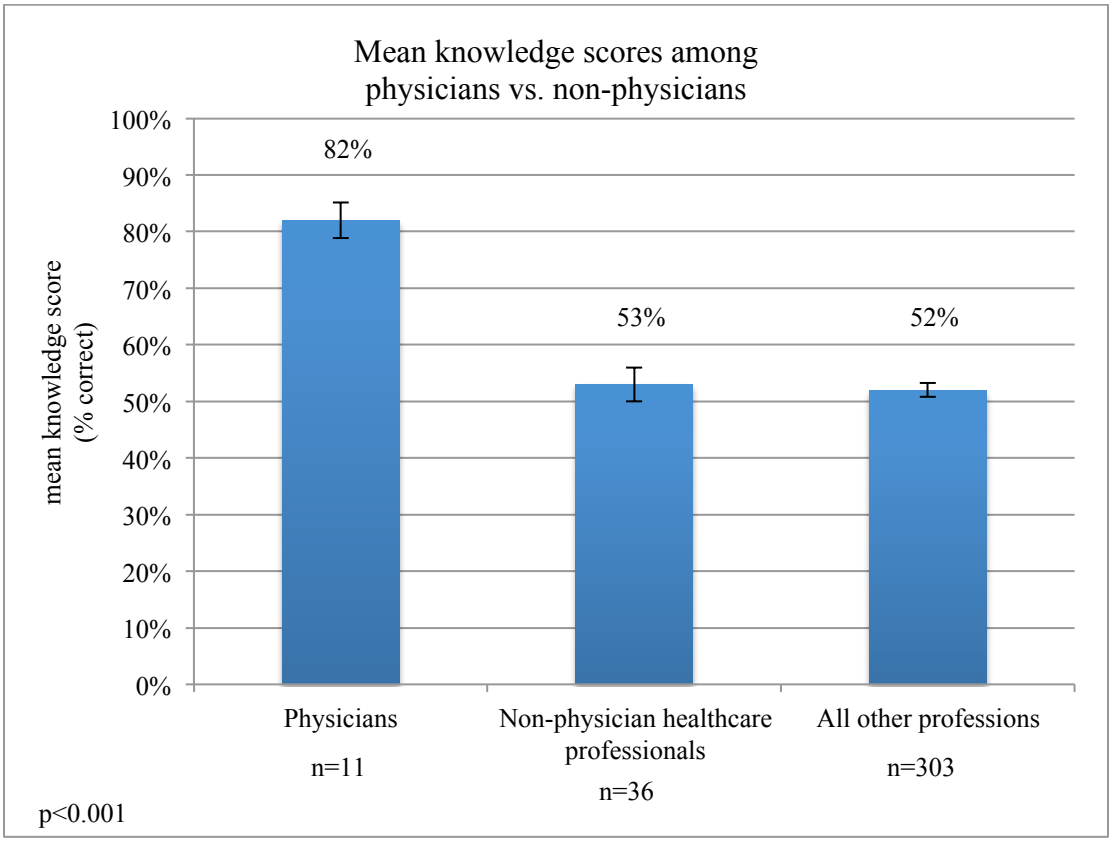
P-values were calculated using one-way analysis of variance. In the profession category, $p<0.001$ for both physicians compared to non-physician healthcare professionals and physicians compared to all other professions.

Table 7: Mean genetic knowledge scores among demographic subgroups				
Demographics	**score %**	**n**	**std. error**	**p-value**
Marital status				**0.022**
Married/Living with partner	56%	168	1.7	
Single	50%	165	1.5	
Divorced/Separated	46%	16	5.1	
Do you have children?				**0.017**
Yes	48%	100	2.0	
No	54%	250	1.4	
Planning on having children within the next 5 years?				**0.532**
Yes	51%	127	1.8	
No	54%	163	1.7	
I am not sure	53%	60	2.7	

P-values were calculated using one-way analysis of variance. For the marital status category, p=0.038 in single vs. single married/living with partner. There was no statistically significant difference between single vs. divorced/separated (p=0.185) or married/living with partner vs. divorced/separated (p=0.748).

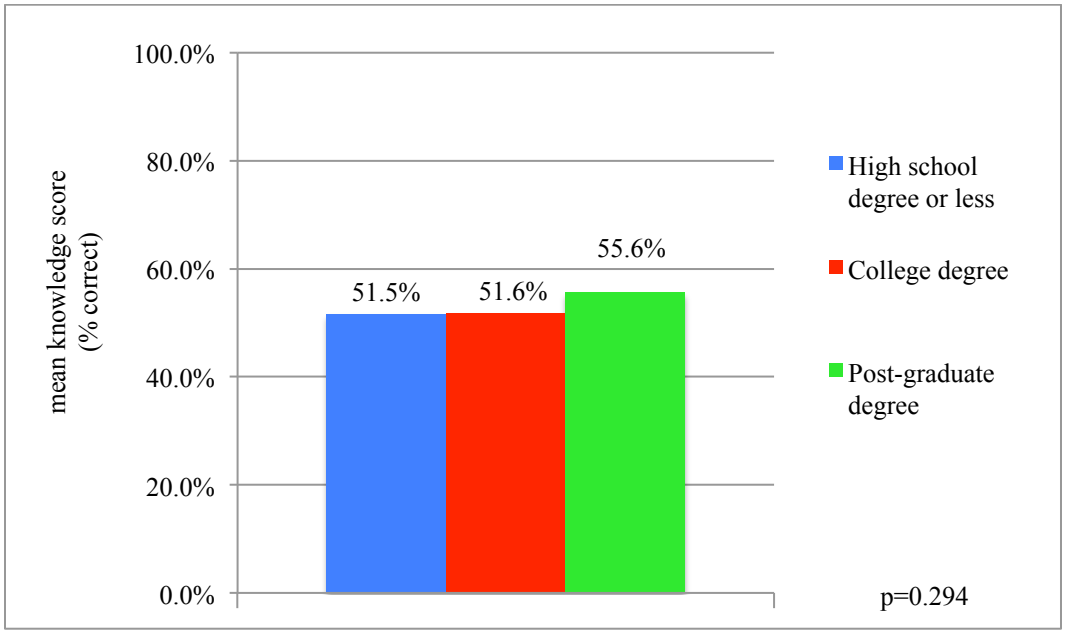
There was a significant difference in mean total genetic knowledge scores of physicians compared to non-physicians (Figure 6, $p<0.001$). The non-physician healthcare professionals had similar mean knowledge scores as compared to all other professions (p=0.869).

Figure 6: Mean genetic knowledge scores of physicians, non-physician healthcare professionals, and all other professions



P-values were calculated using one-way analysis of variance. P<0.001 for both physicians compared to non-physician healthcare professionals and physicians compared to all other professions. P=0.869 for non-physician healthcare professionals compared to all other professions.

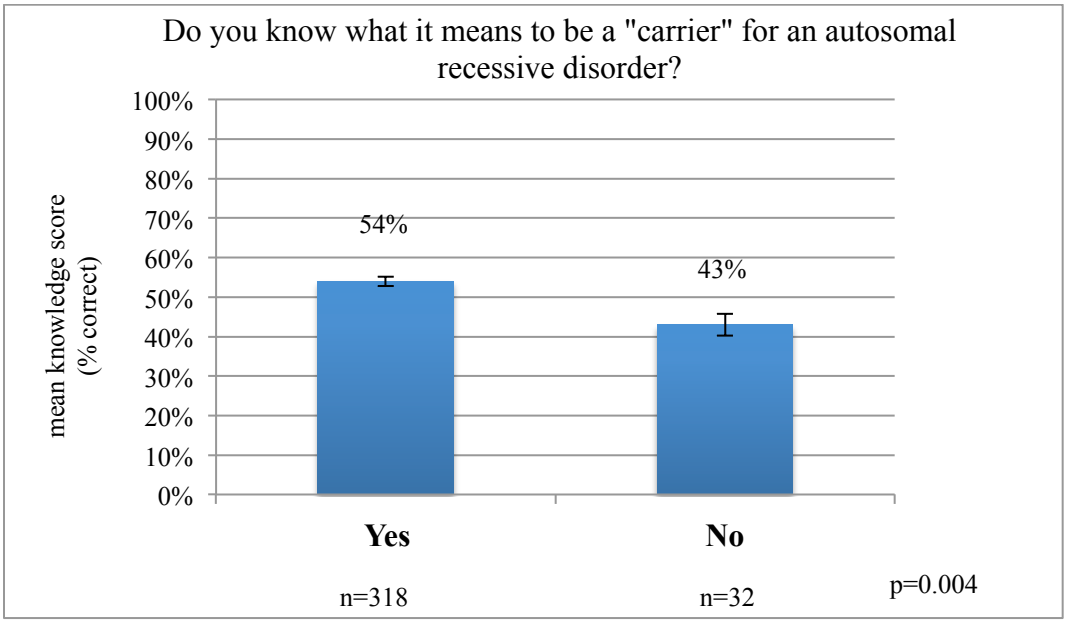
Figure 7: Highest level of education is not significantly associated with increased genetic knowledge scores



3.6 Perceived genetic knowledge compared to actual genetic knowledge

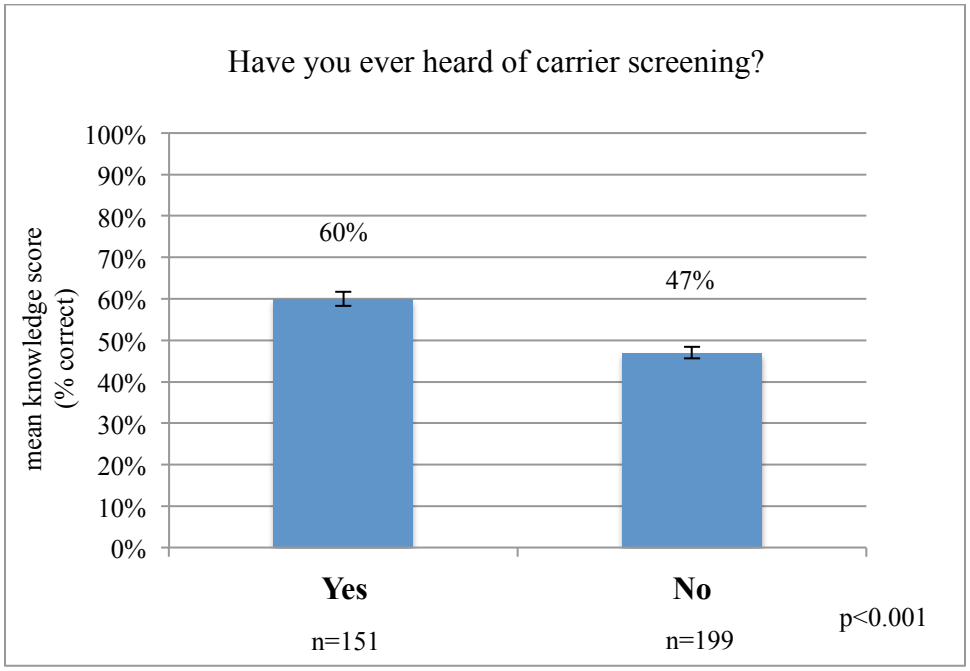
Participants were asked if they knew what it means to be a "carrier" for an autosomal recessive disorder. Participants who responded "yes" had significantly higher genetic knowledge scores (Figure 8, $p=0.004$). Furthermore, participants who had previously heard of carrier screening had higher genetic knowledge scores (Figure 9, $p<0.001$).

Figure 8: Perceived knowledge of the meaning of a "carrier" is associated with higher genetic knowledge scores



P-values were calculated using one-way analysis of variance.

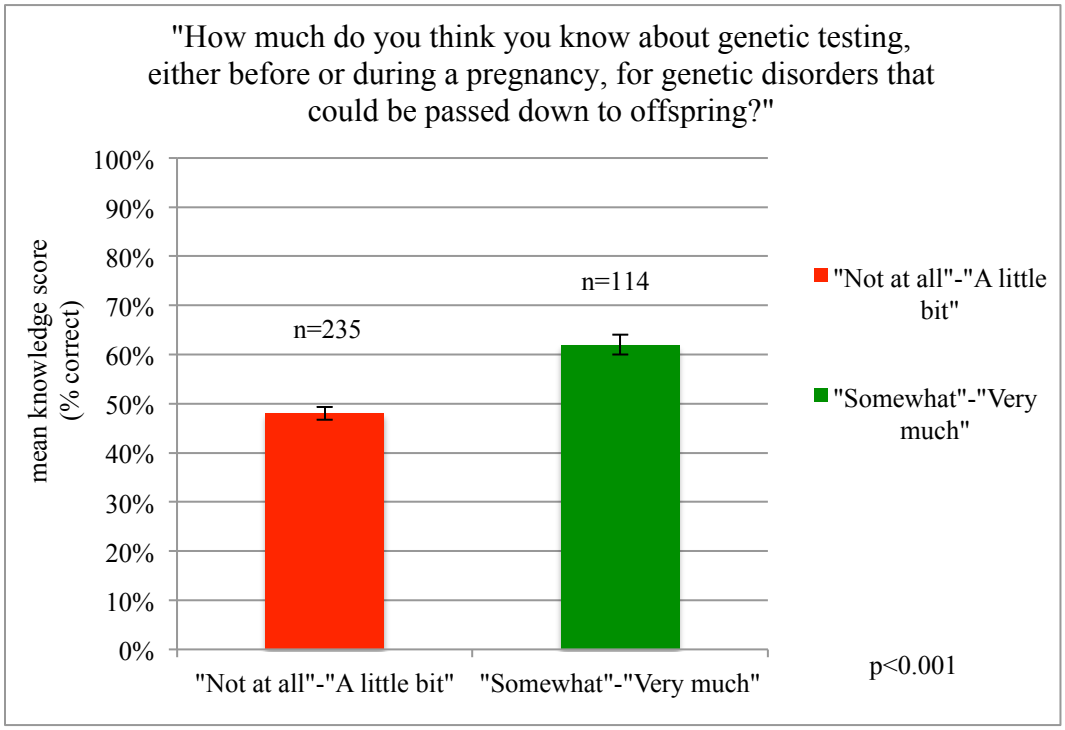
Figure 9: Awareness of carrier screening is associated with higher genetic knowledge scores



P-values were calculated using one-way analysis of variance.

Moreover, participants with higher perceived knowledge about carrier screening did, in fact, have higher genetic knowledge scores. Participants were asked, “How much do you think you know about genetic testing, either before or during pregnancy, for genetic disorders that can be passed down to offspring?” and to rank their answer on a Likert scale from 1-5 (1=”Not at all”; 2=”A little bit”; 3=”Somewhat”; 4=”Quite a bit”; 5=”Very much”). Participants who perceived their knowledge of carrier screening to be between 3-5 had significantly higher genetic knowledge scores than those who answered between 1-2 (Figure 10, $p<0.001$).

Figure 10: Perception of carrier screening knowledge is associated with higher mean genetic knowledge scores



P-values were calculated using one-way analysis of variance.

When participants were asked if Brazilians are at increased risk to be carriers for certain genetic disorders, those with higher perception of carrier risk had significantly higher genetic knowledge scores (Figure 11, p=0.004).

Figure 11: Perceived carrier risk is associated with higher genetic knowledge scores

P-values were calculated using one-way analysis of variance.

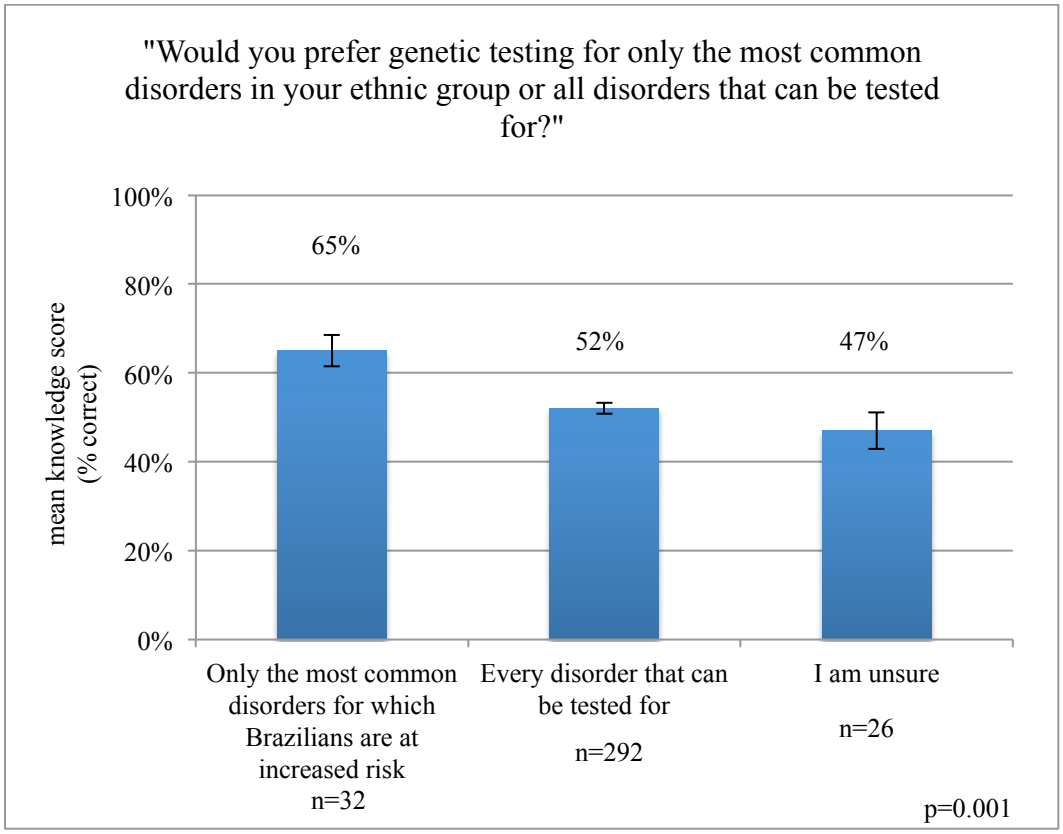
3.7 Interest in carrier screening

Participants were asked how interested they would be in carrier screening and to rank their responses on the same Likert scale from 1-5 (1=”Not at all”; 2=”A little bit”; 3=”Somewhat”; 4=“Quite a bit”; 5=”Very much”). The majority, 78%, selected 4 or 5. Interest in carrier screening was not significantly associated with increased mean genetic knowledge scores (p=0.079, one-way analysis of variance).

Participants were asked if they would prefer carrier screening for only the most common disorders for which Brazilians increased risk to be carriers or for every disorder that can be

tested. A great majority, 83%, preferred the option of testing for every disorder that can be tested, while 9% preferred only the most common disorders for which Brazilians have increased risk, and 7% were not sure. Participants who preferred testing for only the most common disorders for which Brazilians have increased risk had significantly higher mean genetic knowledge scores (Figure 12, $p<0.001$). Since the physicians in this cohort had significantly higher genetic knowledge scores than all other professions, it was important to assess how they responded to this question to determine if their responses skewed the data. In fact, there was an even split between physicians who preferred testing for only the most common disorders in which Brazilians are at increased risk (n=5) and those who preferred testing for every disorder that can be tested (n=6).

Figure 12: Carrier screening preference is associated with genetic knowledge scores



P-values were calculated using one-way analysis of variance.

The participants were asked several questions assessing interest in carrier screening and if interest was dependent on certain circumstances, such as out-of-pocket cost and prognosis of the disorders tested for. Responses were measured on the same Likert scale from 1-5 (1=”Not at all”; 2=”A little bit”; 3=”Somewhat”; 4=”Quite a bit”; 5=Very much”). Responses of 4 or 5 were considered high interest. The distribution of participants who responded with high interest is depicted in Table 8. Eighty-six percent of participants responded with high interest if the carrier screening were free of charge. The high interest group decreased to 59% when prompted that the out-of-pocket cost would be R$500 (approximately $133). When prompted that the cost would be R$1000 (approximately $266), the high interest group dropped to 41%.

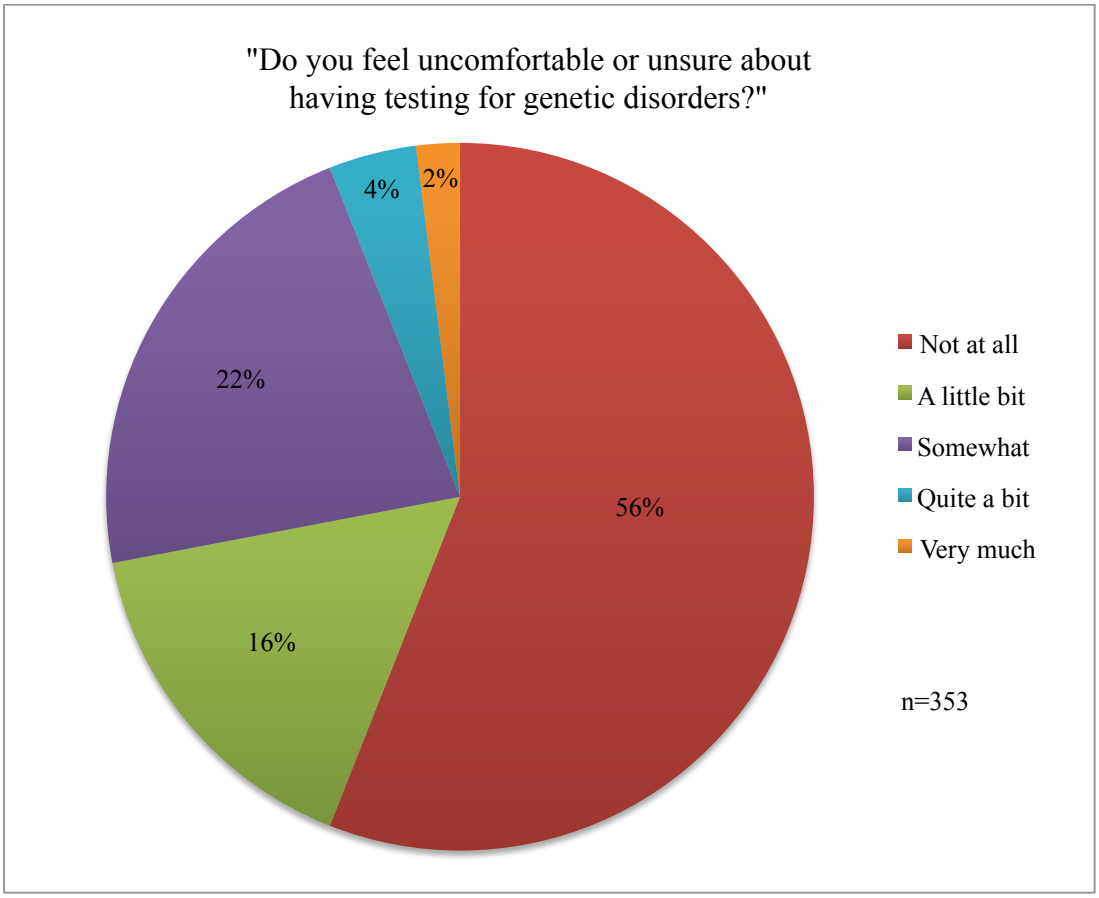
Table 8: Interest in carrier screening

Condition	% total who reported high interest
1. How interested would you be in having genetic testing either before or during a pregnancy for genetic disorders that could be passed down to offspring?	78%
2. How interested would you be in having your partner tested either before or during a pregnancy for genetic disorders that could be passed on to your offspring?	72%
3. How interested would you be in having yourself and your partner both tested either before or during a pregnancy for genetic disorders that could be passed on to your offspring?	78%
4. How likely would you be to have genetic testing to find out if your offspring would be at risk for a life-threatening disorder?	84%
5. How likely would you be to have genetic testing to find out if your offspring would be at risk for a life-threatening disorder if treatment is available?	91%
6. How likely would you be to have genetic testing for disorders that would result in severe physical and/or intellectual disability?	70%
7. How likely would you be to have genetic testing for disorders that would result in mild to moderate physical and/or intellectual disability?	66%
8. How likely would you be to have genetic testing for disorders that would result in a miscarriage?	58%
9. How likely would you be to have genetic testing for severe disorders that could be passed on to your offpsring if it were free of charge?	86%
10. How likely would you be to have genetic testing for severe disorders that could be passed on to your offpsring if the cost to you was R$500?	59%
11. How likely would you be to have genetic testing for severe disorders that could be passed on to your offpsring if the cost to you was R$1000?	41%

In this dataset n=353, except for Q1, Q3, and Q10 n=352. High interest in considered if the respondent answered either 4 or 5 on the Likert scale (4=”Quite a bit”; 5=”Very much”).

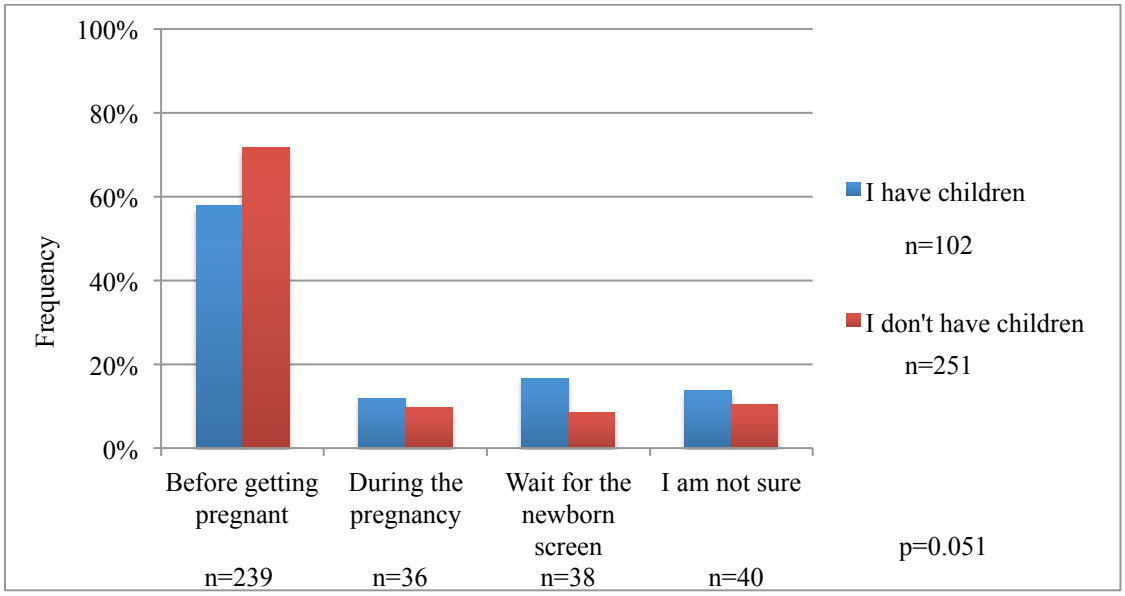
Participants were asked if they felt uncomfortable or unsure about having testing for genetic disorders. The majority of participants (56%) answered “Not at all” (Figure 13). A small percentage of participants answered “Quite a bit” or “Very much”, 4% and 2% respectively.

Figure 13: Participant comfort level about having testing for genetic disorders



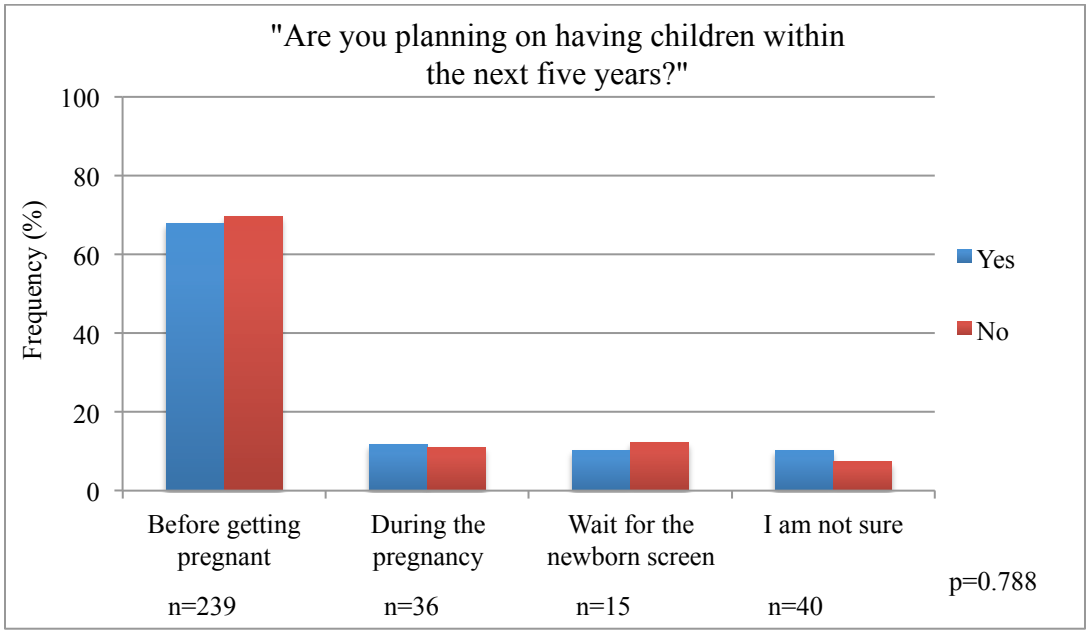
Participants were asked their preference of when to have carrier screening, either before getting pregnant, during a pregnancy, or wait for newborn screening. There was no statistically significant difference between those who currently had children and those who did not (Figure 14, p=0.051).

Figure 14: Carrier screening prior to getting pregnant is preferred by participants both with and without children



There were no significant differences in preference for when to do carrier screening between participants who were planning on having children within the next five years and those who were not; both groups nearly equally preferred carrier screening prior to getting pregnant, 68% and 70%, respectively (Figure 15, p=0.788).

Figure 15: Preference for carrier screening between participants planning on having children within the next five years and those who were not



Participants were asked if they were interested in learning more about carrier screening for sickle-cell anemia, thalassemia, Tay-Sachs disease, and/or cystic fibrosis and to choose one of the following responses: (1) “Definitely interested; (2) “Somewhat interested”; (3) “Not interested in carrier screening”; (4) “I do not know.” Participants who chose “definitely” were considered the high interest group and were compared to participants who chose either “somewhat” or “not interested in carrier screening,” who were considered the low interest group. Those who responded “I do not know” were removed from the analysis. Females were significantly more interested than males (Table 9, p=0.012). However, high interest in carrier screening was not significantly different between females and males in the cohort of participants planning on having children within the next five years (n=127) (p=0.516, Pearson’s Chi-Square). There were significant differences between those who reported “definitely interested” in learning more about carrier screening and those with low interest among participants of different

ethnicities (p=0.032), physicians vs. non physicians (p=0.031), and those planning on having children within the next five years compared to those who were not (p=0.018).

Table 9: Interest in learning more about carrier screening among different demographic subgroups

Demographic subgroups	High interest (n)	%	Low interest (n)	%	p-value
Age					**0.992**
≤ 30	102	59%	71	41%	
> 30	86	59%	60	41%	
Gender					**0.012**
Female	141	64%	81	37%	
Male	47	49%	50	52%	
Ethnicity					**0.032**
White	132	56%	103	44%	
Black/Mulato/Mestiço	47	73%	17	27%	
Other	9	50%	9	50%	
Place of birth					**0.887**
North/Northeast	37	62%	23	38%	
Central-West	12	57%	9	43%	
South/Southeast	139	58%	99	42%	
Highest level of education					**0.253**
High school or less	58	64%	33	36%	
College degree	75	54%	64	46%	
Post-graduate degree	55	63%	33	38%	
Religion					**0.724**
Catholic	51	61%	32	39%	
Non-religious/Atheist	50	56%	40	44%	
Jewish					
Other	86	59%	59	41%	

P-values were calculated using Pearson Chi-Square test.

Table 9: Interest in learning more about carrier screening among different demographic subgroups (continued)

Demographic subgroups	High interest (n)	%	Low interest (n)	%	p-value
Profession					**0.031**
Physician	10	91%	1	9%	
Non-physician healthcare	23	70%	10	30%	
All other professions	155	56%	120	44%	
Marital status					**0.432**
Single	90	58%	66	42%	
Married/Living with partner	90	62%	55	38%	
Divorced/Separated	8	47%	9	0.5	
Have children?					**0.243**
Yes	49	54%	42	46%	
No	139	61%	89	39%	
Planning on having children within the next 5 years					**0.018**
Yes	79	69%	35	31%	
No	79	52%	72	48%	
I don't know	30	56%	24	44%	

P-values were calculated using Pearson Chi-Square test.

4. DISCUSSION

Carrier screening for autosomal recessive disorders to some extent has become part of routine clinical care in the United States. The purpose of carrier screening is to help couples find out if they are at risk to have an affected child with a certain autosomal recessive disorder. Ethnic-based carrier screening, in particular, targets the genetic disorders that tend to be more common in an individual's particular ethnic group. The Brazilian population is heterogeneous, and ethnicities vary greatly in different regions of the country. The purpose of this study was to investigate public awareness of certain autosomal recessive disorders, knowledge of autosomal recessive inheritance, and perception of carrier risk for certain disorders, especially given the complexity of the population's ancestral roots, and to assess the interest for carrier screening in the population of a country where there is currently no preconception or prenatal carrier screening program.

4.1 Awareness and perception of carrier risk for certain autosomal recessive disorders

More than 3/4 of the participants had never heard of thalassemia or Tay-Sachs disease and about 1/3 of the participants had never heard of sickle-cell anemia or cystic fibrosis. There was no significant difference by race among respondents who had previously heard of these disorders and those who had not. Perception of carrier risks was low for sickle-cell anemia, thalassemia, and cystic fibrosis. About 3/4 of participants did not know if there was increased carrier risk, and those who did give an answer were more likely to answer no than yes. Further, there was no significant difference by race in how respondents perceived carrier risks for these disorders. In one study by Siddiqui et al., awareness of sickle cell disease was compared between a group of 150 Dominicans and a group of 58 African Americans from the Northern Manhattan

region of New York City (2012). These two groups were selected based on relatively high carrier rates for sickle cell disease, where African-American carrier frequency is estimated to be 1 in 12, while the carrier frequency in the Dominican population is about 1 in 20. The African-American cohort had significantly higher knowledge than the Dominican group in many realms. Notably, 93% of the African-American participants knew their own carrier status for sickle-cell disease. Also, 76% of the African-American group knew that sickle-cell disease was an inherited blood disorder, compared to only 27% in the Dominican group ($p<0.001$). By comparison, 47% of Brazilian Blacks correctly answered that sickle cell anemia was a hereditary disorder. Only 18% of the Brazilian Blacks in this current study perceived increased carrier risk for sickle cell disease, though the sample size of Black/Mulato participants was small (n=17). Overall, their study demonstrated an information gap between two ethnic groups both at risk to be carriers for sickle-cell disease. Possible explanations for the information gap were the lack of standardized terminology in Spanish for sickle cell disease and sickle cell trait, which could have impacted overall knowledge of the disorder, and the fact that sickle cell disease is traditionally thought of as an "African" disorder, which might impact the perception of risk in other ethnic groups.

In this Brazilian cohort, participants more likely to have heard of these disorders were females compared to males, those over 30 years of age compared to those 30 years of age or less, and those with a college or post-graduate degree compared to those with a high school degree or less, though not all reached statistical significance. Among those who had previously heard of the disorders, participants more likely to perceive carrier risk for these disorders were females compared to males and those 30 years of age or less compared to those over 30, though not all reached statistical significance.

4.2 Genetic knowledge about certain autosomal recessive disorders

The participants in our study were asked if they thought certain disorders were inherited from parents. Of participants who had previously heard of cystic fibrosis (n=248), only 36% believed it was a disorder inherited from parents. A study led by Braido et al. that assessed public awareness of cystic fibrosis in 1,006 Italian adults demonstrated similar findings (2015). The study used a proportional stratified sampling method so that the cohort was representative of the Italian population. They found that 64% of their cohort had previously heard of cystic fibrosis, and of those, only 20% were aware that the disease was hereditary. By comparison, 70% of the entire Brazilian sample in this study had heard of cystic fibrosis, and of those, 36% were aware that it was hereditary. Furthermore, while the majority of the participants in this study were White (75%), only 38% of Whites who had heard of cystic fibrosis correctly answered that it was a disorder inherited from parents. By comparison, of Blacks/Mestiço/mixed participants who had previously heard of cystic fibrosis, 34% correctly answered that it was a disorder inherited from parents.

Public knowledge in genetics and attitudes toward genetic testing have been explored in previous studies in the United States. In a publication by Haga et al., a sample of 300 individuals from Durham, North Carolina completed a survey that assessed their genetic knowledge. Their cohort also had a high representation of White individuals (60%) and individuals with a college degree (65%). The mean genetic knowledge score (based on percentage of correct answers) in their cohort was 84% overall and 90% specifically in the questions pertaining to inheritance and causes of disease. For example, participants were asked if "healthy parents can have a child with a hereditary disease," if "the carrier of a disease gene may be completely healthy," and if "the child of a disease carrier is always also a carrier of the same disease gene." The same survey

instrument was used in a study in a randomly selected Finnish population of 1,216 individuals (Jallinoja et al., 1999). The mean genetic knowledge score in the Finnish cohort was 64%. Moreover, the same survey instrument was used in a study of 306 individuals diagnosed with chronic disease in the Netherlands (Calsbeek et al., 2007). Their mean genetic knowledge score was 46%. This demonstrates that genetic knowledge varies widely in different populations, even when the same survey tools were used. Sampling, cultural differences between the United States and Europe, and differing genetics curricula taught in schools, among others, were postulated as possible reasons for the discrepancy in knowledge scores among those three cohorts.

In this Brazilian sample, the mean genetic knowledge score was 37% in segment #2, pertaining to inheritance of autosomal recessive disorders. In this segment, males scored higher than females ($p=0.010$), individuals 30 years of age or less scored higher than those over 30 ($p=0.022$), and those who were single had higher scores than those who were married/living with partner ($p=0.011$). Mean genetic knowledge scores were higher among participants with higher levels of education, but differences were not statistically significant. Specifically, among participants less than 30 years of age, mean scores were higher in those with higher levels of education. A possible explanation as to why those less than 30 years of age had higher mean knowledge scores than those over 30 might be that they were in school more recently, and if genetic information was included in their curriculum, they may be better at recalling the information compared to those who had been out of school for a longer time. Mean genetic knowledge scores of the physicians in this cohort were significantly higher than all other professions. Notably, the non-physician healthcare professionals had nearly the same genetic knowledge scores as all others in non-physician professions. Since the current plan in Brazil is to train more non-physician healthcare professionals to become genetic counselors, it is informative

to know that, at least in this cohort, the non-physician healthcare professionals' baseline knowledge in differentiating between hereditary disorders vs. non-hereditary disorders and knowledge of autosomal recessive inheritance was similar to other laymen (Passos-Bueno et al., 2014).

The overall lack of knowledge of the inheritance of these disorders and perception of low carrier risks may be in part due to the fact that there is no formal registry of genetic diseases available in Brazil, which limits the availability of concrete information provided to the general public. Another possible explanation could be that there is less media coverage in Brazil regarding topics of genetic testing. In the United States, on the other hand, the frequent media coverage and advertising from commercial laboratories has "mainstreamed" several genetic testing services including expanded carrier screening, which could have an impact on public genetic knowledge. However, studies have shown that familiarity with genetic concepts does not always correlate with understanding of the concepts (Lanie et al., 2004). Importantly, because this cohort is a highly educated sample, it may not be a true representation of the general Brazilian population, and thus the public genetic knowledge in Brazil may be even lower than what was observed in this study.

4.3 Interest in carrier screening

Participants were asked if they would prefer carrier screening for only the disorders for which Brazilians are at increased risk or for all disorders that can be tested. Even though the vast majority preferred expanded carrier screening for all disorders that can be tested, those who preferred the ancestry-based approach had significantly higher genetic knowledge scores. This suggests that education and genetic counseling about the inheritance of these disorders might impact carrier testing preference. Moreover, the majority of participants preferred having carrier

screening prior to getting pregnant rather than during pregnancy or waiting for newborn screening. Though it was not statistically significant, those who did not have children had higher preference in having carrier screening prior to getting pregnant rather than during pregnancy or waiting for newborn screening ($p=0.051$). Importantly, those who were planning on having children within the next five years expressed higher interest in learning more about carrier screening than those who were not ($p=0.018$). Specifically, 69% of the participants who were planning on having children within the next five years ($n=127$) answered that they were "definitely interested" in learning more about carrier screening.

There was a strong interest in carrier screening in this cohort, regardless of demographic background. The interest varied depending on certain circumstances, such as out-of-pocket costs and prognosis of the disorder being tested. Seventy-eight percent of participants in this cohort reported high interest in preconception or prenatal carrier screening. Their interest decreased when prompted that the out-of-pocket cost would be R$500 (approximately $133 U.S.), and even less interest was expressed when the cost was R$1000 (approximately $266 U.S.). This is critical to keep in mind when implementing a program for population-based preconception and prenatal carrier screening. Given the low percentage of the Brazilian population with private health insurance (~25%), it may be most effective if population screening were implemented through the SUS (Sistema Único de Saúde, the government healthcare system) in order to be accessible to all individuals.

4.4 Limitations of the study

One limitation of this study is that the distribution of ethnicity and place of birth is not representative of the general Brazilian population. In this cohort, the distribution of ethnicity was 75% White, 14% Mestiço, 5% Black/Mulato, 4% "other/other mixed ethnicity," 1% Indigenous, and 1% Asian. Based on 2010 census data, the distribution of ethnicity in the general Brazilian population is 48% White, 43% multiracial, 8% Black, 1% Asian, and 0.5% Indigenous (IBGE, 2013). There was a marked underrepresentation of Blacks and mixed respondents in this study (5%). Sickle-cell disease and thalassemia are more prevalent in individuals of African and Mediterranean descent; thus, the representation of Black respondents was central in assessing the survey responses pertaining to sickle-cell disease and thalassemia. Future studies are needed to further explore the knowledge and attitudes toward carrier screening in the Brazilian population. The distribution with respect to region of birth in this cohort also differs from the general Brazilian population. This cohort had an overrepresentation of Brazilians from the South (43%), while the remaining participants were primarily from the Southeast (30%), followed by the Northeast (14.5%), the Central-West (7.4%), and the North (4.8%). The distribution regarding region of birth in the general Brazilian population is highest in the Southeast region (42%), followed by the Northeast (28%), the South (14%), the North (9%), and lastly the Central-West (8%). The overrepresentation of individuals from the South may be an explanation as why there was an overrepresentation of Whites in this cohort, because the South has the highest population of individuals of European descent in Brazil.

Another limitation to this study was the small number of Jewish participants in this cohort (n=7). Interestingly, none of the Jewish participants had ever heard of Tay-Sachs disease, which has the highest carrier rate in that population. This may be explained by the fact that Tay-

Sachs disease carrier screening programs in Brazil are mainly provided in a research setting, though this small sample size limits our ability to draw conclusions. Future studies in the Brazilian Jewish population are warranted to explore awareness of Tay-Sachs disease, interest in carrier screening, and whether they may be concerned that knowledge of their carrier status might affect their ability to marry.

Participation in this survey required respondents to have access to a computer. This recruitment methodology introduces a bias in favor of individuals of higher socioeconomic class. Additionally, participants were required to be proficient in reading and understanding the questions asked in this survey. To mitigate this, we attempted to write the survey at a 6th grade educational level, but some concepts could have been more challenging for individuals of lower reading comprehension and could have introduced a bias. However, the majority of the participants in this study had a college or post-graduate degree, 44% and 28%, respectively. Mean knowledge scores were higher in those with higher levels of education, though it was not statistically significant. Further, this highly educated cohort may not be representative of the general Brazilian population, and therefore, genetic knowledge may be even lower in the general Brazilian population than what was observed in this study.

When participants were asked if they thought there was an increased carrier risk for certain autosomal recessive disorders, the majority reported, “I don’t know” for all of the disorders. When the participants who reported, “I don’t know” were removed from the analysis (along with the participants who had never heard of the disorders), the remaining sample size was small. This made it difficult to test for statistically significant differences among demographic subgroups in their perception of carrier risk. Furthermore, the subgroup that did give a response on carrier risk were not representative of the entire study sample population with

respect to age, gender and geographic distribution, and possibly not representative of the overall Brazilian population. Thus, these results may not truly represent the awareness and carrier risk perception of the general Brazilian population. For example, in perception of carrier risk for sickle-cell anemia, among those who had heard of the disorder, females were significantly more likely than males to answer that they did not know whether or not carrier risk was increased ($p=0.016$). With respect to perception of carrier risk for thalassemia, those born in the Central-West were more likely than participants of other regions of birth to answer that they did not know whether or not carrier risk was increased ($p=0.043$). Participants over 30 years of age were more likely those 30 years of age or less to answer that they did not know whether or not there was increased carrier risk for thalassemia ($p=0.001$).

This study did not use a previously validated questionnaire. Though some of the questions were adapted from other published surveys, all questions were not identical, so our inability to compare the results of this survey directly to the results of others was a limitation of this study.

In summary, though the interest in carrier screening was high in this study, this sample may not be representative of the general Brazilian population because it did not represent the ethnic distribution of the general Brazilian population and was a highly educated population, and the online recruitment methodology may have caused bias in favor of selecting respondents of higher socioeconomic class because it requires proficiency with and access to a computer.

4.5 Future directions

The relatively high carrier frequencies for sickle-cell anemia and thalassemia in individuals of African and Mediterranean ancestry, cystic fibrosis in northern European Caucasians, and Tay-Sachs disease in the Ashkenazi Jewish population are a public health

concern. Carrier screening for autosomal recessive disorders in Brazil is often done for couples only after the birth of an affected child. The ACMG and ACOG have provided well-established protocols and recommendations for population carrier screening in the United States that could be applied in Brazil. The ACMG and ACOG recommend targeted carrier screening, which means testing for disorders based on ethnicity or family history. Commercial laboratories have driven the development of expanded carrier screening, which may test for as many as several hundred disorders and is not ethnicity-based. Expanded carrier screening typically caters to those who are inclined toward information-seeking. Genetic testing laboratories in the United States have serviced patients internationally, especially now that biological specimen preservation during international shipping has become less of a concern.

The goal of this type of genetic screening is for couples to learn of their carrier status in the preconception period, and it is critical that pre- and post-test informed consent, ideally genetic counseling and education, be provided to all individuals undergoing carrier screening. This is especially important in the context of expanded carrier screening. It is nearly impossible to provide detailed information for every disorder included in an expanded carrier screening panel. These disorders vary greatly in terms of the clinical features and variability of expression and severity of symptoms among individuals affected with the same disorder. They also vary in the fact that some disorders may not have well-defined genotype-phenotype correlations, making it difficult to predict the severity of a disorder or the particular clinical features based on the genetic variant identified. Studies have shown that poor health literacy is associated with poor understanding of personal genetic risks (Lea et al, 2011). This emphasizes the importance of public knowledge in genetics. Currently, Brazil has a shortage of genetics specialists but is actively working on training more genetic counselors, which is an important prerequisite to

implementing a population-based genetic screening program. The efficacy of genetic counseling was demonstrated in a publication by Ferreira et al. (2012). The aim of their study was to measure knowledge about autosomal recessive inheritance and the difference between a carrier and an individual affected with an autosomal recessive disorder in a population of sickle cell carriers in Brazil. The Ferreira study showed that only 2.6% of participants had high genetic knowledge scores prior to genetic testing. Post-test genetic counseling was provided, and participants were tested again three months later on their genetic knowledge. Scores were significantly increased; 82% of the participants had improved to high genetic knowledge scores (p-value<0.001), which supports the importance of genetic counseling and education. The participants in the Ferreira study were less educated than the participants in this present cohort; the majority in the Ferreira study reported middle school as their highest level of education. Important to note, the genetic knowledge questions in the present survey were adapted from the Ferreira study.

The outcome of this current study reinforces the need to educate Brazilians regarding the disorders for which the Brazilian population is at increased risk. The study also provides a perspective from a subset of the Brazilian community that the majority would be interested in preconception or prenatal carrier screening if it were available. Specifically, the majority of those planning on having children within the next five years expressed very high interest in learning more about carrier screening. Since we are now in the "age of genomic medicine," it is important to explore topics of genetic screening services in countries where these programs are limited. Further exploration of this topic can help provide information that might positively impact the development of a population-based carrier screening program in Brazil. It is possible that with the evidence of significant interest in carrier screening in this Brazilian cohort, laboratories

abroad may consider expanding their networks to partner with the laboratories and institutions in Brazil in order to provide more services to this population.

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APPENDIX A: Survey in Portuguese

Interesse público em triagem de portadores de mutações genéticas na população brasileira

Informações sobre a pesquisa

Investigador principal
Marina Dutra-Clarke, Estudante pós-graduação de Aconselhamento Genético
Departamento de Pediatria, Divisão de Medicina Genética e Genômica
Universidade da Califórnia, Irvine Centro Médico
101 The City Drive, Orange, CA 92868
telefone: (714) 456-5837
email: mdclarke@uci.edu

Patrocinador da faculdade
Maureen Bocian, MD, FAAP, FACMG, Professor de Pediatria
Departamento de Pediatria, Divisão de Medicina Genética e Genômica
Universidade da Califórnia, Irvine Centro Médico
101 The City Drive, Orange, CA 92868
telefone: (714) 456-6873
email: mebocian@uci.edu

· Pedimos a sua participação em um estudo realizado por pesquisadores da Universidade da Califórnia, Irvine. Participar deste estudo é opcional.

· Se você optar por entrar neste estudo, você irá completar um questionário sobre você e sobre sua compreensão e opiniões de certos serviços de testes genéticos. Esta pesquisa nos ajudará a saber como os indivíduos brasileiros se sentem sobre teste de portador, um tipo de teste genético que pode determinar se os seus filhos podem estar em risco de certas doenças genéticas. A pesquisa levará cerca de 10 a 15 minutos para ser concluída.

· Você será aceito para participar neste estudo se estiver com a idade entre 18 e 45 anos. Por favor preencha o questionário apenas uma vez.

· Você pode ignorar as perguntas que não deseja responder ou interromper a pesquisa a qualquer momento. A pesquisa é anônima, e ninguém será capaz de vincular suas respostas a você. Não inclua seu nome ou outra informação que possa identificá-lo nas suas respostas da pesquisa.

· Se você tiver algum comentário, preocupação ou pergunta sobre a condução desta pesquisa, entre em contato com os pesquisadores listados acima.

· Se você tiver dúvidas ou preocupações sobre seus direitos como participante da pesquisa, você pode entrar em contato com o Conselho de Revisão Institucional da UC Irvine pelo telefone, (949) 824-0665, ou por email no IRB@research.uci.edu ou em 141 Innovation, Suite 250, Irvine, CA 92697.

O que é um IRB? Um Conselho de Revisão Institucional (IRB) é um comitê formado por cientistas e não cientistas. O papel do IRB é proteger os direitos e o bem-estar dos participantes envolvidos na pesquisa. O IRB também assegura que a pesquisa atenda aos regulamentos, leis e políticas institucionais aplicáveis.

· Se você deseja participar deste estudo, por favor clique no botão [OK] para começar a pesquisa.

* 1. Que idade você tem?

2. Qual é o seu sexo?

- ◯ Feminino
- ◯ Masculino
- ◯ Outro

3. Onde você nasceu?

- ◯ Norte
- ◯ Nordeste
- ◯ Centro-oeste
- ◯ Sul
- ◯ Sudeste

4. Qual raça/etnia melhor descreve a sua descendência? (Escolha somente uma resposta.)

- ◯ Branco
- ◯ Negro ou Mulato
- ◯ Mestiço
- ◯ Outra/Várias etnias (especifique)
- ◯ Indígena
- ◯ Asiático

5. Qual o nível de educação mais alto que você concluiu?

- ◯ Ensino Fundamental completo
- ◯ Ensino Médio completo
- ◯ Ensino superior completo
- ◯ Pós-graduação completa
- ◯ Nunca frequentou a escola

6. Qual das opções abaixo melhor descreve sua profissão?

7. Qual é o seu estado civil atual?

- ◯ Solteiro
- ◯ Casado/Morando com o companheiro
- ◯ Divorciado/Separado
- ◯ Viuvo

8. Qual é a sua religião?

- ◯ Budista
- ◯ Católico
- ◯ Hindu
- ◯ Igreja dos Santos dos Últimos Dias
- ◯ Judaico
- ◯ muçulmano
- ◯ Protestante
- ◯ Sem religião / ateista
- ◯ Outro
- ◯ Eu prefiro não responder

9. Você tem filhos?

- ◯ Sim
- ◯ Não

10. Você está planejando ter filhos nos próximos 5 anos?

- ◯ Sim
- ◯ Não
- ◯ Ainda não decidi

11. Você já ouviu falar de anemia falciforme?

- ◯ Sim
- ◯ Não

12. Você já ouviu falar de talassemia?

- ◯ Sim
- ◯ Não

13. Você já ouviu falar da doença de Tay-Sachs?

- ◯ Sim
- ◯ Não

14. Você já ouviu falar de fibrose cística?

- ◯ Sim
- ◯ Não

15. Qual das doenças abaixo é/são herdadas geneticamente dos pais? Assinale todas as respostas corretas

- ☐ Anemia Falciforme
- ☐ Doença de Tay-Sachs
- ☐ Fibrose cística
- ☐ Sarampo
- ☐ Talassemia
- ☐ Tuburculose
- ☐ Zika
- ☐ Nenhuma das citadas acima
- ☐ Não sei

16. Você sabe o que significa para um indivíduo ser "portador" de uma doença genética?

- ◯ Sim
- ◯ Não

17. Qual das opções abaixo descreve como as doenças genéticas recessivas são herdadas?

- ◯ Ambos pais do indivíduo afetado também tem a doença
- ◯ Apenas um dos pais deve ser portador da doença
- ◯ Ambos pais do indivíduo afetado devem ser um portador da doença
- ◯ Não sei
- ◯ Apenas um dos pais deve ter a doença

18. Quando apenas um dos pais é portador de uma doença genética, você sabe se o filho/a pode ter a doença?

- ◯ Sim, porque um dos pais que é portador pode transmitir a doença ao filho/a
- ◯ Não, porque ambos os pais devem ser portadores da doença para transmitir a doença ao filho/a
- ◯ Não sei

19. *Para a seguinte questão, considera estas doenças: fibrose cística, anemia falciforme, doença de Tay-Sachs, e talassemia.*

Um portador de qualquer doença acima citada terá sempre um parente que tenha a doença

- ◯ Verdade
- ◯ Falso
- ◯ Não sei

20. Existe alguma diferença entre um indivíduo ser afetado por uma doença genética recessiva e um indivíduo ser portador da mesma doença genética?

- ◯ Sim, o indivíduo afetada apresenta sintomas, mas o indivíduo que é portador não possui nenhum sintoma
- ◯ Sim, o indivíduo afetada apresenta com muitos sintomas, e o indivíduo que é portador pode ter alguns sintomas
- ◯ Não, não há diferença
- ◯ Não sei

21. Quando ambos os pais são portadores de uma doença genética recessiva, com que frequência sua prole terá a doença?

- ◯ Algumas vezes
- ◯ Sempre
- ◯ Não sei

22. Quando ambos um homem e uma mulher são portadores de uma certa doença genética recessiva, e tem uma criança juntos, qual a chance de a criança poder ter a doença genética?

- ◯ Nenhuma chance de isso acontecer (0%)
- ◯ Menos de 1 em 100 casos (1%)
- ◯ Cerca de 1 em 10 casos (10%)
- ◯ Cerca de 1 em 4 casos (25%)
- ◯ Cerca de 1 em 2 casos (50%)
- ◯ A criança definitivamente terá a doença (100%)
- ◯ Não sei

23. Você acha que os indivíduos brasileiros têm um risco aumentado de serem portadores de anemia falciforme?

- ◯ Sim
- ◯ Não
- ◯ Não sei

24. Você acha que os indivíduos brasileiros têm um risco aumentado de serem portadores de talassemia?

- ◯ Sim
- ◯ Não
- ◯ Não sei

25. Você acha que os indivíduos brasileiros têm um risco aumentado de serem portadores da doença de Tay-Sachs?

- ◯ Sim
- ◯ Não
- ◯ Não sei

26. Você acha que os indivíduos brasileiros têm um risco aumentado de serem portadores de fibrose cística?

- ◯ Sim
- ◯ Não
- ◯ Não sei

27. Você já ouviu falar em triagem de portadores de mutações genéticas?

- ◯ Sim
- ◯ Nunca

28. Quanto você acha que sabe sobre testes genéticos efetuados antes ou durante uma gravidez para doenças genéticas que podem ser transmitidas aos seus filhos?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

29. Quão interessado você estaria em se submeter a testes genéticos antes ou durante uma gravidez para doenças genéticas que poderiam ser transmitidas aos seus filhos?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

30. Quão provável você seria em se submeter a testes genéticos para saber se seus filhos estariam em risco de alguma doença fatal?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

31. Quão provável você seria em se submeter a testes genéticos para saber se seus filhos estariam em risco de alguma doença fatal se o tratamento estiver disponível?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

32. Você se senteria desconfortável ou inseguro em se submeter a testes para doenças geneticas?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

33. Quão interessado você estaria em testar seu parceiro antes ou durante uma gravidez para doenças genéticas que poderiam ser transmitidas para seus filhos?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

34. Você gostaria de serem você e o seu parceiro testados antes ou durante uma gravidez para doenças genéticas que poderiam ser transmitidas para seus filhos?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

35. Quão provável você se submeter a testes genéticos para doenças que poderiam resultar em aborto espontâneo?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

36. Quão provável você se submeteria a testes genéticos para doenças que resultariam em deficiência física e/ou intelectual grave?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

37. Quão provável você se submeteria a testes genéticos para doenças que resultariam em deficiência física e/ou intelectual de leve a moderada?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

38. Quão provável você se submeteria a testes genéticos para doenças graves que poderiam ser transmitidas a seus filhos se os testes fossem grátis?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

39. Quão provável você se submeteria a testes genéticos para doenças graves que poderiam ser transmitidas a seus filhos se o custo para você fosse de até R$500?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

40. Quão provável você se submeteria a testes genéticos para doenças graves que poderiam ser transmitidas a seus filhos se o custo para você fosse de até R$1.000?

De modo nenhum	Muito pouco	Mais ou menos	Bastante	Muito
☆	☆	☆	☆	☆

41. Você preferiria se submeter a testes genéticos antes do casamento ou depois do casamento?

- ◯ Antes do casamento
- ◯ Depois do casamento
- ◯ Não importa quando
- ◯ Eu não teria testes genéticos

42. Se você soubesse que você era portador de uma doença genética antes de se casar, você acha que este fato afetaria sua capacidade de encontrar alguém para se casar?

- ◯ Sim
- ◯ Não
- ◯ Não sei

43. Você preferiria se submeter a testes genéticos apenas para as doenças mais comuns em sua origem étnica ou para todas as doenças que podem ser testadas?

- ◯ Apenas as doenças mais comuns para as quais os brasileiros têm um risco aumentado
- ◯ Todas as doenças que podem ser testadas
- ◯ Não sei

44. Se você estivesse grávida e você e seu parceiro descobrissem que vocês dois eram portadores de anemia falciforme, ou doença de Tay-Sachs, ou fibrose cística, você gostaria de testar seu bebê nascituro?

- ◯ Sim
- ◯ Não
- ◯ Não sei

45. Os bebês recém-nascidos são submetidos rotineiramente a testes para certas doenças genéticas graves, mais conhecido por "teste do pezinho". Você preferiria se submeter a testes genéticos para estas doenças genéticas antes de engravidar, durante a gravidez ou esperar o "teste do pezinho" do recém nascido?

- ◯ Antes de engravidar
- ◯ Durante a gravidez
- ◯ Esperar o "teste do pezinho" do recém-nascidos
- ◯ Não sei

46. Para doenças genéticas que não estão no "teste do pezinhos" de recém-nascidos, você estaria interessado em triagem de portadores de mutações genéticas (testes genéticos para detectar se você é portador de uma doença genética) para descobrir se sua prole corre risco de ter a doença?

- ◯ Sim
- ◯ Não
- ◯ Não sei

47. Se for oferecido, se interessaria em aprender mais sobre triagem de portadores de mutações genéticas para anemia falciforme, talassemia, doença de Tay-Sachs ou fibrose cística?

- ◯ Definitivamente interessada
- ◯ Parcialmente interessada
- ◯ Não estou interessada em triagem de portadores de mutações genéticas
- ◯ Não sei

APPENDIX B: Survey in English (For reference only)

University of California, Irvine
Study Information Sheet
Public interest for carrier screening in the Brazilian population

Lead Researcher
Marina Dutra-Clarke, Genetic Counseling Graduate Student
Department of Pediatrics, Division of Genetic and Genomic Medicine
University of California, Irvine Medical Center
101 The City Drive, Orange, CA 92868
Phone: (714) 456-5837
Email: mdclarke@uci.edu

Faculty Sponsor
Maureen Bocian, MD, FAAP, FACMG, Professor of Pediatrics
Department of Pediatrics, Division of Genetic and Genomic Medicine
University of California, Irvine Medical Center
101 The City Drive, Orange, CA 92868
Phone: (714) 456-6873
Email: mebocian@uci.edu

- We are asking you to take part in a study conducted by researchers at the University of California, Irvine. Participating in this study is optional.
- If you choose to be in the study, you will complete a survey about yourself and about your understanding and opinions of certain types of genetic testing services. This survey will help us learn more about how Brazilian individuals feel about carrier screening, a type of genetic testing that can determine if your children may be at increased risk for certain genetic disorders. The survey will take about 10-15 minutes to complete.
- You are eligible to participate in this study if you are between the ages of 18-45 years. Please fill out this survey only once.
- You may skip questions that you do not want to answer or stop the survey at any time. The survey is anonymous, and no one will be able to link your answers back to you. Please do not include your name or any other information that could be used to identify you in the survey responses.
- If you have any comments, concerns, or questions regarding the conduct of this research, please contact the researchers listed at the top of this form.
- If you have questions or concerns about your rights as a research participant, you can contact the UCI Institutional Review Board by phone, (949) 824-0665, by e-mail at IRB@research.uci.edu or at 141 Innovation, Suite 250, Irvine, CA 92697.

What is an IRB? An Institutional Review Board (IRB) is a committee made up of scientists and non-scientists whose role is to protect the rights and welfare of people involved in research. The IRB also assures that the research complies with applicable regulations, laws, and institutional policies.

- If you want to participate in this study, please click the *OK* button to start the survey.

1. What is your age?

(selected from menu)

2. What is your gender?

Female

Male

Other

3. Where in Brazil were you born?

North

Northeast

Central-West

South

Southeast

4. What is your race?

White

Black or Mulato

Mestiço

Native Indian

Asian

Other/mixed ethnicity

5. What is your highest level of education?

Primary school

Secondary school

College degree or equivalent

Graduate/Professional degree

Never attended school

6. Which of the following best describes your occupation?

Agriculture

Art/Music/Writing

Banking/Finance

Business

Clerical or office work

Construction

Education

Homemaker

Hospitality

Lawyer

Science/Engineering/Technology

Physician

Healthcare professional

Transportation

Retired

Unemployed

Other

7. What is your relationship status?

Single

Married/Living with partner

Divorced/Separated

Widowed

8. What is your religion?

Buddhist

Catholic

Hindu

Latter day Saints

Jewish

Muslim

Protestant

Not religious/Atheist

Other

I prefer not to answer

9. Do you have children?

Yes

No

10. Are you planning to have children within the next 5 years?

Yes

No

I am not sure

11. Have you ever heard of sickle cell anemia?

Yes

No

12. Have you ever heard of Thalassemia?

Yes

No

13. Have you ever heard of Tay-Sachs disease?

Yes

No

14. Have you ever heard of cystic fibrosis?

Yes

No

15. Which of these disorders is/are genetically inherited from parents? Check all that apply

Sickle cell anemia

Tay-Sachs disease

Cystic fibrosis

Measles

Thalassemia

Tuberculosis

Zika

None of the above

I do not know

16. Do you know what it means to be a "carrier" of a genetic disorder?

Yes

No

17. Which of the following options best describes how recessive genetic disorders are inherited?

Both of the individual's parents must also have the disorder

Both of the individual's parents must be a carrier of the disorder

Only 1 parent must have the disorder

Only 1 parent must be a carrier of the disorder

I do not know

18. When only one parent is a carrier of a genetic disorder, can the child have the disorder?

Yes, because one parent who is a carrier can transmit the disorder to their children

No, because both parents must be carriers of the disorder in order to transmit the disorder to their children

I do not know

19. For the following question, refer to these disorders: Cystic fibrosis, sickle cell anemia, Tay-Sachs disease, and thalassemia:

A carrier of any of the above disorders will always have a relative who has the disorder

True

False

I do not know

20. Is there a difference between a person who is affected with a recessive genetic disorder and a person who is a carrier for the same genetic disorder?

Yes, the affected person has symptoms, but the person who is a carrier does not have any symptoms

Yes, the affected person has many symptoms, and the person who is a carrier may have a few symptoms

No, there is no difference

I do not know

21. When both parents are carriers of a recessive genetic disorder, how often will their offspring have the disorder?

Sometimes

Always

I do not know

22. If a man and a woman are both carriers of a certain recessive genetic disorder, and they have a child together, what is the chance that the child could have the genetic disorder?

No chance at all (0%)

Less than 1 in 100 (1%)

About 1 in 10 (10%)

About 1 in 4 (25%)

About 1 in 2 (50%)

The child will definitely have the disorder (100%)

I do not know

23. Do you think that Brazilian individuals have an increased risk to be carriers for sickle cell anemia?

Yes

No

I do not know

24. Do you think that Brazilian individuals have an increased risk to be carriers for thalassemia?

Yes

No

I do not know

25. Do you think that Brazilian individuals have an increased risk to be carriers for Tay-Sachs disease?

Yes

No

I do not know

26. Do you think that Brazilian individuals have an increased risk to be carriers for cystic fibrosis?

Yes

No

I do not know

27. Have you ever heard of carrier screening for genetic disorders?

Yes

Never

Not at all	A little bit	Somewhat	Quite a bit	Very much
1	2	3	4	5

28. How much do you think you know about genetic testing either before or during a pregnancy for genetic disorders that could be passed down to your children?

29. How interested would you be in having genetic testing before or during a pregnancy for genetic disorders that could be passed down to your children?

30. How likely would you be to have genetic testing to find out if your offspring would be at risk for a life-threatening disorder?

31. How likely would you be to have genetic testing to find out if your offspring would be at risk for a life-threatening disorder if treatment is available?

32. Do you feel uncomfortable or unsure about having testing for genetic disorders?

33. How interested would you be in having your partner tested either before or during a pregnancy for genetic disorders that could be passed down to your children?

34. How interested would you be in having both yourself and your partner tested either before or during a pregnancy for genetic disorders that could be passed down to your children?

35. How likely would you be to have genetic testing for disorders that could result in a miscarriage?

36. How likely would you be to have genetic testing for disorders that would result in severe physical and/or intellectual disability?

37. How likely would you be to have genetic testing for disorders that would result in mild to moderate physical and/or intellectual disability?

38. How likely would you be to have genetic testing for severe disorders that could be passed on to your offspring if it were free of charge?

39. How likely would you be to have genetic testing for severe disorders that could be passed on to your offspring if it were R$500?

40. How likely would you be to have genetic testing for severe disorders that could be passed on to your offspring if it were R$1,000?

41. Would you prefer to have genetic testing before marriage or after marriage?

Before marriage

After marriage

It doesn't matter

I would not have genetic testing

42. If you learned that you were a carrier of a genetic disorder before marriage, do you think it would affect your ability to find someone to marry?

Yes

No

I do not know

43. Would you prefer genetic testing for only the most common disorders in your ethnic background or all disorders that can be tested for?

Only the most common disorders for which Brazilians have increased risk

Every disorder that can be tested for

I am not sure

44. If you were pregnant and you and your partner found out you were both carriers of either sickle cell anemia, Tay-Sachs disease, or cystic fibrosis, would you want to test your unborn baby?

Yes

No

I am not sure

45. Newborn babies are routinely screened for certain serious genetic disorders. This is called the "newborn screen." Would you choose to have genetic testing for these serious genetic disorders before getting pregnant, during the pregnancy, or wait for the newborn screen?

Before getting pregnant

During pregnancy

Wait for the newborn screen

I am not sure

46. For genetic disorders that are not included in the newborn screen, would you be interested in "carrier screening" (genetic testing to detect if you are a carrier of a genetic disorder) to find out if your offspring are at risk to have the disorder?

Yes

No

I am not sure

47. If offered, how interested would you be to learn more about carrier screening for sickle cell anemia, thalassemia, Tay-Sachs disease, and/or cystic fibrosis?

Definitely interested

Somewhat interested

Not interested in carrier screening

I do not know

APPENDIX C: Social media advertisement in Portuguese

Olá membros [ ],

Eu sou um estudante de pós-graduação em aconselhamento genético na Universidade da Califórnia, Irvine. Eu estou recrutando participantes de pesquisa para um estudo projetado para explorar como os indivíduos brasileiros se sentem sobre triagem de portadores de mutações genéticas, um tipo de teste genético que pode determinar se sua prole pode estar em risco de certas doenças genéticas.

A pesquisa é completamente anônima e leva cerca de 10 minutos para ser concluída.

<u>Elegibilidade:</u> você deve ter entre 18 e 45 anos para participar desta pesquisa.

Sinta-se à vontade para compartilhar o link da minha pesquisa com qualquer pessoa que você possa conhecer da etnia brasileira.

Sua participação é muito apreciada!

O link: https://pt.surveymonkey.com/r/brazilsurvey1

Muito obrigado,

Marina Dutra-Clarke, B.A.
Estudante de pós-graduação em Aconselhamento Genético
Departamento de Pediatria
Divisão de Medicina Genética e Genômica
Universidade da Califórnia, Irvine
E-mail: mdclarke@uci.edu
telefone: (714) 456-5837

APPENDIX D: Social media advertisement in English (For reference only)

Hello members of [ ]. I am a genetic counseling graduate student at the University of California, Irvine and I am seeking participants for a research study. This study is designed to explore how Brazilian individuals feel about carrier screening, a type of genetic testing that can determine if your offspring may be at risk for certain diseases.

The survey below is completely anonymous and takes about 10 minutes to complete.

Eligibility: You must be between the ages of 18 and 45 and of Brazilian ethnicity to participate in this study.

Please feel free to share my survey to anyone you may know who is of Brazilian ethnicity.

Your participation in this study is greatly appreciated!

[[Link to survey]]

APPENDIX E: Confirmation letter of IRB exempt research registration

UCI University of California, Irvine

CONFIRMATION OF EXEMPT RESEARCH REGISTRATION

November 13, 2017

MARINA DUTRA-CLARKE
DIVISION OF GENETIC AND GENOMIC MEDICINE

RE: HS# 2017-3961 *Public interest for carrier screening in the Brazilian population*

The human subjects research project referenced above has been administratively registered with the UC Irvine Institutional Review Board (UCI IRB) as Exempt from Federal regulations in accordance with 45 CFR 46.101. This exemption is limited to the described activities in the registered UCI IRB Protocol Narrative and extends to the performance of such activities at the sites identified in your UCI IRB Protocol Application. Informed consent from subjects must be obtained unless otherwise indicated below. UCI IRB conditions for the conduct of this research are included on the attached sheet.

Information provided to prospective subjects to obtain their informed consent should, at a minimum, consists of the following information: the subject is being asked to participate in research, what his/her participation will involve, all foreseeable risks and benefits, the extent to which privacy and confidentiality will be protected, that participation in research is voluntary and the subject may refuse to participate or withdraw at any time without prejudice.

Questions concerning registration of this study may be directed to the UC Irvine Office of Research, 141 Innovation Drive, Suite 250, Irvine CA 92697-7600; 949-824-0665 (biomedical committee) or 949-824-6662 (social-behavioral committee).

Level of Review: Adminsitrative Review, Category 2

Cristobal Barrios, MD
Vice Chair, Institutional Review Board
Registration valid from 11/13/2017 to 11/12/2022
UCI (FWA) 00004071, Approved: January 31, 2003

Determinations as Conditions of Exemption:
Informed Consent Requirements:
1. Signed Informed Consent Not Required
    a. Study Information Sheet Required
2. Use of Translated Language Consent[1]

APPENDIX F: IRB e-mod approval letter

UCI University of California, Irvine

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD
PAGE 1 OF 1

February 05, 2018

MARINA DUTRA-CLARKE
DIVISION OF GENETIC AND GENOMIC MEDICINE

RE: HS# 2017-3961 *Public interest for carrier screening in the Brazilian population*

Electronic Modification Request # 23353

The following modification(s) for the human subjects research protocol referenced above has/have been reviewed and approved by Human Research Protections Staff, on behalf of the UC Irvine Institutional Review Board (UCI IRB). Below is a summary of the approved changes requested via e-modification request number 23353**:

Change in Recruitment:

ADD recruitment on Reddit
Reason: To obtain more participants

Other Changes:

Typographical changes to Appendix H and Protocol Narrative. No changes to the study are being proposed.
Reason: Edits provided by Faculty Sponsor to include these changes to sentence structure. No changes to the study are being proposed.

Translated Study Info Sheet and Recruitment Materials. In addition, the Facebook and Reddit recruitment ad were revised to create a single social medial (general) recruitment ad.

**Changes to approved protocols may not be made without prior approval. All changes proposed in the e-modification request may not have been approved. Review the above summary of approved changes and the approved documents released with this letter. If a requested change does not appear in the summary above or in the revised documents, the change was not approved. Please consult with an IRB Administrator for further information.

Note: If the approved modification(s) includes changes to the informed consent document, the approved stamped consent document will be released with this letter. Please discontinue use of any previous versions of the informed consent document and use only the most updated version for enrollment of all new subjects. Questions concerning registration of this study or approval of this modification request may be directed to the UC Irvine Office of Research, 141 Innovation Drive, Suite 250, Irvine CA 92697-7600; 949-824-6068 or 949-824-2125 (biomedical committee) or 949-824-6662 (social-behavioral committee).

Level of Review: Administrative Review

Cristobal Barrios, M.D.,
Vice Chair, Institutional Review Board
Approval Issued: 02/05/2018
Expiration Date: 11/12/2022
UCI (FWA) 00004071, Approved: January 31, 2003