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TÍTULO DEL PROYECTO

STUDY TO UNDERSTAND THE GENETICS OF AN ACUTE RESPONSE TO GLIPIZIDE AND METFORMIN IN PATIENTS WITH THE TYPE 2 DIABETES RISK VARIANT P.E508K OF THE HNF1A GENE

TESIS DE POSGRADO
PARA OBTENER EL TÍTULO DE ESPECIALIDAD EN
ENDOCRINOLOGÍA Y METABOLISMO

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Introduction

It is well known that the risk to develop type 2 diabetes (T2D) is determined in part by the genetic burden of an individual. Furthermore, the variability in the evolution of the disease, the development of complications and drug response in patients with T2D is not entirely explained by environmental factors, thus, genetics may play a cardinal role in the increased prevalence and the relentless worldwide advance of the disease[1].

Population and twin-based studies have shown the contribution of inheritance to the risk of suffering from T2D. Moreover, the difference in the prevalence of the disease across ethnic groups may be related to specific gene polymorphisms that may augment the potential for a detrimental relationship with the environment, generating a complex series of interactions between the genotype and external factors that predispose an individual to impaired glucose tolerance (IGT) or T2D[2].

In recent years, substantial progress in elucidating the genetic nature of T2D has been made with the advent of genome-wide association studies (GWAS). The findings obtained from GWAS have been central in the understanding to the epidemiological differences observed in T2D across populations and in clarifying multiple biological aspects of the condition[3]. However, a large portion of the genetic heritability of T2D is still unexplained.

To further complicate the analysis, it is well known that the response to interventions (*i.e.*, change of life style habits, medications) used to treat T2D is determined in part by genetics. Patients with T2D show great variability in their reaction to antidiabetic drugs, in fact, the difference is greater among the individuals of a population than within a subject or within monozygotic twins. The former represents evidence for the genetic contribution of drug response, which is the main focus of pharmacogenetics and pharmacogenomics[4].

In this study, we explore the drug response of patients that have a gene variant (p.E508K) of the human liver hepatocyte nuclear factor 1α (*HNF-1A*) which increases the risk of T2D in Latinos.

Theoretical framework

For decades, the main theory in T2D has been that the disease results from a combination of genetic and environmental factors that interact and predispose a subject to the development and progression of the disease. While the impact of lifestyle habits and an obesogenic environment is central in the epidemic of T2D, there is a great amount of evidence that links genetics in all aspects of the disease, the understanding of its contribution is essential in the knowledge and ultimately treatment of the condition.

In the following lines, we will explain the role of genetics in several aspects of T2D, including treatment, and why this is important to the current project.

Evidence for type 2 diabetes heritability

Estimates for the heritability of the disease range from 20-80%[2]. The latter observation is obtained from multiple twins and family-based studies, such as the Framingham Offspring study[5]. This report found that the odds ratios (OR) for offspring T2D or IGT was 3.4 (2.3-4.9) for maternal diabetes and 3.5 (2.3-5.2) for paternal diabetes, with an additive effect of 6.1 (2.9-13) with bilineal diabetes, suggesting that the risk of developing the disease is determined in part by the contribution of familial susceptibility. Some twin-based studies have observed that the probandwise concordance of T2D in monozygotic twins is around 50%, whereas for dizygotic twins is close to 43%, furthermore, the concordance in monozygotic twins for IGT appears to be higher (61%)[6]. Similar studies have shown that the concordance in monozygotic twins for T2D may be as high as 75% when twins were followed for 15 years[7]. Also, patients that develop T2D at a younger age (before 45) have a higher degree of familial aggregation of the disease, suggesting that in these patients, the genetic component plays a major role in the appearance of early-onset hyperglycemia, such is the case of monogenic diabetes[8].

Even though most of the twin and family-based studies are confounded by environmental factors or simply by chance, the consistency of the observations is

compelling enough to support the notion that T2D has a considerable genetic component. The basic explanation for the increased risk in family members of a proband with T2D is that they share a vast amount of genes, some of which augment the possibility of inheriting the disease. Thus, the study of this aspect of T2D is paramount in the understanding of the condition.

Evidence of the genetic influence in type 2 diabetes from ethnic groups studies

Current T2D global prevalence stands at 9%[9]. However, estimates vary from one ethnic group to another, even in the same country, thus, people living under similar environmental factors; raising the possibility of genetic aggregation according to ethnic groups. Indeed, the Center for Disease Control (CDC) in the United States reports that in non-Hispanic whites the percentage of age-adjusted T2D is 7.6%, while Asian Americans, Hispanics and American Indians have 9, 12.8 and 15.9% respectively, suggesting that besides cultural and environmental factors, common risk genes among ethnic groups play a part in this difference[10]. Similar observations have been pointed out in the United Kingdom, where minority ethnic populations have contrasting prevalence compared with persons of European descent (*i.e.* South Asians have a three to six-fold increase in T2D *vs* Caucasians)[11].

More data for the genetic predisposition in ethnic groups comes from admixed population studies. A great deal of information regarding admixed populations comes from Mexican-Americans since this group has been extensively mixed with persons of European descent. Chakraborty et al., found a pattern of decreased prevalence in T2D with a higher socioeconomic status (mostly European descent) and a lower Amerindian ancestry in San Antonio, Texas, suggesting that ethnic differences may account for the disparity in T2D risk[12]. Other reports show that native American ancestry was a strong risk factor for T2D in overweight and obese Mexican-American individuals (OR 1.19 for each 10% increase in native American ancestry) as compared to those with mostly European ancestry, underlining the relevance of genetics in the risk of T2D.

Importance of genome-wide association studies in type 2 diabetes

Before the globalization of GWAS, the primary methods to establish a link between genotype and phenotype in T2D were linkage analysis and candidate gene approaches. By using linkage analysis, some causative genetic variants for T2D were identified, such as the *ENPP1*[13] and the *HNF4A*[14] variants. The former is localized in chromosome 6 and increases the risk of IGT and T2D in obese children by generating insulin resistance. Meanwhile, in a Finish cohort of T2D affected siblings, several single nucleotide polymorphisms (SNPs) near the P2 promoter of *HNF4A* (chromosome 20) showed association with T2D and diabetes-related traits. Other groups have used a candidate gene approach to find genetic variants of interest. One of those diabetes-associated genes is the *PPARG* gene. Consistently, it has been established that a change in proline to alanine in codon 12 of *PPARG* increases the risk for insulin deficiency and augments disease severity in people with T2D, even though the same variant may reduce the risk of T2D in the general population[15]. Another gene that has been reported to be associated with T2D is the *KCNJ11* gene which encodes the Kir6.2 ATP-sensitive potassium channel. Mutations in this gene (E23K variant) may cause a defect in insulin secretion of patients with T2D[16]. Finally, polymorphisms in *TCF7L2* (rs7903146) may influence the progression from IGT to T2D due to abnormalities in insulin secretion[17]. Although the former methods have value in identifying rare familial genetic variants that influence certain disease or trait, they are not useful in determining more common genetic variants (with smaller effects) in complex medical conditions such as T2D. GWAS have been useful in filling the hole of information generated by the candidate gene search[3].

In the last ten years, there has been an incredible amount of data obtained by GWAS in T2D. With this approach, it is possible to scan thousands of SNPs across different populations to give us an estimate of genetic risk, especially by finding multiple gene variants with small effects in the disease. The basic premise of a GWAS is to flag a genomic region with a frequent gene variant that may be involved in some way in the pathogenesis of the disease. However, despite the fact that the region may be

associated, it is not possible to ascertain causality by this analysis, thus, they serve as a tool to establish the genetic landscape of a complex condition such as T2D[18].

In 2007, the first GWAS for T2D appeared[19]. This study found that five common loci (*TCF7L2*, *SLC30*, *HHEX*, *LOC387761*, *EXT2*) contributed to a significant portion of the T2D risk (up to 70%). All of these loci seemed to participate in the development of the pancreas and the control of insulin secretion. After this effort, several groups started productive collaborations with the basic premise of increasing the power of the low effect genetic variants by augmenting the sample size. In that way, consortiums such as the Diabetes Genetics Replication and Meta-analysis (DIAGRAM) and the Slim Initiative in Genomic Medicine for the Americas (SIGMA) groups have broadened the number of diabetes-associated genetic variants and given insight on the differences between ethnicities. A meta-analysis of 8 previous T2D GWAS published in 2010 by the DIAGRAM consortium (using data from more than 34,000 T2D patients and more that 59,000 controls of European descent), found 32 T2D susceptibility variants, 12 of which were newly discovered signals that accounted for approximately 10% of the familial clustering of T2D[20]. In an extension of the former project, the DIAGRAM group published another meta-analysis that explored more T2D susceptibility loci in a European and a Pakistani descent cohort and found ten new loci that attained genome-wide significance ($P < 5 \times 10^{-8}$) with the strongest signals at the *ZMIZ1*, *ANK1* and *KLHDC5* loci. Furthermore, using several methods for physiological analysis it was found that the loci have a role in the expression of different diabetes-related traits[21]. A basic characteristic of the DIAGRAM consortium GWAS was that they mainly explored individuals of European ancestry. In a recent study, multiple meta-analyses from four distinct ethnic groups (European, East Asian, south Asian and Mexican American) were aggregated (26,488 cases and 83,964 controls). The results showed a long tail of susceptibility variants with small but homogenous effects across all tested ethnic populations. Seven new loci were found that seem to participate in the β-cell dysfunction that occurs in patients with T2D.

Besides the discovery of genetic risk variants, GWAS have been also useful to evaluate the contribution of T2D susceptibility alleles on glycemic and anthropometric traits. In a study that involved non-diabetic individuals of European ancestry, 20 loci were highly associated with fasting glucose concentrations, 17 loci with fasting insulin levels and 4 additional loci that were associated with the two-hour glucose concentration. These loci accounted for approximately 4.8%, 1.2% and 1.7% of the variance in fasting glucose, fasting insulin level and two-hour glucose concentration, respectively. It is interesting to note that many of the encountered loci overlap with those associated with abdominal obesity[22]. The current epidemic of T2D is partly related to the increase in obesity rates, thus, it is reasonable to assume that many of the susceptibility loci found for obesity have a role in the establishment or progression of T2D. As such, two recent GWAS provide evidence for the genetic contribution in diabetes-related anthropometric traits. A study on body mass index (BMI) from more than 330,000 individuals (primarily European ancestry, but around 17,000 of non-European descent) showed that 97 loci were associated with BMI and that they may be important in several metabolic pathways[23]. Meanwhile, a GWAS on waist-to-hip-ratio adjusted for BMI (as a measure of body fat distribution) of more than 210,000 individuals of European ancestry found 49 loci associated with waist-to-hip-ratio adjusted for BMI. Interestingly, some of the loci seemed to have larger effects on women and others in men. Furthermore, analysis of the SNPs showed associations with diabetes-related traits, fasting and 2-hour glucose. By functional connectivity analysis, it was observed that the variants of risk may impact body fat regulation, insulin sensitivity and glucose levels, all important determinants in the development of T2D[24].

In recent years, there´s mounting evidence that supports T2D risk variants, whether they are located in genes that regulate glycemic or anthropometric traits or affect the heritability and development of the condition. Although much of the data comes from European populations, it is noteworthy to point out that there are also studies of high scientific quality in Latino subjects that confirm the importance of genetic polymorphisms in the appearance of T2D in this particular population.

Evidence of genetic T2D-associated risk variants in Latino populations

T2D has become one of the most important diseases in populations of Mexican and Latin American descent. The prevalence of the condition seems to be higher in these subjects than in other ethnicities. Environmental factors play a significant role accompanied by diabetes-associated risk alleles intrinsic to these populations.

As mentioned before, most of the high-quality evidence that involve risk alleles for T2D is obtained from European populations. Few studies[25] have analyzed Hispanics and most of them have used a small number of subjects which complicates the interpretation of the results. However, studies by the SIGMA consortium have clarified the panorama of T2D risk variants in Latino populations. In a 2014 report of more than 3,000 diabetes patients and 4,000 controls[26], this group replicated the association of previously established T2D susceptibility genes (*TCF7L2* and *KCNQ1*) in Latino individuals, but found new risk loci such as *IGF2* (chromosome 11p15.5) and *SLC16A11* (chromosome 17p13.1). The strongest association observed included one silent mutation and four missense SNPs in *SLC16A11* which was present in more than 50% of the samples coming from Native Americans, while it was considered infrequent in East Asians (10%) and rare in Europeans and Africans. The carriers of the risk haplotype (*SLC16A11*) had an increased risk of developing earlier T2D compared to non-carriers (2.1 years earlier). Also, younger carriers (less than 45 years old) possessed higher OR than older carriers (OR 1.48 *vs* 1.11, respectively). It was estimated that the presence of the risk allele increased the risk of T2D in around 20%. In another project by the SIGMA consortium using whole-exome sequencing in a Latino population, it was shown that the variant p.E508K located in exon 8 of the *HNF-1A* gene had a significant association with T2D. In fact, the effect size of the variant was high (OR 4.96, 95% CI 2.93-8.38) in two replications of independent cohorts. Furthermore, the variant seemed to be specific to Latinos since it was absent from non-Latino cohorts in the replication analysis. Clinical characteristics of the p.E508K variant carriers with T2D patients was not different in terms of adiposity, age of diabetes onset or fasting plasma glucose (FPG) levels compared to T2D patients without the risk mutation. Functional

analysis of the variant demonstrated that the mutation reduces the transcriptional activity of the *HNF-1A* gene promoter. The authors concluded that even though the p.E508K variant was rare, the effect size was considerable, increasing by approximately five times the prevalence of the disease, which would imply that a great number of Hispanic individuals with T2D have the risk variant, a situation that would indeed affect their clinical management[27].

Pharmacogenetics and oral hypoglycemic medications

The term pharmacogenetics refers to the study of genetic factors that influence the clinical outcome or side effects of medications. The aim of pharmacogenetics is to change the rationale of drug prescription from a mechanistic point of view towards an individualized approach[28]. From a practical perspective, the terms pharmacogenetics and pharmacogenomics are used interchangeably, however, in recent years, the word pharmacogenomics has been restricted to the use of genome-wide approaches to clarify the nature of genetic impact in the inter-individual response to drugs, while the concept of pharmacogenetics is based on the presence of gene polymorphisms that modify the metabolism of a particular medication[29].

In pharmacogenetics, multiple SNPs are associated with changes in the absorption, distribution, metabolism and excretion of medications. One of the best studied is the effect of polymorphisms on the cytochrome P450 enzymes. In fact, there´s a difference in the expression of CYP enzymes based on the ethnicity of the subject, for instance, three-quarters of Caucasians have a genetic inability to express CYP3A5, while in African Americans it occurs in about half of the population[28,29]. Furthermore, about it has been estimated that 7% of medications are affected by actionable germline pharmacogenomics. These drugs constitute 18% of the prescriptions in the United States, underlining the importance of an individualized pharmacological regimen[30].

Despite recent advances in the field of pharmacokinetics and pharmacogenomics, not much is known about the distinct pharmacological responses of patients with T2D. It is well established that there is considerable inter-individual variability in the

action of antidiabetic agents and that these differences may be in part explained by the effects of genes. Due to the variable responses of hypoglycemic medications, the clinician makes treatment decisions based on the previously known responses of a group of individuals or based on the side effects profiles of a particular drug. This form of prescription does not take into account the individual reaction of the patient and thus, it may be ineffective, incomplete or unsafe.

As mentioned before, the disparity in the responses to antidiabetic drugs may have a genetic component. Indeed, it has been postulated that genetic polymorphisms in cytochrome P450 enzymes may contribute to the observed differences in drug disposition of the oral hypoglycemic agents. In particular, the cytochrome P450 enzyme CYP2C9 is involved in the metabolism of antidiabetic medications such as glyburide, tolbutamide and glipizide. One study done in healthy volunteers showed that several CYP2C9 genotypes produced variations in the pharmacokinetics and pharmacodynamics of oral glyburide. Some CYP2C9 genotypes generated a slower metabolism of the drug (CYP2C9*3/*3), whilst other genotypes induced a higher clearance rate. Moreover, slow metabolizers of glyburide had an increase in insulin secretion after an oral challenge with the sulfonylurea[31]. Other classes of hypoglycemic agents like pioglitazone and repaglinide are also metabolized by the cytochrome P450 enzymes. Distinct CYP2C9 and CYP2C8 genotypes affect the rate of elimination of both medications[32].

More evidence that links genetics with the effects of antidiabetic medications comes from literature that shows the impact of genotypes in the secretion of insulin provoked by drugs. For instance, the mechanism of action of sulfonylureas (insulin secretagogue) is particularly useful in neonatal diabetes where the deficit in insulin secretion is related to the presence of polymorphisms in the sulfonylurea receptor 1 (SUR1), such as *KCNJ11* and *ABCC8*, located in the ATP-activated potassium channel (KATP)[33,34]. Furthermore, Javorsky et al[32] found that the use of sulfonylureas in Caucasian patients with a heterozygous and homozygous polymorphism (E23K) of the *KCNJ11* gene produced a higher drop in the HbA1c level compared to the non-risk homozygous group (1.04±0.10 *versus* 0.79 ± 0.12%;

p=0.036). In a study performed in Chinese individuals, the sulfonylurea receptor blocker repaglinide decreased significantly the levels of HbA1c in patients homozygous of the *KCNJ11* E23K risk gene (2.65 ± 1.73%) compared to the no-mutation group (1.52 ± 1.03%)[35]. The function of the *KCNJ11* gene may be restricted to the variant E23K since a study on 364 T2D patients from the UKPDS cohort demonstrated that other risk variants in the SUR1 promoter did not augment the risk of T2D[36]. In addition, large-scale association studies have been performed confirming that the *KCNJ11* E23K susceptibility allele is associated with T2D (OR 1.18, CI 1.04-1.34)[37]. Besides the role of the *KCNJ11* gene on the effects of sulfonylureas, some reports have shown that subjects with a mutation in the *ABCC8* gene seem to respond favorably to the treatment with sulfonylureas. Rafiq et al[38] transferred diabetes patients with an *ABCC8* mutation from insulin treatment to glyburide. They found a change from 7.2 to 5.5% in HbA1c with the transfer to glyburide in 85% of the patients with an *ABCC8* mutation. Furthermore, the homozygous state of the variant Ser1369Ala (AA) of the *ABCC8* gene was robustly associated with a decrease in FPG and HbA1c in Chinese T2D patients treated with a course of 8 weeks of glicazide[39]. Apart from the repercussions of the *KCNJ11* and *ABCC8* genes in the action of antidiabetic drugs, the transcription factor 7-like 2 (*TCF7L2*) gene has been proposed as another possible factor in the response to sulfonylureas in T2D patients. Holstein et al[40] showed that patients with the *TCF7L2* risk variant rs7903146 had a higher rate of failure to reduce HbA1c with the use of a sulfonylurea, nonetheless, the lack of success with these drugs may have been related to unbalanced groups. On the other hand, another report suggested that individuals with the genotype of risk of the gene *TCF7L2* could be associated with an improvement in FGP and HbA1c after treatment with glipizide. In addition, it is well established that individuals with maturity-onset diabetes of the young (MODY) resulting from mutations in the transcription factor hepatic nuclear factor-1α (*HNF-1A*) have an augmented sensitivity to sulfonylureas. Patients with this condition often improve their glycemic control with the use of a low dose of sulfonylurea, even producing hypoglycemia events more frequently[40,41]. Even though the results of

studies from antidiabetic drug related-SNPs may yield conflicting results, the total evidence points to a considerable effect of these variants in treatment response.

One of the most used and accepted antidiabetic medications is metformin. This drug also possesses a considerable variability in terms of clinical response. Besides, metformin has a predominant renal metabolism, thus, it is not influenced by the cytochrome P enzyme system and their polymorphisms. As such, the pharmacogenetics of metformin is underrepresented. However, studies in animal models and in healthy volunteers have shown that four allelic variants of the *SLC22A1* gene, that encodes the organic cation transporter 1 (OCT1), participate in the metabolism of metformin. Interestingly, human subjects that carry one or more of these risk variants possess higher metformin levels, nonetheless, the glucose lowering action of the drug is impaired during a glucose tolerance test[42,43]. Zhou et al studied 1,024 metformin users from the GoDARTS cohort and found that 14 SNPs in the *ATM* gene were associated with obtaining a level of HbA1c lower than 7%. The SNP with the strongest association was rs11212617. Subjects in the study with metformin monotherapy had a combined OR for lowering HbA1c of 1.42 (95% CI 1.26 – 1.62, $P = 4 \times 10^{-8}$), moreover, patients with two copies of the C allele at rs11212617 had a three times greater possibility of achieving an HbA1c <7%[44]. Consistent with the notion that variants of the genes that encode OCT 1 may affect metformin metabolism and action; a report showed that polymorphisms in OCT (carriers of 420del) had a greater decrease in fasting glucose level after the addition of metformin in T2D patients treated previously with insulin compared to non-carriers[45]. More information is needed to assess the contribution of genetic polymorphisms in metformin´s action.

Thiazolidinediones are peroxisome proliferator activated receptor-γ ligands (PPARG) that are used to treat patients with T2D due to their effect on the reduction of insulin resistance. There have been studies that prove that variants in the *PPARG* gene affect glucose homeostasis. Indeed, in Hispanic subjects, Wolford et al[46] showed that eight variants of the *PPARG* gene had influence in the response to troglitazone, including three of them that affected diabetes-related traits such as

insulin sensitivity and body weight (rs413526346, rs10510419, rs1152003). Another variant, rs1801282, was associated with a higher HbA1c reduction in T2D subjects exposed to pioglitazone as compared to non-carriers. However in the report by Florez et al, there was no association between this polymorphism and insulin sensitivity of patients treated with troglitazone[47].

The incretin system mimetics are newer antidiabetic medications that try to augment the secretion of endogenous insulin after the ingestion of oral glucose or mixed meals. Initial evidence suggests that genetics affect the response of dipeptidyl-peptidase-4 inhibitors (DPP-4) and the glucagon-like peptide 1 (GLP-1) agonists. In a report of Caucasian subjects, three loci (*CHST3*, *TMEM114*, *CTRB1*/*CTRB2*) showed genome-wide significance for association with the magnitude of GLP-1 stimulated insulin secretion in a modified hyperglycemic clamp. Furthermore, T2D patients with a risk variant near *CTRB1/2* and treated with DPP-4 inhibitors had a lower HbA1c reduction. Similarly, T2D patients with a risk allele in *CHST3* also had a lower response to the treatment with a GLP-1 analog[48].

The development of knowledge in the area of pharmacogenetics and pharmacogenomics of anti-diabetic medications has the potential to improve the variability of response. Moreover, there are still a number of avenues to research that may be important in the treatment of patients with T2D.

From previous lines, it is evident that the panorama of genetics in T2D is vast, moreover, relevant genetic risk variants may have a preponderant role in the response to antidiabetic medications, hence the need to expand the knowledge in this area.

Problem definition

The information of genetics in the effects of antidiabetic drugs in Latino subjects is still limited. As mentioned before, GWAS and other studies have proved that genetic risk variants have a role in the risk, development, and progression of T2D in Latinos. In addition, there are a number of reports that suggest that some risk loci affect the actions of antidiabetic medications. As such information is needed; in this study, we aimed to show the effect of a specific genetic variant in Latinos (p.E508K of the *HNF-1* gene) in the glycemic response to an acute challenge with glipizide and metformin.

Justification

The inter-individual variability in the response to hypoglycemic agents has been attributed to several factors such as the type of diabetes, duration of the disease, comorbidities, drug pharmacokinetics and pharmacological interactions. Nonetheless, recently, it has been recognized that multiple risk alleles contribute to different aspects of T2D. One of the areas that may impact the prognosis of patients with T2D is the role of genetics in the pharmacological response to antidiabetic drugs, therefore, there is a need to generate knowledge regarding the effects of genes in the pharmacological management of the condition. Moreover, the identification of the contribution of genetic risk variants in the action of antidiabetic drugs could help in unveiling relevant pathophysiological mechanisms of the disease that ultimately improve its overall management. The current project expands the previous information of an important polymorphism in Latinos and opens up new research avenues in this group of patients with T2D.

Hypotheses

Null

Subjects with T2D and without T2D that are carriers of the risk variant p.E508K of the *HNF-1A* gene will not have differences in the response to a pharmacological challenge with glipizide and metformin as compared with age, sex, BMI and HbA1c matched non-carriers individuals.

Alternative

Subjects with T2D and without T2D that are carriers of the risk variant p.E508K of the *HNF-1A* gene will have an increased hypoglycemic response to the pharmacological challenge with glipizide and metformin as compared with age, sex, BMI and HbA1c matched non-carriers individuals.

Objectives

Primary

- Establish the pharmacological response to an acute challenge with glipizide in patients with and without T2D according to the carrier status of the p.E508K risk variant of the *HNF-1A* gene.
- Determine the pharmacological response to a short course of metformin in patients with and without T2D according to the carrier status of the p.E508K risk variant of the *HNF-1A* gene.

Secondary

- Examine the events of hypoglycemia between the carriers and non-carriers of the p.E508K risk variant of the *HNF-1A* gene.

Description of the study

This is a prospective, interventional, open label, single group design and pharmacogenetics study done in a cohort of Latino patients with and without T2D that were stratified according to the carrier status of the p.E508K risk variant of the *HNF-1A* gene.

Materials and methods

Individuals were recruited from the Endocrinology department clinics at the National Institute of Medical Sciences and Nutrition Salvador Zubiran and from an existing database of subjects with the p.E508K risk variant of the *HNF-1A* gene. The study was performed in Mexico City, Mexico, between the period of March 2015 and March 2016.

From a previous database, carriers of the p.E508K risk variant of the *HNF-1A* gene were invited to the study and given an informed consent manuscript. Also, non-carriers were selected according to the baseline characteristics of the carriers in order to pair subjects by sex, age, BMI and time of the T2D diagnosis.

After reading the material, doubts were answered if any and then if the subject agreed, the informed consent was formally signed. The prospect was invited to the two portions of the protocol, however, the participation in both portions of the protocol was entirely up to the decision of the candidate.

Subjects were considered eligible to the study if they fulfilled the following inclusion criteria:

a) For carriers and non-carriers of the p.E508K risk variant of the *HNF-1A* gene without T2D.

- Male and non-pregnant female older than 18 years.

b) For carriers and non-carriers of the p.E508K risk variant of the *HNF-1A* gene with T2D.

- Male and non-pregnant female older than 18 years.
- Subjects with T2D that had an HbA1c lower than 7.5% and were treated with ≤ two oral antidiabetic medications.
- Individuals who were deemed safe to undergo a seven day medication washout period before the start of the study.

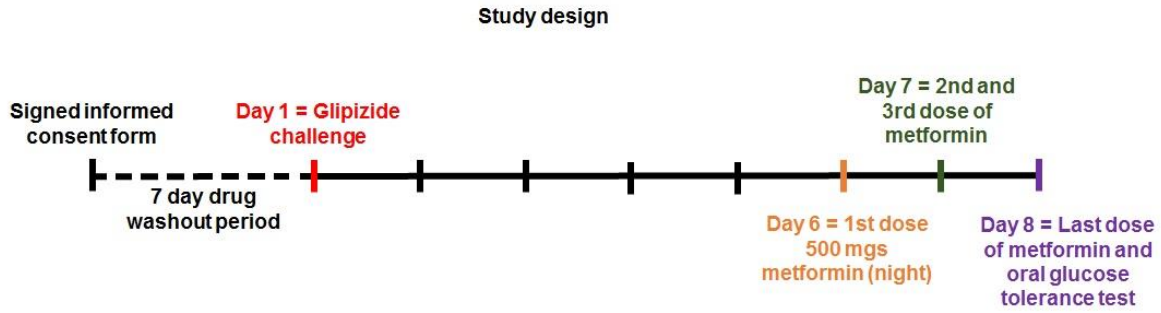
On the other hand, the following exclusion criteria were established:

- Pregnancy, breastfeeding or at risk of becoming pregnant.
- Currently taking any medications for the treatment of diabetes.
- Currently on metformin for any other indication (e.g. polycystic ovary syndrome).
- Onset of diabetes before age 25, with autosomal transmission of diabetes across three generations.
- History of liver or kidney disease.
- Known severe allergic reactions to sulfonylureas or metformin.
- History of porphyria.
- Documented estimated glomerular filtration rate (GFR) < 60 ml/min/1.73 m2, based on the most recent serum creatinine measurement and calculated by the Modification of Diet in Renal Disease (MDRD) equation.
- Currently taking medications known to affect glycemic parameters (e.g. glucocorticoids, growth hormone or fluoroquinolones)
- Established coronary artery disease (CAD), defined as:
    - History of myocardial infarction.
    - History of revascularization.
    - Evidence of ischemia on cardiac stress test.
- History of seizures.
- History of cerebrovascular accident.

Study design

After the informed consent was signed, the patient was scheduled for a Day 1 visit and instructed to undergo a 7 day medication washout period in which all antidiabetics were suspended 7 days prior to the Day 1 visit. During this time, the patient was asked to monitor regularly glucose levels using capillary measurements and to contact the study team in case the glucose levels were above 250 mg/dl. In Figure 1 there is a layout of the study design.

Figure 1. A map of the study design



Day 1, visit 1 (glipizide challenge)

On the first visit, the patient vital signs were checked and anthropomorphic measurements were done. Fasting glucose, insulin, C peptide, serum creatinine, AST/ ALT, glucagon, GLP- 1, lipid panel, DNA were measured.

Glucose results were reviewed in order to determine if the patient could endure the drug challenge. If the result showed a glucose ≥ 100 mg/dl the patient was given 5 mgs of glipizide. In case the glucose was between 80 – 99 mg/dl, the subject was given 2.5 mgs of glipizide. No medication was administered in case the glucose level was below 80 mg/dl.

After the glipizide dose, glucose, proinsulin, insulin, glucagon and GLP- 1 were measured at 30, 60, 90, 120, 180 and 240 minutes. During the challenge, symptoms and signs of hypoglycemia (diaphoresis, tremor, dizziness, tiredness, growing

hunger, confusion, and anxiety) were examined and treated according to the degree of hypoglycemia as follows:

- Asymptomatic subjects and glucose < 50 mg/dl: Measurements of capillary glucose every 5 minutes. If glucose > 50 mg/dl measurements were obtained every 10 minutes.
- Symptomatic individuals and glucose > 50 mg/dl: Measurements of glucose every 10 minutes.
- Symptomatic individuals and glucose < 50 mg/dl: Stop the challenge and administer 120 cc of juice or 50 cc of IV 50% glucose if the patient is unable to drink.

At the end of the study, all individuals were given a meal and stayed for 3 hours at the study facilities to assess any symptom of hypoglycemia if they had a blood glucose level < 80 mg/dl.

Before discharge from the hospital, subjects that agreed to enter the next part of the protocol received proper instructions and three 500 mgs tablets of metformin to take home with thorough instructions on how and when to take them, finally, they were scheduled for visit 2.

Days 2 – 7

All patients were asked to fill a food intake record.

At day 6, all individuals that accepted to be included in the metformin portion of the protocol were instructed to take one 500 mg tablet of metformin at nighttime. At day 7, two doses of 500 mgs of metformin were administered, in the morning and at night. Individuals with the metformin treatment were asked to report any adverse effects with the medication.

Day 8, visit 2 (oral glucose tolerance test)

On arrival to the study facilities, blood glucose levels were obtained. If the glucose level was between 60 and 250 mg/dl, then the patient ingested the fourth dose of

metformin (500 mgs), approximately one hour before the oral glucose tolerance test (OGTT). If the glucose was < 60 mg/dl, metformin was taken 10 minutes after the start of the OGTT. In case the blood glucose was ≥ 250 mg/dl the study was terminated and the patient excluded from the primary analysis.

A superficial vein catheter was placed in the patient and after an hour the subject was given 75 grs of oral glucose. Measurements of glucose, proinsulin, insulin, glucagon and GLP- 1 were made at 5, 10, 15, 30, 60 and 120 minutes.

At the conclusion of the OGTT, the study concluded and the patient was told to resume their previous antidiabetic drugs and report any adverse effects to the study team.

Study medications

Glipizide and metformin were selected on the basis of their pharmacokinetic properties and previous pharmacogenetics studies.

Glipizide is a sulfonylurea that has a complete absorption, 80 – 100% of the oral drug is absorbed. Furthermore, it has a quick onset of action (15 – 30 mins) with a peak at 90 to 120 mins. It binds to plasma proteins (92 - 95%) and is extensively distributed to the liver where it's metabolized by the CYP2C9 cytochrome enzymes. Glipizide average half-life is 4.2 – 5.4 hrs[49]. The selection of glipizide was based on the quick action of the drug and a relative short half-life which diminishes the risk of hypoglycemia while achieving the desired hypoglycemic effect with doses from 2.5 to 5 mg (standard doses used in the treatment of patients with T2D).

Metformin is a biguanide that has a 50 -60% absorption with doses from 0.5 – 1.5 grs. The onset of action is variable, however in average is between two days and a week, with a full hypoglycemic response at 2 weeks. Distribution is extensive and is excreted in urine (35 – 52%) and feces (20 – 33%). Metformin´s glycemic effect is established at a dosage above 1.5 grs. The chosen dosing and timing of administration of metformin was based on the need of a high dose and its onset of action.

Laboratory measurements

Determinations of glucose, insulin, C peptide, serum creatinine, AST/ ALT, glucagon and lipid panel were done according to the protocols established by the site´s central laboratory.

Plasma samples were taken and frozen (-80°C) for GLP-1 measurements in a later time.

Statistical analysis

Anthropometric characteristics and biochemical measurements were analyzed as mean and standard deviation (mean±SD).

The main parameter analyzed was the change of blood glucose level during the glipizide challenge and the OGTT after metformin treatment. Secondary parameters like glucose nadir, area under the curve (AUC, calculated by the trapezoidal method), peak insulin concentration and hypoglycemia events were also reviewed.

Subjects were stratified according to the carrier status of the p.E508K risk variant of the *HNF-1A* gene with and without T2D. Control subjects were paired based on sex, age, BMI and duration of T2D. Four groups were formed. For the sample size calculation we assumed a difference of around 18 mg/dl in the change of glucose between carriers and non-carriers with a standard deviation of 27 mg/dl and $\alpha = 0.05$, which resulted in 41 subjects in each group with a statistical power of 85%.

Normality tests (Shapiro Wilk) were employed to determine if the data had a normal distribution. Independent sample T tests and the U Mann-Whitney test were done according to the previous assessment of normality. Both tests were used to establish statistical differences between groups. Statistical significance was considered as a *P* value below 0.05.

Ethical considerations

The study was approved by the National Institute of Medical Sciences and Nutrition Salvador Zubiran bioethics board and done in accordance to the General Health Law in Medical research and the declaration of Helsinki. All patients included in the study signed the informed consent form.

Results

At the time of analysis for this report, a total of 58 patients were recruited. The distribution of the groups can be seen in Table 1. The recruitment of the carriers with T2D was slower than expected due to the difficulty in finding patients with well-controlled T2D.

Table 1. Distribution of the study individuals according to type 2 diabetes diagnosis and carrier status

	Carriers p.E508K	Non-carriers p.E508	
T2D present	8	15	23
T2D absent	21	14	35
Totals	29	29	58

The mean age of the T2D patients ranged from 54 to 56 years old, while individuals without T2D were younger (41.9 - 46.2). Subjects with T2D were diagnosed at ages 50 to 52 years old and they had a short duration of T2D since their diagnosis (around 3 years).

In terms of anthropometric measurements, carrier patients with T2D had a trend towards lower BMI and waist circumference than non-carriers, however, both parameters did not reach statistical significance. Control non-carriers were thinner than their carrier pairs. Although there was no increase in blood pressure in the entire cohort, carrier individuals with T2D had the lowest mean systolic and diastolic pressures. The analysis of the anthropometrical characteristics of the study can be seen in Table 2.

Table 2. Anthropometrical characteristics of the study subjects

	T2D			Controls		
	pE508 (+) n=8	pE508 (-) N= 15	P	pE508 (+) n= 21	pE508 (-) n= 14	P

Age (years)	56±17.8	54.2±8.17	0.764	41.33±16.87	46.43±11	0.287
Age at diagnosis (years)	53±17.8	50.67±8.58	0.752	___	___	
Duration of diabetes	3±2.9	3.53±4.3	0.945	___	___	
BMI (kg/m^2)	25.54±3.03	30.47±5.6	0.131	28.33±4.85	25.02±4.21	0.045
WAIST (cm)	88.54±4.59	99.78±14.35	0.091	91.5±12.09	87.43±8.52	0.229
HWR	0.91±0.07	0.9±0.1	0.945	0.91±0.08	0.91±0.08	0.7
SBP (mm/Hg)	105±11.41	129.53±13.43	<0.001	113±13	120.28±25.36	0.06
DBP (mm/Hg)	67.28±8.01	81.8±9.6	<0.008	74.28±9.58	75.07±11.91	0.831
HR (beats/min)	66.57±7.13	72.06±8.95	0.123	65.38±7.53	65.71±9.57	0.909
BFM (%)	30.38±8.81	38.26±5.59	0.04	34.28±5.4	29.17±8	0.02

BMI: Body Mass Index; HWR: Hip to waist ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: heart rate; BFM: Body Fat Mass.

Variables are expressed as mean ± standard deviation

After the antidiabetic drugs washout period and before the start of the glipizide challenge baseline biochemical measurements were taken. Carrier patients with T2D had a lower basal insulin secretion and a diminished Homeostatic Model Assessment (HOMA-IR) compared to the non-carriers with T2D, nevertheless, all other biochemical values were not statistically significant in the T2D group. In Table 3 there is a detailed view of the laboratory parameters in all the groups.

Table 3. Biochemical values in the study subjects

	T2D			Controls		
	pE508 (+) n= 8	pE508 (-) n= 15	P	pE508 (+) N= 17	pE508 (-) N= 13	P
Glucose (mg/dl)	118±19.82	136.57±40.15	0.188	97±9.23	102.21±11.8	0.153
Insulin (mu/ml)	5.37±3.18	11.63±7.36	0.01	9.42±6.48	7.6±5.9	0.306
HOMA-IR	1.57±1.05	3.81±2.38	0.005	2.24±1.56	1.99±1.71	0.359
HbA1c (%)	6.13±0.96	6.3±0.72	0.6	5.2±0.49	5.57±0.51	0.097
Creatinine (mg/dl)	0.65±0.1	0.73±0.29	0.636	0.66±0.14	0.83±0.16	0.002
Total Cholesterol (mg/dl)	185.75±42.84	184.53±35.75	0.943	180.71±27.59	191.57±43.71	0.373
Triglycerides (mg/dl)	143.75±25.11	166.86±70.12	0.423	173.61±98.62	168.85±89.46	0.881
HDL-C (mg/dl)	47±16.93	44.13±9.38	0.604	40.85±7.5	41.92±9.05	0.706
LDL-C (mg/dl)	109.95±38.89	107.38±31.27	0.865	98.92±40.22	116.61±41.28	0.474
Apo A1 (mg/dl)	155.02±38.45	149.89±33.48	0.743	141.57±24.1	137.31±23.75	0.610
Apo B (mg/dl)	102.3±24.05	103.44±24.55	0.728	97.9±19.74	105.37±22.54	0.308
AST (Ul/l)	26.37±4.92	37.66±24.21	0.325	31.5±41.7	27.42±7	0.052
ALT (Ul/l)	26±10.11	41.13±23.7	0.045	34.33±51.56	28.71±15.74	0.434
GGT (Ul/l)	29.37±29.79	36.66±28.81	0.466	22.94±24.57	37.92±49.56	0.048

GFR (ml/min)	113.16±32.2	97.77±19.58	0.166	112±18.25	97.6±22.33	0.044

HOMA-IR: Homeostatic model assessment; HbA1c: Glycated hemoglobin; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; ApoA1: Apolipoprotein A1; ApoB: Apolipoprotein B; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma glutamyl transpeptidase; GFR: Glomerular filtration rate. Variables are expressed as mean ± standard.

Glipizide challenge

Patients were given 2.5 or 5 mg of glipizide as an acute challenge after a seven-day washout period. In the group of individuals with T2D, carriers of the p.E508K risk variant had decreased levels of FPG and fasting insulin (102±40.9 *vs* 140.53±41.19, *P*=0.023) in relation to non-carriers. Furthermore, this subgroup had a smaller peak in insulin levels as compared to the non-carriers (15.87±8.41 *vs* 44.54±43.39, *P*=0.016). During the challenge, insulin levels tended to be reduced in the carriers with T2D, but they were only statistically significant in the first 30 minutes of the test. Glucose levels in the p.E508K carriers also showed a trend towards lower values in relation to the non-carriers, however, these differences were not significant for the most part of the challenge. For a graphical depiction see Figure 2. On the other hand, individuals without T2D did not have any differences in terms of fasting glucose, insulin, glucose nadir and insulin peak in the glipizide challenge. For absolute values see Table 4.

Figure 2. Glucose and insulin levels during the glipizide challenge

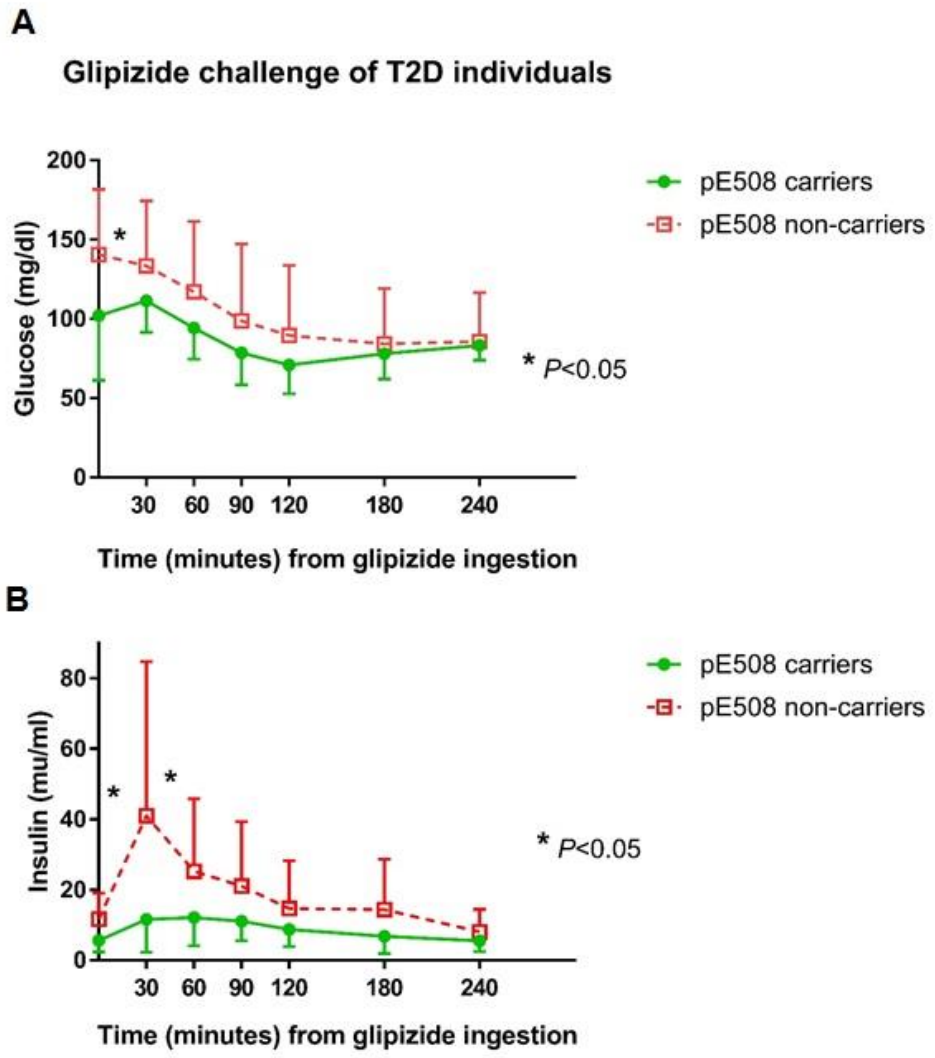


Table 4. Minimum and maximum peak glucose and insulin levels after glipizide

	T2D			Controls		
	pE508 (+) n= 8	pE508 (-) n= 15	p	pE508 (+) N= 21	pE508 (-) N= 14	p
Fasting Glucose (mg/dl)	102±40.9	140.53±41.19	0.023	97±9.18	102.42±11.65	0.139

Fasting Insulin (mu/ml)	5.56±3.24	11.63±7.36	0.01	9.45±6.68	8.02±5.92	0.278
Glucose nadir (mg/dl)	66.5±15.25	77.4±24.63	0.169	55.52±7.46	57.28±10.8	0.571
Insulin peak (mu/ml)	15.87±8.41	44.54±43.39	0.016	40.51±70.62	51.04±65.41	0.943
Glucose nadir: minimum glucose concentration during the curve; Insulin peak: maximum insulin concentration during the curve. Variables are expressed as mean ± standard deviation.						

Carrier individuals with T2D exhibited smaller total and incremental AUC (TAUC and IAUC) in glucose and insulin levels after glipizide administration. Meanwhile in controls, only the TAUC of glucose was statistically different between these subgroups, albeit absolute values of the glucose and insulin AUC were lower in control carriers. The results of the AUC analysis are shown in Table 5

Table 5. Total and incremental area under the curve of glucose and insulin in the glipizide challenge

	T2D			Controls		
	pE508 (+) n=8	pE508(-) n=15	P	pE508(+) n=21	pE508(-) N=14	P
Glucose TAUC	322.16±35.22	388.25±144.97	0.018	289.94±57.48	316.69±21.83	0.039
Glucose IAUC	848.66±91.54	998.66±427.04	0.041	773.17±219.24	856.61±55.94	0.320

Insulin TAUC	29.99±20.26	83.6±65.41	0.041	55.57±54.05	71.79±80.13	0.650
Insulin IAUC	100.98±72.4	284.1±235.16	0.049	184.47±191.5	255.06±300.9	0.536

TAUC: Total Area Under Curve; IAUC: Incremental Area Under Curve. Variables are expressed as mean ± standard deviation.

The magnitude of glipizide´s hypoglycemic effect is shown by the subtraction of the final glucose as compared to the basal glucose value. In carrier T2D subjects, glucose levels dropped only 29.33±9.91 mg/dl, while the change in non-carriers was more pronounced (59.91±39.5), however, this reduction did not reach statistical significance. It is noteworthy that the difference between the minimum peak of glucose during the test and basal glucose was less marked in carrier patients with T2D than non-carriers, although not significantly (-47.33±11.55 vs 65.41±37.84, P=.276). In the meantime, glipizide induced a higher insulin peak in non-carrier T2D subjects compared to the p.E508K carriers (37.37±40.98 *vs* 9.68±7.47, *P*=0.042), although there was no difference in terms of glucose nadir between these subgroups. On the other hand, there was no significant variation in the groups without T2D in terms to the hypoglycemic effect of glipizide. The values of this analysis are shown in Table 6.

Table 6. Magnitude of change (deltas) of glucose and insulin during the glipizide challenge

	T2D			Controls		
DELTAS Glipizide	pE508 (+) n=8	pE508 (-) n=15	P	pE508 (+) N=21	pE508 (-) N=14	P
Final glucose *vs* basal	-29.33±9.91	-56.91±39.5	0.116	-21.29±12.12	-18.46±8.21	0.475

Final insulin *vs* basal	-0.33±2.68	-3.4±6.71	0.180	-.07±5.84	-.63±3.43	0.368
Minimum peak glucose *vs* basal	-47.33±11.55	-65.41±37.84	0.276	-40.88±12.28	-46.92±10.69	0.170
Maximum peak insulin *vs* basal	9.68±7.47	37.37±40.98	0.042	31.06±65.42	43.02±61.01	0.225
Minimum peak glucose *vs* final	-18±10.03	-8.5±9.5	0.067	-19.58±13.99	-28.46±10.26	0.065
Maximum peak insulin *vs* final	10.01±7.15	38.77±44.72	0.143	31.05±68.28	42.38±58.09	0.117
Variables are expressed as mean ± standard deviation						

Hypoglycemia events during the glipizide challenge

As a secondary analysis, and in particular during the glipizide challenge, there were no significant differences in the presence of hypoglycemia in all the studied subgroups as can be seen in Table 7.

Table. Hypoglycemia events in the glipizide challenge

	Reported symptoms of hypoglycemia					
Genotype HNF1α status	DT2 (n=18)			Controls (n=30)		
	yes	**no**	**p**	**yes**	**no**	**p**
pE508K carriers	0	6		1	16	
pE508 non-carriers	1	11	1.000	1	12	1.000

Oral glucose tolerance test with metformin treatment

Individuals that accepted to undergo the metformin portion of the protocol were given 4 doses of 500 mg beginning at day 6 with the last dose taken one hour before de OGTT. Carrier patients with T2D had lower levels of fasting insulin (4.25±2.59 *vs* 11.8±8.04, P<0.005) than the non-carriers, notwithstanding the presence of similar fasting glucose values in both subgroups (111.37±17.5 *vs* 129.42±47.78, P=0.441). Additionally, p.E508K carriers with T2D had a less pronounced insulin peak during the OGTT as compared to their non-carriers counterparts (35.52±25.48 *vs* 122.55±142.43, P=0.042). Likewise, at every time point of the OGTT, there was a trend towards lower levels of glucose and insulin in carriers with T2D in relation to the non-carriers. On the contrary, carriers and non-carriers without T2D (controls) showed a comparable response of glucose and insulin in the OGTT. Figure 3 and Table 7 show the OGTT behavior and the absolute values of fasting glucose and insulin.

Figure 3. Glucose and insulin levels during the oral glucose tolerance test after metformin treatment

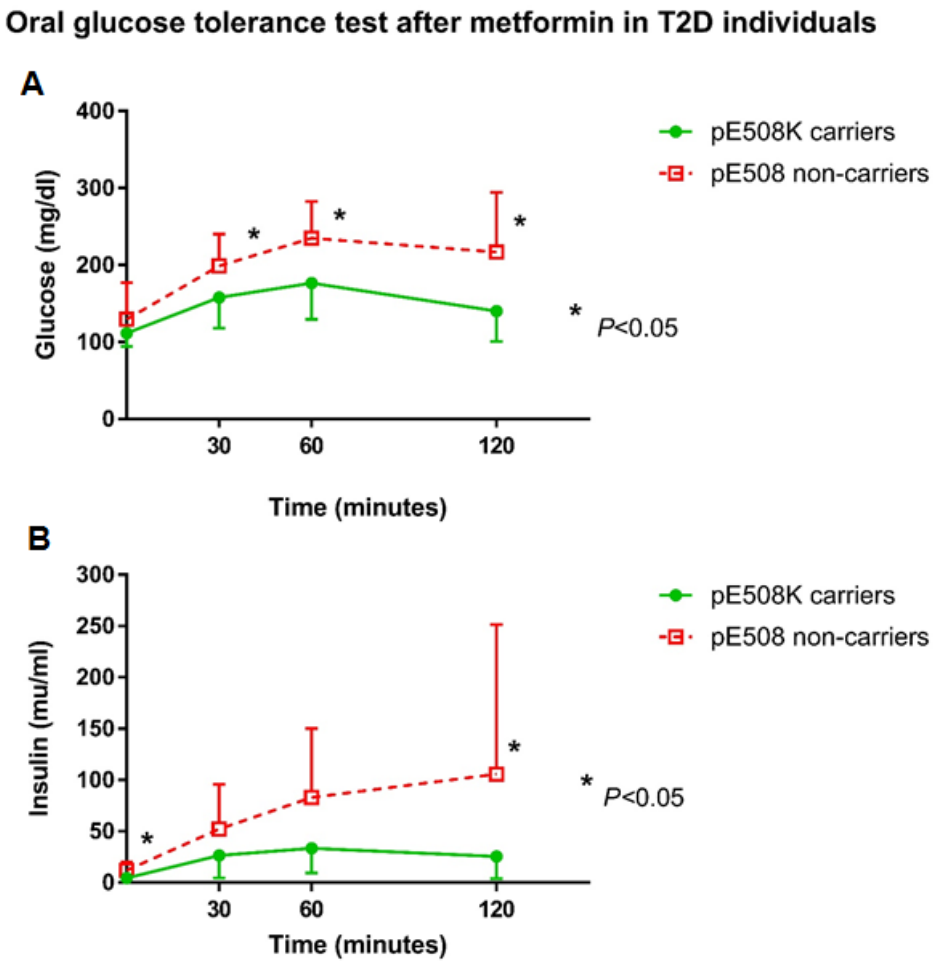


Table 7. Minimum and maximum peak glucose and insulin levels during the oral glucose tolerance test after metformin treatment

	T2D			Controls		
	pE508 (+) n= 8	pE508 (-) n= 15	p	pE508 (+) N= 21	pE508 (-) N= 14	p
Fasting glucose (mg/dl)	111.37±17.5	129.42±47.78	0.441	91.33±8.13	97.57±8.6	0.037

Fasting insulin (mu/ml)	4.25±2.59	11.8±8.04	0.005	8.95±5.74	8.59±9.31	0.309
Glucose nadir (mg/dl)	108.12±14.39	129.42±47.78	0.267	88.7±8.96	94.76±10.92	0.395
Insulin peak (mu/ml)	35.52±25.48	122.55±142.43	0.042	93.23±96.78	103.56±140.13	0.829
Glucose nadir: minimum glucose concentration during the curve; Insulin peak: maximum insulin concentration during the curve. Variables are expressed as mean ± standard deviation.						

Similarly to the observed results in the glipizide challenge, carrier patients with T2D showed a reduced glucose and insulin AUC (in both, TAUC and IAUC) as compared to the non-carriers, achieving statistical significance. On the contrary, control individuals had no difference in their AUC values during the OGTT. Table 8 depicts the results.

Table 8. Total and incremental area under the curve of glucose and insulin in the oral glucose tolerance test

	T2D			Controls		
	pE508 (+) n=8	pE508(-) n=15	P	pE508(+) n=21	pE508(-) n=14	P
Glucose TAUC	300.75±82.2	413.66±103.18	0.032	243.33±52.06	372.94±73.44	0.363

Glucose IAUC	384.16±158.78	566.16±122.07	0.041	302.44±96.17	342.42±142.59	0.363
Insulin TAUC	41.82±37.5	156.77±144.87	0.041	108.37±86.68	132.45±185.11	0.902
Insulin IAUC	77.01±72.08	289.46±274.03	0.041	199.72±164.57	246.8±357.44	1

TAUC: Total Area Under Curve; IAUC: Incremental Area Under Curve. Variables are expressed as mean ± standard deviation.

T2D patients with the p.E508K risk variant showed a smaller rise in the levels of glucose and insulin during the OGTT. The delta of change between the final glucose versus the basal glucose was smaller in carriers with T2D than non-carriers (30.33±42.33 *vs* 87.58±45.61, P=0.013), moreover, the difference between the peak and final value of glucose was also smaller in carriers. Although carrier subjects with T2D showed lower levels of insulin, the difference was not statistically significant. Independently of their risk variant status, control individuals showed no prominent differences in the deltas of glucose and insulin since the plasmatic levels of the molecules were similar during the OGTT.

Discussion

In this study, we examined the effect of a specific genetic variant in Latinos (p.E508K of the *HNF-1A* gene) in the glycemic response to an acute challenge with glipizide and metformin. In a previous report by the SIGMA consortium, it was shown that the variant p.E508K of the *HNF-1A* gene had a significant association with T2D. It was also demonstrated that the variant was specific to the Latino population and that its presence produced a remarkable effect size in the risk of T2D (OR 4.96, 95% CI 2.93-8.38)[27]. Variants in multiple genetic loci have been postulated as one of the mechanisms that influence the high inter-individual variability of antidiabetic drug response[3,4]. The mechanisms by which the former may occur are not completely understood, but it may involve various steps in the pharmacologic action of hypoglycemic agents. There have been reports suggesting that genetic variants produce changes in the metabolism, transport, insulin secretion, insulin resistance and ultimately drug-receptor interaction that affect the action of antidiabetic medication, therefore, the need to properly examine the pharmacological effects of loci variants is warranted since they may change the treatment of T2D patients.

Considering that a genetic risk variant may intervene in glucose metabolism in several ways, the results of the SIGMA consortium[27] and our study suggest that the risk variant p.E508K of the *HNF-1A* gene may alter a few areas of insulin secretion and function. Various studies have shown that 2-3% of the patients with diabetes have monogenic defects of insulin secretion, the so-called MODY-type diabetes, in particular, type 3 MODY is due to mutations in multiple exons of the *HNF-1A* gene. A few polymorphisms in the *HNF-1A* gene demonstrate a high penetrance, with 63% of the carriers affected by T2D before 25 years old[51]. Other reports have proven that mutations in the *HNF-1A* gene that occur at exons 8-10 develop the condition 10 years later than those with variants at exons 1-6 (10-40 years old)[52]. In accordance to the latter, our p.E508K carriers (located at exon 8) were diagnosed with T2D at around 50 years old, on the contrary, type 3 MODY afflicted individuals are diagnosed at a younger age (10-40 years old)[51,52], which would suggest that penetrance of the p.E508K may be lower than other alleles. The observations of the

SIGMA consortium seem to further validate this last point since they showed that p.E508K carriers have incomplete penetrance[27].

Similarly to what has been published previously, our results show that patients with the p.E508K mutation seem to have an almost identical BMI than non-carriers, irrespective of their T2D status. However, a trend towards a lower BMI was seen in the T2D carriers group. This trend may be related to unbalanced grouping or due to the small size of this specific subgroup which complicates proper interpretation of the actual BMI and phenotype in this report. It is interesting to note that carriers, independently of the disease status have diminished values of systolic and diastolic blood pressure which would agree with Bellane et al, who found that hypertension was less common in MODY 3 individuals that in non-MODY 3 patients (genes different of the *HNF-1A* gene)[53], nevertheless, the clinical relevance of this observation is still unknown.

With respect to the basal biochemical parameters, carrier patients with T2D showed a statistically significant lower HOMA-IR of β-cell function and insulin resistance (IR) index and fasting insulin level, with a reduced, but not significant level of fasting glucose. The HOMA-IR index is a method to assess the function of the pancreatic β-cell and insulin resistance calculated from fasting glucose and insulin. This technique has good correlation with the euglycemic clamp in terms of comparing two distinct populations, such as our subjects[54]; hence the presence of lower levels of HOMA-IR and insulin in p.E508K carriers with T2D may be indicative of less IR or an increased insulin sensitivity. The main caveat of the insulin and HOMA results in our population is that they are probably affected by the unbalanced pairing in BMI since the heavier carrier individuals without T2D had higher HOMA-IR and fasting insulin level than the leaner non-carriers. It is necessary to increase the size of the carriers with T2D in order to equilibrate the BMI and establish whether there is a difference in insulin sensitivity in patients with the p.E508K risk variant. Even though series of MODY 3 patients have demonstrated that this group has higher levels of HDL cholesterol[53,55], carriers of the p.E508K variant did not show this relevant

clinical criterion, further attesting to the different effects of the variant in several metabolic areas.

In terms of the overall performance with the pharmacological interventions of the study, carrier patients with T2D had lower levels of fasting glucose and insulin compared to non-carriers, a situation that was also found in carrier patients without T2D, although the latter comparison did not reach statistical significance. During the glipizide challenge, as expected, all patients had a reduction in glucose, with the lowest values encountered in subjects without T2D (55 to 57 mg/dl); meanwhile, glipizide´s hypoglycemic effect in carriers of the p.E508K variant with T2D was characterized by a progressive drop in glucose with a flat profile in insulin secretion. Even though carrier patients with T2D had lower levels of insulin secretion and thus, diminished total and incremental AUC, this was sufficient to produce a significant reduction in total and incremental AUC of glucose during the challenge with the sulfonylurea. Therefore, the results of the glipizide challenge would suggest that the p.E508K variant may not induce an increase in insulin secretion, but it may enhance insulin sensitivity since the glucose values dropped similarly to the other groups. The lower insulin secretion of the carriers with T2D is interesting since glipizide´s mechanism of action is to increase insulin secretion by closing ATP-sensitive potassium channels, thus augmenting the potential of the β-cell membrane and allowing the influx of calcium (Ca^{2+}) by opening voltage-gated calcium channels, which in turn promotes insulin secretion[56]. Similar to our results, Sagen et al found that 7 individuals with an *HNF-1A* mutation compared to 22 healthy controls had an almost identical insulin secretion in response to the acute administration of intravenous tolbutamide[57]. On the other hand, contrary to our findings, Pearson et al found that patients with *HNF-1A* mutations had a robust insulin secretion response to intravenous administration of tolbutamide. In the same study, the authors also showed that patients with *HNF-1A* variants had an increased sensitivity to insulin, which allowed them to attain lower fasting glucose levels with a sulfonylurea treatment (gliclazide)[58]. It is necessary to point out that in our data, the maximum peak of insulin secretion in carrier patients without T2D is 3-fold higher than the carriers with T2D which may indicate that T2D carriers have a reduced insulin

secretion either due to the enhanced sensitivity of insulin given by the variant or due to established damage in the pancreatic β-cell by the progression of the disease. Another factor to consider in this regard is that carrier controls had higher BMI than their diabetes afflicted pairs, hence increased insulin resistance and probably hyperinsulinemia. Despite the administration of glipizide, there was no difference in terms of hypoglycemia events reported during the glipizide test, this may be related to the fact that glucose levels fell moderately (55 – 77 mg/dl) in all subgroups, an aspect that underlines the effect on insulin secretion in carriers. From the previous considerations, sulfonylureas may be considered as the treatment of choice in T2D patients that harbor the p.E508K mutation of the *HNF-1A* gene, since they are capable of inducing the secretion of a sufficient amount of insulin to reduce glucose levels in a patient that seems to have an increased insulin sensitivity.

Coinciding with the glipizide results, the OGTT after metformin treatment showed that p.E508K carriers with T2D have a lower increase in glucose with reduced levels of insulin as compared to non-carriers. T2D carriers have lower peaks of insulin and smaller total and incremental AUC of glucose and insulin. This effect was not seen in patients without T2D. The results suggest that the lower insulin secretion is capable of maintaining lower values of glucose despite the glucose load. Although glucose levels were lower in carrier subjects with T2D than in non-carriers, still they were higher than the controls, this would confirm the observations from previous studies that patients with *HNF-1A* mutations put on metformin treatment have a borderline capacity to maintain euglycemia[59]. Thus, metformin may be an alternative treatment for p.E508K carrier patients with T2D.

The relevance of the study is one of its major strengths. As mentioned before, there are few studies that examine the effect of genetic variants in glucose metabolism. This report expands on the knowledge of a variant that has been found to be encountered in around 2% of Latinos. Taken into account the grand epidemic of T2D in this population, carriers of the p.E508K mutation may account for more than 100,000 cases, thus, correct treatment of their disease with sulfonylureas may improve the overall survival of a great number of patients. Secondly,

pharmacogenetics studies in populations other than Caucasians are sparse, hence, is important to generate new information from this groups, taking into account that there is an estimate of more than 44.3 million of T2D patients in North America, most of them being inadequately treated[60].

The main weakness of the present report is the small sample size, in particular in the subgroup of carriers with T2D. The small sample size may have produce and underestimation of BMI in the carriers with T2D, however, the influence of this factor is yet to be determined. One of the major challenges of the protocol has been the recruitment of well-controlled T2D individuals who can safely endure a washout period, recruitment efforts are ongoing.

To optimize results, the recruitment for this study continues.

Conclusions

It is well established that genetic factors play an important role in the inter-individual variability in the response to antidiabetic medications. The p.E508K variant of the *HNF-1A* gene has been found to increase the risk of T2D in Latinos. We aimed to assess the pharmacological response to a sulfonylurea and metformin in subjects with the risk variant according to their diabetes status. As such, the results show that carriers of the p.E508K with T2D have different pharmacological response profiles to sulfonylureas and metformin in terms of insulin secretion and sensitivity as compared to patients without T2D. Carrier individuals with T2D have lower levels of insulin but they are sufficient to promote a reduction in the levels of glucose during the sulfonylurea and metformin challenge, which may be related to the effect in insulin secretion generated by the genetic variant or to the increase in insulin sensitivity. Furthermore, the results point towards a specific effect in p.E508K carrier patients with T2D, since carrier subjects without T2D seem to behave similarly to their non-carriers counterparts. The reasons why the effects are different in both type of carriers is still unknown. Therefore, p.E508K of the HNF-1A gene carrier patients with T2D may be treated with a sulfonylurea and metformin as an acceptable alternative in order to attain glycemic control. A bigger sample size is needed to adequately pair the carriers versus the non-carriers, recruitment efforts are ongoing.

More pharmacogenetics and pharmacogenomics studies with antidiabetic medications would increase the knowledge on the performance of this medications in patients with T2D. The former could be an important influence to individualize T2D care according to the specific genetic profile of an individual. This study augments the information on the effect of genetic variants in the response to antidiabetic drugs in a group of T2D patients.

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Supplementary material

Informed consent (in Spanish)

CARTA DE CONSENTIMIENTO INFORMADO PARA PARTICIPAR EN EL PROYECTO:

Caracterización de los mecanismos mediante los cuales las variantes de los genes SLC16A11 y HNF1alfa aumentan el riesgo para tener diabetes tipo 2.
Estudio farmacogenómico

Investigador principal: Dr. Carlos A Aguilar Salinas
Dirección del investigador: Departamento de Endocrinología y Metabolismo INCMNSZ. Vasco de Quiroga 15 Col. Sección XVI, C.P. 14000 Del. Tlalpan. México DF.
Teléfono de contacto del investigador (incluyendo uno para emergencias): 55554523, 0445535010502
Investigadores participantes:, Dra. Teresa Tusié Luna, Dra. Ivette Cruz Bautista, Dr. Miguel Herrera Hernández, Dra. Olimpia Arellano Campos, Dra. Paloma Almeda, LN Marco A Melgarejo Hernández
Nombre del patrocinador del estudio: Instituto Carso para la Salud/ Departamento de Endocrinología y Metabolismo del Instituto Nacional de Ciencias Médicas y Nutrición
Versión del consentimiento informado y fecha de su preparación: 20 de abril de 2014

INTRODUCCIÓN:

Por favor, tome todo el tiempo que sea necesario para leer este documento y pregunte al investigador sobre cualquier duda que tenga.
Este consentimiento informado cumple con los lineamientos establecidos en el Reglamento de la Ley General de Salud en Materia de Investigación para la salud, la Declaración de Helsinki y las Buenas Prácticas Clínicas emitidas por la Comisión Nacional de Bioética.
Para decidir si participa o no en este estudio, usted debe tener el conocimiento suficiente acerca de los riesgos y beneficios, con el fin tomar una decisión informada. Este formato de consentimiento informado le dará información detallada acerca del estudio de investigación que podrá comentar con su médico tratante o con algún miembro del equipo de investigadores. Al final se le pedirá que forme parte del proyecto y de ser así, se le invitará a firmar este consentimiento informado, bajo ninguna presión o intimidación.

Procedimiento para dar su consentimiento:

Usted tiene el derecho a decidir si quiere participar en esta investigación, y se puede solicitar todo el tiempo que requiera para considerar esta invitación. El investigador le explicará ampliamente los beneficios y riesgos del proyecto sin ningún tipo de presión y tendrá todo el tiempo que requiera, para pensar solo o con quien usted decida, e informarle acerca de su decisión al investigador. Esta decisión no tendrá efecto alguno sobre su atención médica en el Instituto.

Al final de esta explicación, usted debe entender los puntos siguientes:

I. La justificación y los objetivos de la investigación.
II. Los procedimientos que se utilizarán y su propósito; incluyendo la identificación de que son procedimientos experimentales.
III. Los riesgos o molestias previstos.
IV. Los beneficios que se pueden observar.
V. Los procedimientos alternativos que pudieran ser ventajosos para Usted.
VI. Garantía para recibir respuestas a las preguntas y aclarar cualquier duda sobre los procedimientos, riesgos, beneficios y otros asuntos relacionados con la investigación.
VII. La libertad de retirar su consentimiento en cualquier momento y dejar de participar en el estudio, sin que por ello se interrumpa su atención en el Instituto.
VIII. La seguridad de que no va a ser identificado en ninguna publicación, presentación o divulgación de los resultados del estudio, y que se mantendrá la confidencialidad de la información relativa a su privacidad.
IX. El compromiso de proporcionar información actualizada obtenida durante el estudio, aunque esto pudiera afectar la disposición para continuar su participación.
X. La disponibilidad de tratamiento médico y compensación a la que legalmente tiene derecho por parte de la institución, para que le brinde atención de la salud en el caso de daños, directamente causado por la investigación. Es su derecho, solicitar más tiempo o llevar a casa este formulario antes de proporcionar una decisión final en los próximos días.

INVITACION A PARTICIPAR Y DESCRIPCIÓN DEL PROYECTO
Estimado Sr. (a) _____

El Instituto Nacional de Ciencias Médicas y Nutrición le invitan a participar en un estudio de investigación que tiene como objetivo conocer el efecto que tienen variantes de algunos genes asociados con la diabetes tipo 2 (variantes de SLC16A11, HNF1 alfa, KCNJ11, TCF7L2 y otras que pudieran ser identificadas en el estudio SIGMA) en la respuesta al tratamiento con fármacos de uso común (una dosis única por vía oral de glipizida y al tratamiento a corto plazo con metformin) en el manejo de la hiperglucemia.
La duración del estudio es de dos años. Su participación tendrá una duración aproximada de dos semanas. Se le invita debido a que usted tiene una de estas variantes o porque sus resultados serán usados como referencia.

PROCEDIMIENTOS DEL ESTUDIO

Aquellos participantes que sean incluidos al estudio SIGMA 2 que cumplan con los criterios de inclusión/exclusión serán invitados a participar en el estudio de farmacogenética.

Visita 1: Escrutinio. Dia 1.

Los participantes deberán presentarse en el Departamento de Endocrinología y Metabolismo previo ayuno de 8 a 12 horas. Se obtendrá el consentimiento informado. Se realizará una prueba

de embarazo en las mujeres potencialmente fértiles. Se registrarán variables antropométricas y los signos vitales.

Si la glucemia capilar es mayor de 80 mg/dl, se medirá la respuesta aguda a una dosis oral única de glipizida (5 mg). Si es menor de 80 mg/dl, se repetirá la glucemia capilar una hora después. De persistir la glucemia por debajo de 80 mg/dl se citará al participante en una semana. Durante la prueba, se coloca un catéter endovenoso en un brazo, se toman muestras de sangre de 5ml a los 0, 60, 90, 120, 150, 180 y 240 minutos para la medición de glucosa, insulina, proinsulina y glucagon. En la muestra basal se obtendrá una muestra adicional de 20 ml donde se medirá la concentración de péptido C, creatinina, ALT y AST y se aislará DNA. Se realizará un perfil metabolómico en la muestra basal y a los 60 y 120 minutos de la prueba. Se medirá la glucemia cada 30 minutos para detectar en forma oportuna una hipoglucemia. Si el valor de glucosa es menor de 50 mg/dl en ausencia de síntomas o si existen síntomas de hipoglucemia se incrementará la frecuencia de las mediciones a cada 5 minutos. La prueba se suspende si el valor es menor de 45 mg/dl sin síntomas o de 50 mg/dl con síntomas. Se administrará medio vaso de jugo de fruta por vía oral. Si el paciente no puede deglutir se administrará 50 g de glucosa vía intravenosa. Al término de la prueba se dará un desayuno a los participantes (sin costo). El paciente permanecerá en el centro de estudio tres horas después de la conclusión del estudio para garantizar su seguridad.

Se le pedirá que llene un registro de su alimentación (dos días entre semana y un día del fin de semana). Los registros deberán ser devueltos en la visita siguiente. Se le entregarán cuatro tabletas de metformin (500 mg). Se le pedirá que su actividad física se mantenga inalterada.

Visita 2: Tratamiento de corto plazo con metformin y la realización de una curva de tolerancia oral a la glucosa. Día 8.

Se calculará la tasa de filtración glomerular (usando la fórmula MDRD y el valor de creatinina obtenido en la visita 1). Si el valor es menor de < 60 ml/min/1.73 m^2 no se administrará el metformin. Tampoco se indicará u empleo si el valor de AST o ALT es tres veces mayor al límite superior normal.

De no existir contraindicación, el paciente tomara una tableta de metformin (500 mg) en la tarde del día 6, en la mañana y tarde del día 7. Se presentará en ayunas en la mañana del día 8 en que se realizará una curva de tolerancia oral a la glucosa. La prueba también se llevará a cabo a los pacientes que tengan contraindicación para el empleo del metformin.

Previo a la realización de la prueba de tolerancia oral a la glucosa se medirá la glucosa capilar. Se suspende su realización si el valor es mayor de 250 mg/dl. Si la glucemia capilar es menor de 60, se administra la cuarta dosis de metformin por via oral y la carga de 75 gramos de glucosa se administra en los 10 minutos siguientes. Si la concentración de glucosa es mayor de 60 mg/dl y < 250 mg/dl, la carga de glucosa se administra una hora después de la cuarta dosis de metformin. Durante la prueba el paciente tendrá un catéter endovenoso en un antebrazo. Se obtendrán muestras de 5 mls a los 0, 5, 10,15, 30, 60 y 120 minutos para la medición de GLP1, glucosa, insulina y perfil metabolómico. Un licenciado en nutrición revisará los registros de alimentación. Al término de la prueba el paciente puede retirarse del centro.

RIESGOS E INCONVENIENTES

Los riesgos de la toma de muestra sanguínea son:

1. Posibilidad de sangrado ligero o moretón en el sitio de la punción
2. Mareo o sensación de desmayo

3. Raramente puede producirse punción arterial.

El personal que extraerá la muestra sanguínea está entrenado para realizar una correcta extracción, lo que minimizará los riesgos de complicaciones. Usted será vigilado por un médico durante el estudio, lo que permitirá la corrección oportuna de la concentración de glucosa. Los estudios radiológicos no tienen riesgos inherentes. La cantidad de sangre obtenida en los estudios es menor a 100 mililitros, cantidad que no implica riesgos para su salud. Los datos acerca de su identidad y su información médica no serán revelados en ningún momento como lo estipula la ley, por tanto, en la recolección de datos clínicos usted no enfrenta riesgos mayores a los relativos a la protección de la confidencialidad la cual será protegida mediante la codificación de las muestras y de su información. El volumen de sangre total en la visita 1 es 167 ml y 118 ml en la visita 2

BENEFICIOS POTENCIALES

El estudio permitirá la obtención de nuevos conocimientos sobre los mecanismos que participan en la diabetes. A todos los participantes se les instruirá sobre la adopción de un estilo de vida saludable. A los pacientes con diabetes se ajustará el tratamiento farmacológico con el fin de alcanzar el mejor control glucémico posible.

CONSIDERACIONES ECONÓMICAS

No se cobrará ninguna tarifa por participar en el estudio ni se le hará pago alguno.
Los gastos de traslado a este instituto correrán por su cuenta. Los gastos de sus estudios serán cubiertos por la misma investigación.

COMPENSACION

Si sufre lesiones como resultado de su participación en este estudio, nosotros le proporcionaremos el tratamiento inmediato y lo referiremos, en caso de ameritarlo, al especialista médico que requiera. El Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán no brinda ningún tipo adicional de compensación para cubrir el daño.

ALTERNATIVAS A SU PARTICIPACIÓN:

Su participación es voluntaria. Sin embargo, usted puede elegir no participar en el estudio. El trato y tratamiento no se verán modificados por que usted decida no participar o interrumpir su participación. Usted puede declinar la realización de una o más de las pruebas arriba descritas.

POSIBLES PRODUCTOS COMERCIALES DERIVABLES DEL ESTUDIO:
En este protocolo no se generarán productos comerciales derivables del estudio.

ACCIONES A SEGUIR DESPUÉS DEL TÉRMINO DEL ESTUDIO:

Usted puede solicitar los resultados de sus exámenes clínicos y de las conclusiones del estudio al Dr. Carlos A. Aguilar Salinas (0445535010502) a la Dra. Olimpia Arellano Campos en el INCMNSZ, en el departamento de Endocrinología y Metabolismo o al teléfono 55554523 ext. 2405 y 4525. Cabe

mencionar que la investigación es un proceso largo y complejo, por tanto, el obtener los resultados finales del proyecto puede tomar varios meses.

PARTICIPACIÓN Y RETIRO DEL ESTUDIO:

Su participación es VOLUNTARIA. Si usted decide no participar, no se afectará su relación con el Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán (INCMNSZ) o su derecho para recibir atención médica o cualquier servicio al que tenga derecho.

Si decide participar, tiene la libertad para retirar su consentimiento e interrumpir su participación en cualquier momento sin perjudicar su atención en el Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán (INCMNSZ).

Además, se le informará a tiempo si nueva información es obtenida que pueda afectar su decisión para continuar en el estudio.

CONFIDENCIALIDAD Y MANEJO DE SU INFORMACIÓN

Su nombre no será usado en ninguno de los estudios y las muestras biológicas obtenidas no contendrán ninguna información personal, ya que se codificará con un código numérico para evitar cualquier posibilidad de identificación. El código es un número de identificación que no incluye datos personales.
Por disposición legal, las muestras biológicas, incluyendo la sangre, son catalogadas como residuos peligrosos biológico-infecciosos y por esta razón durante el curso de la investigación su muestra no podrá serle devuelta. Es posible que sus muestras biológicas así como su información médica y/o genética, puedan ser usadas para otros proyectos de investigación análogos relacionados con la enfermedad en estudio. No podrán ser usados para estudios de investigación que no estén relacionados con condiciones distintas a las estudiadas en este proyecto.
Sus muestras podrán ser almacenadas por los investigadores hasta por 4 años. Los códigos que identifican su muestra estarán solo disponibles a los investigadores titulares, quienes están obligados por Ley a no divulgar su identidad. Estos códigos serán guardados en un archivero con llave. Solo los investigadores tendrán acceso. Usted puede solicitar la suspensión del análisis de las muestras y su destrucción. Para ello, deberá ponerse en contacto con el investigador principal y solicitarlo por escrito.

Ninguna información sobre su persona será compartida con otros sin su autorización, excepto:

- Si es necesario para proteger sus derechos y bienestar
  (Por ejemplo, si ha sufrido una lesión y requiere tratamiento de emergencia); o
- Es solicitado por la ley.

Monitores o auditores del estudio podrán tener acceso a la información de los participantes. Todas las hojas de recolección de datos serán guardadas con las mismas medidas de confidencialidad, y solo los investigadores titulares tendrán acceso a los datos que tienen su nombre. Si usted decide retirarse del estudio, podrá solicitar el retiro y destrucción de su material biológico y de su información.
El Comité de Ética en Investigación del Instituto Nacional de Ciencias Médicas y Nutrición ha aprobado la realización de éste estudio. Dicho comité es quien revisa, aprueba y supervisa los estudios de investigación en humanos en el Instituto. En el futuro, si identificamos información que

consideremos importante para su salud, consultaremos con el Comité de ética que supervisa este estudio para que decidamos la mejor forma de darle esta información a usted y a su médico.

Además, le solicitamos que nos autorice contactarlo de nuevo, en caso de ser necesario, para solicitarle información que podría ser relevante para el desarrollo de este proyecto.

Los datos científicos obtenidos como parte de este estudio podrían ser utilizados en publicaciones o presentaciones médicas. Su nombre y otra información personal serán eliminados antes de usar los datos. Si usted lo solicita su médico de cabecera será informado sobre su participación en el estudio.

Su material genético no será usado con fines distintos a los mencionados en este documento. Si el investigador desea usarlo con fines distintos deberá notificarlo y solicitarle su firma en un documento similar al que usted está leyendo. Los resultados de los estudios genéticos no serán incluidos en su expediente del Instituto, a menos que tengan implicaciones para su tratamiento. Además los resultados de estudios genéticos podría ser causa de discriminación para las personas que tengan alguna anormalidad que los predisponga para sufrir una enfermedad. Tomaremos las acciones necesarias para evitar que su información sea conocida por terceros que pudieran tomar acciones discriminatorias contra usted.

IDENTIFICACIÓN DE LOS INVESTIGADORES:

En caso de que usted sufra un daño relacionado al estudio o tiene preguntas sobre el estudio, por favor póngase en contacto con Dr. Carlos A. Aguilar Salinas o a la Dra. Olimpia Arellano Campos en el INCMNSZ, en el departamento de Endocrinología y Metabolismo o al teléfono 55554523 ext. 2405 y 4525.

Si usted tiene preguntas acerca de sus derechos como participante en el estudio, puede hablar con el Presidente del Comité de Ética en Investigación del INCMNSZ (Dr. Arturo Galindo. Teléfono: 54870900 ext. 4524).

DECLARACIÓN DEL CONSENTIMIENTO INFORMADO

He leído con cuidado este consentimiento informado, he hecho todas las preguntas que he tenido y todas han sido respondidas satisfactoriamente. Para poder participar en el estudio, estoy de acuerdo con todos los siguientes puntos:

- Estoy de acuerdo en participar en el estudio descrito anteriormente. Los objetivos generales, particulares del reclutamiento y los posibles daños e inconvenientes me han sido explicados a mi entera satisfacción.
- Estoy de acuerdo en donar de forma voluntaria mis muestras biológicas de sangre y tejidos para ser utilizadas en éste estudio. Así mismo, mi información médica y biológica podrá ser utilizada con los mismos fines.
- Estoy de acuerdo, en caso de ser necesario, que se me contacte en el futuro si el proyecto requiere colectar información adicional o si encuentran información relevante para mi salud.
- Mi firma también indica que he recibido un duplicado de este consentimiento informado.

Por favor responda las siguientes preguntas

		SÍ (marque por favor)	**NO (marque por favor)**
a.	¿Ha leído y entendido la forma de consentimiento informado, en su lenguaje materno?	☐	☐
b.	¿Ha tenido la oportunidad de hacer preguntas y de discutir este estudio?	☐	☐
c.	¿Ha recibido usted respuestas satisfactorias a todas sus preguntas?	☐	☐
d.	¿Ha recibido suficiente información acerca del estudio y ha tenido el tiempo suficiente para tomar la decisión?	☐	☐
e.	¿Entiende usted que su participación es voluntaria y que es libre de suspender su participación en este estudio en cualquier momento sin tener que justificar su decisión y sin que esto afecte su atención médica o sin la pérdida de los beneficios a los que de otra forma tenga derecho?	☐	☐
g.	¿Entiende los posibles riesgos, algunos de los cuales son aún desconocidos, de participar en este estudio?	☐	☐
h.	¿Entiende que puede no recibir algún beneficio directo de participar en este estudio?	☐	☐
i.	¿Ha discutido usted otras opciones de tratamiento con el médico participante en el estudio y entiende usted que otras opciones de tratamiento están a su disposición?	☐	☐
j.	¿Entiende que no está renunciando a ninguno de sus derechos legales a los que es acreedor de otra forma como sujeto en un estudio de investigación?	☐	☐
k.	¿Entiende que el médico participante en el estudio puede retirarlo del mismo sin su consentimiento, ya sea debido a que Usted no siguió los requerimientos del estudio o si el médico participante en el estudio considera que médicamente su retiro es en su mejor interés?	☐	☐

		SÍ (marque por favor)	**NO (marque por favor)**
l.	¿Entiende que el estudio puede ser suspendido por el patrocinador del estudio en cualquier momento?	☐	☐
m.	¿Entiende que usted recibirá un original firmado y fechado de esta Forma de Consentimiento, para sus registros personales?	☐	☐

Declaración del paciente: Yo, _____ declaro que es mi decisión participar en el estudio. Mi participación es voluntaria. He sido informado que puedo negarme a participar o terminar mi participación en cualquier momento del estudio sin que sufra penalidad alguna o pérdida de beneficios. Si suspendo mi participación, recibiré el tratamiento médico habitual al que tengo derecho en el Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán (INCMNSZ) y no sufriré perjuicio en mi atención médica o en futuros estudios de investigación. Yo puedo solicitar información adicional acerca de los riesgos o beneficios potenciales derivados de mi participación en el estudio. Puedo obtener los resultados de mis exámenes clínicos si los solicito. Si usted tiene preguntas sobre el estudio, puede ponerse en contacto Dr. Carlos A. Aguilar Salinas o a la Dra. Olimpia Arellano Campos en el INCMNSZ, en el departamento de Endocrinología y Metabolismo o al teléfono 55554523 ext. 4525. Si usted tiene preguntas sobre sus derechos como participante en el estudio, problemas, preocupaciones o preguntas, obtener información, y ofrecer información que puede hablar con el Presidente del Comité de Ética de Investigación de INCMNSZ (Dr. Arturo Galindo Tel: 54870900. Ext. 6101).Debo informar a los investigadores de cualquier cambio en mi estado de salud (por ejemplo, uso de nuevos medicamentos, cambios en el consumo de tabaco) o en la ciudad donde resido, tan pronto como sea posible. He leído y entendido toda Ia información que me han dado sobre mi participación en el estudio. He tenido la oportunidad para discutirlo y hacer preguntas. Todas las preguntas han sido respondidas a mi satisfacción. He entendido que recibiré una copia firmada de este consentimiento informado.

_____ _____ _____

Nombre del Participante Firma del Participante Fecha

Coloque su huella digital si no sabe escribir

_____ _____ _____

Nombre del representante legal (Si aplica) | Firma del representante legal | Fecha

_____ _____ _____

Nombre del Investigador que explicó el documento | Firma del Investigador | Fecha

_____ _____ _____

Nombre del Testigo 1 | Firma del Testigo 1 | Fecha

Relación con el participante:_____

Dirección:_____

_____ _____ _____

Nombre del Testigo 2 | Firma del Testigo 2 | Fecha

Relación con el participante:_____

Dirección:_____

Lugar y Fecha:_____

(El presente documento es original y consta de 9 páginas)