UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE MEDICINA

Departamento de Medicina

TESIS DOCTORAL

Interaction by known beta cell loci on the association of anti-hypertensive drug therapies with hyperglycemia in the Framingham Offspring Study

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

José María de Miguel y Yanes

Co-directed by

James B. Meigs
Jesús Millán Núñez Cortés

Madrid, 2013

UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE MEDICINA

DEPARTAMENTO DE MEDICINA

DOCTORAL THESIS

INTERACTION BY KNOWN BETA CELL LOCI ON THE ASSOCIATION OF ANTI-HYPERTENSIVE DRUG THERAPIES WITH HYPERGLYCEMIA IN THE FRAMINGHAM OFFSPRING STUDY

INTERACCIÓN DE LOS LOCI CONOCIDOS DE CÉLULA BETA SOBRE LA ASOCIACIÓN DE FÁRMACOS ANTIHIPERTENSIVOS CON LA HIPERGLUCEMIA EN EL ESTUDIO DE LA DESCENDENCIA DE FRAMINGHAM

Presented by:

José María de Miguel y Yanes

Co-Directed by:
Professor James B. Meigs
Professor Jesús Millán Núñez-Cortés

Madrid, 2012

UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE MEDICINA
PROGRAMA DE DOCTORADO EN MEDICINA INTERNA

INTERACTION BY KNOWN BETA CELL LOCI ON THE ASSOCIATION OF ANTI-HYPERTENSIVE DRUG THERAPIES WITH HYPERGLYCEMIA IN THE FRAMINGHAM OFFSPRING STUDY

MEMORIA PARA LA OBTENCIÓN DEL GRADO DE DOCTOR

JOSÉ MARÍA DE MIGUEL Y YANES

Madrid, 2012

INTERACTION BY KNOWN BETA CELL LOCI ON THE ASSOCIATION OF ANTI-HYPERTENSIVE DRUG THERAPIES WITH HYPERGLYCEMIA IN THE FRAMINGHAM OFFSPRING STUDY

Tesis doctoral realizada por D. José María de Miguel y Yanes para optar al Grado de Doctor en Medicina por la Universidad Complutense de Madrid

Co-Directores:

Prof.: James B. Meigs, M.D., M.P.H., F.A.H.A.
Associate Professor in Medicine, Harvard Medical School
Boston, MA. USA

Prof.: Jesús Millán Núñez-Cortés, M.D., Ph.D., Pharm.D., F.R.C.P., F.A.C.P.
Catedrático de Medicina
Facultad de Medicina
Universidad Complutense de Madrid

Madrid, 2012

Departamento de Medicina
Facultad de Medicina
Pabellón I, 1.ª planta
Universidad Complutense
28040 Madrid

Prof. Jesús Millán Núñez-Cortés
Catedrático de Medicina Interna
Facultad de Medicina
Universidad Complutense de Madrid

CERTIFICA:

Que la presente Tesis Doctoral titulada "INTERACTION BY KNOWN BETA CELL LOCI ON THE ASSOCIATION OF ANTI-HYPERTENSIVE DRUG THERAPIES WITH HYPERGLYCEMIA IN THE FRAMINGHAM OFFSPRING STUDY" ha sido realizada bajo su dirección por el Licenciado en Medicina y Cirugía Don José María de Miguel y Yanes.

Quien suscribe considera que dicho trabajo reúne todas y cada una de las condiciones para su presentación, lectura y defensa como Tesis Doctoral, mostrando su conformidad a tal fin.

A tales efectos, se extiende la presente certificación en Madrid, a 20 de Junio de 2012.

Universidad Complutense
SaludMadrid
Departamento de Medicina
Hospital General Universitario Gregorio Marañón

Prof. Jesús Millán Núñez-Cortés

Prof. James B. Meigs
Associate Professor of Medicine
Harvard Medical School

CERTIFIES:

The project that supports the present thesis “INTERACTION BY KNOWN BETA CELL LOCI ON THE ASSOCIATION OF ANTI-HYPERTENSIVE DRUG THERAPIES WITH HYPERGLYCEMIA IN THE FRAMINGHAM OFFSPRING STUDY” has been carried out under my supervision by Mr. José María de Miguel y Yanes, M.D..

This work fulfills all the necessary requirements to qualify as a doctoral thesis in Medicine, and I give my consent for its presentation as an oral dissertation aimed to obtain a Doctorate Degree.

For academic purposes, signed in Boston, Massachusetts, June 20, 2012.

Prof. James B. Meigs

Prof. José C. Flórez
Associate Professor of Medicine
Harvard Medical School

CERTIFIES:

The project that supports the present thesis “INTERACTION BY KNOWN BETA CELL LOCI ON THE ASSOCIATION OF ANTI-HYPERTENSIVE DRUG THERAPIES WITH HYPERGLYCEMIA IN THE FRAMINGHAM OFFSPRING STUDY” has been carried out by Mr. José María de Miguel y Yanes, M.D..

This work fulfills all the necessary requirements to qualify as a doctoral thesis in Medicine, and can therefore be presented to obtain a Doctorate Degree.

For academic purposes, signed in Boston, Massachusetts, July 02, 2012.

Jose C Florez

Prof. José C. Flórez

MASSACHUSETTS
GENERAL HOSPITAL

HARVARD
MEDICAL SCHOOL

Massachusetts General Hospital
General Medicine Division
50 Staniford St. 9th Floor
Boston MA 02114-2698
phone: 617-724-3203
fax: 617-724-3544
email: jmeigs@partners.org

JAMES B. MEIGS, M.D., M.P.H.
Associate Professor of Medicine
Harvard Medical School

August 18th, 2010

To whom it may concern,

Dr. Jose M. de Miguel-Yanes, MD, MBA, has spent one year (August 20, 2009 until August 19, 2010) as a Research Fellow in Medicine in the General Internal Medicine Division at the Massachusetts General Hospital and Harvard Medical School in Boston, USA. He has been working in the fields of Clinical and Genetic Epidemiology applied to type 2 diabetes.

Dr. de Miguel has actively participated in the academic activities of the General Internal Medicine Division at MGH, in the meetings of the Framingham Study Diabetes and Genetic Epidemiology Group at the Boston University and has regularly attended the Primer Sessions in Genetics and the lectures of the Program in Medical and Population Genetics at the Broad Institute of Harvard and MIT.

As his main activity, he has been fully integrated in the following research programs in our Laboratory:

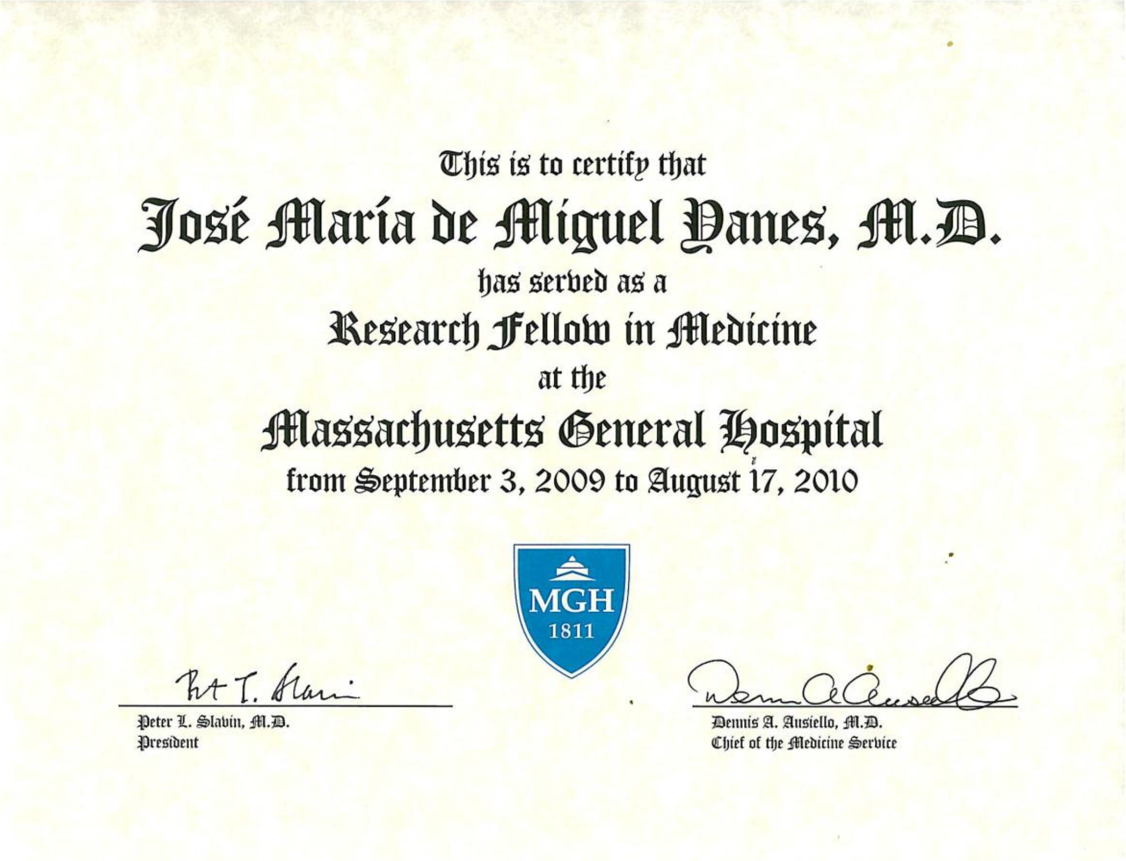
1. Metabolic Trait Levels and Common Variation in Endocannabinoid Pathway Genes.
2. Insulin Resistance, Endothelial Dysfunction, and Risk for Type 2 Diabetes.
3. Type 2 Diabetes and Cancer Epidemiology in Partners.
4. Common genetic variation and diabetes traits in Framingham SHARe (Interaction by single-nucleotide polymorphisms at beta cell loci on the association of antihypertensive drug therapies with glycemic traits and risk of type 2 diabetes in Framingham).

Dr. de Miguel has gained a substantial experience in research activities, such as elaboration of documents for IRB approval, comprehensive evaluation of datasets, interpretation of research results and the process of writing original research manuscripts. His performance has been outstanding and he has been very productive, having presented posters at the AHA Cardiovascular Disease Epidemiology and Prevention Scientific Sessions and the Annual Meeting of the American Diabetes Association and published several original scientific papers. Additionally, he has attended several training courses during his stay, has reviewed manuscripts for different peer-review journals, such as *Diabetes Care*, and is committed to write his Spanish Doctoral Thesis under my supervision. He has been able to build excellent collaborative professional and personal relationships.

The goals and expectations that we had at the beginning of his one-year fellowship have been accomplished. He is well-positioned to initiate an independent research career at his institution in Spain. It has been a pleasure having Jose working with us this year.

Sincerely,

James B Meigs MD MPH



This is to certify that

José María de Miguel Yanes, M.D.

has served as a

Research Fellow in Medicine

at the

Massachusetts General Hospital

from September 3, 2009 to August 17, 2010

MGH
1811

Peter L. Slavin, M.D.
President

Dennis A. Ausiello, M.D.
Chief of the Medicine Service

THIS WORK IS DEDICATED…

…to my parents, Jose and Pili, because I owe them everything that I am. My father passed on me the vocation to become a clinician, which in turn he received from his father; my mother was always there to make the way easier.

…to Alicia, my wife, who gave me the most valuable gifts.

…to Ana and Marta, my daughters, the *leitmotiv* to improve every single day. In their memories I will remain the day I am no longer here.

…to Pilar, Marta and Alfonso, my sisters and brother, who remind me that sometime, long ago, we were kids.

ACKNOWLEDGMENTS

I wish to give endless thanks to the following people and institutions, which made it possible that I could carry out this project:

To Dr. James B. Meigs, M.D., M.P.H., outstanding mentor, and friend; promoter and director of this project, and of other projects I took part in; he understood and corrected my weaknesses and leveraged my strengths; having worked with him meant a change in my life as a researcher and as a physician.

To José Carlos Flórez, M.D., Ph.D., superb researcher, a model for success for any Spaniard; he helped me take the first steps for traveling to the USA, and shared with me his passion for and knowledge of Genetics and diabetes.

To Jesús Millán Núñez-Cortés, M.D., Ph.D., co-director of this thesis, for his help and advice in the process of writing this work and for his dedication to teaching in Medicine.

To Carlos Pérez de Oteyza, M.D., Ph.D., who gave me personal and institutional support for travelling to the USA, and gave me valuable advice to write this Thesis.

To Bianca Porneala M.S., and Peter Shrader, M.S., who patiently and conveniently did most of the statistical work; without them, this project would not have reached its end.

To Josée Dupuis, Ph.D., and Michael J. Pencina, Ph.D., who always provided wise advice; they patiently dedicated their energy and time to review the data and to the development of some of the statistical methods used for this project.

To Angelo Volandes, M.D., M.P.H., and Aretha D. Davis, J.D., M.D., for their friendship, advice and support during my Research Fellowship at the Massachusetts General Hospital.

To Javier Muñoz González, M.D., my former mentor, outstanding physician and cultivated person, one of the people who have influenced me most as a person and as a physician.

To Víctor González Ramallo, M.D., Ph.D., Juan Antonio Andueza Lillo, M.D., and Luis Pastor Gómez-Cornejo, M.D., great internists and reliable friends, who always helped me to achieve my professional goals.

To Pedro Conthe Gutiérrez, M.D., Ph.D., for his continuous help in my professional growth.

To Jonna Grimsby, Ph.D., and Kelsey O´Brien B.S., for sharing their enthusiasm with me in their daily work, and for their friendship.

To the patients and staff people of the Framingham Offspring Study. The altruism of the former and the dedication of the latter made this work possible.

To the General Internal Medicine Division at the Massachusetts General Hospital, Harvard Medical School and the Broad Institute of the MIT and Harvard, whose unique conditions favor the best quality research and make it accessible to foreign investigators.

To the Departments of Internal Medicine and Emergency Medicine at the "Gregorio Marañón" Hospital, whose staff people relieved me in my duties during my research period in the USA.

And to the "Instituto de Investigación Biomédica" at the "Gregorio Marañón" Hospital, for its support for research development at this Institution.

[…] If you can fill the unforgiving minute
with sixty seconds' worth of distance run,
yours is the Earth and everything that's in it,
and –which is more– you'll be a Man, my son!

–Rudyard Kipling

ABSTRACT

Background: Common beta cell–associated single nucleotide polymorphisms (SNPs) and hypertension or its treatment might interact in their positive associations with change over time in fasting glucose (ΔFG) or incident type 2 diabetes mellitus (T2DM).

Methods: We pooled data from 3,471 Framingham Offspring Study participants into 6 ~4-yr periods (15,852 person-exams; mean age 52 years, 54% women). We defined three hypertension exposures: 1) hypertension vs. no-hypertension; 2) treated vs. untreated hypertension; 3) five mutually exclusive anti-hypertension drug categories (beta-blockers, thiazides, renin-angiotensin system agents, combinations, others) vs. untreated hypertension; and two genetic exposures reflecting total beta-cell genetic risk burden: 16 FG-SNP and 33 T2DM-SNP additive genetic scores. We tested ~4-year mean ΔFG or odds of T2DM by hypertension category and per-risk allele change in genetic scores, seeking hypertension-by-genetic-score significant ($p<0.05$) interaction.

Results: Versus no hypertension, hypertension conferred higher ΔFG (2.6 vs. 1.7 mg/dl; $p<0.0001$) and T2DM risk (OR=2.9; 95% CI 2.8-3.0; $p<0.0001$). Versus untreated hypertension, treated hypertension conferred higher ΔFG (3.4 vs. 3.0 mg/dl; $p<0.0001$) and T2DM risk (OR=1.4; 95% CI 1.3-1.5; $p=0.02$). Beta-blockers (OR=1.6; 95% CI 1.1-2.4), combination treatment (OR=1.6; 95% CI 1.1-2.5) and others (OR=2.0; 95% CI 1.4-2.9) increased T2DM risk (all $p<0.02$). Genetic scores increased both ~4-yr ΔFG (0.6 mg/dl per risk allele; $p=8.9\times10^{-16}$) and T2DM risk (~17% per risk allele; $p=2.1\times10^{-7}$). In joint models including interaction terms, all hypertension category-by-genetic score interaction terms were $p>0.05$. In joint models without interaction, hypertension

($p<0.0001$) and FG-SNP or T2DM-SNP genetic scores independently increased ΔFG or T2DM risk (both $p<0.001$).

Conclusion: Hypertension, hypertension treatment and common FG- and T2DM-SNP genetic scores independently predicted ΔFG and T2DM incidence, but did not modify each other's association with ΔFG or T2DM risk.

RESUMEN EN ESPAÑOL

Antecedentes

Las tasas de prevalencia de la diabetes mellitus tipo 2 y de la hipertensión arterial están alcanzando proporciones alarmantes. Se estima que cerca de una tercera parte de la población de los países desarrollados es hipertensa, y que alrededor de una quinta parte recibe medicación antihipertensiva.

La hipertensión es un factor que predispone, *per se*, al desarrollo de la diabetes mellitus tipo 2. Adicionalmente, numerosos estudios sugieren que algunos fármacos antihipertensivos pueden empeorar el control glucémico e incrementar el riesgo de diabetes mellitus tipo 2. Las tiazidas podrían hacerlo mediante la inhibición directa de la función de la célula beta pancreática, a través de mecanismos de inflamación, o bien, de forma secundaria a la hipopotasemia que inducen. Asimismo, los beta-bloqueantes podrían empeorar el metabolismo de los hidratos de carbono disminuyendo la sensibilidad a la insulina, favoreciendo la ganancia de peso o mediante cambios en la acción que la insulina ejerce a nivel celular.

De forma opuesta, se preconiza que los fármacos inhibidores del eje renina-angiotensina-aldosterona, esto es, los inhibidores del enzima de conversión de angiotensina (IECA) y los antagonistas del receptor de angiotensina II (ARA-II), podrían mejorar el control glucémico y reducir el riesgo de diabetes mellitus tipo 2; mecanismos facilitadores podrían ser la kaliuria inducida, el incremento potencial de la perfusión de la célula beta o la influencia beneficiosa que dichos fármacos parecen ejercer sobre el estrés oxidativo y la adipogénesis. No obstante, no hay pruebas concluyentes e irrefutables que apoyen los argumentos arriba mencionados acerca del efecto que los fármacos antihipertensivos ejercen sobre el control glucémico y el riesgo de diabetes mellitus tipo

2, quizá debido a la limitación que supone el sesgo de la confusión por indicación fuera del ámbito de los ensayos clínicos aleatorizados.

Hasta la fecha, se han descrito cerca de 40 polimorfismos de nucleótido simple ("SNPs") relacionados con el riesgo de padecer diabetes mellitus tipo 2 en estudios de asociación a lo ancho del genoma (GWAS), y 16 polimorfismos (8 de los cuales están incluidos en esa lista de 40 polimorfismos asociados con el riesgo de padecer diabetes mellitus tipo 2) que influyen en los niveles de glucosa basal plasmática. La mayoría de estos polimorfismos parecen modificar el control glucémico y el riesgo de padecer diabetes mellitus tipo 2 modificando la función de la célula beta pancreática.

Si bien es aceptado que el factor que supone la presencia de hipertensión y el uso de algunos de sus tratamientos, por un lado, y que factores genéticos, por el otro, modifican el control glucémico y el riesgo de padecer diabetes mellitus tipo 2, no se sabe hasta qué punto la presencia de cualquiera de ellos modifica la acción que el otro factor ejerce sobre la glucemia y sobre el riesgo de desarrollar diabetes mellitus tipo 2.

Nuestra hipótesis principal fue demostrar que aquellos polimorfismos comunes de nucleótido simple ("SNPs") que se asocian con los niveles de glucosa basal plasmática o con el riesgo de sufrir diabetes mellitus tipo 2, y que aparentemente lo hacen a través de su influencia sobre la función de la célula beta pancreática, presentaban un efecto de interacción con la hipertensión arterial -o sus tratamientos- en la asociación de ésta -o de sus tratamientos- con la variación en el tiempo de los niveles de la glucosa basal plasmática y con el riesgo de desarrollar diabetes mellitus tipo 2.

Métodos y procedimientos

Población del estudio

Utilizamos los datos del Estudio de la Descendencia de Framingham. Esta población ha sido evaluada mediante exámenes periódicos cada 3-4 años aproximadamente, salvo por el lapso entre el primer y el segundo examen (cerca de 8 años). Para conseguir la homogeneidad en la interpretación de los resultados, los datos provenientes de este primer intervalo de tiempo no fueron incluidos. Se dispuso de información genética para 3.471 de los participantes.

Definiciones

Definimos tres clasificaciones de exposición al factor "hipertensión": En cada examen, clasificamos a los participantes en: 1. hipertensión frente a no hipertensión; 2. hipertensión tratada frente a hipertensión no tratada; y 3. los hipertensos tratados fueron categorizados en 5 grupos, según los tratamientos antihipertensivos específicos recibidos (beta-bloqueantes, tiazidas, IECA/ARA-II, combinaciones de los previos, y "otros"). Definimos la hipertensión arterial como la presencia de presión arterial sistólica ≥140 mm Hg ó diastólica ≥90 mm Hg en el examen físico, o bien, recibir tratamiento antihipertensivo. Definimos la diabetes mellitus ante una glucosa basal plasmática ≥ 126 mg/dl, o bien, recibir tratamiento con antidiabéticos. No usamos la HbA1C para el diagnóstico (sólo disponible en los exámenes 5 y 7). El 99% de los casos de diabetes mellitus en el Estudio de la Descendencia de Framingham fueron diabetes mellitus tipo 2. Analizamos la asociación de hipertensión, hipertensión tratada y las 5 categorías específicas de exposición a fármacos antihipertensivos con la variación en el tiempo de los niveles de glucosa basal plasmática y el riesgo de desarrollar diabetes tipo 2.

Para controlar otros factores de confusión recogimos datos sobre variables adicionales: edad (en años), sexo, índice de masa corporal (kg/m^2), estado de fumador (actual -al menos 1 cigarrillo/día en el último año- frente a no fumador), niveles de lipoproteínas plasmáticas de alta densidad (HDL) y triglicéridos (ambos en mg/dl), y el consumo de alcohol referido por el propio sujeto.

Variación genética común asociada a la glucosa basal plasmática y al riesgo de sufrir diabetes mellitus tipo 2

Para evaluar la influencia genética sobre la variación en el tiempo de la glucosa basal plasmática, nos servimos de los 16 polimorfismos para los que se ha descrito su asociación con la glucosa basal plasmática, dado que todos ellos parecen influir a través de su acción sobre la célula beta pancreática. Para analizar el riesgo de diabetes mellitus tipo 2, solamente usamos 33 de los 40 polimorfismos para los que se ha descrito su asociación con el riesgo de diabetes tipo 2 aparentemente mediado por su acción sobre la célula beta pancreática, excluyendo a los polimorfismos en los que se sospecha una asociación a través de una resistencia a la acción insulínica. Construimos un “tanteo” (“score”) genético aditivo, ponderado según los efectos descritos en la literatura para cada polimorfismo, usando los 16 polimorfismos asociados con los niveles de glucosa basal plasmática y, de forma similar, un “tanteo” genético aditivo, ponderado, usando los 33 polimorfismos asociados al riesgo de desarrollar diabetes mellitus tipo 2. Evaluamos la asociación del tanteo genético asociado a los niveles de glucosa basal plasmática con la variación a ~4 años de la glucosa basal plasmática, y la asociación del tanteo genético asociado al riesgo de desarrollar diabetes mellitus tipo 2 con el riesgo a ~4 años de aparición de diabetes mellitus tipo 2.

Métodos estadísticos

Nuestra hipótesis principal fue analizar si los polimorfismos comunes asociados con la glucosa basal plasmática o con el riesgo de diabetes mellitus tipo 2, pero exclusivamente aquellos que parecen ejercer su influencia a través de la función de la célula beta, interaccionan con la hipertensión arterial o con los fármacos antihipertensivos en sus asociaciones positivas con: 1) la variación a ~4 años de la glucosa basal plasmática (calculada como la diferencia entre el valor al final menos el valor al principio de cada período de tiempo) y 2) el riesgo de incidencia a ~4 años de diabetes mellitus tipo 2.

Para la estadística descriptiva empleamos el test de chi-cuadrado para variables categóricas y la prueba T para la comparación de medias en el caso de variables cuantitativas. Evaluamos la variación en el tiempo de la glucosa basal plasmática y el riesgo de desarrollar diabetes mellitus tipo 2 como una función de la exposición a la hipertensión arterial o sus tratamientos y la predisposición genética usando una aproximación de regresión "agrupada" o "de fondo común" ("pooled"): agrupamos los datos clínicos de los 2.922 participantes no diabéticos de los que se disponía de información sobre todas las variables en todos los exámenes en 6 períodos de tiempo (exámenes 2-3, 3-4, 4-5, 5-6, 6-7 y 7-8). De esta forma, generamos 15.852 personas-observaciones pertenecientes a 6 períodos de tiempo, con una longitud media de período de tiempo de 4,15 años, a lo largo de un máximo de 28,3 años de seguimiento. Mediante esta metodología, la información de cada individuo al inicio de cada período de tiempo contribuyó de forma independiente a la información del mismo individuo perteneciente a otro período de tiempo, en el que las condiciones de exposición pueden haber cambiado.

Este método de regresión produce estimaciones de punto y varianzas similares al método de regresión de Cox con el tiempo como variable dependiente.

Excluimos los casos de diabetes al inicio de cada periodo de observación. Para evaluar la variación en el tiempo de la glucosa basal plasmática, excluimos también a los pacientes que habían iniciado tratamiento antidiabético durante el periodo de tiempo precedente. Usamos el método de ecuaciones estimativas generalizadas ("GEE") con la función de enlace de identidad familiar en modelos ajustados para la edad, el sexo y el nivel de glucosa basal plasmática al principio del periodo de tiempo para el análisis de la variación en el tiempo de la glucosa basal plasmática. Para el análisis del riesgo de desarrollar diabetes mellitus tipo 2, usamos también el método de ecuaciones estimativas generalizadas con la función de enlace *logit* en modelos ajustados para la edad y el sexo.

Para demostrar un efecto de interacción entre la hipertensión arterial o las categorías terapéuticas, por un lado, y los tanteos genéticos, por el otro, que condicionara una modificación de la variación en el tiempo de la glucosa basal plasmática o del riesgo de desarrollar diabetes mellitus tipo 2 más allá de los efectos principales, incluimos en los modelos términos de interacción de primer orden entre hipertensión, hipertensión tratada o exposiciones específicas a tratamientos antihipertensivos y cada tanteo genético. Los términos de interacción adoptan la forma: variable dependiente = $\beta0 + \beta c*\text{covariables} + \beta1*E + \beta2*SNP + \beta3*E*SNP + \varepsilon$, donde E representa la exposición a la hipertensión arterial o al fármaco y "SNP" denota la contribución genética. El resultado de interés es el valor de *p* para el test: $H0: \beta3 = 0$.

En análisis secundarios especificados *a priori*, evaluamos modelos conjuntos introduciendo las variables, pero sin incluir términos de interacción, para evaluar la hipótesis que, en caso que no se demostrara un efecto de interacción, la hipertensión arterial o sus tratamientos y los tanteos genéticos se asociaran, de forma independiente, a la variación en el tiempo de la glucosa basal plasmática o al riesgo de desarrollar diabetes mellitus tipo 2.

En análisis secundarios adicionales, exploramos las asociaciones en modelos con ajustes añadidos para factores asociados al riesgo de desarrollar diabetes mellitus tipo 2 medidos al principio de cada período (presión arterial media, índice de masa corporal, tabaquismo, valores de colesterol HDL y triglicéridos, y consumo de alcohol), para descartar que estos factores de confusión explicaran las asociaciones observadas.

Los análisis estadísticos se realizaron mediante el sistema SAS, versión 9.2. Se consideró presente la significación estadística para los términos de interacción o los efectos principales ante valores de $p<0.05$.

Poder estadístico para detectar interacción hipertensión-tanteo genético y exposición a antihipertensivos-tanteo genético

Tuvimos una potencia estadística del 80%, con un error de tipo I de 0,05 en análisis con dos colas, para frecuencias de alelos entre el 20% y el 80%, para poder detectar coeficientes de regresión entre 0,04 y 0,05 para el test de interacción entre hipertensión y el tanteo genético, y entre 0,10 y 0,12 para el test de interacción entre la exposición a beta-bloqueantes y el tanteo genético, en el caso del análisis de la variación en el tiempo de la glucosa basal plasmática; y coeficientes de regresión entre 1,07 y 1,08 para el test de interacción entre hipertensión y el tanteo genético, y entre 1,19 y 1,23 para el test de interacción entre la exposición a beta-bloqueantes y el tanteo genético, en el caso del análisis del riesgo de desarrollar diabetes mellitus tipo 2.

Resultados

Características basales de la población del estudio

La media de edad fue de 51,9 años y el 53,8% de la población estaba compuesta por mujeres. Con el envejecimiento de la población del estudio, el índice de masa corporal aumentó, y tanto la hipertensión como la hipertensión tratada se hicieron más prevalentes. Las personas con hipertensión arterial tendían a ser mayores, más frecuentemente hombres, con índices de masa corporal más altos y con perfiles lipídicos de mayor riesgo que las personas sin hipertensión. Los participantes con hipertensión no tratada eran más jóvenes, más frecuentemente hombres, y con valores más elevados de presión arterial que sus homólogos tratados. Las diferencias basales entre las diferentes exposiciones a antihipertensivos y el grupo de referencia con hipertensión no tratada fueron nominalmente significativas para la edad, el sexo, el índice de masa corporal y el hábito tabáquico.

Asociación entre hipertensión, hipertensión tratada y exposiciones específicas a antihipertensivos con la variación en el tiempo de la glucosa basal plasmática

Frente a la ausencia de hipertensión arterial, el diagnóstico de hipertensión arterial se asoció a una variación en el tiempo de la glucosa basal plasmática 0,9 mg/dl mayor (p<0,0001). Frente a la hipertensión no tratada, la hipertensión tratada se asoció a una variación en el tiempo de la glucosa basal plasmática 0,4 mg/dl mayor (p<0,0001). Frente a la hipertensión no tratada, los IECA/ARA-II (p=0,037) y el tratamiento combinado (p=0,046) se asociaron a una mayor variación en el tiempo de la glucosa basal plasmática; la asociación de la variación en el tiempo de la glucosa basal plasmática con la exposición a beta-bloqueantes y tiazidas no fue estadísticamente significativa.

Asociación entre hipertensión, hipertensión tratada y exposiciones específicas a antihipertensivos con el riesgo de desarrollar diabetes mellitus tipo 2

Frente a la ausencia de hipertensión, el diagnóstico de hipertensión se asoció a una probabilidad ~3 veces mayor de desarrollar diabetes mellitus tipo 2 (Odds Ratio (OR) = 2,9; 95% CI 2,8-3,0). Frente a la hipertensión no tratada, la hipertensión tratada confirió una probabilidad un 40% mayor de desarrollar diabetes mellitus tipo 2 (OR = 1,4; 95% CI 1,3-1,5). Frente a la hipertensión no tratada, el tratamiento con beta-bloqueantes (OR=1,6; 95% CI 1,1-2,4), el tratamiento combinado (OR=1,6; 95% CI 1,1-2,5) y el tratamiento con "otros" (OR=2,0; 95% CI 1,4-2,9) se asociaron a un mayor riesgo de desarrollar diabetes mellitus tipo 2.

Asociación de los tanteos genéticos aditivos con la variación en el tiempo de la glucosa basal plasmática y el riesgo de desarrollar diabetes mellitus tipo 2

Los efectos individuales de los polimorfismos genéticos de nucleótido simple en la variación en el tiempo de la glucosa basal plasmática y el riesgo de desarrollar diabetes mellitus tipo 2, en modelos ajustados para la edad y el sexo, fueron mayoritariamente en la misma dirección y de efectos similares a los originalmente descritos en los estudios de asociación a lo ancho del genoma. Por alelo genético ponderado de riesgo, la variación en el tiempo de la glucosa basal plasmática aumentó 0,55 mg/dl ($p=8,9 \times 10^{-16}$), y la probabilidad de desarrollar diabetes mellitus tipo 2 aumentó un 16,6% (95% CI 10,0-

23,6), mostrando aquellos sujetos que debutaron con diabetes mellitus tipo 2 tanteos genéticos medios más altos que los que no lo hicieron (17,3 vs. 16,8; p=2,1x10^{-7}).

Efecto de interacción entre la hipertensión arterial o sus categorías de tratamiento y los tanteos genéticos

Ninguno de los valores de *p* en los modelos conjuntos que incluyeron términos de interacción para la hipertensión arterial, la hipertensión tratada o los tratamientos con fármacos antihipertensivos específicos x tanteos genéticos, prediciendo la variación en el tiempo de la glucosa basal plasmática o el riesgo de desarrollar diabetes mellitus tipo 2, fue estadísticamente significativo (todas los valores de p>0,3).

Modelos conjuntos incluyendo hipertensión arterial, la hipertensión tratada o los tratamientos con fármacos antihipertensivos específicos y los tanteos genéticos sin términos de interacción

En los modelos conjuntos sin interacción, la hipertensión arterial, el uso de tiazidas y de IECA/ARA-II, pero no la hipertensión tratada o el uso de beta-bloqueantes, y el tanteo genético asociado a glucosa basal plasmática predijeron, de forma independiente, la variación en el tiempo de la glucosa basal plasmática. En los modelos conjuntos sin interacción, la hipertensión arterial, la hipertensión tratada y el tanteo genético asociado a diabetes mellitus tipo 2 predijeron, de forma independiente, el riesgo de desarrollar diabetes mellitus tipo 2. Ninguna de las exposiciones específicas a fármacos antihipertensivos (todos los valores de p>0,19) predijo la incidencia de diabetes.

Análisis secundarios

En análisis secundarios adicionales usando modelos con ajuste adicional para factores de confusión de riesgo de desarrollar diabetes mellitus tipo 2, los resultados estadísticamente significativos hallados en los modelos con ajuste para edad, sexo, y adicionalmente para glucosa basal plasmática en el caso de la variación en el tiempo de la glucosa basal plasmática, fueron mayoritariamente reproducidos.

Conclusiones

1. En el Estudio de la Descendencia de Framingham, con el envejecimiento de la población, el índice de masa corporal aumentó, y tanto la hipertensión como la hipertensión tratada se hicieron más prevalentes.
2. Frente a la ausencia de hipertensión, la hipertensión arterial se asoció a una mayor variación en el tiempo (~4 años) de la glucosa basal plasmática y a una mayor probabilidad de desarrollar diabetes mellitus tipo 2.
3. Frente a la hipertensión no tratada, la hipertensión tratada confirió una mayor variación en el tiempo (~4 años) de la glucosa basal plasmática y una mayor probabilidad de desarrollar diabetes mellitus tipo 2.
4. El uso de IECA/ARA-II se asoció nominalmente a una mayor variación en el tiempo (~4 años) de la glucosa basal plasmática. El uso de beta-bloqueantes se asoció nominalmente a una mayor probabilidad de desarrollar diabetes mellitus tipo 2.
5. La variación en el tiempo (~4 años) de la glucosa basal plasmática y la probabilidad de desarrollar diabetes mellitus tipo 2 se vieron significativamente aumentadas por alelo genético ponderado de riesgo.

6. Los sujetos que debutaron con diabetes mellitus tipo 2 presentaron tanteos genéticos medios más altos que los que no lo hicieron.
7. La hipertensión arterial y el tanteo genético (por alelo genético ponderado de riesgo asociado a glucosa basal o a diabetes mellitus tipo 2) predijeron, de forma independiente, la variación en el tiempo (~4 años) de la glucosa basal plasmática y la probabilidad de desarrollar diabetes mellitus tipo 2.
8. La hipertensión arterial o los tratamientos específicos antihipertensivos, y la variación genética común asociada a los niveles de glucosa basal y al riesgo de diabetes mellitus tipo 2 mediado por su acción sobre la célula beta pancreática, no se influyeron mutuamente en su asociación con la variación en el tiempo de la glucosa basal plasmática o la probabilidad de desarrollar diabetes mellitus tipo 2.
9. Por tanto, la influencia que la hipertensión arterial o su tratamiento ejerció sobre la variación en el tiempo de la glucosa basal plasmática o la probabilidad de desarrollar diabetes mellitus tipo 2 fue homogénea independientemente de la susceptibilidad genética conferida por la variación genética común asociada a los niveles de glucosa basal o al riesgo de diabetes mellitus tipo 2.
10. Recíprocamente, el efecto que la variación genética común asociada a los niveles de glucosa basal plasmática o al riesgo de diabetes mellitus tipo 2 ejerció sobre la variación en el tiempo de la glucosa basal plasmática o la probabilidad de desarrollar diabetes mellitus tipo 2 no fue mayor en la presencia de hipertensión arterial, de hipertensión tratada o de tratamientos específicos antihipertensivos.
11. Los posibles factores de confusión adicional de riesgo de desarrollar diabetes mellitus tipo 2 (presión arterial media, índice de masa corporal, tabaquismo,

niveles de colesterol HDL y triglicéridos, y consumo de alcohol) no parecieron explicar la mayoría de las asociaciones estadísticamente significativas encontradas en los modelos ajustados para la edad y el sexo, con ajuste adicional para el valor de glucosa basal plasmática en el análisis de la variación en el tiempo de la glucosa basal plasmática.

12. Este trabajo permite asomarnos a la posibilidad de analizar efectos de interacción entre polimorfismos individuales x categorías específicas de fármacos antihipertensivos en muestras poblacionales más grandes, o bien agrupando polimorfismos de acuerdo a las vías biológicas a través de las cuales se ha descrito que operan, para responder a la cuestión de si puede haber fundamento biológico para un peor control de la glucosa basal plasmática o un mayor riesgo de desarrollar diabetes mellitus tipo 2 asociados a los tratamientos antihipertensivos.

INDEX

1. BACKGROUND

1.A. The magnitude of the problem: The global burden of type 2 diabetes mellitus

According to the World Health Organization (WHO), diabetes mellitus is defined by plasma fasting glucose levels ≥126 mg/dl (7.0 mmol/l), or by the results of a 75 g oral glucose tolerance test (OGTT), having settled the diabetes diagnosis cut-off point at 200 mg/dl (11.1 mmol/l) for plasma glucose levels 2 hours after the glucose intake (1). Also according to the WHO, we can define "impaired fasting glucose" when fasting glucose levels are 110 mg/dl (6.1 mmol/l) or higher, but lower than 126 mg/dl (7 mmol/l). "Impaired glucose tolerance" can be diagnosed if OGTT 2-hour glucose levels are 140 mg/dl (7.8 mmol/l) or higher, but lower than 200 mg/dl. Both impaired fasting glucose and impaired glucose tolerance are metabolic intermediate stages between the normal and the pathological state. A 2003 follow-up report from the American Diabetes Association (ADA) revisited the lower value of the range of plasma fasting glucose values used to define impaired fasting glucose: this was lowered from ≥110 (6.1 mmol/l) to ≥100 (5.6 mmol/l), to make it more comparable with the impaired glucose tolerance test values (2) (**Table 1**). However, the WHO has not changed its threshold level. Additionally, the last recommendations from the ADA include hemoglobin A1C (HbA1C) value as another criterion for the diagnosis of diabetes mellitus, with a cut-off point of ≥6.5% (3,4).

Table 1. Current ADA definitions for diabetes mellitus (only one criterion is needed).

1. A1C ≥6.5% in a certified laboratory and using a standardized to the DCCT assay
2. Fasting plasma glucose ≥126 mg/dl (7.0 mmol/l)
3. 2-hour plasma glucose ≥200 mg/dl (11.1 mmol/l) during an oral load test with 75 g of anhydrous glucose dissolved in water
4. A random plasma glucose ≥200 mg/dl (11.1 mmol/l) in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis
Unless unequivocal hyperglycemia, criteria 1-3 should be confirmed by repeat testing

Type 2 diabetes mellitus (T2DM) is characterized by chronic hyperglycemia due to insulin resistance of peripheral tissues (skeletal muscle, liver, adipose tissue and, probably, also the brain), and insufficient compensatory insulin secretion by pancreatic β-cells (5). In contrast to insulin resistance, the decline in β-cell function is considered a late event (6), and has been shown to be caused, at least in part, by an irreversible loss of the β-cell mass (7). T2DM patients do not strictly need insulin for survival and there is no autoimmune destruction of the beta cells. Secondary T2DM may result as a consequence of different diseases, such as acromegaly, Cushing's syndrome, thyrotoxicosis, pheochromocytoma, chronic pancreatitis, cancer and drugs.

The incidence and prevalence rates of T2DM are reaching alarming proportions. Among the adults older than 30 years old from the United States of America (USA), T2DM prevalence has been reported to be 13.7% for men (95% Confidence Interval (CI): 12.0-15.4%) and 11.7% for women (95% CI: 10.4-13.0%) in the pooled 2003-2006 National Health and Nutrition Examination Survey (NHANES) (8). Moreover, approximately one third of all the T2DM cases in 2003-2006 were estimated to remain undiagnosed. In parallel, the economic impact of T2DM is enormous, with an estimated health cost of 132 billion USA dollars during 2002 in the USA (9). These data support the notion that preventive measures contributing to ameliorate this enormous burden would therefore have a very favorable impact in terms of population health, resources utilization and financial costs.

In Spain, the prevalence rates of T2DM are quite similar to the data reported from the USA. The Spanish Insulin Resistance Study recruited 2,949 subjects aged 34-69 from diverse geographical sites and found a prevalence rate of 10.2% (95% CI not reported) for T2DM, according to the 1,999 WHO T2DM diagnostic criteria (10). More recent cross-sectional studies including subjects from different regions have revealed prevalence rates ranged between 10% and 15%, finding higher rates in males and in older people (11). Several factors may be responsible for the increasing prevalence rates, namely aging populations, adverse environmental factors of the Occidental lifestyle (*i.e.*, high-caloric diets, physical inactivity, and sedentary habits), higher obesity rates, the higher life expectancy for people with cardiovascular risk factors, and the increasingly higher incidence rates of T2DM in the population (12). The most recent epidemiological data from Spain point to an overall 13.8% (95% CI: 12.8-14.7%) prevalence rate of T2DM, and an additional 9.2% (95% CI: 8.2-10.2%) prevalence rate of abnormal glucose tolerance (13).

1.B. Hypertension: the other epidemic

Hypertension is defined as a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg, according to the American Heart Association (14). High blood pressure is a universal problem: Overall, 26.4% (95% CI: 26.0-26.8%) of the adult population in 2000 had hypertension (26.6% of men (95% CI: 26.0-27.2%) and 26.1% of women (95% CI: 25.5-26.6%)), and 29.2% (95% CI: 28.8-29.7%) overall were projected to have this condition by 2025 (15). The estimated total number of adults with hypertension in 2000 was 972 million (95% CI: 957-987 million); 333 million (95% CI: 329-336 million) in economically developed countries and 639 million (95% CI: 625-654 million) in economically developing countries. The number of adults with hypertension in 2025 was predicted to increase ~60%, up to 1.56 billion (95% CI: 1.54-1.58 billion).

In the USA, data from the National Health and Nutrition Examination Survey (NHANES) 2003–2006 indicate that 33.6% of US adults older than 20 years of age have hypertension (16). This amounts to an estimated 74,500,000 US adults with hypertension. The prevalence of hypertension is nearly equal between men and women. African-American adults have among the highest rates of hypertension in the world, at ~43%. Among hypertensive adults, approximately 78% are aware of their condition, 68% are using antihypertensive medication, and only 44% of those treated had their hypertension controlled. It has been reported that as many as 45 million people in the USA are following antihypertensive treatment (14).

In Spain, the estimated prevalence of hypertension in the overall population is around 45%, with higher prevalence rates in older people, and in males than in females (17). Only one fourth of this population is strictly controlled.

1.C. The association of hypertension with hyperglycemia and type 2 diabetes mellitus.

Solid evidence suggests that there is a close association between hypertension and incident T2DM. Longitudinal studies have shown that higher blood pressure is associated with increased risk of T2DM (18). Using data from the Atherosclerosis Risk In Communities (ARIC) Study, the Coronary Artery Risk Development in Young Adults (CARDIA) Study, and the Framingham Heart Study, Wei et al. reported that a higher blood pressure was a risk factor for new-onset T2DM in middle-aged African American and white persons in the community (19). In African Americans, the higher diabetes incidence among hypertensive individuals might be explained by body mass index (BMI), fasting glucose, triglyceride, and HDL cholesterol. In whites, however, pre-hypertension and hypertension were associated with greater risk of diabetes, beyond that explained by other risk factors. Nonetheless, African Americans, regardless of blood pressure, had greater risks of developing diabetes than whites. Other additional longitudinal studies using prospectively followed-up cohorts have shown an association between blood pressure and risk of developing T2DM (20,21).

1.D. Dysglycemic effects of the antihypertensive drugs

The possible influence due to antihypertensive drugs on glycemic control and T2DM onset has been examined in the medical literature for nearly half a century: thiazides (22,23) and beta-blockers (24,25) have long been considered to be associated with T2DM onset and deleterious effects upon glycemic control. Other diuretics have not been definitely implicated in T2DM onset in the medical literature. Renin-angiotensin system agents (angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB)) have been reported to have protective effects in this regard.

1.D.1. Hyperglycemic effects of thiazides

Thiazides deliver a high sodium load in the distal convoluted tubule, with a resulting increase in potassium excretion. Moreover, they activate the renin-angiotensin system (RAS) through volume depletion, which in turn leads to increased aldosterone secretion and further enhanced potassium excretion (26). One of the hypotheses that could explain the risk of T2DM associated with thiazides use is that the diuretic-induced hypokalemia leads to a higher secretion ratio of proinsulin to insulin -biologically less active than insulin-, which might impair glucose homeostasis. This idea has been reinforced by recent reports of the association between serum and dietary potassium and the risk of incident T2DM (27).

Nevertheless, the exact reason why thiazides confer a higher risk of T2DM remains to be proven: whereas recent studies have failed to show a direct association among changes in potassium levels during diuretic treatment with new-onset T2DM (28), other authors continue to suggest the possibility of preventing new-onset T2DM due to thiazides through potassium supplementation (29). However, additional factors have been proposed as causal mechanisms: hypomagnesaemia (30), direct pancreatic beta cell damage due to free fatty acids (31), visceral fat redistribution, liver fat accumulation, low-grade inflammation, and aggravated insulin resistance (32). Adipose tissue gene expression of serum amyloid A and serum levels of E selectin were higher after hydrochlorothiazide treatment compared to candesartan (33), what suggests that additional factors may be operating.

Most old epidemiological studies and recent empowered clinical trials and meta-analyses (34-36) support an association between thiazides and risk of incident T2DM. Nonetheless, previous work had failed to demonstrate a significant association (37-39). Disparities in the results may be due to: differences in the particular design of every study (intention-to-treat analyses vs. actual on-therapy; randomized clinical trial vs. observational; primary aim vs. post-hoc analyses); differences among population samples (i.e., whole population vs. only the elderly); lack of statistical power; failure to appropriately adjust for confounders (blood pressure, drug doses or body mass index (BMI) (40)); and use of different criteria for diagnosing T2DM. At last, the percentage of the variability of glucose levels as a response to thiazides explained by ethnicity has been estimated to be about 13% (41), which means that genetic factors should also be accounted for.

Twenty-five years ago, Wilson et al. highlighted the higher risk of incident T2DM associated with thiazides treatment in the Framingham Heart Study (23): thiazides use was significantly associated with T2DM onset in the univariate analysis ($P<0.001$). The association still remained significant for women in the multivariate regression model ($P<0.01$), but that was not the case for men. In the "Health Professionals Follow-up

Study", another different longitudinal prospectively follow-up cohort study, Taylor et al. showed that the risk of developing T2DM was higher for subjects who received thiazides for hypertension than for subjects who received no thiazides, in fully-adjusted multivariate regression models (20%, 45% and 36% higher for old women, young women and men, respectively) (35).

Some criticism has been brought against data from longitudinal follow-up cohort studies, in which the possibility of allocation bias or confounding by indication is a major concern (42). As additional randomized clinical trials and meta-analyses that include data exclusively from randomized clinical trials have been published, further evidence supports an association between thiazides and risk of T2DM. As an example, the "Intervention as a Goal in Hypertension Trial" (INSIGHT) found fewer cases of new onset T2DM with nifedipine compared to the potassium-sparing/thiazide combination coamilozide (4.3% vs. 5.6%, respectively; P=0.023) (43).

The widespread use of meta-analyses has also overcome the limitation of low statistical power, which has traditionally precluded from drawing definitive conclusions. This limitation was an issue in the "Systolic Hypertension in the Elderly Program Trial" (SHEP), where the difference in new-onset T2DM incidence among those subjects treated with chlorthalidone vs. placebo did not reach statistical significance (8.6% vs. 7.5%; hazard ratio (HR): 1.2; 95% confidence interval (CI): 0.9-1.5; P=0.25) (39).

Contrarily, as cited above, in a post hoc subgroup analyses from the "Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial" (ALLHAT) among non-diabetic participants who were randomized to receive treatment with chlorthalidone (n=8,419), amlodipine (n=4,958), or lisinopril (n=5,034), and who were observed for a mean 4.9-year period, the odds ratios (ORs) of developing T2DM at 2 years with lisinopril (OR=0.55; 95% CI: 0.43-0.70) or amlodipine (OR=0.73; 95% CI: 0.58-0.91) vs. chlorthalidone were significantly lower than 1.0 (P<0.001 and P=0.006, respectively) (34).

Furthermore, in a network meta-analysis of 22 randomized clinical trials involving 143,153 participants, Elliot et al. reported a statistically significant 23% (95% CI: 6-37%) higher risk of T2DM onset in hypertensive subjects treated with thiazides as compared to placebo (P=0.009) (**Figure 1**) (36).

Figure 1: **Odds ratios (ORs) for new-onset T2DM after anti-hypertensive drug exposures according to Elliot et al.** (36).

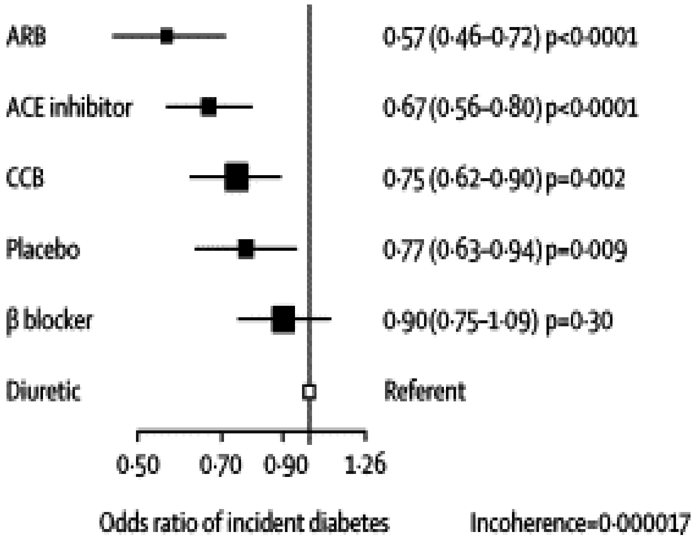


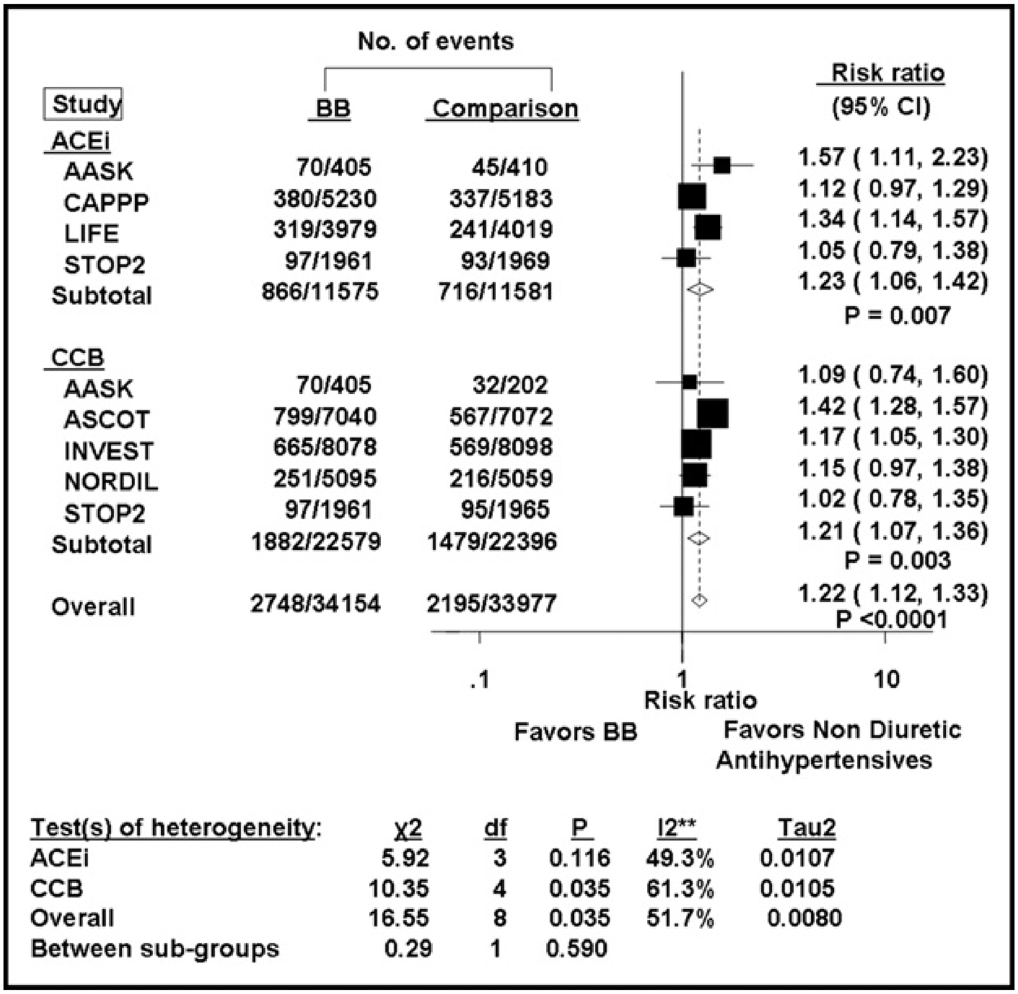
Figure 1 is comparing each anti-hypertensive exposure group with diuretics as a reference group (OR=1). Diuretics, but not beta-blockers, pose a higher risk than placebo or ACEI/ARB. Taken from Elliot et al. (with permission).

1.D.2. Hyperglycemic effects of beta-blockers

In regard to beta-blockers, whereas a few studies did not support an association between beta-blockers therapy for hypertension and T2DM onset (44), most of the classical, non-randomized older studies did (24,25). Gress et al., for instance, reported a 28% excess risk for T2DM onset in hypertensive subjects treated with beta-blockers in the longitudinal ARIC Study (“Atherosclerosis Risk In Communities”) in multi-adjusted regression models (37). Again, in an attempt to avoid the issue of confounding by indication, randomized clinical trials and meta-analyses of the randomized clinical trials that evaluated beta-blockers use in hypertensive patients have been carried out. One example is the one conducted by Bangalore et al., which showed a 22% (95% CI: 12%-33%) excess risk of T2DM onset with the use of beta-blockers for hypertension as compared to calcium channel blockers (CCB) or angiotensin converting enzyme (ACE) inhibitors ($p<0.0001$) (**Figure 2**) (45). In addition, some recent randomized clinical trials and meta-analyses report a higher risk of T2DM onset when the combination of diuretics and beta-blockers is used to treat hypertension, such as the study by Gupta et al. (46), which showed that use of atenolol, either alone or along with a diuretic, was one of the major determinants of new onset T2DM in hypertensive patients. Another meta-analysis published by Mason et al., which included 76,949 treated individuals, reported a higher risk of new-onset T2DM for the combined use of old beta-blockers and thiazides (RR: 1.23; 95% CI: 1.16-1.30) (47).

Plausible mechanisms that could explain the contribution of beta-blockers to the development of T2DM include weight gain, attenuation of the beta-receptor–mediated release of insulin from pancreatic beta cells, decreased insulin sensitivity, changes in the level of aerobic exercise or subtle changes in the cellular actions of insulin (48). Intra-class variation, or different incidence rates of T2DM with the use of different formulations of beta-blockers, has been reported, with higher T2DM incident rates for old drugs and metoprolol than for carvedilol (49).

Figure 2: **Risk ratios for new-onset T2DM in randomized clinical trials comparing beta-blockers vs. other anti-hypertensive therapies, after excluding diuretics, as reported by Bangalore et al.** (44).



Study	No. of events BB	No. of events Comparison	Risk ratio (95% CI)
ACEi			
AASK	70/405	45/410	1.57 (1.11, 2.23)
CAPPP	380/5230	337/5183	1.12 (0.97, 1.29)
LIFE	319/3979	241/4019	1.34 (1.14, 1.57)
STOP2	97/1961	93/1969	1.05 (0.79, 1.38)
Subtotal	866/11575	716/11581	1.23 (1.06, 1.42) P = 0.007
CCB			
AASK	70/405	32/202	1.09 (0.74, 1.60)
ASCOT	799/7040	567/7072	1.42 (1.28, 1.57)
INVEST	665/8078	569/8098	1.17 (1.05, 1.30)
NORDIL	251/5095	216/5059	1.15 (0.97, 1.38)
STOP2	97/1961	95/1965	1.02 (0.78, 1.35)
Subtotal	1882/22579	1479/22396	1.21 (1.07, 1.36) P = 0.003
Overall	2748/34154	2195/33977	1.22 (1.12, 1.33) P <0.0001

Test(s) of heterogeneity:	χ2	df	P	I2**	Tau2
ACEi	5.92	3	0.116	49.3%	0.0107
CCB	10.35	4	0.035	61.3%	0.0105
Overall	16.55	8	0.035	51.7%	0.0080
Between sub-groups	0.29	1	0.590		

In Figure 2 it is shown that most studies report a higher T2DM risk for beta-blockers than for ACE inhibitors or CCB. Taken from Bangalore et al. (with permission).

1.D.3. Protective glycemic effects of angiotensin converting enzyme inhibitors and the angiotensin receptor blockers

On the other hand, ACE inhibitors and ARB have been proposed to have a protective effect in regard to T2DM onset and glycemic control (37), beyond the neutral effects attributed to calcium channel blockers (51). The first study to report a lower incidence of new-onset T2DM with the use of for treating hypertension with ACE inhibitors was the CAPPP ("Captopril Prevention Project") (52), which evaluated the effects of captopril versus conventional antihypertensive therapy in patients with diastolic hypertension. The relative risk (RR) for T2DM onset associated with use of captopril was 0.86 (95%CI: 0.74-0.99), as compared to conventional treatment, which included beta-blockers and diuretics (P=0.04).

In the HOPE ("Heart Outcomes Prevention Evaluation") study, the hypertensive subjects treated with ramipril showed a lower risk of in new-onset T2DM in secondary analyses (RR: 0.66; 95% CI: 0.52-0.84; P<0.001) (53). Similar results were observed in the ANBP-2 ("Australian National Blood Pressure-2") survey: the relative risk (RR) for new-onset T2DM in the ACE inhibitor-treated versus the diuretic-treated group was 0.69 (95% CI: 0.56-0.85; P<0.001) (54).

In the VALUE ("Valsartan Anti-hypertensive Long-term Use Evaluation) study, the RR for T2DM onset was 0.81 (95% CI: 0.74-0.83) in patients treated with valsartan compared to patients treated with amlodipine (55). The results of the VALUE and ALLHAT (34) studies directly comparing use of ARB with CCB, which are thought to be metabolically neutral, suggest a true beneficial effect of ARB on T2DM development. This effect seems to be directly related to the blockade of the biological effects of angiotensin II beyond the "relative" benefit exclusively driven by comparing them with drugs with deleterious effects in regard to risk of T2DM onset, such as thiazides or beta-blockers.

The LIFE ("Losartan Intervention For Endpoint reduction") study was a double-blind, randomized, parallel-group trial to compare the effects of losartan and atenolol on cardiovascular morbidity and mortality in 9,193 high-risk hypertensive patients (systolic BP: 160-200 mmHg; or diastolic BP: 95-115 mmHg), with left ventricular hypertrophy. This study provided further evidence that new-onset T2DM was less frequent (RR: 0.75; 95% CI: 0.64-0.89; P<0.001), and insulin-sensitivity was higher with losartan than with atenolol (56).

In the CHARM ("Candesartan in Heart failure Assessment of Reduction in Mortality and morbidity") study, patients with chronic heart failure were randomly assigned to receive candesartan or placebo in addition to standard anti-heart failure therapy (57). Significantly fewer patients in the candesartan-treated group developed new-onset T2DM compared to the placebo group (RR: 0.81; 95% CI: 0.66-0.99; P=0.02). Notwithstanding the previous result, a recent paper has shown no evidence that the addition of the ARB telmisartan to usual care prevents incident T2DM or leads to regression of impaired fasting glucose or impaired glucose tolerance in people at high risk for cardiovascular disease but free from diabetes (58).

Combining several individual studies, in a meta-analysis including more than 103,000 patients, Burke et al. found a significant 27% (95% CI: 7-51%) higher risk for calcium channel blockers and a 47% (95% CI: 12-95%) higher risk for "other hypertensive treatments" of developing T2DM than for subjects treated with ACE inhibitors alone (P=0.007 and P=0.006, respectively) (59). Jandeleit-Dahm et al. performed another meta-analysis including data from randomized clinical trials including more than 65,000 individuals evaluating the incidence of T2DM in subjects with renin-angiotensin axis blockade vs. non-blockade, finding an overall risk ratio = 0.78 (95% CI: 0.74-0.83) of T2DM onset for subjects with axis blockage (**Figure 3**) (60). Similar results have been reported by Gillespie et al. (61).

Figure 3: **Risk ratios for new-onset T2DM in comparative outcome trials involving the use of renin-angiotensin axis blockade vs. non-blockade, as reported by Jandeleit-Dahm et al.** (60).

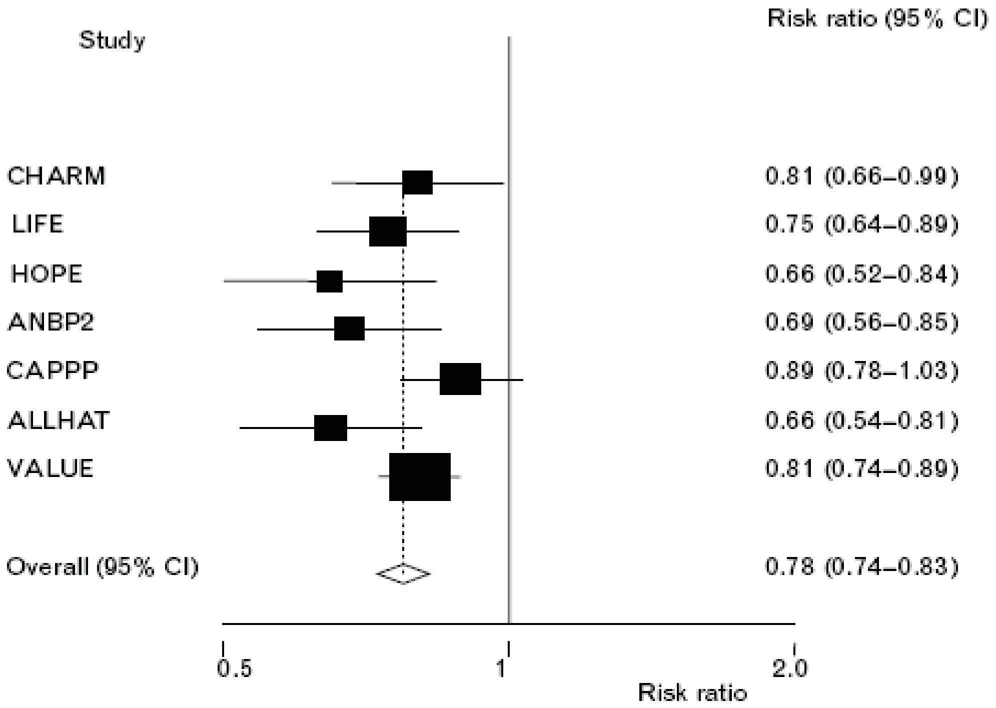


Figure 3 shows a lower risk of incident T2DM with the use of ACE inhibitors/ARB. Taken from Jandeleit-Dahm et al. (with permission).

More recently, the results from the NAVIGATOR ("Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research") study have been published (62). This study is a double-blind, randomized clinical trial with a 2-by-2 factorial design including 9,306 patients with impaired glucose tolerance and established cardiovascular disease or cardiovascular risk factors. The intervention consisted of the administration of valsartan (up to 160 mg daily) or placebo, in addition to lifestyle modification, with a five-year follow up. The cumulative incidence of T2DM was 33.1% in the valsartan group, as compared with 36.8% in the control group (HR=0.86; 95% CI: 0.80-0.92; $P<0.001$). Valsartan treatment increased glucose-stimulated insulin release and insulin sensitivity in normotensive subjects with impaired glucose metabolism in a recent report by van der Zijl et al. (63).

The way ACE inhibitors and ARB reduce the risk of T2DM remains uncertain, though several mechanisms, such as decreased renal potassium wasting, and improved islet blood flow and pancreatic beta cell perfusion by reducing angiotensin II-mediated vasoconstriction in the pancreas, have been proposed (60).

1.E. Genetic contribution to the risk of T2DM

T2DM is a multi-factorial disease. Genetic background is partially responsible for T2DM risk. This has long been thought, as some T2DM patients have normal weight, and reciprocally, many obese subjects never develop T2DM, what suggests that T2DM is not exclusively caused by environmental factors. Moreover, certain ethnic minorities do show high T2DM prevalence (up to 21% in Pima Indians) (64). Also, T2DM clusters in families: first-degree relatives have, compared with the general population, more than 3-fold higher risk of developing the disease (65). It has also been reported a higher concordance for T2DM in monozygotic compared with dizygotic twins (≈70% *vs*. 10%) (66).

T2DM is considered to be polygenic, since it does not follow a Mendelian pattern of transmission. It follows a "complex pattern of genetic transmission", caused by the simultaneous occurrence of common (minor allele frequencies >5%) DNA sequence variations in many genes (67). The sum of the individual modest effects of all the intervening genes confers an increased susceptibility toward adverse environmental factors. Single nucleotide polymorphisms (SNPs), exchanges of single base pairs, cover approximately 90% of the sequence variation within the human genome (68), and are regarded as the major determinants of the individual predisposition to complex diseases. Thus, the term "genetic polymorphism" defines monogenic traits that exist in the normal population in at least two phenotypes, neither of which is rare (69).

There has been a huge international effort for the identification of the genetic predisposition to T2DM. Two main approaches have been followed to achieve this aim: candidate gene studies and the hypothesis-free genome-wide association scan approach

(GWAS). With the former, candidate genes are combed through for common genetic variants, and these variants' allele frequencies are finally analyzed for being altered in T2DM cases compared with healthy controls. Candidate genes are identified from different sources: 1. Basic research of cell and mouse studies, which was the case for *PPARg* (on chromosome 3p25) (70) and *KCNJ11* (on chromosome 11p15.1) (71,72); 2. Genetics of rare monogenic forms of human diabetes, whose causal genes' mutations are known to exert strong effects and cause monogenic forms of diabetes (maturity onset diabetes of the young (MODY), Wolfram syndrome, etc.); this was the case for *HNF1B* (chromosome 17q12) (73) and *WFS1* (chromosome 4p16.1) (74); and 3. Human family linkage studies, such as diabetes-associated variants in *TCF7L2* (chromosome 10q25.3) (75,76).

The most recent and successful approach to identify novel risk alleles or replicate those previously reported to be associated with T2DM is the systematic genotyping of several hundred thousand SNPs in tens of thousands of cases and controls using high-density SNP arrays (GWAS), through a technology that has become widespread and affordable in recent times. In these GWAS, the frequency of all these genotyped SNPs is then compared between cases and controls, and alleles significantly more frequent in cases than in controls (at an arbitrarily established level of genome-wide statistical significance to correct for multiple testing) are considered risk alleles. In early 2007, Sladek et al. (77) were the first, not only to confirm *TCF7L2* as a T2DM risk genetic locus, but also to identify additional novel T2DM risk loci, namely *SLC30A8* (chromosome 8q24.11) and *HHEX* (chromosome 10q23.33). Next, *FTO* (chromosome 16q12.2) was characterized as a T2DM risk gene through its action upon obesity (78,79).

The aforementioned associations for *HHEX*, *SLC30A8*, *TCF7L2*, *FTO*, *KCNJ11*, and *PPARG* were replicated in large GWAS, which also revealed three novel T2DM risk loci: *CDKAL1* (chromosome 6p22.2), *IGF2BP2* (chromosome 3q27.2) and a genomic region between *CDKN2A* and *CDKN2B* on chromosome 9p21 (80-83). In 2008, a meta-analysis of GWAS comprising approximately 60,000 subjects reported six additional risk loci: *JAZF1* (chromosome 7p15.2-p15.1), *THADA* (chromosome 2p21), *ADAMTS9* (chromosome 3p14.1), *NOTCH2* (chromosome 1p13-p11), and two inter-genic regions, one between *CDC123* and *CAMK1D* on chromosome 10p13 and another between *TSPAN8* and *LGR5* on chromosome 12q21-q22 (84). All the common variants had low effect sizes ORs for T2DM risk (in the range 1.09-1.13). In this study, *DCD*, on chromosome 12q13.1, approached genome-wide statistical significance and *VEGFA*, on chromosome 6p12, which had shown a significant signal in a previous GWAS (81), did too.

More recently, confirmed diabetes risk alleles of *KCNQ1* on chromosome 11p15.5 were reported in Asian GWAS that also included European replication cohorts (85). Moreover, a meta-analysis of 13 GWAS (>83,000 subjects) revealed common variation in the *MTNR1B* gene on chromosome 11q21-q22 that conferred an increased risk for T2DM (86), and this was verified in subsequently published studies (87). Additionally, Rung et al. reported that a genetic variant near *KIAA1486/IRS1*, on chromosome 2q36, was associated with T2DM, insulin resistance and hyper-insulinemia (88).

After that, two additional GWAS have enlarged the list of T2DM associated SNPs: in the first one, Dupuis et al., using data from MAGIC ("Meta-Analyses of

Glucose and Insulin-related traits Consortium"), identified additional common variants associated with fasting glucose levels (see below, section 1.F). In their study, they also tested these loci for association with incident T2DM as a dichotomous trait in up to 40,655 cases and 87,022 non-diabetic controls, demonstrating that the fasting glucose-raising alleles at 7 loci (in or near *ADCY5* (chromosome 3q21.1), *PROX1* (chromosome 1q32.2-q32.3), *GCK* (chromosome 7p15.3-p15.1), *GCKR* (chromosome 2p23) and *DGKB-TMEM195* (chromosome 7p21.2), and the known T2DM genes *TCF7L2* and *SLC30A8* were robustly associated ($P<5\times10^{-8}$) with increased risk of T2DM (89).

In the other study, Voight et al., for the DIAGRAM + ("Diabetes Genetics Replication And Meta-analysis") Consortium, combined genome-wide association data from 8,130 T2DM cases and 38,987 controls of European descent and followed up novel meta-analysis signals in a sample including 34,412 cases and 59,925 controls, identifying 12 novel T2D-association signals with combined $P<5x10^{-8}$ (90). These included a second independent signal at the *KCNQ1* locus, the first report of a X-chromosomal association (near *DUSP9*), and a further instance of overlap between loci implicated in monogenic and multifactorial forms of diabetes (*HNF1A).* The two signals at *KCNQ1* were in low linkage disequilibrium (LD), which is defined as the non-random association of alleles of SNPs that reside one close to another. The remaining SNPs are in or near the following loci: *HMGA2* (chromosome 12q15), *CENTD2* (chromosome 11q13.4), *KLF14* (chromosome 7q32.2), *PRC1* (chromosome 15q26.1), *TP53INP1* (chromosome 8q22), *ZBED3* (chromosome 5q14.1), *ZFAND6* (chromosome 15q25.1), *BCL11A* (chromosome 2p16.1) and *CHCHD9* (chromosome 9q21.31). Two more SNPs have recently been reported to be associated with T2DM: in or near *HCCA2,* on chromosome 11p15.5 (91) and in or near *RBMS1/ITGB6*, on chromosome 2q24.2 (92). These genetic research efforts of the last decade have led to the identification of around 41 T2DM-associated susceptibility loci as of June 2009, comprising 40 autosomal loci and 1 locus in the sexual X chromosome (**Table 2**). At last, a very recent report suggests that there may be another signal showing genome-wide statistical significance at the vicinity of *GATAD2A/CILP2/PBX4*, to be further replicated (93).

Remarkably, most of the aforementioned SNPs are tagging genetic loci known to influence pancreatic beta cell function. A summary of the different biologic pathways that these beta cell genetic loci may be related to is shown in **Table 3**. Proposed mechanisms per loci discovered in recent GWAS that influence beta cell function include beta-cell development, insulin maturation, glucose transport, and insulin secretion (i.e., incretin-mediated secretion) or release (i.e., through the activation of the potassium channel). For this study, we decided not to exclude those loci for which an effect on insulin secretion could not be definitely ruled out owing to the fact that they regulate molecular pathways that may be indirectly related to beta-cell function, such as signal transduction, zinc finger protein regulation, mitogenic activity and others. However, for six of the T2DM-SNPs, in or near *IRS1*, *PPARγ*, *ADAMTS9*, *KLF14*, *HMGA2* and *FTO*, mechanisms other than those related to the beta cell function have been proposed (namely, insulin-resistance as opposed to beta cell function) (90,94). We therefore excluded these SNPs that had been suggested to modify T2DM risk through insulin action (as opposed to insulin secretion), or through other well-defined non beta-cell mechanisms (namely, obesity in case of *FTO*). We also removed from the dataset SNP rs2237892, at *KCNQ1*, associated with T2DM risk mostly in Asian populations. In addition, no genotyped or imputed data were available in Framingham for rs1800574, at *TCF1/HNF1A*, or for *DUSP9*, in chromosome X. Thus, we finally used an updated list - by September 2010- of 33 SNPs that reportedly influence beta cell function for the T2DM analyses.

Table 2. Common genetic variation in autosomal genetic loci associated with T2DM in published GWAS.

SNP	Genetic locus	Chromosome	Risk allele	Beta cell SNP	GWAS (citation)
rs10923931	*NOTCH2*	1	T	Yes	Zeggini (84)
rs340874	*PROX1*	1	C	Yes	Dupuis (89)
rs780094	*GCKR*	2	C	Yes	Dupuis (89)
rs7578597	*THADA*	2	T	Yes	Zeggini (84)
rs243021	*BCL11A*	2	A	Yes	Voight (90)
rs7593730	*RBMS1/ITGB6*	2	C	Possibly	Qi (92)
rs7578326	*KIAA1486/IRS1*	2	A	No	Rung (88)
rs1801282	*PPARg*	3	C	No	Saxena (80)
rs4607103	*ADAMTS9*	3	C	No	Zeggini (84)
rs11708067	*ADCY5*	3	A	Yes	Dupuis (89)
rs1470579	*IGF2BP2*	3	C	Yes	Saxena (80)
rs10010131	*WFS1*	4	G	Yes	Sandhu (74)
rs4457053	*ZBED3*	5	G	Yes	Voight (90)
rs7754840	*CDKAL1*	6	C	Yes	Saxena (80)
rs9472138	*VEGFA*	6	T	Possibly	Zeggini (84)
rs2191349	*DGKB/TME195*	7	T	Yes	Dupuis (89)
rs864745	*JAZF1*	7	T	Yes	Zeggini (84)
rs4607517	*GCK*	7	A	Yes	Dupuis (89)
rs972283	*KLF14*	7	G	No	Voight (90)
rs896854	*TP53INP1*	8	T	Yes	Voight (90)
rs13266634	*SLC30A8*	8	C	Yes	Saxena (80)
rs10811661	*CDKNA/2B*	9	T	Yes	Saxena (80)
rs13292136	*TLE4/CHCHD9*	9	C	Yes	Voight (90)
rs12779790	*CDC123,CAMK1D*	10	G	Yes	Zeggini (84)
rs1111875	*HHEX*	10	C	Yes	Saxena (80)
rs7903146	*TCF7L2*	10	T	Yes	Grant (75)
rs2334499	*HCCA2*	11	T	Possibly	Kong (91)
rs231362	*KCNQ1*	11	G	Yes	Voight (90)
rs2237892	*KCNQ1*	11	C	Yes	Unoki (85)
rs5215	*KCNJ11*	11	C	Yes	Saxena (80)
rs1552224	*CENTD2*	11	A	Yes	Voight (90)
rs10830963	*MTNR1B*	11	G	Yes	Prokopenko (86)
rs1153188	*DCD*	12	A	Possibly	Zeggini (84)
rs1531343	*HMGA2*	12	C	No	Voight (90)
rs7961581	*TSPAN8,LGR5*	12	C	Yes	Zeggini (84)
rs7957197	*OASL/TCF1(HNF1A)*	12	T	Yes	Voight (90)
rs11634397	*ZFAND6*	15	G	Yes	Voight (90)
rs8042680	*PRC1*	15	A	Yes	Voight (90)
rs9939609	*FTO*	16	A	No	Van Hoeck (78)
rs757210	*HNF1B/TCF2*	17	T	Yes	Winckler (73)

SNP: single nucleotide polymorphism. GWAS: genome-wide association scan.

Table 3. Proposed patho-physiological mechanisms that could explain impairment of beta cell function mediated by common genetic variation (95).

SNP	Chr	Nearest gene	Biologic pathways	Generic function
rs10923931	1	*NOTCH2*	Pancreatic organogenesis. Cell growth	Beta cell development
rs340874	1	*PROX1*	Encodes HNK4, necessary for beta cell development	Beta cell development
rs7578597	2	*THADA*	Apoptosis. Transport activity. Not well known	Diverse/unknown
rs243021	2	*BCL11A*	HbF levels. Beta cell differentiation	Beta cell development
rs780094	2	*GCKR*	Inhibits glucokinase in beta cell. MODY	Insulin secretion, not incretins
rs7593730	2	*RBMS1/ITGB6*	RNA processing/integration; inflammation, emphysema	Diverse/unknown
rs11708067	3	*ADCY5*	Adenylate cyclase. GLP1, AMPc and insulin secretion	Incretins. Insulin secretion
rs1470579	3	*IGF2BP2*	Pancreatic development. Morphogenesis. Protein binding	Beta cell development
rs10010131	4	*WFS1*	GLP1-induced insulin secretion	Incretins. Insulin secretion
rs4457053	5	*ZBED3*	Encodes Zn finger. Unknown	Diverse/unknown
rs7754840	6	*CDKAL1*	Signal transducer. Down regulates insulin expression	Diverse/unknown
rs9472138	6	*VEGFA*	Vascular endothelial growth factor. Angiogenesis. Unknown	Diverse/unknown
rs864745	7	*JAZF1*	Zn finger protein. Encodes a nuclear receptor	Diverse/unknown
rs2191349	7	*DGKB/TMEM195*	Protein kinase and insulin secretion	Insulin secretion, not incretins
rs4607517	7	*GCK*	Phosphorilates glucose through glycolysis. MODY	Diverse/unknown
rs13266634	8	*SLC30A8*	Zn transporter for insulin maturation	Insulin maturation
rs896854	8	*TP53INP1*	Tumor protein p53	Diverse/unknown
rs10811661	9	*CDKNA/2B*	Pancreatic regenerative capacity	Beta cell development
rs13292136	9	*TLE4/CHCHD9*	Transcription regulator. Unknown	Diverse/unknown
rs1111875	10	*HHEX*	Pancreatic organogenesis. Insulin secretion (glucose mediated)	Insulin secretion, not incretins
rs12779790	10	*CDC123, CAMK1D*	Cell division cycle (*CDC123*)/calmodulin protein kinase ID	Insulin secretion, not incretins
rs7903146	10	*TCF7L2*	Response to incretins. Chromatin integrity. Signal transduction	Incretins. Insulin secretion
rs5215	11	*KCNJ11*	Potassium channel. Insulin release. Glucagon secretion	Glucose transport
rs1552224	11	*CENTD2*	Regulates movement of actine filaments in cell cycle	Diverse/unknown
*rs2237892	11	*KCNQ1*	Potassium channel. Insulin release	Glucose transport
rs231362	11	*KCNQ1*	Potassium channel. Insulin release	Glucose transport
rs10830963	11	*MTNR1B*	Melatonin, circadian rhythm	Diverse/unknown
rs2334499	11	*HCCA2*	Protein interactor, Zn binder, liver neoplasm	Diverse/unknown
rs1153188	12	*DCD*	Defense against bacteria. Unknown	Diverse/unknown
rs7961581	12	*TSPAN8,LGR5*	Protein glycosilation and signal transducer activity. Neoplasms	Diverse/unknown
rs7957197	12	*OASL/TCF1/HNF1A*	Transport activity, apoptosis. Neoplasms	Diverse/unknown
rs8042680	15	*PRC1*	Regulate cytokinesis. Associated to tumors	Diverse/unknown
rs11634397	15	*ZFAND6*	Zn finger. Unknown	Diverse/unknown
rs757210	17	*HNF1B/TCF2*	MODY. Transcription regulator. Neoplasms	Diverse/unknown

SNP: single nucleotide polymorphism. Chr: chromosome. MODY: maturity-onset diabetes of the young. GLP-1: glucagon-like peptide 1.* rs2237892, at *KCNQ1*, was not included in the dataset.

1.F. Genetic contribution to plasma fasting glucose level regulation

The study by Prokopenko et al. (86) also tested the association of one variant at *MTNR1B* with plasma fasting glucose as a quantitative trait. It might seem intuitive that those SNPs associated with T2DM would also be associated with fasting glucose, but it has been shown that this is not necessarily true: a large effect size on T2DM does not always translate into an equivalently large fasting glucose effect in non-diabetic subjects. For instance, the risk allele at *TCF7L2*, the genetic locus with the largest effect on T2DM, has a small effect on fasting glucose levels. Additionally, not every locus associated with fasting glucose within the 'physiological' range needs to be associated with 'pathological' fasting glucose levels and T2DM risk. We can then conclude that variation in fasting glucose levels in healthy individuals is not necessarily an endo-phenotype for T2DM (89).

It was precisely the "MAGIC" GWAS which shed light on the association between common genetic variation across the human genome and fasting glucose levels (89). In this study, variants in or near *ADCY5*, *MADD* (chromosome 11p11.2), *ADRA2A* (chromosome 10q24-q26), *CRY2* (chromosome 11p11.2), *FADS1* (chromosome 11q12.2-q13), *GLIS3* (chromosome 9p24.2), *SLC2A2* (chromosome 3q26.1-q26.2), *PROX1* and *C2CD4B* (chromosome 15) showed a genome-wide level significant association with fasting glucose levels, and one SNP near *IGF1* (chromosome 12q22-q23), an association with fasting insulin and HOMA-IR (homeostasis model assessment of insulin resistance). Also, it confirmed the previously known associations between variants in *GCK*, *GCKR*, *G6PC2* (chromosome 2q24.3) and *MTNR1B* with fasting glucose levels, and showed genome-wide statistical significance for the association between fasting glucose levels and the recently reported *DGKB-TMEM195* locus, and the T2DM-associated loci at *TCF7L2* and *SLC30A8*. All these fasting glucose-associated variants seem to act by influencing beta cell function, including *C2CD4B* (96). To complete the overall view of glycemic traits-associated common SNPs, Saxena et al. performed a meta-analysis of nine genome-wide association studies (n=15,234 non-diabetic individuals) and a follow-up of 29 independent loci (n=6,958-30,620), and identified variants at *GIPR* (chromosome 19q13.3), *ADCY5*, *VPS13C* (chromosome 15q22.2), *GCKR* and *TCF7L2* to be associated with results from a 2-h oral glucose tolerance test (OGTT) (97) (**Table 4**). For the change in fasting glucose analyses, we used the full 16-fasting glucose SNPs list as reported by Dupuis et al. (89) (**Table 4,** in bold).

Table 4. Genetic loci associated with T2DM-related quantitative traits in published GWAS.

SNP	Chr	Nearest gene	Trait	T2DM loci	Risk allele	Beta cell	Citation
rs340874	1	*PROX1*	FG	Yes	C	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs560887	2	*G6PC2*	FG	No	C	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs11708067	3	*ADCY5*	FG	Yes	A	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs11920090	3	*SLC2A2*	FG	No	T	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs2191349	7	*DGKB/TMEM195*	FG	Yes	T	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs4607517	7	*GCK*	FG	Yes	A	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs13266634	8	*SLC30A8*	FG	Yes	C	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs7034200	9	*GLIS3*	FG	No	A	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs10885122	10	*ADRA2A*	FG	No	G	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs7903146	10	*TCF7L2*	FG	Yes	T	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs11605924	11	*CRY2*	FG	No	A	Possibly	Dupuis J. Nat Genet 2010. MAGIC
rs174550	11	*FADS1*	FG	No	T	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs7944584	11	*MADD*	FG	No	A	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs10830963	11	*MTNR1B*	FG	Yes	G	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs11071657	15	*FAM148B(C2CD4B)*	FG	No	A	Possibly	Dupuis J. Nat Genet 2010. MAGIC
rs780094	2	*GCKR*	FG, FI	Yes	C	Yes	Dupuis J. Nat Genet 2010. MAGIC
rs35767	12	*IGF1*	FI	No	G	No	Dupuis J. Nat Genet 2010. MAGIC
rs17271305	15	*VPS13C*	2hrG only	No	G	Possibly	Saxena R. Nat Genet 2010. MAGIC
rs10423928	19	*GIPR*	2hrG only	No	A	Yes	Saxena R. Nat Genet 2010. MAGIC

Chr: chromosome. FG: fasting glucose. FI: fasting insulin. 2hrG only: association only with two-hour glucose levels after an oral glucose tolerance test (OGTT). In bold, the 16 fasting glucose-associated SNPs.

1.G. Gene-hypertension and gene-drug interaction and risk of incident T2DM

1.G.1. The concept of gene-environment interaction

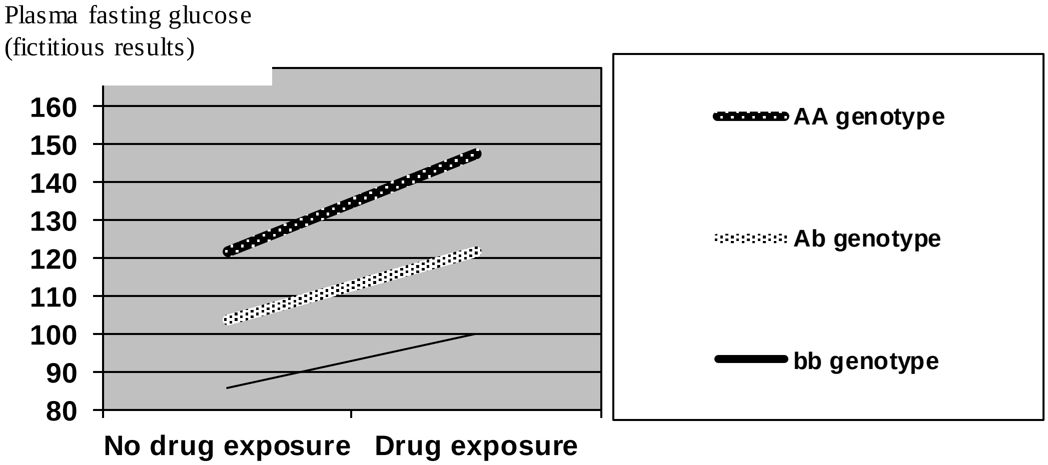
It is important to note that, in different contexts, statisticians, biologists, clinicians and geneticists can mean somewhat different things when they refer to “interaction”. Sometimes, “gene-environment interaction” is used in a very loose sense, meaning some sort of interplay between genetic and environmental factors. It does not necessarily convey a specific mode of joint action, or even a precise relationship between statistical risks. Other times, it is even used to express that several factors are contributing to disease risk, without excluding the possibility of complete independence among them. In these cases, using the term ‘joint action’ instead of “interaction” would be preferable (98).

We define “biological interaction” as the joint effect of two factors that act together in a direct physical or chemical reaction; it summarizes the co-participation of two or more factors in the same causal mechanism of disease development (99). However, the term “statistical interaction” should be reserved for those cases where some heterogeneity of effects is present, that is, a departure from additivity of effects on a specific outcome scale (100). We could rather take a multiplicative model as the null hypothesis; that is, the relative risk of disease in individuals with both the genetic and environmental risk factors is the product of the relative risks of each separately. Therefore, any joint effect that differs from these predictions can be viewed as a “statistical interaction”, and as a matter of fact, the multiplicative model of departure as the null hypothesis is also commonly evaluated in Epidemiology (101).

If only one factor is present, we would call “main effect” its eventual effect on the risk of disease. In case two or more risk factors are present, the “marginal effect” of a risk factor would then be its average effect across all levels of the other risk factors. The risk factors are said to “interact” if the effect of one risk factor depends on the level of the other risk factor. The joint effect of two risk factors would then refer to both their marginal effects and their interaction effect. So, this joint effect between a gene and an environmental factor that cannot be readily explained by their separate marginal effects is what we will call gene-environment interaction (101). The joint effect can vary from less than additive (“sub-additive”) to more than multiplicative (“supra-multiplicative”) of the individual marginal effects. The visual representation of the presence of statistical interaction between the presence of 0, 1 or 2 risk alleles at a genetic locus and a drug exposure for determining a hypothetical level of a quantitative trait (i.e., plasma fasting glucose) is shown in **Figure 4**.

It has been reported that the detection of an interaction effect requires a sample size at least four times larger than that one required for the detection of a main effect of comparable magnitude. However, a range of interaction effect sizes can be detected in a GWAS by testing for interaction or a genetic effect in an environmental subgroup, even when the marginal effects are not detectable (101).

Figure 4: **Visual representation of a hypothetical statistical gene-environment interaction effect.**



Note: Even if a main effect would be present, the three lines would be in parallel if a statistical interaction effect was not present. Original representation.

We must make a distinction between "interaction" and "confounding" among environment and genetic factors. "Confounding" refers to a mixing of extraneous effects with the effect of interest, in such a way that an undetected correlation between the genetic and the environmental risk factors actually accounts for the effect, giving the appearance of an interaction effect; this can happen in populations with latent stratification, or when genes influence on behaviors that modify the incidence of some diseases. Yet, for most situations, this will not be a matter of concern, as genotype and environmental risk factors are usually independent and genetic background is not influenced by the environment (concept of "Mendelian randomization") (102).

It has been proposed that the relevant research questions that could be addressed by a gene-environment interaction study, beyond simply analyzing the main gene effects, include (97,103):

a. The clinical relevance of a gene-environment interaction, where the interaction itself is of interest; the aim of an initial study is primarily hypothesis-generating (exploratory), yet in other cases it will be hypothesis-testing (confirmatory); eventual associations should further be replicated in different populations and ideally validated in randomized controlled trials.

b. The ability to predict individual risk of disease or prognosis, and potential changes in risk in relation to modifiable environmental factors.

c. The possibility of choosing the best treatment for an individual to maximize response or minimize side effects based on genetic predisposition ("pharmacogenetics"). The idea that the term "pharmacogenetics" conveys is more general than "pharmacogenomics", which may rather be considered as "the whole-genome application of pharmacogenetics".

d. There may also underlie an interesting aspect of public health impact, depending on the prevalence of the conditions studied (high both for hypertension and diabetes), the strength of the interaction, and the allele frequencies in the population.

1.G.2. Gene-environment interaction in the field of anti-hypertensive drugs and diabetes onset

There seems to be overall agreement that, to reduce the rate of false discovery, search for gene-environment interactions should be restricted to *a priori* biologically hypotheses-driven approaches (104).

Several reports in the medical literature describe the effect that genetic polymorphisms have on different endpoints (atherosclerosis, micro-albuminuria, nephropathy, blood pressure responsiveness, renal hemodynamics or even re-hospitalization) in the diabetic populations as a response to anti-hypertensive drugs (105,106). Moreover, there is published work on how common genetic variation modifies the hypoglycemic response at the exposure to different oral hypoglycemic drugs (107,108).

Other additional studies have focused on the interaction effects between genes and other environmental factors on T2DM incidence. Thus, *PPAR-γ* has been a thoroughly studied genetic locus: some -but not all- studies have demonstrated an interaction effect between Pro12Ala genotype and the composition of dietary fat intake on insulin resistance, BMI, or glycemia (109); also, results of gene–exercise interactions have been more consistent in showing Ala-allele carriers as being more sensitive to the metabolic effects of exercise (110). However, certainty about whether environmental or behavioral factors modify the association between variation in *TCF7L2* and T2DM and diabetes-related quantitative traits remains to be established (76).

Reports concerning increased or decreased T2DM incidence as secondary effects of antihypertensive drugs due to common genetic variation are lacking. The possible modifying effect of common genetic variation on the secondary hyperglycemic effects of antihypertensive drugs has been only occasionally reported in the medical literature. Whereas hypertension and hypertension treatment, on the one hand, and genetic background, on the other hand, seem to increase fasting glucose and T2DM risk, it is not known whether these factors can modify each other's association with glycemia. Eventual demonstration of gene-drug interactions might make genetic information useful when selecting anti-hypertensive drugs for patients at risk for developing adverse metabolic effects.

Here, we tested the hypothesis that the subsets of common SNPs associated with fasting glucose or T2DM that appear to influence beta-cell function interact with hypertension or antihypertensive drugs in their positive associations with change over time in fasting glucose (ΔFG) or incident T2DM.

2. SUMMARIZED HYPOTHESES

Recent well-powered studies and meta-analyses support an association for both thiazides and beta-blockers with risk of T2DM. Contrarily, ACE inhibitors and ARB appear to have neutral or even protective effects in regard to T2DM risk. Pathophysiological responsible mechanisms are largely unknown.

Common genetic variation has been convincingly associated with T2DM risk in the literature. Thus, it is a biologically plausible hypothesis that common genetic variation in T2DM-associated SNPs that influence beta-cell function could either enhance or attenuate the deleterious or beneficial effects that antihypertensive drugs exert on risk of T2DM or fasting glucose levels.

We proposed to analyze the 33 autosomal SNPs so far convincingly associated with T2DM and the 16 SNPs so far associated with fasting glucose levels (8 overlap with T2DM risk), which apparently influence pancreatic beta-cell function (**Table 2**). Each one of these SNPs allows for testing drug-gene interactions associated with T2DM risk or change over time in fasting glucose levels, respectively.

Using data on antihypertensive drug exposures, occurrence of T2DM and plasmatic levels of fasting glucose from the Framingham Offspring Cohort, in this observational, longitudinal, cohort study, we thus proposed to test the following hypotheses:

PRELIMINARY HYPOTHESES

- Hypertension increases T2DM risk and change over time in plasma fasting glucose levels as compared to no-hypertension.
- Overall hypertension treatment increases T2DM risk and change over time in plasma fasting glucose levels as compared to untreated hypertension.
- Treatment with thiazides or beta-blockers increases T2DM risk and change over time in plasma fasting glucose levels as compared to untreated hypertensive patients.
- Treatment with ACE inhibitors or ARB decreases T2DM risk and change over time in levels of plasma fasting glucose as compared to untreated hypertensive patients.
- Common beta cell function-influencing fasting glucose associated- and T2DM associated-SNPs confer higher change over time in fasting glucose and T2DM risk.

MAIN HYPOTHESIS

- Variation in novel MAGIC, DIAGRAM + and other previously reported beta cell function-influencing SNPs would modify the association between hypertension or specific hypertension treatments and T2DM risk or change over time in plasma fasting glucose levels.

3. METHODS

3.A. Population: The Framingham Offspring Study

The original Framingham Heart Study was started in 1948. It recruited 5,209 individuals, among whom there were 3,288 from 1,644 husband-wife pairs (111). The children of these couples and the children's spouses were invited to participate in the Framingham Offspring Study (112). The offspring were thought to be a desirable target population for several reasons: Firstly, a very high proportion of the offspring were thought to reside indefinitely in the New England area and would therefore be accessible for study. Also, many members of the offspring were close to the same age their parents were when they were first examined in the original Framingham Heart Study (1948-1952) (113,114).

Records from the first Framingham Heart Study exam were utilized to construct a roster of families to be contacted. Attempts were made to contact the parent by telephone to obtain address information on all children. When no contact could be made with a parent, relatives, friends or neighbors were asked to supply this information about the children. In only 42 instances (2.6%) was it impossible to determine whether there were descendants of the FHS spouse pair. An additional 198 pairs were known to be childless. Thus, 1,404 couples were known to have had at least one child and 1,202 of these families (86%) are represented by at least one child in the study. The 1,202 mothers gave birth to or adopted 3,717 children, of which 2,656 (71%) were examined in this study. All offspring of eligible parents were asked to participate, regardless of the size of the family (112).

Invitations to participate in the study were also sent to a special group of children who had one parent in the Framingham Heart Study who had either coronary heart disease or who exhibited abnormal lipoprotein patterns at the tenth biennial examination of the original cohort. This resulted in the examination of an additional 899 offspring of 378 parents. Also, 1,644 spouses of examined offspring were invited for examination. Of these, both members of 144 spouse pairs are offspring of the original cohort (112). This added up to 5,124 people who agreed to participate. For this analysis, we only initially included 2,922 non-diabetic individuals for whom complete clinical and genotype data were available. Participants were essentially of white European ancestry.

Examinations of the offspring started in September, 1971, and were completed in July, 1975. Participants have been seen in the clinic about every 4 years (up to 8 exams), except for the ~8-year period between exams 1 and 2, to receive a complete medical history and physical exam, including anthropometric measurement and blood sample collection. Subjects were asked about their medical history, use of medication, alcohol consumption and history and current use of tobacco. All subjects reporting a history of cardiovascular disease or specific symptoms of coronary heart disease or peripheral vascular disease were examined by a cardiologist (115). The outcomes of this exam as well as previous hospital or physician records were used in the standardized diagnosis of cardiovascular disease (113,114). Over 80% of the eligible offspring were still living in New England 7 years after the constitution of the study cohort.

Electrocardiography (ECG) measurements, anthropometric measurements, blood pressure and skin fold measurements were also obtained from all subjects. Fasting blood specimens were used for lipoprotein quantification including electrophoresis and hematological observations. Whenever possible, the study protocol called for procedures identical to those used in the recent examinations of the FHS cohort (112).

3.B. Data collection and variables coding in the Framingham Offspring Study

When subjects were examined in the Framingham Offspring Study, the information collected for each exam did not exactly overlap in previous exams: whereas the first exam reported 183 items, the seventh exam requested information on 712 variables, as information concerning exposure to medications, physical activity, behavior, laboratory data and outcomes became more detailed. Exam 8 focused on outcomes.

3.B.1. Drug exposures

To understand the variability of the information collected at each exam, especially in the oldest ones, we can compare how information was retrieved from exams 1 and 7 in regard to anti-hypertensive medication (**Appendix 1**). For instance, detailed information on drug exposures is missing for the first exam; in subsequent exams, a more thorough collection of information on drug exposures was done. Complete information on use of beta-blockers and other anti-hypertensive drugs became available at exam 2, and on ACE inhibitors at exam 3. This information is summarized in **Table 5**.

Table 5. Anti-hypertensive drugs exposures at each exam in the Framingham Offspring Study.

	Exam 1	Exam 2	Exam 3	Exam 4	Exam 5	Exam 6	Exam 7	Exam 8
Hipertension	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Systolic BP	Level	Level	Level	Level	Level	Level	Level	Level
Treatment for high BP	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Diuretics	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
Thiazides			Y/N	Y/N	Y/N	Y/N	Y/N	
Other diuretics			Y/N	Y/N	Y/N	Y/N	Y/N	
Potassium supplement			Y/N	Y/N	Y/N	Y/N	Y/N	
Beta-blockers		Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	
Type of beta-blocker		Y/N			Y/N	Y/N	Y/N	
Calcium-channel blockers			Y/N	Y/N	Y/N	Y/N	Y/N	
ACE inhibitors or ARB			Y/N	Y/N	Y/N	Y/N	Y/N	
"Other hypotensives"	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N

Blank spaces: information not available. Y/N: information coded as "Yes" or "No". BP: blood pressure.
Interval between exam 1 and 2 was excluded from our analysis.

3.B.2. Covariates

Tables 6-8 show the information on some of the covariates that is available at each exam:

Table 6. Information on clinical covariates available in the Framingham Offspring Study.

	Age, sex	Parents in FHS	Parental T2DM	Hypertension	BP	Diabetes	BMI	Waist
Exam1	YES	YES		YES	YES	YES	YES	
Exam2	YES			YES	YES	YES	YES	
Exam3	YES			YES	YES	YES	YES	
Exam4	YES			YES	YES	YES	YES	YES
Exam5	YES			YES	YES	YES	YES	YES
Exam6	YES		YES	YES	YES	YES	YES	YES
Exam7	YES			YES	YES	YES	YES	YES
Exam8	YES		YES	YES	YES	YES	Weight	

FHS: Framingham Heart Study. BP: blood pressure levels. BMI: body mass index. Waist: waist perimeter measured.

"YES" denotes data available for that variable at that particular exam.

Table 7. Information on behavior patterns and other covariates in the Framingham Offspring Study.

	Physical activity	Smoking	Alcohol	Potassium supplementation	Use of steroids
Exam 1		YES	YES	YES	
Exam 2	YES	YES	YES	YES	
Exam 3		YES	YES	YES	YES
Exam 4	YES	YES	YES	YES	YES
Exam 5	YES	YES	YES	YES	YES
Exam 6		YES	YES	YES	YES
Exam 7	YES	YES	YES	YES	YES
Exam 8		YES			

Alcohol intake is measured in ounces per week. HDL: high density lipoprotein. TG: triglycerides.

"YES" denotes data available for that variable at that particular exam.

Table 8. **Information on some basic laboratory data in the Framingham Offspring Study.**

	Glucose	HbA1C	OGTT	HDL	TG	eGFR
Exam1	YES			YES	YES	
Exam2	YES			YES	YES	
Exam3	YES			YES	YES	
Exam4	YES			YES	YES	
Exam5	YES	YES	YES	YES	YES	YES
Exam6	YES			YES	YES	
Exam7	YES	YES	YES	YES	YES	
Exam8	YES				YES	

OGTT: oral glucose tolerance test. HDL: high density lipoprotein. TG: triglycerides. eGFR: estimated glomerular filtration rate.

"YES" denotes data available for that variable at that particular exam.

For co-morbidities, information was collected on those previously known coexisting conditions that might have made physicians preferentially use some anti-hypertensive medications over others. These previous conditions included coronary heart disease, renal insufficiency, chronic heart failure, stroke and lower limb intermittent claudication. We used the dataset called "sequence of events" in the Framingham Study, specifically constructed to compile information on the following outcomes: coronary heart disease, cerebrovascular accident, chronic heart failure, intermittent claudication and death (for instance, coronary heart disease was coded as shown in **Appendix 2**). By Dec 31, 2008, a total of 3,980 cardiovascular events in 1,915 subjects had been reported in the Framingham Offspring Study.

3.C. Genotype and imputation

3.C.1. Genotype in Framingham

While pursuing the Framingham Study's established research goals, the NHLBI and the Framingham investigators expanded their research mission into the study of genetic factors underlying cardiovascular disease and other disorders. Over the past two decades, DNA has been collected from blood samples and from immortalized cell lines obtained from Original Cohort participants, members of the Offspring Cohort and the Third Generation Cohort. Genome-wide linkage analysis has been conducted using genotypes of approximately 400 microsatellite markers that have been completed in over 9,300 subjects in all three generations. For other recent collaborative projects, thousands of SNP have been genotyped for candidate gene regions in subsets of FHS subjects with available DNA (116).

In 2007, the FHS conducted the genotyping for the FHS SHARe (SNP Health Association Resource) project, using approximately 550,000 SNPs (Affymetrix 500K mapping array plus Affymetrix 50K supplemental array) in over 9,300 subjects from the three generations of subjects (117). The SHARe database is housed at NCBI's dbGaP and contains all 550,000 SNPs as well as SNP and microsatellite genotyping conducted previously in the Framingham Heart Study. The phenotype database contains a vast array of phenotype information available in all three generations. These include: quantitative measures of the major risk factors, such as systolic blood pressure, total and HDL cholesterol, fasting glucose, and cigarette use; anthropomorphic measures, such as body mass index; and so on. Many of these measures have been collected repeatedly in the original and Offspring cohorts. It is interesting to highlight that genome-wide data are publicly available in Framingham.

Genotypes for this study were obtained from the Affymetrix array data available in the FHS-SHARe dataset (116) or from *de novo* genotyping on the iPLEX (Sequenom) platform (118), when not included in the Affymetrix array. Albeit there is evidence of population sub-stratification in Framingham, there was no evidence of inflation of the type-I error rate due to population stratification for most traits in previous studies (89).

3.C.2. Blood samples treatment for DNA extraction in Framingham

Treatment of blood samples for DNA extraction followed standard procedures, which have been reported elsewhere (119) (here, summarized in **Appendix 3**).

3.C.3. Control filters for genotype quality in Framingham

There are well-established control filters for quality of genotyping. "Minor allele frequency" (MAF) is the frequency of the SNP's less frequent allele in a given population. Common genetic variants have MAF >5%, whereas rare variants have MAF<5%, or even <1%. The "call rate" is another control filter, which is defined as the proportion of samples for which genotypes are obtained for a converted marker (120). The genotyping-calling algorithm used in Framingham for Affymetrix was BRLMM ("Bayesian robust linear modeling using Mahalanobis distance") (89), which did not appear to have a calling bias against heterozygotes (121).

An additional control filter is the "Hardy-Weinberg equilibrium" (HWE), defined as the stable frequency distribution of genotypes (AA, Aa, and aa) in the proportions p^2, 2pq, and q^2, respectively (where p and q are the frequencies of the alleles, A and a) that is a consequence of random mating, in the absence of mutation, migration, natural selection or random drift (122). Lastly, as an additional control filter in our sample, individuals with more than 1,000 Mendelian errors were excluded. For the SNPs genotyped, MAF were >5% and minimum call rates were 97% for Affymetrix and 96.9% for iPLEX SNPs. All SNPs were in HWE ($P>10^{-6}$ for Affymetrix and $P>0.02$ for iPLEX).

3.C.4. SNP imputation in Framingham

For several candidate SNPs, genotyping was actually not done; "proxies" can be alternatively used for these SNPs, which are other SNPs in the same genetic region that are in linkage disequilibrium (LD) with the putative SNPs. However, it is not uncommon that a proxy for the candidate SNP has not been assayed either, what makes it necessary to use some techniques as an attempt to predict which alleles the candidate SNP can actually have (123). With this aim, the information provided by the HapMap2 haplotypes has been very valuable for samples with a similar ancestry to populations included in the HapMap panels, the reference ones (124).

Imputation in Framingham has been performed with MACH software (123). In **Tables 9** and **10**, we can see which SNPs were actually genotyped or imputed for T2DM and fasting glucose in Framingham. Quality of predictions from imputations can easily be measured by initially masking the genotypes and then evaluating the results (123). The most likely predicted genotype above some threshold can be compared with the true genotype and a plot of the percentage discordance versus the percentage of missing genotypes can be constructed for a range of thresholds to illustrate performance (125).

The error rate of the "best guess" genotype for various methods can thus be estimated (126). Our imputation method has been shown to produce well-calibrated probabilities (127). Some issues have been raised from previous studies comparing the performance of the several imputation methods, however: firstly, imputation error rate increases as the minor allele frequency decreases (124); and secondly, differences in genetic diversity between the study population and the reference panel can also influence imputation accuracy (128).

Table 9. Genotyped and imputed beta cell T2DM-associated SNPs.

SNP	Chromosome	Nearest gene	Genotyped/Imputed	Platform	Variance ratio
rs10923931	1	*NOTCH2*	I	iPLEX	1.010411
rs340874	1	*PROX1*	I	iPLEX	0.711865
rs780094	2	*GCKR*	G	Affymetrix	
rs7578597	2	*THADA*	G	Affymetrix	
rs243021	2	*BCL11A*	I	iPLEX	0.967461
rs7593730	2	*RBMS1/ITGB6*	G	Affymetrix	1.009913
rs11708067	3	*ADCY5*	I	iPLEX	0.936832
rs1470579	3	*IGF2BP2*	I	iPLEX	1.0221
rs10010131	4	*WFS1*	I	iPLEX	1.010443
rs4457053	5	*ZBED3*	I	iPLEX	0.888613
rs7754840	6	*CDKAL1*	G	Affymetrix	
rs9472138	6	*VEGFA*	G	Affymetrix	
rs2191349	7	*DGKB/TMEM195*	I	iPLEX	0.973185
rs864745	7	*JAZF1*	G	Affymetrix	
rs4607517	7	*GCK*	I	iPLEX	0.994926
rs896854	8	*TP53INP1*	I	iPLEX	0.954928
rs13266634	8	*SLC30A8*	I	iPLEX	0.328816
rs10811661	9	*CDKNA/2B*	G	Affymetrix	
rs13292136	9	*TLE4 (CHCHD9)*	I	iPLEX	0.953989
rs12779790	10	*CDC123,CAMK1D*	I	iPLEX	0.768427
rs1111875	10	*HHEX*	I	iPLEX	0.996042
rs7903146	10	*TCF7L2*	I	iPLEX	0.989887
rs2334499	11	*HCCA2*	I	iPLEX	0.738763
rs231362	11	*KCNQ1*	I	iPLEX	0.680838
rs5215	11	*KCNJ11*	G	Affymetrix	
rs1552224	11	*CENTD2*	I	iPLEX	0.919218
rs10830963	11	*MTNR1B*	I	iPLEX	0.696892
rs1153188	12	*DCD*	G	Affymetrix	
rs7961581	12	*TSPAN8,LGR5*	G	Affymetrix	
rs7957197	12	*OASL/TCF1/HNF1A*	I	iPLEX	0.974055
rs11634397	15	*ZFAND6*	I	iPLEX	0.921906
rs8042680	15	*PRC1*	I	iPLEX	1.029531
rs757210	17	*HNF1B/TCF2*	I	iPLEX	0.202485

G: actually genotyped. I: imputed. Only one SNP (rs757210) had a variance ratio <0.3 (highlighted in red).

As a control filter for imputation, it is common practice to refer to the variance ratio, which is defined as the ratio of the empirically observed variance (based on the imputation) to the expected binomial variance p (1-p), where p is the minor allele frequency (129); for the imputed SNPs in our study, the variance ratio was >0.3, except for rs757210, a T2DM SNP, at *HNF1B* (variance ratio=0.20) (**Table 9**); the median variance ratio was 0.94 for the T2DM SNPs and 0.98 for the quantitative-traits SNPs.

Table 10. Genotyped and imputed beta cell fasting glucose-associated SNPs.

SNP	Chromosome	Nearest gene	Genotyped/Imputed	Platform	Variance ratio
rs340874	1	*PROX1*	I	iPLEX	0.711865
rs560887	2	*G6PC2*	I	iPLEX	0.991368
rs780094	2	*GCKR*	G	Affymetrix	
rs11708067	3	*ADCY5*	I	iPLEX	0.936832
rs11920090	3	*SLC2A2*	I	iPLEX	0.995684
rs2191349	7	*DGKB/TMEM195*	I	iPLEX	0.973185
rs4607517	7	*GCK*	I	iPLEX	0.994926
rs13266634	8	*SLC30A8*	I	iPLEX	0.328816
rs7034200	9	*GLIS3*	I	iPLEX	0.986822
rs10885122	10	*ADRA2A*	I	iPLEX	0.948183
rs7903146	10	*TCF7L2*	I	iPLEX	0.989887
rs11605924	11	*CRY2*	I	iPLEX	0.998308
rs7944584	11	*MADD*	G	Affymetrix	
rs174550	11	*FADS1*	I	iPLEX	0.991725
rs10830963	11	*MTNR1B*	I	iPLEX	0.696892
rs11071657	15	*FAM148B (C2CD4B)*	I	iPLEX	0.674072

G: actually genotyped. I: imputed. In bold, overlapping fasting glucose- and T2DM-SNPs.

3.D. Statistical methods

3.D.1. Definitions

We defined diabetes mellitus as having a fasting plasma glucose ≥126 mg/dl (7.0 mmol/l) at any exam or diabetes treatment at any exam. We did not use HbA1C to define diabetes. More than 99% of the cases of diabetes among the participants in the Framingham Offspring Study are T2DM (130). We defined a positive self-reported family history of diabetes as a report that one or both parents had diabetes; this definition is more than 56% sensitive and 97% specific for confirmed parental diabetes (131).

Hypertension was defined as either following treatment for hypertension or elevated blood pressure at any exam, according to the recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg) (14). In the Framingham Offspring Study, blood pressure was determined by at least two separate measurements carried out by physicians -for exams 1 and 2, a blood pressure measurement by a nurse was also available-, and the mean value was calculated; subjects had been seated for at least 5 minutes before blood pressure measurement (132). At each exam, we defined "no hypertension" as not on treatment and normal blood pressure, according to the recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (systolic blood pressure <140 mm Hg or diastolic blood pressure <90 mm Hg) (14). Untreated hypertension was defined as not on treatment for hypertension but with elevated blood pressure (systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg).

Current smoking was defined as consuming at least 1 cigarette per day within 1 year of the index examination. Weight, measured to the nearest pound, was obtained with the participant wearing a gown without slippers or shoes. To calculate body mass index (BMI), we divided weight in kilograms by height in meters squared.

3.D.2. Laboratory determinations

Fasting glucose was measured in fresh plasma with a hexokinase reagent kit (A-gent glucose test; Abbott, South Pasadena, CA). Glucose assays were run in duplicate; intra-assay coefficients of variation were around 3% (133). The fasting total plasma cholesterol and triglycerides were measured enzymatically, and the HDL cholesterol fraction was measured after precipitation of low-density and very low-density lipoproteins with dextran sulfate-magnesium (134).

Insulin was measured in EDTA plasma as total immunoreactive insulin (Coat-A-Count Insulin; Diagnostic Products, Los Angeles, CA). Cross-reactivity of this assay with proinsulin at midcurve is around 40%, the intra-assay and inter-assay coefficients of variation ranged from 5.0 to 10.0%, and the lower limit of sensitivity was 8 pmol/l. HbA1C was measured only at exam 5 (1991-1994) by high-performance liquid chromatography after an overnight dialysis against normal saline to remove the labile fraction.

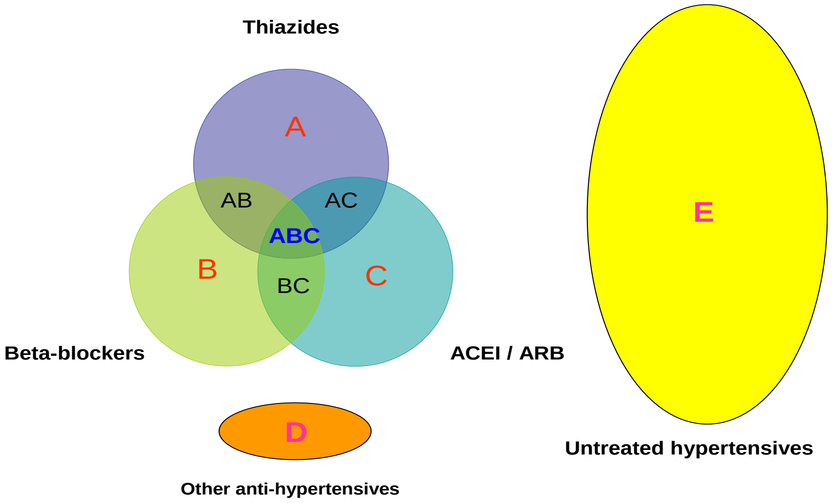
3.D.3. Statistical analyses

3.D.3.1. Sequence of actions

Step 1: We defined three "**hypertension exposure**" **classifications**. We classified individuals at each exam as:

- Having or not having hypertension.
- Having medication-treated hypertension or untreated hypertension.
- Among those treated, into medication treatment groups: we classified individuals on hypertensive treatment into five mutually exclusive antihypertensive drug exposure groups: thiazides only; beta-blockers only; ACE inhibitors/ARB only; any combination of the previous three classes; and "other" hypertensive therapies (calcium-channel blockers, reserpine derivatives, methyldopa, alpha-1 agonists, alpha-2 blockers, and peripheral vasodilators) (**Figure 5**).

Figure 5. **Visual representation of the anti-hypertensive drug exposures in the hypertensive subjects**



We believed that the comparison between “pure” exposures (thiazides only; beta-blockers only; ACE inhibitors/ARB only; any combination of the previous three classes; and “other”) vs. untreated hypertensive subjects would yield the most interesting results on a clinical basis, though at the expense of possibly jeopardizing the statistical power of our sample. Alternative hypotheses could have been to compare “mixed” exposures (i.e., for thiazides, [A+AB+AC+ABC] in **Figure 5**) vs. untreated hypertensive subjects; or to compare each exposure drug vs. treated hypertensive subjects receiving alternative treatments (i.e., thiazides exposure vs. treatments other than thiazides: [A+AB+AC+ABC] vs. [B+BC+C+D] in **Figure 5**; and so on).

Step 2: We evaluated the **baseline characteristics** of the population included in our study (natural, demographic, coexisting conditions), using the chi-square test to compare dichotomous variables and the T-test to compare means for continuous variables.

Step 3: We next defined the **genetic exposures**. To assess genetic influences on change in fasting glucose levels, we used 16 fasting glucose-associated SNPs reported to influence beta-cell function (89,96). For risk of T2DM, we only used 33 out of 40 T2DM-associated SNPs thought to influence beta-cell function (80-92). As mentioned earlier, all SNPs were selected from large studies showing robust, replicated statistical association.

In order to overcome the issues of limited statistical power and multiple testing, we decided to use the two lists of SNPs to build a16-fasting glucose-beta cell SNP genetic risk score, and a 33 T2DM-beta cell SNP genetic score (8 SNPs overlapped), modeled as additive weighted genetic scores. According to our main hypothesis, we evaluated the association of the fasting glucose-SNP genetic score with change over time in fasting glucose, and the association of the T2DM-SNP genetic score with risk of incident T2DM.

For the construction of the 16-fasting glucose SNP and the 33-T2DM SNP additive weighted genetic risk scores, we counted risk alleles (0,1,2) for each genotyped SNP -or its dosage, when imputed- and multiplied each SNP genotype by its published beta coefficient for unit increase in fasting glucose or in diabetes risk (89,90). We used the ORs for T2DM risk from the replication stages of the large GWAS, when the associations were replicated or found in GWAS; for those SNPs that were not replicated in the large GWAS, we used the ORs from the replication stages of the discovery publications. For FG, we used the beta effects reported by Dupuis et al. (89).

We added up the product of that multiplication at each SNP, divided the sum by twice the sum of the betas and multiplied the result by the number of SNPs. The genetic scores can be interpreted as a measure of beta-cell frailty due to having been built by means of counting SNPs with deleterious effects on glycemia putatively mediated by impaired beta cell biology. This approach by building genetic risk scores has been used extensively in previous work (135).

Step 4: We tested the association of hypertension, hypertension treatment and specific antihypertensive drug exposures with the **outcomes**: change over time in fasting glucose levels (calculated as fasting glucose end minus beginning of each time period) or risk of T2DM. We analyzed change in fasting glucose and T2DM risk as a function of baseline hypertension category and genetic status using a **pooled regression** approach, in age-, sex-adjusted models (136): we pooled clinical data from the 2,922 non-diabetic individuals for whom complete clinical data for all variables were available at every exam into 6 time (~4-year) periods (exams 2-3, 3-4, 4-5, 5-6, 6-7 and 7-8). We thus generated **15,852 person-observations** pertaining to 6 time periods, with a mean pool length of 4.15 years over a maximum of 28.3 years of study follow up.

As a result of pooling, the information from each individual for the start point of each time period contributed independently from the information belonging to a different time period. This way, the possibility of switching treatments and changes in covariates over time -like, for instance, body weight- could be accounted for. This method, named "pooled logistic regression" or "logistic regression with pooled repeated measures" has been widely used in Framingham (135,137) and has been shown to produce similar point estimates and variances as time-dependent covariate Cox regression analysis, provided that the exams are separated by relatively short time periods, that the outcome is relatively uncommon, and that people who develop the outcome are excluded from the following observation period (138). Odds Ratios can overestimate the relative risks in case disease prevalence increases.

To test change over time (~4-year) in fasting glucose levels, we used age-, sex-, baseline fasting glucose-adjusted Generalized Estimating Equations (GEE) models with the FAM IDENTITY link function (139). For ~4-year risk of incident T2DM, we used age-, sex-adjusted pooled logistic regression GEE models (LOGIT link). Use of GEE with family identity as the clustering unit allowed us to account for correlated observations in individuals within families. GEE method simultaneously analyzes the cross-sectional relationship between each of the independent variables and change in fasting glucose or incident T2DM, and the relationship between changes in these variables and changes in fasting glucose or incident T2DM over time. The REPEATED and SUBJECT options in PROC GENMOD allow accounting for correlated observations in both family and individual identities.

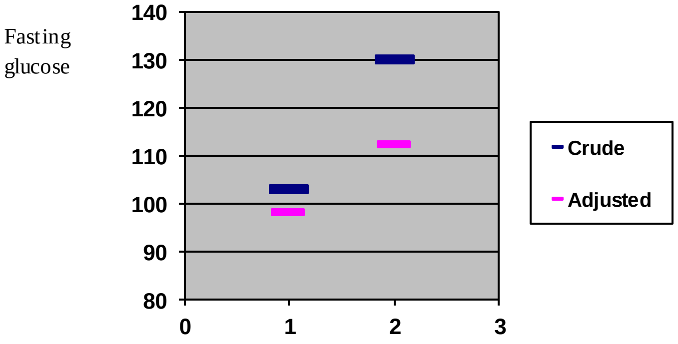
We excluded diabetes cases at baseline of each time period. For the change over time in fasting glucose analyses, we additionally excluded people who had started diabetes treatment within a given time period.

Step 5: Our main hypothesis was to evaluate whether common SNPs associated with fasting glucose or T2DM that appear to influence beta-cell function interact with hypertension or antihypertensive drugs in their positive associations with: 1) ~4-year change in fasting glucose, and 2) ~4-year risk of incident T2DM.

Thus, to analyze whether hypertension categories and genetic scores interact to influence change over time in fasting glucose or incident T2DM beyond main effects, we tested **first-order interaction terms** between hypertension, antihypertensive treatment or specific drug-exposures and each genetic score. Interaction terms took the form: trait = $\beta0 + \beta c*covariates + \beta1*E + \beta2*SNP + \beta3*E*SNP + \varepsilon$, where E represented drug exposure and SNP denoted genetic contributions. The result of interest was the P value for the test H0: $\beta3 = 0$. In this way, we tested our primary hypothesis that the aggregate genetic risk for β-cell dysfunction interacted with drug exposure to determine change in fasting glucose and risk of T2DM. Visual representations of hypothetical interaction effects between a drug exposure and an individual SNP or a genetic risk score on fasting glucose levels are shown in **figure 6**.

Figure 6: Visual representation of the proposed hypothetical interaction effects between an anti-hypertensive treatment and an individual SNP or the genetic risk score, for fasting glucose levels.

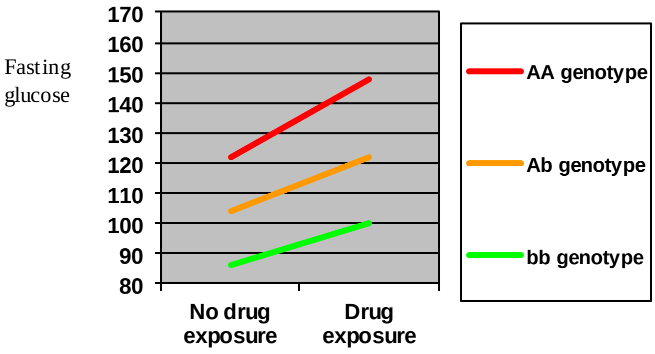
Figure 6.1. Association drug exposure-trait before and after genotype-adjustment



Crude: $FG=\alpha+\beta_1 Rx$
Adj. for genotype: $FG: \alpha+\beta_1 Rx+\beta_2 Ab+\beta_3 AA$
We test Ho: $\beta_2=\beta_3=0$

When the model is additionally adjusted for covariates that can explain part of the effect (i.e., genotype), the marginal effect is weaker (thus, the slope for the pink marks is flatter than for the blue marks).

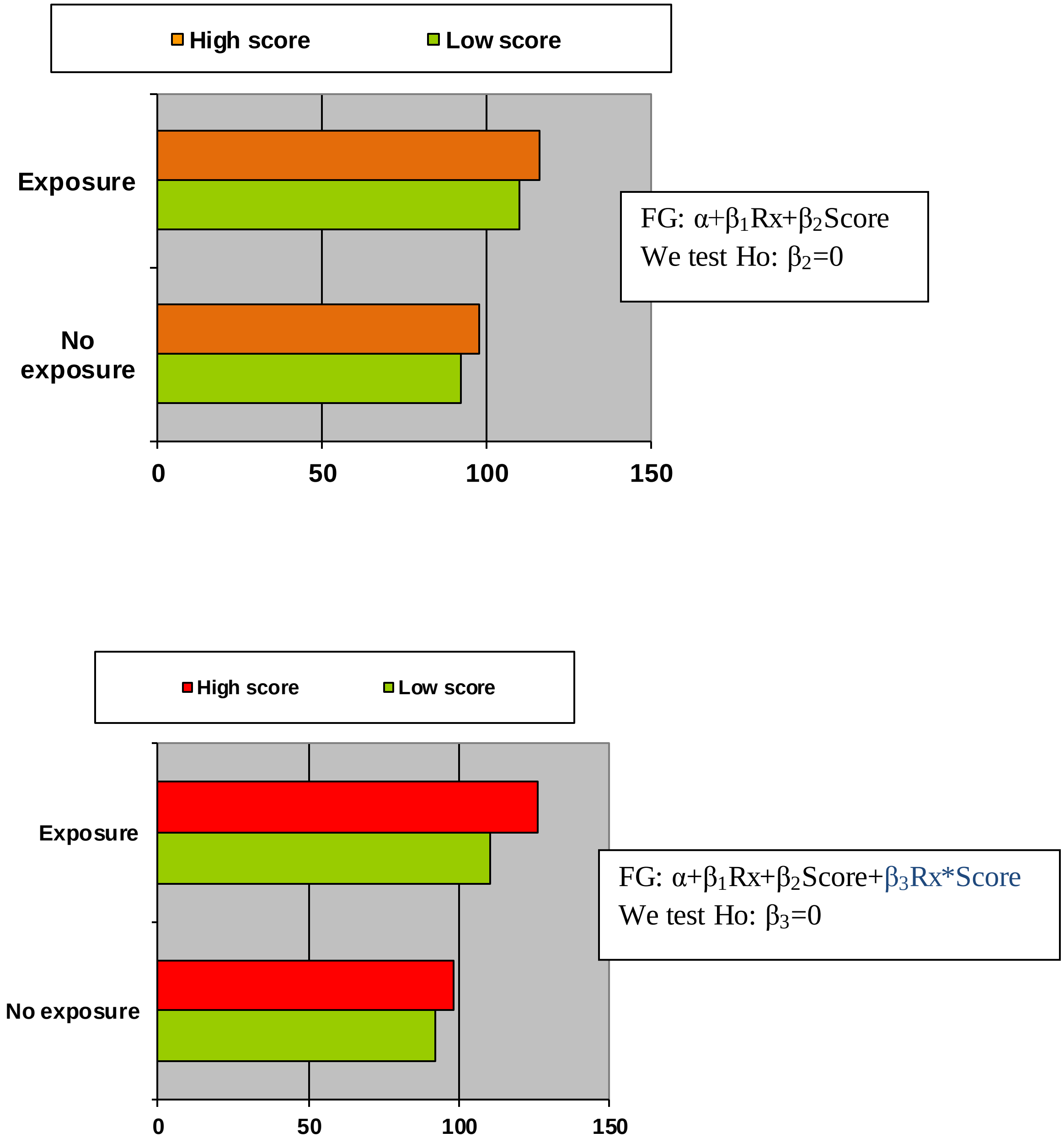
Figure 6.2. Interaction among drug exposure and SNP



$FG: \alpha+\beta_1 Rx+\beta_2 Ab+\beta_3 AA+\beta_4 Rx*Ab+\beta_5 Rx*AA$
We test Ho: $\beta_4=\beta_5=0$
New interaction terms (in blue) are entered in the regression formula

Across the spectrum of different values of the genotype, the effect that the exposure exerts on the trait differs. This is an example of statistical interaction: depicted lines are not parallel.

Figure 6.3. An additive interaction effect is present between drug exposure and the genetic risk score in the lower pannel (abscissa axis fasting glucose in mg/dl).



FG: $\alpha+\beta_1 Rx+\beta_2 Score$
We test Ho: $\beta_2=0$

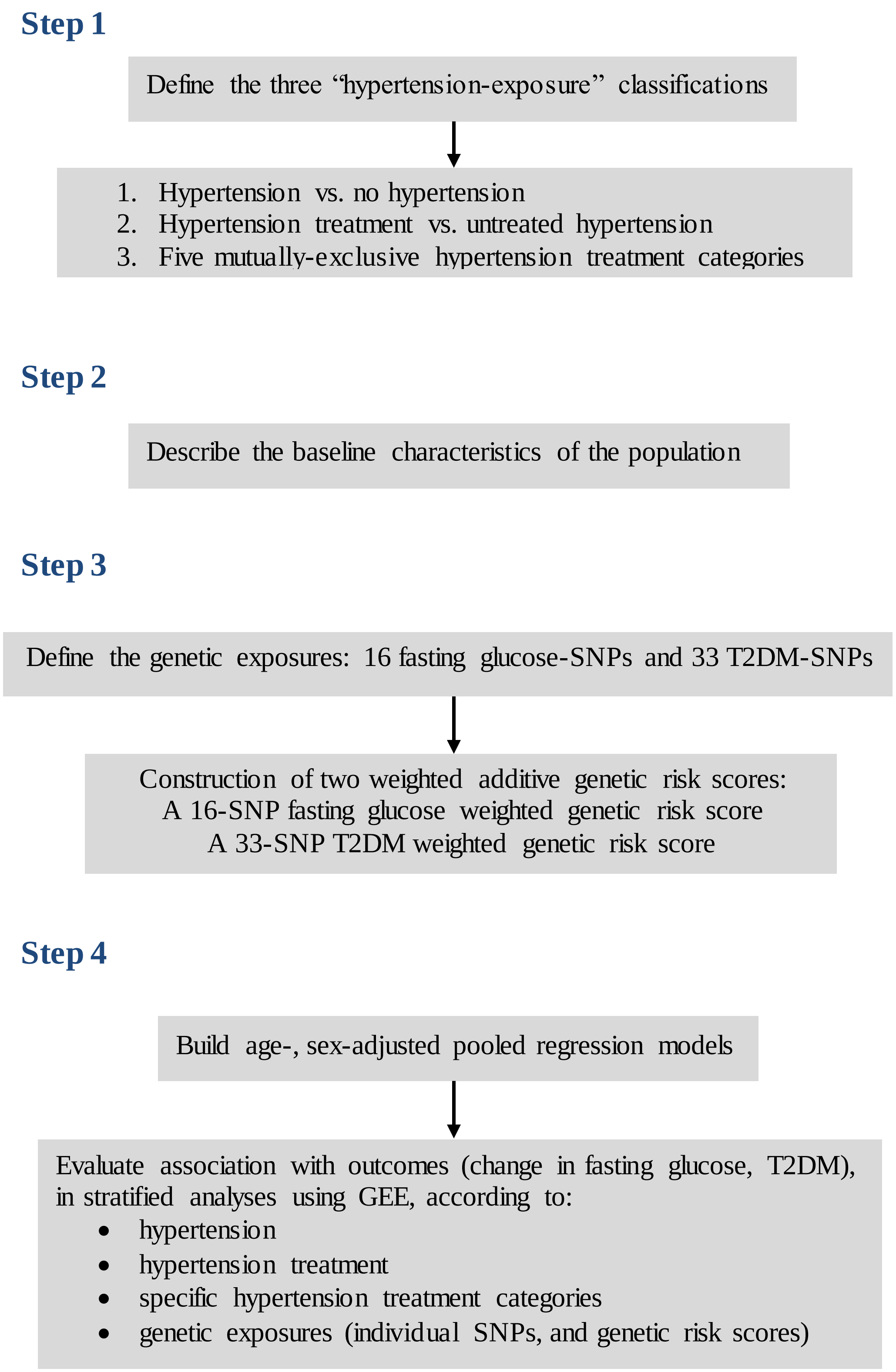
FG: $\alpha+\beta_1 Rx+\beta_2 Score+\beta_3 Rx*Score$
We test Ho: $\beta_3=0$

Step 6: In *a priori* specified secondary analyses, we evaluated **joint models without interaction terms** to test the hypotheses that, in case no interactions were found, hypertension or antihypertensive treatments and the genetic scores had independent associations with ΔFG, or incident T2DM.

Step 7: In additional secondary analyses, we tested associations in models with **additional adjustment** for T2DM risk factors measured at the baseline of each period (mean blood pressure, body mass index, smoking, plasma high-density lipoprotein (HDL) and triglyceride (TG) levels (both per mg/dl), and self-reported alcohol consumption (ounces per week)) to see whether these confounders altered observed associations.

Statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Cary, NC). An empirical p value <0.05 indicated statistical significance for interaction terms or main effects, as we established one hypothesis for change over time in fasting glucose, and a separate one for T2DM risk.

Figure 7. Flow-chart showing the sequential steps in the statistical analysis.



Step 1

Define the three “hypertension-exposure” classifications

1. Hypertension vs. no hypertension
2. Hypertension treatment vs. untreated hypertension
3. Five mutually-exclusive hypertension treatment categories

Step 2

Describe the baseline characteristics of the population

Step 3

Define the genetic exposures: 16 fasting glucose-SNPs and 33 T2DM-SNPs

Construction of two weighted additive genetic risk scores:
A 16-SNP fasting glucose weighted genetic risk score
A 33-SNP T2DM weighted genetic risk score

Step 4

Build age-, sex-adjusted pooled regression models

Evaluate association with outcomes (change in fasting glucose, T2DM), in stratified analyses using GEE, according to:

- hypertension
- hypertension treatment
- specific hypertension treatment categories
- genetic exposures (individual SNPs, and genetic risk scores)

Step 5

Seek first-order interaction effects between:

Hypertension, hypertension treatment, or specific drug exposures
*
16-fasting glucose SNP or 33-T2DM SNP genetic risk scores
on
~4-year change in fasting glucose levels, or ~4-year risk of incident T2DM

Step 6

Look for independent associations of:

hypertension or its treatments and genetic exposures
in joint models without interaction terms
with change in fasting glucose or T2DM risk

Step 7

Secondary analyses in fully-adjusted models for
additional risk factors for T2DM

3.D.4. Statistical power to detect hypertension-genetic score and drug exposure-genetic score interaction

Only for very weak marginal effects (OR≈1.2) and at least moderate interactions (OR≈1.5), interactions are detectable with smaller sample sizes than the marginal effects. Rather, usually larger sample sizes are needed to detect interaction effects.

We performed estimations of the study power for the gene-environment interaction tests using Quanto software, version 1.2.4 (140), prior to conducting the association analyses, in an attempt to calculate the statistical power that we had with the Framingham Offspring Study population. We estimated statistical power for the hypertension vs. no hypertension analysis, and for the beta-blockers exposure vs. untreated hypertensive, as an example of specific drug exposure analysis. In age-, sex-adjusted models, sample sizes in our study varied between 14,673 for change in fasting glucose and 15,852 for T2DM risk in the hypertension vs. no hypertension analyses, and between 4,492 for change in fasting glucose and 4,900 for T2DM risk in the specific drug exposure analyses.

According to previously published data, we assumed an additive genetic model using an average per-allele effect size of 0.45 mg/dl on fasting glucose (89), and an average per-allele relative risk of 1.15 for incident T2DM (90), interacting with binary environmental exposures with effect sizes of 3.24 mg/dl for hypertension and of 0.011 mg/dl for beta-blockers exposure in the change in fasting glucose analyses, and relative risks of 2.4 for hypertension, and 1.28 for beta-blockers exposure in the T2DM analyses (37).

We had 80% statistical power, with a type I error of 0.05 in two-tailed analyses, and for allele frequencies between 20% and 80%, to detect regression coefficients ranging from 0.04 to 0.05 for the genetic score-hypertension interaction tests, and from 0.10 to 0.12 for the genetic score-beta blockers exposure interaction test in the change in fasting glucose analyses, and coefficients ranging from 1.07 to 1.08 for the genetic score-hypertension interaction tests, and from 1.19 to 1.23 for the genetic score-beta blockers exposure interaction test in the T2DM analyses.

3.D.5. Permissions and institutional review board approval

All participants gave written informed consent. The study protocols were approved by the Institutional Review Board at the Boston University Medical Center. Use of SHARe information by this author was allowed by the National Center for Biotechnology Information Genotypes and Phenotypes Database (NCBI dbGaP) Data Access Request system at the National Institutes of Health (141), on May 3, 2010.

4. RESULTS

4.A. Base-line characteristics of the study population

Mean age was 51.9 years, and women constituted 53.8% of our study population. As people in the Framingham Offspring Study aged, BMI increased and hypertension and hypertension treatment became more prevalent, but less people were smokers (**Table 11**).

Table 11. Base-line characteristics of the Framingham Offspring Study population at each exam (exam 1 excluded).

	Exam 2 (1979-1983)	**Exam 3** (1983-1987)	**Exam 4** (1987-1991)	**Exam 5** (1991-1995)	**Exam 6** (1995-1998)	**Exam 7** (1998-2001)
	Mean or n (§SD or %)	Mean or n (SD or %)	Mean or n (SD or %)	Mean or n (SD or %)	Mean or n (SD or %)	Mean or n (SD or %)
Number of subjects (n)	2,622	2,841	2,867	2,681	2,581	2,273
Age, years	43.4 (9.9)	47.6 (9.9)	50.6 (9.8)	54.0 (9.8)	57.9 (9.6)	59.8 (9.1)
Male sex, (%)	1,243 (47.4)	1,353 (47.6)	1,339 (46.7)	1,218 (45.4)	1,167 (45.2)	1,000 (44.0)
Family history of diabetes	469 (17.9%)	508 (17.9%)	515 (18.0%)	482 (18.0%)	468 (18.1%)	388 (17.1%)
*Systolic blood pressure	121 (16)	122 (16)	125 (18)	125 (18)	127 (9)	125 (18)
Diastolic blood pressure	77 (10)	79 (10)	79 (10)	74 (10)	76 (5)	74 (10)
Blood pressure drugs (%)	232 (8.9%)	400 (14.1%)	432 (15.1%)	428 (16.0%)	631 (24.5%)	640 (28.2%)
Hypertension, (%)	520 (19.8%)	775 (27.3%)	910 (31.8%)	819 (30.6%)	973 (37.7%)	903 (39.8%)
†BMI, kg/m^2	25.7 (4.2)	26.0 (4.5)	26.6 (4.8)	27.1 (4.7)	27.5 (4.9)	27.7 (5.0)
Smoking, (%)	955 (36.5%)	887 (31.3%)	729 (25.5%)	509 (19.0%)	450 (17.5%)	333 (14.9%)
‡HDL cholesterol, mg/dl	49.0 (13.4)	51.9 (14.8)	50.2 (14.7)	51.0 (15.0)	52.2 (16.1)	55.4 (17.0)
Triglycerides, mg/dl	100.5 (83.2)	115.5 (89.7)	117.2 (88.1)	140.7 (105.3)	134.3 (88.6)	128.6 (80.4)
Fasting glucose, mg/dl	95.9 (9.4)	91.1 (9.2)	90.8 (9.1)	94.8 (9.6)	96.7 (9.8)	96.8 (9.6)
Alcohol, ounces/week	3.6 (4.8)	3.3 (4.7)	2.9 (4.2)	2.6 (3.7)	2.4 (3.6)	2.7 (3.7)

* Blood pressure: in mmHg. †BMI: body mass index. ‡HDL: high density lipoprotein. §SD: standard deviation.

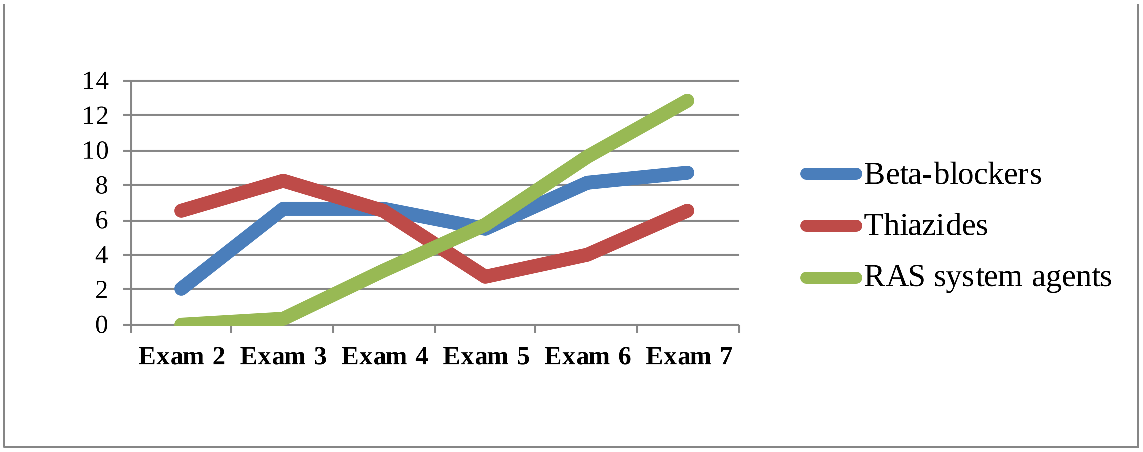
Also, the prevalence of co-existing medical conditions increased with aging (**Table 12**). The pattern of anti-hypertensive drug treatment showed two peaks in the case of beta-blockers and thiazides, and RAS system agents use followed a lineally ascending trend (**Figure 8**).

Table 12. Co-existing conditions and incident T2DM in Framingham Offspring Study at each exam (exam 1 excluded).

	Exam 2 (1979-1983)	**Exam 3** (1983-1987)	**Exam 4** (1987-1991)	**Exam 5** (1991-1995)	**Exam 6** (1995-1998)	**Exam 7** (1998-2001)
	Mean or n (‡SD or %)	Mean or n (SD or %)	Mean or n (SD or %)	Mean or n (SD or %)	Mean or n (SD or %)	Mean or n (SD or %)
Cardiovascular disease, (%)	74 (2.8%)	146 (5.1%)	161 (5.6%)	177 (6.6%)	209 (8.1%)	197 (8.7%)
Coronary heart disease, (%)	54 (2.1%)	107 (3.8%)	117 (4.1%)	118 (4.4%)	143 (5.5%)	132 (5.8%)
Chronic heart failure, (%)	1 (0.0%)	4 (0.1%)	4 (0.1%)	8 (0.3%)	15 (0.6%)	12 (0.5%)
Intermitent claudication, %	16 (0.6%)	31 (1.1%)	37 (1.3%)	50 (1.9%)	52 (2.0%)	45 (2.0%)
Beta-blockers users, (%)	56 (2.1%)	190 (6.7%)	189 (6.6%)	147 (5.5%)	214 (8.3%)	252 (11.1%)
Thiazides users, (%)	170 (6.5%)	235 (8.3%)	186 (6.5%)	73 (2.7%)	102 (4.0%)	148 (6.5%)
RAS users, (%)	-	8 (0.3%)	90 (3.1%)	152 (5.7%)	246 (9.6%)	291 (12.8%)
Incident diabetes cases	32	39	75	91	70	115

*Denote prevalence, not incidence. †RAS: renin-angiotensin agents. ‡SD: standard deviation.

Figure 8. Pattern of use of anti-hypertensive drugs along time (percentages).



People with hypertension were older, more frequently male and had higher BMIs and worse lipid profiles than people without hypertension (**Table 13**).

Table 13. Base-line characteristics stratified by hypertension status.

	Hypertension status		
	Yes (*HTN=1)	No (HTN=0)	
Number of subjects (n)	4,900	10,952	
	Mean (§Std. Dev.)	**Mean (Std. Dev.)**	**p-value**
Age, years	57.8 (10.0)	49.3 (10.7)	<.0001
Male sex, %	52.1 (0.5)	43.4 (0.5)	<.0001
†BMI, kg/m^2	28.5 (5.1)	26.0 (4.3)	<.0001
Smoking, %	19.1 (0.4)	26.9 (0.4)	<.0001
‡HDL cholesterol, mg/dl	49.8 (15.7)	52.3 (15.0)	<.0001
Triglycerides, mg/dl	148.0 (96.1)	111.2 (85.9)	<.0001
Alcohol, ounces/week	3. 4 (4.9)	2.7 (3.8)	<.0001

*HTN: Hypertension. †BMI: Body mass index. ‡HDL: High density lipoprotein. §Std. Dev.: Standard Deviation

People with untreated hypertension were younger, more frequently male, and had higher blood pressure values than their treated counterparts (**Table 14**).

Table 14. Base-line characteristics stratified by hypertension treatment status.

	All hypertensive		
	Treated hypertensive	Untreated hypertensive	
Number of subjects (n)	2,763	2,137	
	Mean (§Std. Dev.)	**Mean (Std. Dev.)**	**p-value**
Age, years	59.5 (9.4)	55.7 (10.3)	<.0001
Male sex, %	50.6 (0.5)	54.1 (0.5)	0.013
Mean *BP, mmHg	98 (11)	107 (8)	<.0001
†BMI, kg/m^2	28.7 (5.0)	28.4 (5.2)	0.06
Smoking, %	16.6 (0.4)	22.4 (0.4)	<.0001
‡HDL cholesterol, mg/dl	49.2 (15.5)	50.7 (16.0)	0.001
Triglycerides, mg/dl	149.1 (89.2)	146.7 (104.3)	0.40
Alcohol, ounces/week	3.2 (4.6)	3.7 (5.3)	0.0003

*BP: Blood pressure. †BMI: Body mass index. ‡HDL: High density lipoprotein. §Std. Dev.: Standard Deviation. Mean BP = ([2xdiastolic BP] + systolic BP) / 3.

Base-line differences among people treated with different anti-hypertensive drugs and the untreated hypertensive group are shown in **Table 15**. Differences with the reference group were nominally significant in regard to age, sex, BMI and smoking status.

Table 15. Base-line characteristics stratified by specific hypertension treatment drug category (reference group: untreated hypertensive)

	Untreated hypertensive	**Taking beta-blockers only**		**Taking thiazides only**		**Taking ||ACE-I/ARB only**	
Nº of subjects	2,137	676		527		516	
	Mean (§Std Dev)	**Mean (Std Dev)**	**p-value**	**Mean (Std Dev)**	**p-value**	**Mean (Std Dev)**	**p-value**
Age, years	55.7 (10.3)	60.2 (9.3)	<.0001	55.4 (9.4)	<.0001	60.6 (8.8)	<.0001
Male sex, %	54.1 (0.5)	59.0 (0.5)	0.0001	42.3 (0.5)	<.0001	56.8 (0.5)	0.03
Mean *BP	107 (8)	98 (12)	<.0001	101 (10)	0.04	96(11)	<.0001
†BMI, kg/m^2	28.4 (5.2)	28.2 (4.6)	0.03	28.5 (5.2)	0.83	28.7 (4.8)	0.48
Smoking, %	22.4 (0,42)	16.0 (0,37)	0.02	22.4 (0.4)	0.06	11.0 (0,31)	<.0001
‡HDL	50.7 (16.0)	46.0 (13.3)	<.0001	50.3 (16.3)	0.49	49.7 (15.3)	0.86
Triglycerides	146.7 (104.3)	155.9 (94.6)	0.02	140.9 (73.8)	0.03	143.9 (79.8)	0.23
Alcohol	3.7 (5.3)	3.4 (5.1)	0.89	3.1 (4.4)	0.15	3.2 (4.3)	0.32

*BP: Blood pressure. Mean BP = ([2xdiastolic BP] + systolic BP)/3, in mmHg. Alcohol: ounces/week.

†BMI: Body mass index. ‡HDL: High density lipoprotein cholesterol. HDL and triglycerides, in mg/dl.

§Std Dev: Standard Deviation.

Drug exposure groups: subjects treated with more than drug have been excluded.

NOTE: Uncorrected for multiple testing. Initiation of RAS systemagents use started later in time. Thus, caution should be exercised when drawing conclusions.

4.B. Association of hypertension, hypertension treatment and specific drug exposures with change over time in fasting glucose

Versus no hypertension, hypertension was associated with 0.9 mg/dl higher ~4-year change in fasting glucose (**Table 16**).

Table 16: Association of hypertension with change over time in fasting glucose

	Age-, sex-adjusted: hypertensive vs. non-hypertensive		
	Hypertension status		
	Yes	No	
Number of subjects (n) (person-exams)	4,492	10,181	
	Mean (*Std. Error)	Mean (Std. Error)	p-value
Age, years	57.8 (10.0)	49.3 (10.7)	<.0001
Male sex, %	52.1 (0.5)	43.5 (0.5)	<.0001
	Estimates (Std. Error)	Estimates (Std. Error)	p-value
Fasting glucose, mg/dl	96.5 (0.1)	93.0 (0.1)	<.0001
Change in fasting glucose, mg/dl	**2.6** (0.2)	**1.7** (0.1)	**<.0001**

Fasting glucose and change in fasting glucose are mean values across all exams.

Change in fasting glucose was additionally adjusted for base-line fasting glucose at the beginning of each time period.

Subjects who started anti-diabetic treatment during a given time period were excluded from the fasting glucose analysis.

*Std Error: standard error.

Versus untreated hypertension, overall treated hypertension conferred 0.4 mg/dl higher change in fasting glucose, what might suggest that treating hypertension without even accounting for specific drug exposures, further increases change over time in fasting glucose (**Table 17**).

Table 17: Association of hypertension treatment with change over time in fasting glucose.

	Age-, sex-adjusted: treated vs. untreated hypertensive		
	All hypertensive		
	Treated hypertensive	Untreated hypertensive	
Number of subjects (n) (person-exams)	2,517	1,985	
	Mean (Std. Error)	Mean (Std. Error)	p-value
Age, years	59.5 (9.4)	55.7 (10.3)	<.0001
Male sex, %	50.6 (0.5)	54.1 (0.5)	0.013
	Estimates (Std. Error)	Estimates (Std. Error)	p-value
Fasting glucose, mg/dl	98.4 (0.2)	96.7 (0.2)	<.0001
Change in fasting glucose, mg/dl	**3.4** (0.3)	**3.0** (0.3)	**<.0001**

Fasting glucose and change in fasting glucose are mean values across all exams.

Change in fasting glucose was additionally adjusted for base-line fasting glucose at the beginning of each time period.

Subjects who started anti-diabetic treatment during a given time period were excluded from the fasting glucose analysis.

*Std Error: standard error.

Versus untreated hypertension, RAS agents and combination treatment were associated with a higher absolute ~4-year change in fasting glucose, whereas beta-blockers, thiazides and "others" were not (**Tables 18A**, **18B**, **18C**, **18D**, **18E**).

Table 18A: Association of beta-blockers exposure with change over time in fasting glucose.

	Age-, sex-, fasting glucose-adjusted models		
	Untreated hypertension	**Beta-blockers only**	
Number of subjects (person-exams)	1,985	618	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.2 (9.2)	<.0001
Male sex,%	54.1 (0.5)	59.0 (0.5)	0.0001
	Estimates (†Std. Error)	Estimates (Std. Error)	p-value
Fasting glucose, mg/dl	96.7 (0.2)	98.8 (0.4)	<.0001
Change in fasting glucose, mg/dl	2.8 (0.3)	3.4 (0.5)	0.351

†Std. error: Standard error.

Table 18B: Association of thiazides exposure with change over time in fasting glucose.

	Age-, sex-, fasting glucose-adjusted models		
	Untreated hypertension	**Thiazides only**	
Number of subjects (person-exams)	1,985	495	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	55.4 (9.4)	<.0001
Male sex,%	54.1 (0.5)	42.3 (0.5)	<.0001
	Estimates (†Std. Error)	Estimates (Std. Error)	p-value
Fasting glucose, mg/dl	96.7 (0.2)	98.0 (0.4)	0.008
Change in fasting glucose, mg/dl	2.8 (0.3)	2.0 (0.6)	0.237

†Std. error: Standard error.

Table 18C, D and E: Association of ACE-inhibitors/ARB, “combination” and “other” exposures with change over time in fasting glucose (†Std. error: Standard error)**.**

	Age-, sex-, fasting glucose-adjusted models		
	Untreated hypertension	**ACE-I / ARB only**	
Number of subjects (person-exams)	1,985	457	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.5 (8.8)	<.0001
Male sex,%	54.1 (0.5)	56.8 (0.5)	0.025
	Estimates (†Std. Error)	Estimates (Std. Error)	p-value
Fasting glucose, mg/dl	96.7 (0.2)	96.5 (0.5)	0.633
Change in fasting glucose, mg/dl	2.8 (0.3)	4.2 (0.6)	**0.037**
	Untreated hypertension	**“Combination”**	
Number of subjects (person-exams)	1,985	448	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.0 (9.4)	<.0001
Male sex,%	54.1 (0.5)	47.3 (0.5)	0.023
	Estimates (†Std. Error)	Estimates (Std. Error)	p-value
Fasting glucose, mg/dl	96.7 (0.2)	100.5 (0.5)	<.0001
Change in fasting glucose, mg/dl	2.8 (0.3)	4.2 (0.6)	**0.046**
	Untreated hypertension	**“Others”**	
Number of subjects (person-exams)	1,985	499	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.9 (9.0)	<.0001
Male sex,%	54.1 (0.5)	45.1 (0.5)	0.0005
	Estimates (†Std. Error)	Estimates (Std. Error)	p-value
Fasting glucose, mg/dl	96.7 (0.2)	98.4 (0.4)	0.0008
Change in fasting glucose, mg/dl	2.8 (0.3)	4.0 (0.6)	0.06

4.C. Association of hypertension, hypertension treatment and specific drug exposures with T2DM risk.

Versus no hypertension, hypertension was significantly associated with ~3-fold increased odds of T2DM (OR=2.9; 95% CI 2.8-3.0) (**Table 19**).

Table 19: Association of hypertension with estimated incident risk of T2DM

	Age-, sex-adjusted: hypertensive vs. non-hypertensive		
	Hypertension status		
	Yes	No	
Number of subjects (n) (person-exams)	4,900	10,952	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	57.8 (10.0)	49.3 (10.7)	<.0001
Male sex, %	52.1 (0.5)	43.5 (0.5)	<.0001
Incidence of diabetes model, %	**3.6%** (1.5%)	**1.2%** (0.5%)	**<.0001**

Note: Subjects who started anti-diabetic treatment during a period were excluded from the fasting glucose analysis, so numbers of subjects for the fasting glucose analyses were lower than for T2DM.

Versus untreated hypertension, treated hypertension significantly conferred 40% increased odds of T2DM (OR=1.4; 95% CI 1.3-1.5) (**Table 20**).

Table 20: Association of hypertension treatment with estimated incident risk of T2DM

	Age-, sex-adjusted: treated vs. untreated hypertensive		
	All hypertensive		
	Treated hypertensive	Untreated hypertensive	
Number of subjects (n) (person-exams)	2,763	2,137	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	59.5 (9.4)	55.7 (10.3)	<.0001
Male sex, %	50.6 (0.5)	54.1 (0.5)	0.013
Incidence of diabetes model, %	**4.9%** (0.7%)	**3.6%** (0.5%)	**0.023**

Again, results in **Table 20** suggest that treating hypertension without even accounting for specific drug exposures, further increases risk of incident T2DM. Versus untreated HTN, beta-blockers (OR=1.6; 95% CI 1.1-2.4) (**Table 3**), combination treatment (OR=1.6; 95% CI 1.1-2.5) and others (OR=2.0; 95% CI 1.4-2.9) were associated with increased odds of T2DM, unlike thiazides or ACE-inhibitors / ARB.

Table 21A: Association of beta-blockers exposure with estimated risk of incident T2DM.

	Age-, sex-adjusted.		
	Untreated hypertension	**Beta-blockers only**	
Number of subjects (person-exams)	2,137	676	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.2 (9.2)	<.0001
Male sex,%	54.1 (0.5)	59.0 (0.5)	0.0001
Incidence of diabetes (%)	4.1%	6.6%	**0.010**
Odds Ratio, vs. untreated hypertension	NA	**1.6**	

Table 21B: Association of thiazides exposure with estimated risk of incident T2DM.

	Age-, sex-adjusted.		
	Untreated hypertension	**Thiazides only**	
Number of subjects (person-exams)	2,137	527	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	55.4 (9.4)	<.0001
Male sex,%	54.1 (0.5)	42.3 (0.5)	<.0001
Incidence of diabetes (%)	4.1%	5.8%	0.205
Odds Ratio, vs. untreated hypertension	NA	1.3	

Table 21C: Association of ACE-inhibitors / ARB exposure with estimated risk of incident T2DM.

	Age-, sex-adjusted.		
	Untreated hypertension	**ACE-inhibitors/ARB only**	
Number of subjects (person-exams)	2,137	516	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.5 (8.8)	<.0001
Male sex,%	54.1 (0.5)	56.8 (0.5)	0.025
Incidence of diabetes (%)	4.1%	5.7%	0.239
Odds Ratio, vs. untreated hypertension	NA	1.3	

Table 21D: Association of "combination" exposure with estimated risk of incident T2DM.

	Age-, sex-adjusted.		
	Untreated hypertension	**"Combination" only**	
Number of subjects (person-exams)	2,137	499	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.0 (9.4)	<.0001
Male sex,%	54.1 (0.5)	47.3 (0.5)	0.023
Incidence of diabetes (%)	4.1%	6.3%	**0.019**
Odds Ratio, vs. untreated hypertension	NA	1.6	

Table 21E: Association of "others" exposure with estimated risk of incident T2DM.

	Age-, sex-adjusted.		
	Untreated hypertension	**"Others" only**	
Number of subjects (person-exams)	2,137	545	
	Mean (Standard error)	Mean (Standard error)	p-value
Age, years	55.7 (10.3)	60.9 (9.0)	<.0001
Male sex,%	54.1 (0.5)	45.1 (0.5)	0.0005
Incidence of diabetes (%)	4.1%	7.8%	**0.0003**
Odds Ratio, vs. untreated hypertension	NA	2.0	

4.D. Association of the additive genetic scores with change over time in fasting glucose or risk of T2DM

The effects of individual SNPs upon fasting glucose in the Framingham Offspring Study were mostly in the same direction as in GWAS and of expected effect sizes (**Table 22**).

Table 22. Fasting glucose-associated SNPs thought to act via beta cell biology, and their per allele increase in fasting glucose (FG) according to published and Framingham Offspring Study data.

SNP	*Chr	Position	Closest gene	†T2DM locus	Risk allele	Published beta effects, mg/dl (Standard Error)	FG-adjusted beta effects in Framingham	p value
rs340874	1	212225879	*PROX1*	YES	C	0.23 (0.05)	0.32	0.02
rs560887	2	169471394	*G6PC2*	No	C	1.35 (0.05)	0.28	0.05
rs780094	2	27594741	*GCKR*	YES	C	0.52 (0.05)	0.35	0.004
rs11708067	3	124548468	*ADCY5*	YES	A	0.49 (0.05)	0.35	0.03
rs11920090	3	172200215	*SLC2A2*	No	T	0.36 (0.07)	0.23	0.17
rs2191349	7	15030834	*DGKB/TMEM195*	YES	T	0.54 (0.05)	0.05	0.70
rs4607517	7	44202193	*GCK*	YES	A	1.12 (0.07)	0.48	0.003
rs13266634	8	118253964	*SLC30A8*	YES	C	0.49 (0.07)	0.20	0.41
rs7034200	9	4279050	*GLIS3*	No	A	0.32 (0.05)	0.13	0.29
rs10885122	10	113032083	*ADRA2A*	No	G	0.40 (0.07)	0.30	0.18
rs7903146	10	114748339	*TCF7L2*	YES	T	0.41 (0.07)	0.52	0.001
rs11605924	11	45829667	*CRY2*	No	A	0.27 (0.05)	-0.02	0.89
rs174550	11	61328054	*FADS1*	No	T	0.31 (0.05)	-0.12	0.40
rs7944584	11	47292896	*MADD*	No	A	0.38 (0.05)	0.003	0.98
rs10830963	11	92348358	*MTNR1B*	YES	G	1.21 (0.05)	0.55	0.001
rs11071657	15	60221254	*FAM148B/C2CD4B*	No	A	0.14 (0.05)	0.11	0.48

The model was adjusted for base-line fasting glucose at each time period. *Chr: Chromosome.

†T2DM locus: if "YES", overlapping SNP for T2DM. Published beta effects expressed in mg/dl: Based on Dupuis et al. (89). They represent the increase of the trait in mg/dl per risk allele. FG-adjusted beta effects in Framingham = the increase of the trait in mg/dl per risk allele in our data with FG adjustment only.

The effects of individual SNPs upon T2DM in the Framingham Offspring Study were mostly in the same direction as in the original GWAS and of expected effect sizes, too (**Table 23**).

Table 23. T2DM-associated SNPs thought to act via beta cell biology, and their per allele increase in odds of incident T2DM according to published and Framingham Offspring Study data.
Analysis in the whole sample of participants (n=15,852 person-exams).

SNP	*Chr	Position	Closest gene	†FG locus	Risk allele	Published ‡ORs	T2DM Framingham ORs	p value
rs10923931	1	120319482	*NOTCH2*	No	T	1.14	1.03	0.79
rs340874	1	212225879	*PROX1*	YES	C	1.07	1.16	0.08
rs243021	2	60438323	*BCL11A*	No	A	1.08	1.09	0.23
rs7578597	2	43586327	*THADA*	No	T	1.15	1.22	0.12
rs780094	2	27594741	*GCKR*	YES	C	1.06	1.20	0.01
rs7593730	2	160879700	*RBMS1/ITGB6*	No	C	1.11	1.14	0.12
rs1470579	3	187011774	*IGF2BP2*	No	C	1.14	1.03	0.68
rs11708067	3	124548468	*ADCY5*	YES	A	1.12	1.17	0.10
rs10010131	4	6343816	*WFS1*	No	G	1.11	0.96	0.54
rs4457053	5	76460705	*ZBED3*	No	G	1.08	0.96	0.65
rs7754840	6	20769229	*CDKAL1*	No	C	1.18	1.05	0.51
rs9472138	6	43919740	*VEGFA*	No	T	1.06	1.16	0.06
rs864745	7	28147081	*JAZF1*	No	T	1.20	0.89	0.08
rs2191349	7	15030834	*DGKB/TMEM195*	YES	T	1.06	1.14	0.07
rs4607517	7	44202193	*GCK*	YES	A	1.07	1.05	0.55
rs13266634	8	118253964	*SLC30A8*	YES	C	1.15	0.97	0.85
rs896854	8	96029687	*TP53INP1*	No	T	1.06	0.97	0.68

rs10811661	9	22124094	*CDKN2A/2B*	No	T	1.19	1.37	0.002
rs13292136	9	81141948	*TLE4/CHCHD9*	No	C	1.11	1.25	0.20
rs12779790	10	12368016	*CDC123, CAMK1D*	No	G	1.09	1.02	0.85
rs1111875	10	60221254	*HHEX*	No	C	1.17	1.07	0.33
rs7903146	10	114748339	*TCF7L2*	YES	T	1.40	1.32	0.0001
rs5215	11	17365206	*KCNJ11*	No	C	1.09	1.06	0.45
rs231362	11	2648047	*KCNQ1*	No	G	1.08	0.98	0.77
rs10830963	11	92348358	*MTNR1B*	YES	G	1.13	1.24	0.02
rs1552224	11	72110746	*CENTD2*	No	A	1.14	0.98	0.85
rs2334499	11	1653425	*HCCA2*	No	T	1.35	1.10	0.24
rs1153188	12	53385263	*DCD*	No	A	1.08	0.92	0.29
rs7961581	12	69949369	*TSPAN8, LGR5*	No	C	1.11	1.02	0.84
rs7957197	12	119945069	*OASL/TCF1(HNF1A)*	No	T	1.07	1.12	0.23
rs11634397	15	78219277	*ZFAND6*	No	G	1.06	1.05	0.1
rs8042680	15	89322341	*PRC1*	No	A	1.07	1.06	0.46
rs757210	17	33170628	*HNF1B*	No	T	1.12	0.94	0.72

*Chr: Chromosome. †FG (fasting glucose) locus: if "YES", overlapping SNP for the fasting glucose analysis. ‡OR: Odds Ratio. Published ORs: Based on Voight et al. (90). T2DM OR represents the OR of incident T2DM for every SNP individually in our data with no further adjustments in the model.

Per-weighted genetic score risk allele, change in fasting glucose significantly increased by 0.55 mg/dl per risk allele ($P=8.9x10^{-16}$) in the age-, sex-, fasting glucose-adjusted models (**Table 24**).

Table 24: Effect of each weighted fasting glucose risk allele on change over time in fasting glucose

Age-, sex-, fasting glucose-adjusted	Change in fasting glucose, in mg/dl	
	Estimate	p-value
Age, years	0.2064	<.0001
Sex, % male	1.7059	<.0001
Fasting Glucose, mg/dl	-0.3503	<.0001
Fasting glucose 16-SNP Genetic Risk Score	**0.5488**	**8.88178E-16**

Per-weighted genetic score risk allele, odds of T2DM increased 16.6% (95% CI 10.0-23.6) per risk allele. Individuals who developed diabetes showed significantly higher mean genetic scores than those who did not (17.3 vs. 16.8; $p=2.1x10^{-7}$) (**Table 25**).

Table 25: Mean weighted genetic risk scores in subjects who developed T2DM vs. subjects who did not, and additional estimated risk of incident T2DM per weighted risk allele.

Age-, sex-adjusted model	T2DM		No T2DM		†OR	p-value
	Mean	*Std. Dev	Mean	Std. Dev		
Number of Subjects	422		15,430			
Age, years	57.87	9.57	51.78	11.18	1.053	<.0001
Sex, % male	54.7%		45.9%		1.460	0.0002
Mean (CI) T2D 33-SNP Genetic Risk Score	17.25	1.73	16.84	1.71	**1.166**	**2.08E-07**

*Std. Dev: Standard Deviation. †OR: Odds ratio, per risk-allele.

4.E. Interaction effect between hypertension or its treatment categories and the genetic scores to predict change over time in fasting glucose or incident T2DM

All interaction P values in joint models including interaction terms for hypertension, hypertension treatment or specific antihypertensive drug exposures by genetic scores (in bold), predicting change in fasting glucose or T2DM, were non-significant (all p>0.3) (**Tables 26-28**).

Table 26. Interaction effect by fasting glucose- or T2DM-genetic risk scores on hypertension for change over time in fasting glucose or incident T2DM risk.

	Change in fasting glucose		**T2DM**	
	***Beta, per mg/dl**	**p-value**	**†OR (GEE)**	**p-value**
Number of subjects	14,683		15,852 (422 diabetes cases)	
Age, years	0.18	<.0001	1.03	<0.0001
Sex, male	1.60	<.0001	1.34	0.004
Fasting glucose, mg/dl	-0.36	<.0001		
Hypertension (HTN)	1.25	0.35	4.76	0.14
Mean fasting glucose / T2DM genetic score	0.54	$3.2x10^{-11}$	1.19	0.0004
HTN x Mean fasting glucose / T2DM genetic score	0.10	0.53	0.98	0.70

Age-, sex-adjusted models, with additional baseline fasting glucose levels-adjustment for change over time in fasting glucose. Subjects who started anti-diabetic treatment during a given time period were excluded from the change in fasting glucose analyses.

*Beta effect represents the mg/dl increase in fasting glucose levels per unit increase in the covariate.

†OR: Odds ratio. GEE: generalized estimating equations.

Table 27. Interaction effect by fasting glucose- or T2DM-genetic risk scores on hypertension treatment for change over time in fasting glucose or incident T2DM risk.

	Change in fasting glucose		T2DM	
	*Beta, per mg/dl	p-value	†OR (GEE)	p-value
Number of subjects	4,502		4,900 (272 diabetes cases)	
Age, years	0.09	<.0001	1.01	0.06
Sex, male	0.70	0.07	1.22	0.11
Fasting glucose, mg/dl	-0.22	0.0002		
Treated hypertensive	1.69	0.46	1.18	0.90
Mean fasting glucose / T2DM genetic score	0.52	0.01	1.14	0.038
HTN Treated x Mean ‡FG / T2DM genetic score	-0.11	0.67	1.02	0.83

Age-, sex-adjusted models, with additional baseline fasting glucose levels-adjustment for change over time in fasting glucose. Subjects who started anti-diabetic treatment during a given time period were excluded from the change in fasting glucose analyses.

*Beta effect represents the mg/dl increase in fasting glucose levels per unit increase in the covariate.

†OR: Odds ratio. GEE: generalized estimating equations. ‡FG: fasting glucose.

Table 28. Interaction effect by fasting glucose- or T2DM-genetic risk scores on hypertension specific drug exposures for change over time in fasting glucose or incident T2DM risk.

	Change in fasting glucose		**T2DM**	
	***Beta, per mg/dl**	**p-value**	**†OR (GEE)**	**p-value**
Number of subjects	4,502		4,900 (272 diabetes cases)	
Age, years	0.08	<.0001	1.01	0.10
Sex, male	0.63	0.10	1.23	0.10
Fasting glucose, mg/dl	-0.22	<.0001		
Mean fasting glucose / T2DM genetic score	0.52	0.004	1.14	0.038
Hypertensive exposed to beta-blockers only‡	1.01	0.,77	2.80	0.58
Beta-blockers x Mean fasting glucose / T2DM genetic score	-0.05	0.91	0.97	0.76
Hypertensive exposed to thiazides only‡	-1.30	0.74	0.26	0.56
Thiazides x Mean fasting glucose / T2DM genetic score	0.06	0.90	1.10	0.48
Hypertensive exposed to ACE-I/ARB only‡	2.39	0.57	2.47	0.69
ACE-I/ARB x Mean fasting glucose / T2DM genetic score	-0.11	0.82	0.96	0.77

Age-, sex-adjusted models, with additional baseline fasting glucose levels-adjustment for change over time in fasting glucose. Subjects who started anti-diabetic treatment during a given time period were excluded from the change in fasting glucose analyses.

*Beta effect represents the mg/dl increase in fasting glucose levels per unit increase in the covariate.

†OR: Odds ratio. GEE: generalized estimating equations.

‡Specific drug exposures compared to the reference untreated hypertensive group. ACE-I: Angiotensin converting enzyme inhibitors. ARB: angiotensin receptor blockers. Data for “combination” and “others” not shown.

4.F. Joint models including hypertension, its treatments and genetic scores without interaction terms to predict change over time in fasting glucose or incident T2DM

In joint models without interaction, hypertension, thiazides and renin-angiotensin agents, and per fasting glucose-SNP risk allele independently predicted change over time in fasting glucose (**Tables 29** and **30**). However, neither hypertension treatment nor beta-blockers predicted change over time in fasting glucose.

Table 29. Joint models including hypertension or hypertension treatment and the genetic risk scores upon change over time in fasting glucose without interaction terms.

	Change in fasting glucose	
Joint model: hypertension, genetic score; age-, sex-, fasting glucose-adjusted model	***Beta per mg/dl**	**p-value**
Number of subjects	14,683	
Age, years	0.18	<.0001
Sex, male	1.60	<.0001
Fasting glucose, mg/dl	-0.36	<.0001
Hypertension	2.10	**<.0001**
Mean fasting glucose 16-SNP genetic score	0.57	**<.0001**
Joint model: hypertension treatment, genetic score; age-, sex-, fasting glucose-adjusted model	***Beta per mg/dl**	**p-value**
Number of subjects	4,502	
Age, years	0.09	<.0001
Sex, male	0.70	0.07
Fasting glucose, mg/dl	-0.22	<.0001
Treated hypertensive	0.72	0.07
Mean fasting glucose 16-SNP genetic score	0.45	**0.0008**

We show change in fasting glucose in mg/dl by each unit increase in the covariates (*betas).
Subjects who started antidiabetic treatment during a given time period have been excluded from the fasting glucose analysis.

Table 30. Joint models including specific antihypertensive drug exposures and the genetic risk scores upon change over time in fasting glucose without interaction terms.

Joint model: specific drug, genetic score; age-, sex-, fasting glucose-adjusted model	Change in fasting glucose *Beta per mg/dl	p-value
Number of subjects	4,502	
Age, years	0.09	<.0001
Sex, male	0.67	0.08
Fasting glucose, mg/dl	-0.21	<.0001
Hypertensive exposed to beta-blockers only†	0.17	0.75
Mean fasting glucose 16-SNP genetic score	0.44	**0.0009**
Age, years	0.09	<.0001
Sex, male	0.60	0.12
Fasting Glucose, mg/dl	-0.21	<.0001
Hypertensive exposed to thiazides only†	-1.34	**0.02**
Mean fasting glucose 16-SNP genetic score	0.45	**0.0008**
Age, years	0.09	<.0001
Sex, %male	0.64	0.09
Fasting Glucose, mg/dl	-0.21	<.0001
Hypertensive exposed to ‡ACE-I/ARB only†	1.11	**0.03**
Mean fasting glucose 16-SNP genetic score	0.45	**0.0008**

We show change in fasting glucose in mg/dl by each unit increase in the covariates (*betas).

Subjects who started antidiabetic treatment during a given time period have been excluded from the fasting glucose analysis.

†Specific drug exposures compared to the reference untreated hypertensive group. ACE-I: Angiotensin converting enzyme inhibitors. ARB: angiotensin receptor blockers. Data for “combination” and “others” not shown.

In joint models without interaction, hypertension, hypertension treatment, and T2DM-genetic scores all independently predicted incident T2DM (**Table 31**). No specific hypertension drug predicted incident T2DM in joint models without interaction terms (all p values >0.19).

Table 31. Joint models including hypertension, hypertension treatment or specific drug exposure categories and the genetic scores upon T2DM without interaction terms.

	T2DM	
Joint model: hypertension, genetic score; age-, sex-adjusted model	***OR**	**p-value**
Number of subjects	15,852 (422 diabetes cases)	
Age, years	1.03	<0.0001
Sex, male	1.34	0.004
Hypertension	3.16	**<.0001**
Mean T2DM 33-SNP genetic score	1.17	**$1.2\text{x}10^{-7}$**
Joint model: hypertension treatment, genetic score; age-, sex-adjusted model	***OR**	**p-value**
Number of subjects	4,900 (272 diabetes cases)	
Age, years	1.01	0.06
Sex, male	1.22	0.11
Treated hypertensive	1.57	**0.0004**
Mean T2DM 33-SNP genetic score	1.15	**0.0002**
Joint model: specific drug, genetic score; age-, sex-adjusted model	***OR**	**p-value**
Number of subjects	4,900 (272 diabetes cases)	
Age, years	1.02	0.02
Sex, male	1.20	0.14
Hypertensive exposed to beta-blockers only†	1.25	0.19
Mean T2DM 33-SNP genetic score	1.15	**0.0002**
Age, years	1.02	0.01
Sex, male	1.22	0.11
Hypertensive exposed to thiazides only†	1.03	0.9
Mean T2DM 33-SNP genetic score	1.15	**0.0001**
Age, years	1.02	0.01
Sex, male	1.22	0.11
Hypertensive exposed to ‡ACE-I/ARB only†	0.96	0.84
Mean T2DM 33-SNP genetic score	1.15	**0.0001**

*OR: Odds ratio, the effect on the odds of incident T2DM by each unit increase in each covariate.

†People exposed to each specific antihypertensive drug vs. reference untreated hypertensive group. ACE-I: Angiotensin converting enzyme inhibitors. ARB: angiotensin receptor blockers. Data for “combination” and “others” not shown.

4.G. Secondary analyses

In secondary analyses using models with further adjustment for a full set of possible confounding T2DM risk factors (age, sex, mean blood pressure, body mass index, smoking, high density lipoprotein cholesterol, triglycerides levels, alcohol consumption, with additional adjustment for fasting glucose levels in the change in fasting glucose analyses), the statistically significant results seen in the age-, sex-adjusted models were mostly reproduced (**Appendices 3-9**).

5. DISCUSSION

5.A. Summary of findings. Putting them in the context of previous knowledge

5A.1. Results for the hypertension, hypertension categories and genetic scores exposures.

Preliminarily, it should be noted that the Framingham Offspring Study was started up recruiting subjects who were 35.5 years on average at exam 1 (data not shown). Thus, new onset diabetes rate, number of incident cardiovascular events and number of coexisting conditions were low till the latest stages of follow-up. We decided to exclude diabetes cases at the beginning of each time period to give more robustness to our "pooled" logistic method; we were aware of the possibility of losing statistical power for the change over time in fasting glucose analyses, since diabetic patients may be more vulnerable to hyperglycemic effects attributable to antihypertensive drugs; however, we might have had to deal with the issue of antidiabetic drugs and their confounding effects on the results of the fasting glucose analyses.

We found that hypertension and overall hypertension treatment conferred higher ~4-year change over time in plasma fasting glucose and risk of incident T2DM in non-diabetic subjects in the Framingham Offspring Study. Previous work had shown positive associations of hypertension with higher fasting glucose and T2DM risk (37). These associations had been reported to be stronger in European-ancestry Americans, such as Framingham Study's participants, whereas in African Americans additional risk factors apparently confounded the associations (19). Additionally, several studies had also demonstrated that genetic variation predicted the individual systolic blood pressure response to antihypertensive drugs (143).

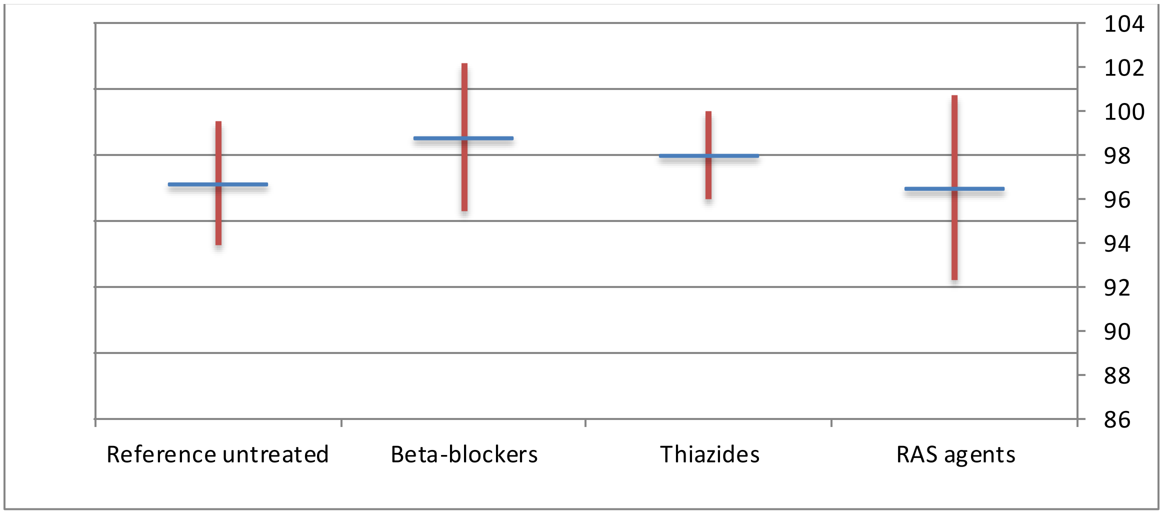
It is worth to mention that treated hypertension appeared to confer higher change over time in plasma fasting glucose and risk of T2DM despite the lower blood pressure values found in people receiving medication to treat high blood pressure. This may suggest that overall hypertension treatment exerts deleterious effects on glycemic traits.

The pattern of historical use of antihypertensive drugs reflects the lack of availability of more recently discovered drugs in the early stages of follow-up. Appearance of RAS agents initially meant lower prescription rates of thiazides and beta-blockers, but participants went on being prescribed these drugs along time as hypertension became more severe.

Renin-angiotensin system agents were nominally associated with change over time in plasma fasting glucose. This finding came as a surprise, since in the literature they have been proposed to be neutral or even have a protective effect in regard to T2DM incidence. Moreover, in our study they conferred a non-significant 30% higher risk of incident T2DM than in the untreated hypertensive group. This may have simply reflected confounding by indication, as physicians may have preferentially prescribed RAS agents more frequently due to physician-perceived higher risks of diabetes in some of the patients.

Also, we must underscore that we calculated change over time in plasma fasting glucose as the absolute difference between fasting glucose levels at the end of the time-period minus at the beginning, a higher “absolute” difference may have just reflected higher “volatility around the line”, as shown in **Figure 9**; that is, many people with significant changes in fasting glucose levels, yet some people with final increased values, whereas others with actually decreased values. In fact, average final fasting glucose values in people treated with RAS agents did not differ from those found in the reference untreated hypertensive group.

Figure 9. Representation of final fasting glucose levels (blue horizontal lines) and change over time in fasting glucose (red lines), in mg/dl.



The increased change in fasting glucose is statistically significant for RAS agents (data for "combination" and "others" not shown).

Exposure to beta-blockers increased ~4-year T2DM risk in the Framingham participants, replicating previous findings in the literature (37), though this association should be interpreted with caution, as the lower boundary of the 95% CI for the beta-blockers' OR includes the ORs for thiazides and RAS agents, which are non-significantly associated with T2DM risk. Confounding by indication is usually not an issue for beta-blockers, as physicians tend to avoid them at a higher perceived risk of diabetes. The significant association between beta-blockers exposure and T2DM risk was still significant even after accounting for the full set of diabetes risk factor (**Appendix 6A**). Anyway, the possible increased T2DM risk must be weighed against the proven cardiovascular benefits of this class of drugs.

Thiazides have shown significant associations with T2DM risk in old papers, but less convincingly than beta-blockers; in the old papers, no adjustment was done for high blood pressure, a strong confounder that could explain their association with T2DM risk. The lack of association in our study may simply reflect lack of enough statistical power to show a true association, though.

Users of "any combinations" of beta-blockers, thiazides and RAS agents showed a higher T2DM risk, as well; this possibility has already been highlighted in the literature, specifically when a combination of a beta-blocker plus a thiazide is used, whereas use of a RAS agent in combination seems to lower new onset T2DM risk (142). However, both the "combination" and "others" exposure groups were too heterogeneous to draw definitive conclusions.

Weighted genetic risk scores derived from fasting glucose- and T2DM-associated SNPs conferred higher ~4-year change over time in plasma fasting glucose and risk of incident T2DM. These associations remained statistically significant in all the models. We opted for evaluating beta-cell influencing loci only to avoid alternative different mechanisms that might have obscured any significant associations. The genotyped SNPs successfully passed all the standard quality filters. Even though many of the SNPs used were actually imputed, quality of imputation was good for the SNPs tested in this work.

We preferred to use somewhat loose criteria to select the "beta-cell function-related" genetic loci, despite the possibility that having included loci with unclear or unproven mechanisms leading to change over time in plasma fasting glucose or increased T2DM risk may have subtracted power from the study. As an example, we decided to include *GCKR*, formerly thought to act mainly through liver-mediated insulin resistance, because a recent report suggested possible mechanisms influencing beta-cell function, still to be confirmed (144).

5A.2. Results for the joint models including hypertension or hypertension categories and beta-cell common genetic variation.

As our main result, we detected no interaction effect for any hypertension-treatment category-by-genetic risk score. This means that hypertension or antihypertensive specific treatments, and fasting glucose- or T2DM-associated common genetic variation influencing beta-cell function, did not modify each other's association with change over time in plasma fasting glucose or T2DM risk.

Nevertheless, hypertension and genetic scores were independently associated with glycemic outcomes in joint models without including interaction terms, which means that main and marginal effects explain all the associations between the environmental factor (hypertension and its treatments) and the genetic background, apparently leaving no place for eventual interaction effects.

Albeit others had evaluated the interaction effects between genetic background and environmental factors for T2DM incidence, few have focused on the possible modifying role of common genetic variation on the effects derived from antihypertensive drugs upon glycemic-related traits. To our knowledge, the interaction effect between T2DM-associated genetic loci and T2DM risk has mostly been tested for oral antidiabetic drugs, and only exceptionally for other kinds of drugs (145).

The previously described possible crosstalk between insulin and renin-angiotensin-aldosterone signaling systems (146) and the plausible anti-diabetic mechanisms of the ACE inhibitors and the ARB (147) have prompted some researchers to study the interaction effects upon glucose metabolism. Thus, one case-control study of 497 cases of diabetes has recently reported that the risk of T2DM due to thiazides use was not increased among *AR1* (angiotensin receptor-1 gene) 1166CC (rs5186) homozygous subjects (OR: 0.63; 95% CI: 0.28-1.40); however, homozygous *ACE* GG subjects (rs1799752) and *ACE* C allele carriers both had an increased risk of T2DM associated with thiazides use; this risk was higher for *ACE* GG subjects (synergy index (SI) for the interaction: 1.70 (95% CI: 1.08-2.66)) (148). In another publication from the same group, homozygous 1166CC carriers of *AR1* (rs5186) who followed treatment with ARB had an increased risk of T2DM as compared to 1166A carriers (interaction OR: 5.3; 95% CI: 1.8-16.1) (149).

Becker et al. have studied how common genetic variation modifies diabetes onset in people treated with calcium-channel blockers (150): calcium-channel blockers users with the TT genotype in rs10494366 at *NOS1AP* had a higher risk of incident T2DM than calcium-channel blockers users with the TT genotype (HR 1.78; 95% CI: 1.03-3.03). The lower risk was exclusively found in patients treated with calcium-channel blockers. These findings were later replicated in another study (151), in which an association between rs10494366 in *NOS1AP* and incident T2DM was observed among white calcium-channel blockers users (HR=1.75; 95% CI: 1.08-3.12; p=0.016) for the T allele. Again for calcium-channel blockers, Irving et al. more recently reported that SNP rs2228576 polymorphism, at the amiloride-sensitive epithelial sodium channel *SCNN1A* locus, modified the association between fasting glucose levels and amlodipine vs. chlortalidone treatment (p<0.001) (152).

According to our results, the influence that hypertension or its treatment exerted on change over time in plasma fasting glucose and T2DM risk was homogeneous irrespectively of the genetic susceptibility conferred by fasting glucose- or T2DM-associated common genetic variation. And reciprocally, the effect of fasting glucose- and T2DM-associated common genetic variation on change over time in plasma fasting glucose and T2DM risk was not greater in the presence of hypertension or hypertension treatment.

Had we found significant interaction effects in our study, the clinical implication could have been that, for similar efficacy rates, clinicians might preferentially opt for using drugs with less adverse metabolic effects. These adverse effects may be more easily anticipated by knowing the genetic susceptibility of the patients and the possible modifying effect of hypertension treatment to increase over time their fasting glucose levels or make them more prone to develop T2DM.

Our main hypothesis of seeking interaction effects between fasting glucose- and T2DM-associated SNPs and hypertension upon change over time in plasma fasting glucose and T2DM risk thus seemed plausible and based on clinical applications. Notwithstanding the previous idea, some authors have lately proposed that the therapeutic or adverse effects of any pharmacological action on glucose metabolism may not be adequately detected by using change over time in plasma fasting glucose or T2DM alone, since the interplay of multiple physiologic systems to maintain glycemia in a narrow range may blunt the final effects, and perhaps other phenotypes more closely related to the action of the drug should be defined and tested (153).

5.B. Strengths of our work.

Confounding by indication, which is a matter of concern in observational studies of drug therapies, has traditionally precluded claiming irrefutable evidence for the effect of hypertension and its treatment on glycemic control and risk of incident diabetes. Thus, the fact that genetics do not confound by indication ("Mendelian randomization") gives strength to our study.

The quality of the data from the Framingham studies is outstanding: clinical and laboratory data have been thoroughly collected in every exam, and dozens of papers in the literature are based on this methodology.

The pooled analyses gave robustness to our analyses and partly offset the limited sample size. Having replicated the results from the age-, sex-adjusted models in the fully-adjusted models for the whole set of T2DM clinical risk factors gives strength to our results and accounts for the possibility of residual confounding. We were able to account for correlation effects within individuals and within families, which is an issue in these pooled analyses carried out in populations where familial relationships cannot be fully ruled out.

The specific drug-exposures analyses with mutually-exclusive drug categories allowed an orthodox analysis of the true effects found for every medication group.

At last, a "clean", detailed, step-by-step statistical analysis was planned and performed for a logical interpretation of the results.

5.C. Limitations of this work.

Firstly, if we had observed evidence for a gene-environment interaction, its biological plausibility should have been further critically discussed and potential confounders or intermediate pathways would need to be explored. We might not have been able to confirm or reject conclusions dealing with biological mechanisms by epidemiological data alone. Only in light of additional lines of evidence, such as large randomized controlled trials and functional experiments, may the inferences toward causality be extended (154). Thus, even though the potential clinical relevance or impact of a reported gene-environment interaction might have been discussed, potential implications should have been evaluated in subsequent studies designed for that special purpose.

Secondly, some methodological limitations should be further pointed out: we only analyzed common genetic variants; eventual incorporation of rare variants with larger effects could have further enhanced our ability to detect significant interaction effects by constructing more powerfully discriminative genetic scores. Furthermore, genome wide association studies SNPs only tag putative functional variants; had we had knowledge of the functional variants at each locus, we might have been able to account for a more precise genetic effect and detect interaction. Moreover, Framingham Offspring Study subjects were mostly white and of European ancestry. Had we analyzed populations with a different ancestry, we might have seen different results.

Additionally, in the Framingham Offspring Study, we were unable to ascertain exact exposure duration or a precise date of T2DM onset in the participants, since this information was retrieved only at subsequent visits during the scheduled follow-up of the participants. However, we used a robust pooled-regression analysis method that accounted for this limitation.

Lastly, we only had power to detect relatively large regression coefficients for the interaction effects with our sample size. Greater power might be needed to unmask very subtle interaction effects, and even more to look at specific SNP effects. Although SNP level effects might be mechanistically more precise, our sample size prohibited single SNP analyses.

5.D. Future directions.

Trials are under way to explore and identify the genetic determinants of the antihypertensive and adverse metabolic responses to antihypertensive drugs: for instance, the PEAR Study has been started to evaluate the genetic effects on the response to a thiazide diuretic (hydrochlorothiazide), a β-blocker (atenolol), and their combination (155).

Even though we have found no significant interaction effects with models using genetic scores, this study hints at the possibility of analyzing individual SNP-by-specific drug category interaction tests in larger samples, or by clustering SNPs according to the biological pathways through which they reportedly operate. Researchers should next design this kind of studies to address whether there is some biological basis for fasting glucose impairment or T2DM risk associated with anti-hypertensive therapies.

Here we describe a potential approach for future studies, using beta-blockers exposure as an example: we have shown a higher incident T2DM risk in hypertensive people who were exposed to beta-blockers treatment. As mentioned in the background section, potential mechanisms that could explain the association include weight gain, attenuation of the beta-receptor–mediated release of insulin from pancreatic beta cells, decreased insulin sensitivity or changes in the level of aerobic exercise or subtle changes in the cellular actions of insulin (48).

According to the findings from an extensive search in the literature, it is reasonable to select the following SNPs, as potential candidates to test the interaction effects between individual SNPs and beta-blockers exposure on T2DM risk:

In chromosome 3, we have tested rs1470579, at *IGF2BP2*, related to pancreatic development and protein binding; according to Groenewoud et al., variants at this genetic locus attenuate the first phase of insulin secretion (156). Propranolol has been shown to inhibit insulin exocytosis (157). So, it is a plausible hypothesis that propranolol exposure might interact with genetic variation at this genetic locus to modify incident T2DM risk. In a similar fashion, in chromosome 6, rs7754840, at *CDKAL1*, which regulates a signal transducer and downregulates insulin expression, should also be included in the beta-blockers analyses, due to the effect of genetic variation at this locus on first-phase insulin exocytosis mediated through potassium (ATP) channel responsiveness (158).

In another recent report, Park et al. argue that propranolol completely abolished induction of *VEGF* expression in cancer cells (159); thus, rs9472138, at *VEGFA*, which regulates a vascular endothelial growth factor, but whose mechanistic link with diabetes onset has not been readily explained yet, should also be included in the list of specific beta-blockers exposure SNPs.

In chromosome 7, at *DGKB/TMEM195*, rs2191349 was included in our T2DM analyses. This locus seems to regulate diacylglycerol-kinase mediated insulin secretion; it has been reported that, in small arteries, diacylglycerol kinase activity is increased by adrenergic stimulation implying a role in vascular smooth muscle responses (160). Then, we might also want to include this SNP in our beta-blockers exposure list of SNPs.

There is an interesting locus at chromosome 11, *KCNQ1*, linked to potassium channel-mediated insulin release, for which we analyzed rs231362 as a T2DM associated SNP. It has been suggested that expanded gene survey may be required in *KCNQ1*-related patients who are resistant to beta-blockers therapies in regard to the long QT interval syndrome (161). There seems to be a modifying effect on behalf of beta-blocker therapies in this disease, as heterogeneity has been described for genetic variation at this locus. Lastly, rs10830963, at *MTNR1B*, in chromosome 11, related to melatonin and circadian rhythm, should also be tested for an interaction effect between beta-blockers exposure and genetic variation at this locus, since a very recent report underscores the relevance of catecholamines to explain the relationship between insulin and melatonin in regard to T2DM risk (162).

This way, we would pick 6 SNPs, for which proposed biological actions could somehow interact with beta-blockers exposure in hypertensive patients to confer a heterogeneous risk of developing T2DM. And in a similar fashion we might proceed with thiazides and RAS agents' exposures, and also for the change in fasting glucose set of analyses.

It can be argued that these tests are largely exploratory, but it can be replied that with this design, in individual SNPs analyses, and also by clustering SNPs in a way that accounts for similar biological pathways through which they reportedly operate, researchers could attempt to address whether there is some biological basis for fasting glucose impairment or T2DM risk associated with anti-hypertensive therapies.

Use of larger samples and eventual incorporation of new genetic information with rare variants could enhance our ability to detect interaction effects upon glycemia on behalf of antihypertensive therapies. In fact, by the time this work was about to be printed, an updated list of over 63 SNPs have been associated with T2DM (163), and an updated list of 36 SNPs have been associated with fasting glucose levels (164), and these numbers are expected to increase due to the synergistic research efforts in the field.

6. CONCLUSIONS

1. In the Framingham Offspring Study, as people aged, body mass index increased and both hypertension and hypertension treatment became more prevalent.
2. Versus no hypertension, hypertension was associated with higher ~4-year change in plasma fasting glucose and increased odds of type 2 diabetes.
3. Versus untreated hypertension, treated hypertension conferred higher ~4-year change in plasma fasting glucose and increased odds of type 2 diabetes.
4. Use of renin-angiotensin system agents was nominally associated with higher change over time in plasma fasting glucose. Use of beta-blockers was nominally associated with increased odds of type 2 diabetes.
5. Change over time (~4-year) in plasma fasting glucose and odds of type 2 diabetes significantly increased per weighted genetic risk score allele.
6. People who developed type 2 diabetes had higher weighted genetic risk scores tan people who did not.
7. Hypertension and the genetic risk scores (per-fasting glucose-SNP- or per-type 2 diabetes-weighted genetic risk allele) independently predicted change over time (~4-year) in plasma fasting glucose or type 2 diabetes risk.
8. Hypertension or antihypertensive specific treatments, and fasting glucose- or type 2 diabetes-associated common genetic variation influencing beta-cell function, did not modify each other's association with change over time in plasma fasting glucose or type 2 diabetes risk.
9. Therefore, the influence that hypertension or its treatment exerted on change over time in plasma fasting glucose or type 2 diabetes risk was homogeneous

irrespectively of the genetic susceptibility conferred by fasting glucose- or type 2 diabetes-associated common genetic variation.

10. Reciprocally, the effect of fasting glucose- and type 2 diabetes-associated common genetic variation on change over time in plasma fasting glucose or type 2 diabetes risk was not greater in the presence of hypertension, treated hypertension or hypertension specific treatments.
11. Additional possible confounding type 2 diabetes risk factors (mean blood pressure, body mass index, smoking, high density lipoprotein cholesterol, triglycerides levels, and alcohol consumption) did not seem to explain most of the statistical associations found in the age-, sex-adjusted models, with further adjustment for fasting glucose levels in the change over time in plasma fasting glucose analyses.
12. This study hints at the possibility of analyzing individual SNP-by-specific drug category interaction tests in larger population samples, or by clustering SNPs according to the biological pathways through which they reportedly operate, to address whether there is some biological basis for fasting glucose impairment or type 2 diabetes risk associated with anti-hypertensive therapies.

7. ABBREVIATIONS AND ACRONYMS

2hrG: 2 hour glucose levels

ACE: angiotensin converting enzyme

ADA: American Diabetes Association

ALLHAT: antihypertensive and lipid-lowering treatment to prevent heart attack trial

ANBP-2: Australian national blood pressure-2

AP: angina pectoris

AR1: angiotensin receptor 1

ARB: angiotensin receptor blocker

ARIC: atherosclerosis risk in communities

ATP: adenosine triphosphate

BMI: body mass index

BP: blood pressure

BRLMM: bayesian robust linear modeling using Mahalanobis distance

CAPPP: captopril prevention project

CCB: calcium channel blocker

CHARM: candesartan in heart failure assessment of reduction in mortality and morbidity

CI: confidence interval

DNA: desoxi-ribonucleic acid

ECG: electrocardiogram

FG: fasting glucose

FI: fasting insulin

GEE: Generalized Estimating Equations

GLP-1: glucagon-like peptide 1

GWAS: genome-wide association scan

HapMap: haplotype map

HOMA-IR: homeostasis model assessment of insulin resistance

HOPE: heart outcomes prevention evaluation

HR: hazard ratio

INSIGHT: intervention as a goal in hypertension trial

Kb: kilobase

LD: linkage disequilibrium

LIFE: losartan intervention for endpoint reduction

MAF: minor allele frequency

MODY: maturity-onset diabetes of the young

NAVIGATOR: nateglinide and valsartan in impaired glucose tolerance outcomes research

NHANES: National Health and Nutrition Examination Survey

NHLBI: National Heart, Lung and Blood Institute

OGTT: oral glucose tolerance test

OR: odds ratio

RAS: renin-angiotensin system

RR: relative risk

SHEP: systolic hypertension in the elderly program

SI: synergy index

SNP: single-nucleotide polymorphism

T2DM: type 2 diabetes mellitus

USA: United States of America

VALUE: valsartan anti-hypertensive long-term use evaluation

WHO: World Health Organization

8. SUMMARY OF TABLES, FIGURES AND APPENDICES

TABLES

Table 15. Base-line characteristics stratified by specific hypertension treatment drug category.

Table 16: Association of hypertension with change over time in fasting glucose.

Table 17: Association of hypertension treatment with change over time in fasting glucose.

Tables 18A-E: Association of specific anti-hypertensive drug exposures with change over time in fasting glucose.

Table 19: Association of hypertension with estimated risk of incident diabetes.

Table 20: Association of hypertension treatment with estimated risk of incident diabetes.

Table 21A-E: Association of specific anti-hypertensive drug exposures with estimated risk of incident diabetes.

Table 22: Fasting glucose-associated SNPs thought to act via beta cell biology, and their per allele increase in fasting glucose according to published and Framingham Offspring Study data.

Table 23: T2DM-associated SNPs thought to act via beta cell biology, and their per allele increase in odds of incident T2DM according to published and Framingham Offspring Study data.

Table 24: Effect of each weighted fasting glucose risk allele on change over time in fasting glucose.

Table 25: Mean weighted genetic risk scores in subjects who developed T2DM vs. subjects who did not, and additional estimated risk of incident T2DM per weighted risk allele.

Table 26: Interaction effect by fasting glucose- or T2DM-genetic risk scores on hypertension for change over time in fasting glucose or incident T2DM risk.

Table 27: Interaction effect by fasting glucose- or T2DM-genetic risk scores on hypertension treatment for change over time in fasting glucose or incident T2DM risk.

Table 28: Interaction effect by fasting glucose- or T2DM-genetic risk scores on hypertension specific drug exposures for change over time in fasting glucose or incident T2DM risk.

Table 29: Joint models including hypertension or hypertension treatment and the genetic risk scores upon change over time in fasting glucose without interaction terms.

Table 30: Joint models including specific antihypertensive drug exposures and the genetic risk scores upon change over time in fasting glucose without interaction terms.

Table 31: Joint models including hypertension, hypertension treatment or specific drug exposure categories and the genetic scores upon T2DM without interaction terms.

FIGURES

Figure 1: OR for new-onset T2DM after anti-hypertensive drug exposure according to Elliot et al. (36).

Figure 2: Risk ratios for new-onset T2DM in randomized clinical trials comparing beta-blockers vs. other anti-hypertensive therapies, after excluding diuretics, as published by Bangalore et al. (44).

Figure 3: Risk ratios for new-onset T2DM in comparative outcome trials involving the use of renin-angiotensin axis blockade vs. non-blockade, as reported by Jandeleit-Dahm et al. (60).

Figure 4: Visual representation of a hypothetical statistical gene-environment interaction.

Figure 5. Visual representation of the anti-hypertensive drug exposures in hypertensive subjects.

Figure 6: Visual representation of the proposed hypothetical interaction effects between an anti-hypertensive treatment and an individual SNP or a genetic risk score, to determine fasting glucose levels.

Figure 7. Flow-chart of the sequential steps in the statistical analysis.

Figure 8. Pattern of use of anti-hypertensive drugs along time.

Figure 9. Representation of final fasting glucose levels and change over time in fasting glucose, in mg/dl.

APPENDICES

Appendix 1. Variables coding in Framingham. Example: anti-hypertensive drugs exposure.

Appendix 2. Coding of outcomes in the Framingham Offspring Study: coronary heart disease.

Appendix 3. Blood samples treatment for DNA extraction in Framingham.

Appendix 4. Replication of the results shown in Tables 13, 16 and 19 in full T2DM risk factors-adjusted models: association of hypertension with change in fasting glucose and incident T2DM.

Appendix 5. Replication of the results shown in Tables 14, 17 and 20 in full T2DM risk factors-adjusted models: association of hypertension treatment with change in fasting glucose and incident T2DM.

Appendix 6. Replication of the results shown in Tables 15, 18 and 21 in full T2DM risk factors-adjusted models: association of specific anti-hypertensive drug exposures with change in fasting glucose and incident T2DM.

Appendix 7. Effect of each weighted fasting glucose risk allele on change over time in fasting glucose in the full T2DM risk factors-adjusted model.

Appendix 8. Mean weighted genetic risk scores in subjects who developed T2DM vs. subjects who did not, and additional estimated risk of incident T2DM per weighted risk allele in full T2DM risk factors-adjusted models.

Appendix 9. Interaction effect by fasting glucose-genetic risk score on hypertension, hypertension treatment or specific drug exposures for change over time in fasting glucose in the full set of T2DM risk factors-adjusted models.

Appendix 10. Interaction effect by T2DM-genetic risk score on hypertension, hypertension treatment or specific drug exposures for T2DM in the full set of T2DM risk factors-adjusted models.

9. REFERENCES

1. World Health Organization. Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications: Report of a WHO Consultation. Part 1. Diagnosis and Classification of Diabetes Mellitus. Geneva, World Health Org, 1999.
2. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care. 2003; 26:3160-3167.
3. International Expert Committee Report on the Role of the A1C Assay in the Diagnosis of Diabetes. Diabetes Care. 2009; 32:1327-1334.
4. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010; 33:S62-S69.
5. Matthaei S, Stumvoll M, Kellerer M, Haring HU. Pathophysiology and pharmacological treatment of insulin resistance. Endocr Rev. 2000; 21:585-618.
6. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR. Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. Lancet. 1992; 340:925-929.
7. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta cell deficit and increased β-cell apoptosis in humans with type 2 diabetes. Diabetes. 2003; 52:102-110.
8. Cowie CC, Rust KF, Ford ES, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care. 2009; 32:287-294.
9. Hogan P, Dall T, Nikolov P, on behalf of the American Diabetes Association. Diabetes Care. 2003; 26:917-932.
10. Lorenzo C, Serrano-Ríos M, Martínez-Larrad M, et al. Was the historic contribution of Spain to the Mexican gene pool partially responsible for the higher prevalence of type 2 diabetes in Mexican origin populations? The Spanish Insulin Resistance Study Group, the San Antonio Heart Study and the Mexico City Diabetes Study. Diabetes Care. 2001; 24:2059-2064.

11. Valdés S, Rojo-Martínez G, Soriguer F. Evolución de la prevalencia de la diabetes tipo 2 en población adulta española. Med Clin (Barc). 2007; 129:352-355.
12. Tuomilehto J, Lindstrom J, Eriksson JG, et al. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med. 2001; 344:1343-1350.
13. Soriguer F, Goday A, Bosch-Comas A, et al. Prevalence of diabetes mellitus and impaired glucose regulation in Spain: the Di@bet.es Study. Diabetologia. 2012; 55:88-93.
14. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. JAMA. 2003; 289:2560-2572.
15. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005; 365:217-223.
16. Lloyd-Jones D, Adams RJ, Brown TM, et al. Executive summary: heart disease and stroke statistics--2010 update: a report from the American Heart Association. Circulation. 2010; 121:948-954. Erratum in: Circulation. 2010; 121:e259.
17. Banegas JR, Rodríguez Artalejo F, de la Cruz Troca JJ, Guallar Castillón P, del Rey Calero J. Blood pressure in Spain: distribution, awareness, control, and benefits of a reduction in average pressure. Hypertension. 1998; 32:998-1002.
18. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation. 2005; 112:3066-3072.
19. Wei GS, Coady SA, Goff DC Jr, et al. Blood pressure and the risk of developing diabetes in African Americans and Whites: ARIC, CARDIA, and the Framingham Heart Study. Diabetes Care. 2011; 34:873-879.
20. Golden SH,WangNY, Klag MJ,Meoni LA, Brancati FL. Blood pressure in young adulthood and the risk of type 2 diabetes in middle age. Diabetes Care. 2003; 26:1110-1115.

21. Conen D, Ridker PM, Mora S, Buring JE, Glynn RJ. Blood pressure and risk of developing type 2 diabetes mellitus: the Women's Health Study. Eur Heart J. 2007; 28:2937-2943.
22. Shapiro AP, Benedek TG, Small JL. Effect of thiazides on carbohydrate metabolism in patients with hypertension. N Engl J Med. 1961; 265:1028-1033.
23. Wilson PW, Anderson KM, Kannel WB. Epidemiology of diabetes mellitus in the elderly. The Framingham Study. Am J Medicine. 1986; 80:3-9.
24. Mykkanen L, Kuusisto J, Pyorala K, Laakso M, Haffner SM. Increased risk of non-insulin-dependent diabetes mellitus in elderly hypertensive subjects. J Hypertens. 1994; 12:1425-1432.
25. Samuelsson O, Hedner T, Berglund G, Persson B, Andersson OK, Wilhelmsen L. Diabetes mellitus in treated hypertension: incidence, predictive factors and the impact of non-selective beta-blockers and thiazide diuretics during 15 years treatment of middle-aged hypertensive men in the Primary Prevention Trial Göteborg, Sweden. J Hum Hypertens. 1994; 8:257-263.
26. Gorden P, Sherman BM, Simopoulos AP. Glucose intolerance with hypokalemia: an increased proportion of circulating proinsulin-like component. J Clin Endocrinol Metab. 1972; 34:235-240.
27. Chatterjee R, Yeh HC, Shafi T, et al. Serum and dietary potassium and risk of incident type 2 diabetes mellitus: The Atherosclerosis Risk In Communities (ARIC) study. Arch Intern Med. 2010; 170:1745-1751.
28. Bakris G, Molitch M, Hewkin A, et al. Differences in glucose tolerance between fixed-dose antihypertensive drug combinations in people with metabolic syndrome. Diabetes Care. 2006; 29:2592–2597. Erratum in: Diabetes Care. 2007; 30:1329.
29. Helderman JH, Elahi D, Andersen DK, et al. Prevention of the glucose intolerance of thiazide diuretics by maintenance of body potassium. Diabetes. 1983; 32:106-111.
30. Cocco G, Iselin HU, Strozzi C, Cesana B, Baumeler HR. Magnesium depletion in patients on long-term chlorthalidone therapy for essential hypertension. Eur J Clin Pharmacol. 1987; 32:335-338.

31. Ayvaz G, Balos Toruner F, Karakoc A, Yetkin I, Cakir N, Arslan M. Acute and chronic effects of different concentrations of free fatty acids on the insulin secreting function of islets. Diabetes Metab. 2002; 28:3S7-12.
32. Eriksson JW, Jansson PA, Carlberg B, et al. Hydrochlorothiazide, but not candesartan, aggravates insulin resistance and causes visceral and hepatic fat accumulation: the mechanisms for the diabetes preventing effect of Candesartan (MEDICA) Study. Hypertension. 2008; 52:1030-1037.
33. Palming J, Jansson PA, Renström F, et al. Hydrochlorothiazide compared to candesartan treatment increases adipose tissue gene expression and circulating levels of serum amyloid A in hypertensive patients. Horm Metab Res. 2011; 43:319-324.
34. Barzilay JI, Davis BR, Cutler JA, et al. Fasting glucose levels and incident diabetes mellitus in older nondiabetic adults randomized to receive 3 different classes of antihypertensive treatment: a report from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). Arch Intern Med. 2006; 166:2191-2201.
35. Taylor EN, Hu FB, Curhan GC. Antihypertensive medications and the risk of incident type 2 diabetes. Diabetes Care. 2006; 29:1065-1070.
36. Elliott WJ, Meyer PM. Incident diabetes in clinical trials of antihypertensive drugs: a network meta-analysis. Lancet. 2007; 369:201-207.
37. Gress TW, Nieto FJ, Shahar E, Wofford MR, Brancati FL, for the Atherosclerosis Risk in Communities Study. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. Atherosclerosis Risk in Communities Study. New Eng J Med. 2000; 342:905-912.
38. Neaton JD, Grimm RH Jr, Prineas RJ, et al. Treatment of Mild Hypertension Study: final results. JAMA. 1993; 270:713-724.
39. Savage PJ, Pressel SL, Curb JD, et al. Influence of long-term, low-dose, diuretic-based, antihypertensive therapy on glucose, lipid, uric acid, and potassium levels in older men and women with isolated systolic hypertension: the Systolic Hypertension in the Elderly Program. SHEP Cooperative Research Group. Arch Intern Med. 1998; 158:741-751.

40. Donahue RP, Abbott RD, Wilson PW. Effect of diuretic use on the development of diabetes mellitus. The Framingham study. Horm Metab Res Suppl. 1990; 22:46-48.
41. Maitland-van der Zee AH, Turner ST, Schwartz GL, Chapman AB, Klungel OH, Boerwinkle E. Demographic, environmental, and genetic predictors of metabolic side effects of hydrochlorothiazide treatment in hypertensive subjects. Am J Hypertens. 2005; 18:1077-1083.
42. Feinstein AR, Landis JR. The role of prognostic stratification in preventing the bias permitted by random allocation of treatment. J Chronic Dis. 1976; 29:277-284.
43. Mancia G, Brown M, Castaigne A, et al. Outcomes with nifedipine GITS or co-amilozide in hypertensive diabetics and nondiabetics in Intervention as a Goal in Hypertension (INSIGHT). Hypertension. 2003; 41:431-436.
44. Padwal R, Mamdani M, Alter DA, et al. Antihypertensive therapy and incidence of type 2 diabetes in an elderly cohort. Diabetes Care. 2004; 27:2458-2463.
45. Bangalore S, Parkar S, Grossman E, Messerli FH. A meta-analysis of 94,492 patients with hypertension treated with beta blockers to determine the risk of new-onset diabetes mellitus. Am J Cardiol. 2007; 100:1254-1262.
46. Gupta AK, Dahlof B, Dobson J, Sever PS, Wedel H, Poulter NR; Anglo-Scandinavian Cardiac Outcomes Trial Investigators. Determinants of new-onset diabetes among 19,257 hypertensive patients randomized in the Anglo-Scandinavian Cardiac Outcomes Trial--Blood Pressure Lowering Arm and the relative influence of antihypertensive medication. Diabetes Care. 2008; 31:982-988.
47. Mason JM, Dickinson HO, Nicolson DJ, Campbell F, Ford GA, Williams B. The diabetogenic potential of thiazide-type diuretic and beta-blocker combinations in patients with hypertension. J Hypertens. 2005; 23:1777-1781.
48. Sowers JR, Bakris GL. Antihypertensive Therapy and the Risk of Type 2 Diabetes Mellitus. New Eng J Med. 2000; 342:969-970.
49. Torp-Pedersen C, Metra M, Charlesworth A, et al. Effects of metoprolol and carvedilol on pre-existing and new onset diabetes in patients with chronic heart

failure: data from the Carvedilol Or Metoprolol European Trial (COMET). Heart. 2007; 93:968-973.

50. Holzgreve H, Nakov R, Beck K, Janka HU. Antihypertensive therapy with verapamil SR plus trandolapril versus atenolol plus chlorthalidone on glycemic control. Am J Hypertens. 2003; 16:381-386.
51. Pepine CJ, Handberg EM, Cooper-DeHoff RM, et al. A calcium antagonist vs. a non-calcium antagonist hypertension treatment strategy for patients with coronary artery disease. The International Verapamil–Trandolapril Study (INVEST): a randomized controlled trial. JAMA. 2003; 290: 2805-2816.
52. Niklason A, Hedner T, Niskanen L, Lanke J. Development of diabetes is retarded by ACE inhibition in hypertensive patients – a subanalysis of the Captopril Prevention Project (CAPPP). J Hypertens. 2004; 22:645-652.
53. Heart Outcomes Prevention Evaluation Study Investigators. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICROHOPE substudy. Lancet. 2000; 355:253-259. Erratum in: Lancet. 2000; 356:860.
54. Reid CM, Johnston CI, Ryan P, Wilson K, Wing LM. Diabetes and cardiovascular outcomes in elderly subjects treated with ACE-inhibitors or diuretics: findings from the 2nd Australian National Blood Pressure Study. Am J Hypertens. 2003; 16:A11.
55. Julius S, Kjeldsen SE, Weber M, et al. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: the VALUE randomised trial. Lancet. 2004; 363:2022-2031.
56. Lindholm LH, Ibsen H, Borch-Johnsen K, et al. LIFE study group risk of new-onset diabetes in the Losartan Intervention For Endpoint reduction in hypertension study. J Hypertens. 2002; 20:1879-1886.
57. Pfeffer MA, Swedberg K, Granger CB, et al. Effects of candesartan on mortality and morbidity in patients with chronic heart failure: the CHARM-Overall programme. Lancet. 2003; 362:759-766. Erratum in: Lancet. 2009; 362:1744.

58. Barzilay JI, Gao P, Rydén L, et al. Effects of telmisartan on glucose levels in people at high risk for cardiovascular disease but free from diabetes: the TRANSCEND study. Diabetes Care. 2011; 34:1902-1907.
59. Burke TA, Sturkenboom MC, Ohman-Strickland PA, Wentworth CE, Rhoads GG. The effect of antihypertensive drugs and drug combinations on the incidence of new-onset type-2 diabetes mellitus. Pharmacoepidemiol Drug Saf. 2007; 16:979-987.
60. Jandeleit-Dahm KA, Tikellis C, Reid CM, Johnston CI, Cooper ME. Why blockade of the renin-angiotensin system reduces the incidence of new-onset diabetes. J Hypertens. 2005; 23:463-473.
61. Gillespie EL, White CM, Kardas M, Lindberg M, Coleman CI. The impact of ACE inhibitors or angiotensin II type 1 receptor blockers on the development of new-onset type 2 diabetes. Diabetes Care. 2005; 28:2261-2266.
62. NAVIGATOR Study Group; McMurray JJ, Holman RR, et al. Effect of valsartan on the incidence of diabetes and cardiovascular events. N Engl J Med. 2010; 362:1477-1490. Erratum in: N Engl J Med. 2010; 362:1748.
63. van der Zijl NJ, Moors CC, Goossens GH, Hermans MM, Blaak EE, Diamant M. Valsartan improves beta-cell function and insulin sensitivity in subjects with impaired glucose metabolism: a randomized controlled trial. Diabetes Care. 2011; 34:845-851.
64. Knowler WC, Bennett PH, Hamman RF, Miller M. Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. Am J Epidemiol. 1978; 108:497-505.
65. Weires MB, Tausch B, Haug PJ, Edwards CQ, Wetter T, Cannon-Albright LA. Familiality of diabetes mellitus. Exp Clin Endocrinol Diabetes. 2007; 115:634-640.
66. Elbein SC. The genetics of human noninsulin-dependent (type 2) diabetes mellitus. J Nutr. 1997; 127:1891S-1896S.
67. Collins FS, Guyer MS, Charkravarti A. Variations on a theme: cataloging human DNA sequence variation. Science 1997; 278:1580-1581.
68. http://www.ornl.gov/sci/techresources/Human_Genome/faq/snps.shtml

69. Meyer UA. Genotype or phenotype: the definition of a pharmacogenetic polymorphism. Pharmacogenetics. 1991; 1:66-67.
70. Hara K, Okada T, Tobe K, et al. The Pro12Ala polymorphism in *PPARγ2* may confer resistance to type 2 diabetes. Biochem Biophys Res Commun. 2000; 271:212-216.
71. Hani EH, Boutin P, Durand E, et al. Mis-sense mutations in the pancreatic islet β-cell inwardly rectifying K+ channel gene (*KIR6.2/BIR*): a meta-analysis suggests a role in the polygenic basis of type II diabetes mellitus in Caucasians. Diabetologia. 1998; 41:1511-1515.
72. Florez JC, Burtt N, de Bakker PI, et al. Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. Diabetes 2004; 53:1360-1368.
73. Winckler W, Weedon MN, Graham RR, et al. Evaluation of common variants in the six known maturity-onset diabetes of the young (MODY) genes for association with type 2 diabetes. Diabetes. 2007; 56:685-693.
74. Sandhu MS, Weedon MN, Fawcett KA, et al. Common variants in *WFS1* confer risk of type 2 diabetes. Nat Genet. 2007; 39:951-953.
75. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nat Genet. 2006; 38:320-323.
76. Florez JC, Jablonski KA, Bayley N, et al. *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med. 2006; 355: 241-250.
77. Sladek R, Rocheleau G, Rung J, et al. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007; 445:881-885.
78. van Hoek M, Dehghan A, Witteman JC, et al. Predicting type 2 diabetes based on polymorphisms from genome-wide association studies: a population-based study. Diabetes. 2008; 57:3122-3128.
79. Frayling TM, Timpson NJ, Weedon MN, et al. A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. Science. 2007; 316:889-894.

80. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University and Novartis Institutes of BioMedical Research, Saxena R, et al. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science. 2007; 316:1331-1336.
81. Zeggini E, Weedon MN, Lindgren CM, et al. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. Science. 2007; 316:1336-1341. Erratum in: Science. 2007; 317:1035-1036.
82. Scott LJ, Mohlke KL, Bonnycastle LL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science. 2007; 316:1341-1345.
83. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, et al. A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. Nat Genet. 2007; 39:770-775.
84. Zeggini E, Scott LJ, Saxena R, et al. Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet. 2008; 40:638-645.
85. Unoki H, Takahashi A, Kawaguchi T, et al. SNPs in *KCNQ1* are associated with susceptibility to type 2 diabetes in East Asian and European populations. Nat Genet. 2008; 40:1098-1102.
86. Prokopenko I, Langenberg C, Florez JC, et al. Variants in *MTNR1B* influence fasting glucose levels. Nat Genet. 2009; 41:77-81.
87. Lyssenko V, Nagorny CL, Erdos MR, et al. Common variant in *MTNR1B* associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat Genet. 2009; 41:82-88.
88. Rung J, Cauchi S, Albrechtsen A, et al. Genetic variant near *IRS1* is associated with type 2 diabetes, insulin resistance and hyperinsulinemia. Nat Genet. 2009; 41:1110-1115. Erratum in: Nat Genet. 2009; 41:1156.
89. Dupuis J, Langenberg C, Prokopenko I, et al. New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet. 2010; 42:105-116.

90. Voight BF, Scott LJ, Steinthorsdottir V, et al. Twelve type 2 diabetes susceptibility loci identified through large-scale association analysis. Nat Genet. 2010; 42:579-589.
91. Kong A, Steinthorsdottir V, Masson G, et al. Parental origin of sequence variants associated with complex diseases. Nature. 2009; 462:868-874.
92. Qi L, Cornelis MC, Kraft P, et al. Genetic variants at 2q24 are associated with susceptibility to type 2 diabetes. Hum Mol Genet. 2010; 19:2706-2715.
93. Saxena R, Elbers CC, Guo Y, al. Large-scale gene-centric meta-analysis across 39 studies identifies type 2 diabetes loci. Am J Hum Genet. 2012; 90:410-425.
94. Florez JC. Newly identified loci highlight beta cell dysfunction as a key cause of type 2 diabetes: where are the insulin resistance genes? Diabetologia. 2008; 51:1100-1110.
95. http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly
96. Boesgaard TW, Grarup N, Jørgensen T, et al. Variants at *DGKB/TMEM195*, *ADRA2A*, *GLIS3* and *C2CD4B* loci are associated with reduced glucose-stimulated beta cell function in middle-aged Danish people. Diabetologia. 2010; 53:1647-1655.
97. Saxena R, Hivert MF, Langenberg C, et al. Genetic variation in *GIPR* influences the glucose and insulin responses to an oral glucose challenge. Nat Genet. 2010; 42:142-148.
98. Dempfle A, Scherag A, Hein R, Beckmann L, Chang-Claude, Schafer H. Gene–environment interactions for complex traits: definitions, methodological requirements and challenges. European Journal of Human Genetics. 2008; 16: 1164-1172.
99. Yang Q, Khoury MJ. Evolving methods in genetic epidemiology. III. Gene-environment interaction in epidemiologic research. Epidemiol Rev. 1997; 19:33-43.
100. Rothman KJ, Greenland S: Modern Epidemiology. Philadelphia: Lippincott-Raven, 1998.
101. Thomas D. Gene–environment-wide association studies: emerging approaches. Nat Rev Genet. 2010; 11:259-272.

102. Davey Smith G, Ebrahim S. Mendelian randomization: can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003; 32:1- 22.
103. Daly AK. Genome-wide association studies in pharmacogenomics. Nat Rev Genet. 2010; 11:241-246.
104. Grarup N, Andersen G. Gene-environment interactions in the pathogenesis of type 2 diabetes and metabolism. Curr Opin Clin Nutr Metab Care. 2007; 10:420-426.
105. Ahluwalia TS, Ahuja M, Rai TS, et al. *ACE* variants interact with the RAS pathway to confer risk and protection against type 2 diabetic nephropathy. DNA Cell Biol. 2009; 28:141-150.
106. Konoshita T, Kato N, Fuchs S, et al. Genetic variant of the renin-angiotensin system and diabetes influences blood pressure response to angiotensin receptor blockers. Diabetes Care. 2009; 32:1485-1490.
107. Zhou K, Donnelly L, Burch L, et al. Loss-of-function *CYP2C9* variants improve therapeutic response to sulfonylureas in type 2 diabetes: a Go-DARTS study. Clin Pharmacol Ther. 2010; 87:52-56.
108. Zhou K, Donnelly LA, Kimber CH, et al. Reduced-function *SLC22A1* polymorphisms encoding organic cation transporter 1 and glycemic response to metformin: a GoDARTS study. Diabetes. 2009; 58:1434-1439.
109. Franks PW, Mesa J-L, Harding AH, Wareham NJ. Gene-lifestyle interaction on risk of type 2 diabetes. Nutr Metab Cardiovasc Dis. 2007; 17:104-124.
110. Adamo KB, Sigal RJ, Williams K, et al. Influence of Pro12Ala peroxisome proliferator-activated receptor gamma2 polymorphism on glucose response to exercise training in type 2 diabetes. Diabetologia. 2005; 48:1503-1509.
111. The Framingham Study. An Epidemiological Investigation of Cardiovascular Disease. DHEW Publication. Washington, DC, US GPO. 1974; No. (NIH) 74-478.
112. Kannel WB, Feinleib M, McNamara JR, Garrison RJ, Castelli WP. An investigation of coronary heart disease in families: the Framingham Offspring Study. Am J Epidemiol. 1979; 110:281-290.

113. Dawber TR, Moore FE, Mann GV. Coronary heart disease in the Framingham Study. Am J Public Health. 1957; 47:4-24.
114. Feinleib M, Kannel WB, Garrison RJ, McNamara PM, Castelli WP. The Framingham Offspring Study. Design and preliminary data. Prev Med. 1975; 4:518-525.
115. Kannel WB, Dawber TR, McNamara PM. Vascular disease of the brain—epidemiologic aspects: The Framingham Study. Am J Public Health. 1965; 55:1355-1366.
116. http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000007.v9.p4
117. http://www.affymetrix.com/support/help/faqs/mapping_500k/index.jsp
118. http://www.sequenom.com/Home/Products---Services/Genetic-Analysis/Applications/SNP-Genotyping-with-iPLEX-Gold
119. http://media.affymetrix.com/support/technical/datasheets/500k_datasheet.pdf
120. Moorhead M, Hardenbol P, Siddiqui F, et al. Optimal genotype determination in highly multiplexed SNP data. Eur J Hum Genet. 2006; 14:207-215. Erratum in: Eur J Hum Genet. 2006; 14:976.
121. http://www.broadinstitute.org/gen_analysis/genotyping/brlmm_affy_ncrr.html
122. http://www.biochem.northwestern.edu/holmgren/Glossary/Definitions/Def-H/Hardy-Weinberg_equilibrium.html
123. Marchini J, Howie B. Genotype imputation for genome-wide association studies. Nat Rev Genet. 2010; 11:499-511.
124. Frazer K, Ballinger D, Cox D, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007; 449:851-861.
125. Pei YF, Li J, Zhang L, Papasian CJ, Deng HW. Analyses and comparison of accuracy of different genotype imputation methods. PLoS One. 2008; 3:e3551. Epub 2008 Oct 29.
126. Hao K, Chudin E, McElwee J, Schadt EE. Accuracy of genome-wide imputation of untyped markers and impacts on statistical power for association studies. BMC Genet. 2009; 10:27. doi:10.1186/1471-2156-10-27.

127. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. Nat Genet. 2007; 39:906-913.
128. Huang L, Li Y, Singleton AB, et al. Genotype-imputation accuracy across worldwide human populations. Am J Hum Genet. 2009; 84:235-250.
129. de Bakker PI, Ferreira MA, Jia X, Neale BM, Raychaudhuri S, Voight BF. Practical aspects of imputation-driven meta-analysis of genome-wide association studies. Hum Mol Genet. 2008; 17:R122-8.
130. Meigs JB, Cupples LA, Wilson PWF. Parental transmission of type 2 diabetes mellitus: the Framingham Offspring Study. Diabetes. 2000; 49:2201-2207.
131. Murabito JM, Nam BH, D'Agostino RB Sr, Lloyd-Jones DM, O'Donnell CJ, Wilson PW. Accuracy of offspring reports of parental cardiovascular disease history: the Framingham Offspring Study. Ann Intern Med. 2004; 140:434-440.
132. Kannel WB, Wolf PA, Garrison RJ. The Framingham Study: An Epidemiological Investigation of cardiovascular Disease: Section 34: Some Risk Factors Related to the Annual Incidence of Cardiovascular Disease and Death Using Pooled Repeated Biennial Measurements: Framingham Heart Study, 30-Year-Follow-Up. Bethesda, Md: National Heart, Lung, and Blood Institute, 1987.
133. Meigs JB, D'Agostino RB Sr, Nathan DM, Rifai N, Wilson PW; Framingham Offspring Study. Longitudinal association of glycemia and microalbuminuria: the Framingham Offspring Study. Diabetes Care. 2002; 25:977-983.
134. McNamara JR, Schaefer EJ. Automated enzymatic standardized lipid analyses for plasma and lipid lipoprotein fractions. Clin Chim Acta. 1987; 166:1-8.
135. Meigs JB, Shrader P, Sullivan LM, et al. Genotype score in addition to common risk factors for prediction of type 2 diabetes. N Engl J Med. 2008; 359:2208-2219. Erratum in: N Engl J Med. 2009; 360:648.
136. D'Agostino RB, Lee ML, Belanger AJ, Cupples LA, Anderson K, Kannel WB. Relation of pooled logistic regression to time dependent Cox regression analysis: the Framingham Heart Study. Stat Med. 1990; 9:1501-1515.

137. Polak JF, Pencina MJ, Pencina KM, O'Donnell CJ, Wolf PA, D'Agostino RB Sr. Carotid-wall intima-media thickness and cardiovascular events. N Engl J Med. 2011; 365:213-221.

138. Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr. Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. Arch Intern Med. 2007; 167:1068-1074.

139. Diggle PJ, Liang KY, Zeger SL. Analysis of longitudinal data. Oxford University Press, New York, first edition, 1994.

140. Gauderman WJ, Morrison JM. QUANTO 1.1: A computer program for power and sample size calculations for genetic-epidemiology studies. 2006; http://hydra.usc.edu/gxe

141. dbgap-help@ncbi.nlm.nih.gov

142. Liou YS, Ma T, Tien L, Lin CM, Jong GP. The relationship between antihypertensive combination therapies comprising diuretics and/or beta-blockers and the risk of new-onset diabetes: a retrospective longitudinal cohort study. Hypertens Res. 2009; 32:496-499.

143. van Rijn-Bikker PC, Mairuhu G, van Montfrans GA, et al. Genetic factors are relevant and independent determinants of antihypertensive drug effects in a multiracial population. Am J Hypertens. 2009; 22:1295-1302.

144. Bi M, Kao WH, Boerwinkle E, et al. Association of rs780094 in *GCKR* with metabolic traits and incident diabetes and cardiovascular disease: the ARIC Study. PLoS One. 2010; 5:e11690.

145. Holstein A, Hahn M, Körner A, Stumvoll M, Kovacs P. *TCF7L2* and therapeutic response to sulfonylureas in patients with type 2 diabetes. BMC Med Genet. 2011; 12:30. doi: 10.1186/1471-2350-12-30.

146. Muscogiuri G, Chavez AO, Gastaldelli A, et al. The crosstalk between insulin and renin-angiotensin-aldosterone signaling systems and its effect on glucose metabolism and diabetes prevention. Curr Vasc Pharmacol. 2008; 6:301-312.

147. Kurtz TW, Pravenec M. Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. J Hypertens. 2004; 22:2253-2261.

148. Bozkurt O, de Boer A, Grobbee DE, et al. Variation in renin-angiotensin system and salt-sensitivity genes and the risk of diabetes mellitus associated with the use of thiazide diuretics. Am J Hypertens. 2009; 22:545-551.
149. Bozkurt O, de Boer A, Grobbee DE, et al. Renin-angiotensin system polymorphisms and the association between use of angiotensin II receptor blockers or angiotensin-converting enzyme inhibitors and the risk of diabetes. J Renin Angiotensin Aldosterone Syst. 2009; 10:101-108.
150. Becker ML, Visser LE, Newton-Cheh C, et al. Genetic variation in the *NOS1AP* gene is associated with the incidence of diabetes mellitus in users of calcium channel blockers. Diabetologia. 2008; 51:2138-2140.
151. Chu AY, Coresh J, Arking DE, et al. *NOS1AP* variant associated with incidence of type 2 diabetes in calcium channel blocker users in the Atherosclerosis Risk in Communities (ARIC) study. Diabetologia. 2010; 53:510-516.
152. Irvin MR, Lynch AI, Kabagambe EK, et al. Pharmacogenetic association of hypertension candidate genes with fasting glucose in the GenHAT Study. J Hypertens. 2010; 28:2076-2083.
153. Watanabe RM. Drugs, diabetes and pharmacogenomics: the road to personalized therapy. Pharmacogenomics. 2011; 12:699-701.
154. Thompson WD. Effect modification and the limits of biological inference from epidemiologic data. J Clin Epidemiol.1991; 44:221-232.
155. Johnson JA, Boerwinkle E, Zineh I, et al. Pharmacogenomics of antihypertensive drugs: Rationale and design of the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study. Am Heart J. 2009; 157:442-449.
156. Groenewoud MJ, Dekker JM, Fritsche A, et al. Variants of *CDKAL1* and *IGF2BP2* affect first-phase insulin secretion during hyperglycaemic clamps. Diabetologia. 2008; 51:1659-1663.
157. Cerasi E, Luft R, Efendić S. Effect of adrenergic blocking agents on insulin response to glucose infusion in man. Acta Endocrinol (Copenh). 1972; 69:335-346.

158. Ohara-Imaizumi M, Yoshida M, Aoyagi K, et al. Deletion of *CDKAL1* affects mitochondrial ATP generation and first-phase insulin exocytosis. PLoS One. 2010; 5:e15553.

159. Park SY, Kang JH, Jeong KJ, et al. Norepinephrine induces *VEGF* expression and angiogenesis by a hypoxia-inducible factor-1α protein-dependent mechanism. Int J Cancer. 2011; 128:2306-2316.

160. Ohanian J, Ohanian V. Lipid second messenger regulation: the role of diacylglycerol kinases and their relevance to hypertension. J Hum Hypertens. 2001; 15:93-98.

161. Kobori A, Sarai N, Shimizu W, et al. Additional gene variants reduce effectiveness of beta-blockers in the LQT1 form of long QT syndrome. J Cardiovasc Electrophysiol. 2004; 15:190-199.

162. Peschke E, Hofmann K, Pönicke K, Wedekind D, Mühlbauer E. Catecholamines are the key for explaining the biological relevance of insulin-melatonin antagonisms in type 1 and type 2 diabetes. J Pineal Res. 2012; 52:389-396.

163. the DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium, Morris AP, Voight BF, et al. Large-scale association analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet. 2012; 44:981-990.

164. Scott RA, Lagou V, Welch RP, et al. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. Nat Genet. 2012; 44:991-1005.

.

10. APPENDICES

Appendix 1. Variables coding in Framingham. Example: anti-hypertensive drugs exposure.

For exam 1, exposure to anti-hypertensive medication was coded this way:

A70 C7/14 History of Hypertension
0 no
1 yes
. unknown

A78 C7/22 Diuretics For Blood Pressure
3 no
4 yes (now)
5 yes (not now)
. unknown

A79 C7/23 Hypotensives (excluding diuretics)
6 no
7 yes (now)
8 yes (not now)
. unknown

For exam 7, the items that collect information on blood pressure were:

G008 IN THE INTERIM HAVE YOU TAKEN MEDICATION FOR THE TREATMENT OF HYPERTENSION?
0 NO
1 YES
2 YES, NOT NOW
. UNKNOWN

G013 CARDIOVASCULAR MEDICATIONS: CALCIUM CHANNEL BLOCKERS (NIFEDIPINE, VERAPAMIL, DILTIAZEM)
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G017 CARDIOVASCULAR MEDICATIONS: BETA BLOCKERS
0 NO
1 YES
2 YES, NOT NOW
3 MAYBE
. UNKNOWN
NOTE: CHOICE OF "2 YES, NOT NOW" AND "3 = MAYBE" WAS ADDED DURING EXAM 7 DATA COLLECTION

G018 BETA BLOCKER GROUP
0 DOES NOT TAKE BETA BLOCKERS
01 PROPRANOLOL
02 TIMOLOL
03 NADOLOL
04 ATENOLOL
05 METOPROLOL
06 PINDOLOL
07 CARVEDILOL
08 LABETALOL
09 OTHER
. UNKNOWN

G019 BETA BLOCKER GROUP: DOSE (MG/DAY) OF BETA BLOCKER
0 DOES NOT TAKE BETA BLOCKERS
3 - 400
. UNKNOWN

G020 CARDIOVASCULAR MEDICATIONS: LOOP DIURETICS (LASIX, ETC.)
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G022 CARDIOVASCULAR MEDICATIONS: THIAZIDE DIURETICS
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G023 CARDIOVASCULAR MEDICATIONS: K-SPARING DIURETICS
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G024 CARDIOVASCULAR MEDICATIONS: POTASSIUM SUPPLEMENTS
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G025 CARDIOVASCULAR MEDICATIONS: RESERPINE DERIVATIVES
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE

. UNKNOWN

G026 CARDIOVASCULAR MEDICATIONS: METHYLDOPA (ALDOMET)
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G027 CARDIOVASCULAR MEDICATIONS: ALPHA-1 AGONIST
(CLONIDINE, WYTENSIN, GUANABENZ)
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G028 CARDIOVASCULAR MEDICATIONS: ALPHA-2 BLOCKERS
(PRAZOSIN, TERAZOSIN, DOXAZOSIN)
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G029 CARDIOVASCULAR MEDICATIONS: RENIN-ANGIOTENSIN BLOCKING DRUGS
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G031 CARDIOVASCULAR MEDICATIONS: ANGIOTENSIN II ANTAGONISTS
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G030 CARDIOVASCULAR MEDICATIONS: PERIPHERAL VASODILATORS
(HYDRALAZINE, MINOXIDIL, ETC.)
0 NO
1 YES, NOW
2 YES, NOT NOW
3 MAYBE
. UNKNOWN

G032 CARDIOVASCULAR MEDICATIONS: OTHER ANTI-HYPERTENSIVES
0 NO
1 YES, NOW
2 YES, NOT NOW

	3 MAYBE . UNKNOWN
G708	TREATMENT FOR BLOOD PRESSURE 0 NO 1 YES (BY MEDS AND PHYSICIAN OPINION) . UNKNOWN
G711	HYPERTENSION 0 NO 1 YES BY ELEVATED BP OR TREATMENT FOR BP . UNKNOWN

Appendix 2. Coding of outcomes in the Framingham Offspring Study: coronary heart disease.

01-09 CHD

01 MI RECOGNIZED, WITH DIAGNOSTIC ECG

02 MI RECOGNIZED, WITHOUT DIAGNOSTIC ECG, WITH ENZYMES AND HISTORY

03 MI RECOGNIZED, WITHOUT DIAGNOSTIC ECG, WITH AUTOPSY EVIDENCE, NEW EVENT (SEE ALSO CODE 09)

04 MI UNRECOGNIZED, SILENT

05 MI UNRECOGNIZED, NOT SILENT

NOTES on MI: Recognized means at time of MI occurrence, an MI was known to have taken place. Unrecognized means MI was not known to have taken place until later. Silent MI means at time of MI, there were no symptoms similar to MI symptoms. Not silent means that symptoms were present.

Unrecognized MIs are dated using the midpoint between the dates of the diagnostic ECGs. Hierarchy for coding MI is based on strongest evidence: EVENT=01 > EVENT=02 > EVENT=03.

06 AP, FIRST EPISODE ONLY
If date of was unavailable, AP is dated as midpoint of the interval between medical encounters (FHS exams, hospitalizations or MD office visits). If AP occurred with MI or CI, then use same date as MI or CI.

Before 1987, if AP occurred just before MI or CI, then AP date was assigned 3 months before MI or CI. If AP occurred just after MI or CI, then AP date was assigned 3 months after MI or CI.

Beginning in 1987, if approximate or exact date of new angina was available, then that date was assigned as date of AP.

07 CI, DEFINITE BY BOTH HISTORY AND ECG.

08 QUESTIONABLE MI AT EXAM 1
Date same date as Exam 1 for Original cohort and Offspring cohort. For the other cohort groups, a date other than the Exam 1 date is used if available. Any questionable MI episode at Exam 1: definite MI history without enzymes or autopsy, or possible MI on ECG. Does not include questionable AP.

09 MI ACUTE AUTOPSY, NOT A NEW EVENT.
This is coded when there has been a previously coded MI (1 or 2) and that MI shows on autopsy.

CHD	Coronary Heart Disease
MI	Myocardial Infarction
ECG	Electrocardiogram
AP	Angina Pectoris
CI	Coronary Insufficiency

Appendix 3. Blood samples treatment for DNA extraction in Framingham.

In summary, buffy coats of nucleated cells obtained from anti-coagulated blood (ACD or EDTA) were re-suspended in 15 ml polypropylene centrifugation tubes with 3 ml of nuclei lysis buffer. The cell lysates were digested overnight. After digestion was complete, 1 ml of saturated NaCl (approximately 6M) was added to each tube and shaken vigorously for 15 seconds, followed by centrifugation at 2500 rpm for 15 minutes. The precipitated protein pellet was left at the bottom of the tube and the supernatant containing the DNA was transferred to another 15 ml polypropylene tube. Exactly 2 volumes of room temperature absolute ethanol were added and the tubes inverted several times until the DNA precipitated. The precipitated DNA strands were removed with a plastic spatula or pipette and transferred to a 1.5 ml micro-centrifuge tube containing 100-200 pi TE buffer. The DNA was allowed to dissolve 2 hours at 37°C before being quantified.

The Mapping 500K Array Set builds on the proven and simple approach for reducing genomic complexity that is employed by the GeneChip Human Mapping 10K Array and the GeneChip Mapping 100K Set (1). Total genomic DNA (250 ng) is digested with a restriction enzyme (Nsp I or Sty I) and ligated to adaptors that recognize the cohesive four base-pair overhangs. All fragments resulting from restriction enzyme digestion, regardless of size, were substrates for adaptor ligation. A generic primer that recognized the adaptor sequence was used to amplify adaptor-ligated DNA fragments. Polymerase chain reaction conditions have been optimized to preferentially amplify fragments in the 200 to 1,100 base-pair size range. The amplified DNA was then fragmented, labeled, and hybridized to a GeneChip Human Mapping 250K Array.

All SNPs on the GeneChip Human Mapping 500K Array Set went through a rigorous screening and validation process. Optimal SNPs were selected and tiled on arrays based on accuracy, call rate, and linkage disequilibrium analysis in three populations across the genome. The median physical distance between SNPs was 2.5 kilo-bases (kb) and the average distance between SNPs was 5.8 kb. The average heterozygosity of these SNPs was 0.30. Eighty-five percent of the human genome is within 10 kb of a SNP.

The technology for iPLEX was based on the fact that parent ion masses could be easily determined from the spectrum without the need for complex data processing and were accessible as numerical data for direct processing (2). The mass spectrometric approach enabled direct analyte detection with 100% specificity and needed no redundancy (3). This accuracy and efficacy were combined with sample miniaturization, bioinformatics, and chip-based technologies for parallel processing of numerous samples (4). Using a proprietary algorithm, masses -as well as signal intensities- were automatically analyzed and interpreted. After completion of analysis, the results were transferred to a database and stored as accessible genetic information. The database also provided a tool for visual control and comparison of spectra with theoretically expected results (5).

References for methods (blood samples treatment)

1. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16:1215.

2. Jurinke C, van den Boom D, Cantor CR, Köster H. Automated Genotyping Using the DNA MassArray Technology. Methods in Molecular Biology, vol. 170: DNA Arrays: Methods and Protocols. Edited by: J. B. Rampal. Humana Press Inc., Totowa, NJ. 2008; 170:103-116.
3. Ross P, Hall L, Smirnov I, Haff L. High level multiplex genotyping by MALDI-TOF mass spectrometry. Nat Biotech. 1998; 16:1347-1351.
4. Buetow KH, Edmonson M, MacDonald R, et al. High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Proc Natl Acad Sci. 2001; 98:581-584.
5. Braun A, Little DP, Reuter D, Muller-Mysock B, Köster H. Improved analysis of microsatellites using mass spectrometry. Genomics. 1997; 46:18-23.

Appendix 4. Replication of the results shown in Tables 13, 16 and 19 in full T2DM risk factors-adjusted models: association of hypertension with change in fasting glucose and incident T2DM.

HYPERTENSIVE VS. NON-HYPERTENSIVE, fully-adjusted model

	Hypertension status				
	Yes (HTN=1)		No (HTN=0)		
Number of Subjects (N)	4,900		10,952		
	Mean	**Std Dev**	**Mean**	**Std Dev**	**p-value**
Age, years	57.83	9.96	49.31	10.68	<.0001
Sex, % male	52.12	0.49	43.45	0.50	<.0001
BMI, kg/m^2	28.53	5.10	25.94	4.32	<.0001
Smoking, %	19.11%	0.39	26.85%	0.44	<.0001
HDL cholesterol, mg/dl	49.83	15.69	52.34	15.03	<.0001
Triglycerides, mg/dl	148.00	96.10	111.20	85.93	<.0001
Alcohol, ounces/week	3.38	4.88	2.71	3.83	<.0001
Number of Subjects (N)	4,445		10,045		
Traits	**Mean**	**Std. Error**	**Mean**	**Std. Error**	**p-value**
Fasting glucose, mg/dl	95.55	0.14	93.42	0.09	<.0001
FG_adj. change in fasting glucose, mg/dl	2.25	0.18	1.82	0.11	<.0001

Incidence of diabetes	2.81%	3.36%	1.41%	1.86%	<.0001

Appendix 5. Replication of the results shown in Tables 14, 17 and 20 in full T2DM risk factors-adjusted models: association of hypertension treatment with change in fasting glucose and incident T2DM.

TREATED VS. UNTREATED HYPERTENSIVE, fully-adjusted model

	All hypertensive				
	Treated Hypertensives		Untreated Hypertensives		
Number of Subjects (N)	2,763		2,137		
	Mean	**Std Dev**	**Mean**	**Std Dev**	**p-value**
Age, years	59.46	9.37	55.72	10.30	<.0001
Sex, % male	50.56	0.50	54.14	0.50	0.013
Mean BP, mmHg, ([2xDBP]+SBP) / 3	98.39	11.13	107.30	7.52	<.0001
BMI, kg/m^2	28.65	4.98	28.37	5.24	0.06
Smoking, %	16.58	0,37	22.39	0.42	<.0001
HDL cholesterol, mg/dl	49.17	15.47	50.68	15.93	0.001
Triglycerides, mg/dl	149.10	89.21	146.70	104.30	0.40
Alcohol, ounces/week	3.15	4.54	3.68	5.29	0.0003
Number of Subjects (N)	2,494		1,951		
Traits	**Mean**	**Std. Error**	**Mean**	**Std. Error**	**p-value**
Fasting glucose, mg/dl	98.27	0.19	96.92	0.21	<.0001
FG_adj. change in fasting glucose, mg/dl	3.24	0.26	3.09	0.30	<.0001

Incidence of diabetes	4,94%	3.98%	3,39%	2,90%	0,015

Appendix 6. Replication of the results shown in Tables 15, 18 and 21 in full T2DM risk factors-adjusted models: association of specific anti-hypertensive drug exposures with change in fasting glucose and incident T2DM.

(*p-values: compared with the untreated hypertensive reference group).

6.A. BETA-BLOCKERS EXPOSURE, fully-adjusted model

	Untreated hypertensive		Taking Beta Blockers ONLY		
Number of Subjects (N)	2,137		676		
	MEAN	**STD DEV**	**MEAN**	**STD DEV**	***p-value**
Age, years	55.72	10.3	60.24	9.25	<.0001
Sex, % male	54.1	0.50	59.0	0.49	0.0001
Mean BP, mmHg, ([2xDBP]+SBP) / 3	107.30	7.51	97.46	11.47	<.0001
BMI, kg/m^2	28.37	5.24	28.17	4.58	0.032
Smoking, %	22.39	0.42	15.97	0.37	0.018
HDL cholesterol, mg/dl	50.68	15.93	46.00	13.25	<.0001
Triglycerides, mg/dl	146.7	104.3	155.90	94.63	0.023
Alcohol, ounces/week	3.68	5.29	3.41	5.05	0.888
Number of Subjects (N)	1,951		609		
Traits	**Param Estimates**	**Std. Error**	**Param Estimates**	**Std. Error**	**p-value**
Fasting glucose, mg/dl	96.93	0.23	98.63	0.39	0.0002
FG_adj. change in fasting glucose, mg/dl	2.88	0.31	3.17	0.53	0.643

Incidence of diabetes	4.10%	6.64%	0.010

6.B. THIAZIDES EXPOSURE, fully-adjusted model

	Untreated hypertensive		Taking Thiazides ONLY		
Number of Subjects (N)	2,137		527		
	MEAN	**STD DEV**	**MEAN**	**STD DEV**	***p-value**
Age, years	55.72	10.3	55.43	9.37	<.0001
Sex, % male	54.1	0.50	42.3	0.50	<.0001
Mean BP, mmHg, ([2xDBP]+SBP) / 3	107.30	7.51	101.30	10.06	0.036
BMI, kg/m^2	28.37	5.24	28.49	5.17	0.835
Smoking, %	22.39	0.42	22.39	0.42	0.055
HDL cholesterol, mg/dl	50.68	15.93	50.28	16.34	0.491
Triglycerides, mg/dl	146.70	104.3	140.90	73.82	0.026
Alcohol, ounces/week	3.68	5.29	3.12	4.35	0.150
Number of Subjects (N)	1,951		493		
Traits	**Param Estimates**	**Std. Error**	**Param Estimates**	**Std. Error**	**p-value**
Fasting glucose, mg/dl	96.93	0.23	98.12	0.42	0.013
FG_adj. change in fasting glucose, mg/dl	2.88	0.31	2.07	0.57	0.212

Incidence of diabetes	4.10%	5.77%	0.111

6.C. ACE-INHIBITORS/ARB EXPOSURE, fully-adjusted model

	Untreated hypertensive		Taking ACE-I/ARB ONLY		
Number of Subjects (N)	2,137		516		
	MEAN	**STD DEV**	**MEAN**	**STD DEV**	***p-value**
Age, years	55.72	10.3	60.55	8.84	<.0001
Sex, % male	54.1	0.50	56.8	0.49	0.025
Mean BP, mmHg, ([2xDBP]+SBP) / 3	107.30	7.51	96.40	10.45	<.0001
BMI, kg/m^2	28.37	5.24	28.67	4.77	0.479
Smoking, %	22.39	0.42	10.96	0.31	<.0001
HDL cholesterol, mg/dl	50.68	15.93	49.71	15.27	0.855
Triglycerides, mg/dl	146.70	104.3	143.90	79.82	0.232
Alcohol, ounces/week	3.68	5.29	3.20	4.26	0.317
Number of Subjects (N)	1,951		451		
Traits	**Param Estimates**	**Std. Error**	**Param Estimates**	**Std. Error**	**p-value**
Fasting glucose, mg/dl	96.93	0.23	96.35	0.45	0.266
FG_adj. change in fasting glucose, mg/dl	2.88	0.31	4.04	0.61	0.097

Incidence of diabetes	4.10%	5.65%	0.141

Appendix 7: Effect of each weighted fasting glucose risk allele on change over time in fasting glucose in the full T2DM risk factors-adjusted model.

Age-, sex-, fasting glucose-adjusted	**Change in fasting glucose, in mg/dl**	
	Estimate	p-value
Age, years	0.185	<.0001
Sex, % male	0.805	0.0003
Fasting glucose, mg/dl	-0.417	<.0001
Mean BP, mmHg, ([2xDBP]+SBP) / 3	0.017	0.054
BMI, kg/m^2	0.375	<.0001
Smoking, %	0.171	0.438
HDL cholesterol, mg/dl	-0.018	0.016
Triglycerides, mg/dl	0.009	<.0001
Alcohol, ounces/week	-0.015	0.534
Fasting glucose 16-SNP Genetic Risk Score	0.623	1E-29

Appendix 8: Mean weighted genetic risk scores in subjects who developed T2DM vs. subjects who did not, and additional estimated risk of incident T2DM per weighted risk allele in full T2DM risk factors-adjusted models.

Full T2DM risk factors-adjusted model	**T2DM**		**No T2DM**		**†OR**	**p-value**
	Mean	*Std. Dev	Mean	Std. Dev		
Number of Subjects	403		14,997			
Age, years	57.87	9.57	51.78	11.18	1.056	<.0001
Sex, % male	54.74%		45.90%		0.962	0.7546
Mean BP, mmHG, ([2xDBP]+SBP) / 3	99.09	11.58	92.25	11.10	1.138	<.0001
BMI, kg/m^2	31.51	5.49	26.61	4.64	1.138	<.0001
Smoking, %	0.22	0.42	0.25	0.43	1.179	0.2104
HDL cholesterol, mg/dl	43.66	13.52	51.77	15.27	0.975	<.0001
Triglycerides, mg/dl	192.80	148.80	120.70	87.92	1.002	<.0001
Alcohol, ounces/week	2.79	4.64	2.92	4.18	1.000	0.9827
Mean (CI) T2D 33-SNP Genetic Risk Score	17.25	1.73	16.84	1.71	1.201	4.41E-09

*Std. Dev: Standard Deviation. †OR: Odds ratio, per risk-allele.

Appendix 9. Interaction effect by fasting glucose-genetic risk score on hypertension, hypertension treatment or specific drug exposures for change over time in fasting glucose in the full set of T2DM risk factors-adjusted models.

Interaction_HTNxSNP, multi-adjusted model	**Change in fasting glucose, mg/dl**	
	Estimate	p-value
Number of subjects	**14,490**	
Age, years	0.17	<.0001
Sex, % male	0.84	0.0006
Fasting glucose, mg/dl	-0.42	<.0001
BMI, kg/m^2	0.36	<.0001
Smoking, %	0.22	0.39
HDL cholesterol, mg/dl	-0.02	0.02
Triglycerides, mg/dl	0.01	1.60E-12
Alcohol, ounces/week	-0.02	0.40
Hypertension, %	1.04	0.43
Mean FG 16-SNP Genetic Risk Score	0.62	1.82E-14
HTN x Mean (CI) FG 16-SNP Genetic Risk Score	0.03	0.85

Interaction_TreatmentxSNP, multi-adjusted model	**Change in fasting glucose, mg/dl**	
	Estimate	p-value
Number of subjects	**4,445**	
Age, years	0.12	6.7E-9
Sex, % male	0.37	0.44
Fasting glucose, mg/dl	-0.28	<.0001
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	-0.0057	0.76
BMI, kg/m^2	0.33	4.4E-16
Smoking, %	0.33	0.57
HDL cholesterol, mg/dl	-0.03	0.03
Triglycerides, mg/dl	0.01	0.0003
Alcohol, ounces/week	-0.07	0.10
Treated hypertensive	2.06	0.37
Mean FG 16-SNP GR Score	0.56	0.004
HTN x Mean (CI) FG GR Score	-0.18	0.50

Interaction_Beta-blockersxSNP, multi-adjusted model	**Change in fasting glucose, mg/dl**	
	Estimate	p-value
Number of subjects	**4,445**	
Age, years	0.11	<.0001
Sex, % male	0.28	0.55
Fasting glucose, mg/dl	-0.28	<.0001
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	-0.0001	1.00
BMI, kg/m^2	0.32	<.0001
Smoking, %	0.37	0.53
HDL cholesterol, mg/dl	-0.03	0.03
Triglycerides, mg/dl	0.01	0.0005
Alcohol, ounces/week	-0.07	0.10
Hypertensive exposed to beta-blockers ONLY	1.27	0.71
Mean FG 16-SNP Genetic Risk Score	0.56	0.01
Beta-blockers x Mean (CI) FG 16-SNP Genetic Risk Score	-0.11	0.79
Interaction_ThiazidesxSNP, multi-adjusted model	**Change in fasting glucose, mg/dl**	
Number of subjects	**4,445**	
Age, years	0,11	<.0001
Sex, % male	0,28	0,55
Fasting glucose, mg/dl	-0,28	<.0001
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	-0,0001	1,00
BMI, kg/m^2	0,32	<.0001
Smoking, %	0.37	0.53
HDL cholesterol, mg/dl	-0.03	0.03
Triglycerides, mg/dl	0.01	0.0005
Alcohol, ounces/week	-0.07	0.10
Hypertensive exposed to thiazides ONLY	-0.48	0.90
Mean FG 16-SNP Genetic Risk Score	0.56	0.01
Thiazides x Mean (CI) FG 16-SNP Genetic Risk Score	-0.04	0.93
Interaction_ACE-inhibitors/ARBxSNP, multi-adjusted model	**Change in fasting glucose, mg/dl**	
Number of subjects	**4,445**	
Age, years	0.11	<.0001
Sex, % male	0.28	0.55
Fasting glucose, mg/dl	-0.28	<.0001
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	-0.0001	1.00
BMI, kg/m^2	0.32	<.0001
Smoking, %	0.37	0.53
HDL cholesterol, mg/dl	-0.03	0.03
Triglycerides, mg/dl	0.01	0.0005
Alcohol, ounces/week	-0.07	0.10
Hypertensive exposed to ACE-inhibitors/ARB ONLY	3.12	0.45
Mean FG 16-SNP Genetic Risk Score	0.56	0.01
ACE-inhibitors/ARB x Mean (CI) FG 16-SNP Genetic Risk Score	-0.22	0.64

Appendix 10. Interaction effect by T2DM-genetic risk score on hypertension, hypertension treatment or specific drug exposures for risk of incident T2DM in the full set of T2DM risk factors-adjusted models.

Multi-adjusted model	**T2DM**	
	OR	**p-value**
Number of subjects	**15,392** (DM=1 N=403)	
Age, years	1.05	2.2E-16
Sex, % male	1.00	1.00
BMI, kg/m^2	1.14	<.0001
Smoking, %	1.20	0.18
HDL cholesterol, mg/dl	0.98	3.5E-6
Triglycerides, mg/dl	1.00	9.8E-8
Alcohol, ounces/week	1.00	0.88
Hypertension, %	4.39	0.18
Mean (CI) T2D 33-SNP GR Score	1.23	4.7E-5
HTN x Mean (CI) T2D 33-SNP Genetic Risk Score	0.96	0.50
Multi-adjusted model	**T2DM**	
	OR	**p-value**
Number of subjects	**4,757** (DM=1 HTN=1 N=260)	
Age, years	1.03	2.3E-5
Sex, % male	1.09	0.61
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	1.01	0.27
BMI, kg/m^2	1.11	<.0001
Smoking, %	1.23	0.22
HDL cholesterol, mg/dl	0.98	0.003
Triglycerides, mg/dl	1.00	0.0003
Alcohol, ounces/week	0.98	0.25
Treated hypertensive	2.80	0.48
Mean (CI) T2D 33-SNP Genetic Risk Score	1.19	0.01
HTN_treated x Mean (CI) T2D 33-SNP GR Score	0.97	0.71

Multi-adjusted model	**T2DM**	
	OR	**p-value**
Number of subjects	**4,757** (DM=1 HTN=1 N=260)	
Age, years	1.03	0,0001
Sex, % male	1.10	0,564
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	1.01	0,283
BMI, kg/m^2	1.11	<.0001
Smoking, %	1.23	0,226
HDL cholesterol, mg/dl	0.98	0,003
Triglycerides, mg/dl	1.00	0,0005
Alcohol, ounces/week	0.98	0,254
Treated hypertensive	10.76	0,222
Mean (CI) T2D 33-SNP Genetic Risk Score	1.19	0,013
Beta-blockers_treated x Mean (CI) T2D 33-SNP GR Score	0.90	0,336

Multi-adjusted model	**T2DM**	
	OR	**p-value**
Number of subjects	**4,757** (DM=1 HTN=1 N=260)	
Age, years	1.03	0,0001
Sex, % male	1.10	0,564
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	1.01	0,283
BMI, kg/m^2	1.11	<.0001
Smoking, %	1.23	0,226
HDL cholesterol, mg/dl	0.98	0,003
Triglycerides, mg/dl	1.00	0,0005
Alcohol, ounces/week	0.98	0,254
Treated hypertensive	0.61	0,84
Mean (CI) T2D 33-SNP Genetic Risk Score	1.19	0,013
Thiazides_treated x Mean (CI) T2D 33-SNP GR Score	1.05	0,72

Multi-adjusted model	**T2DM**	
	OR	**p-value**
Number of subjects	**4,757** (DM=1 HTN=1 N=260)	
Age, years	1.03	0,0001
Sex, % male	1.10	0,564
Mean blood pressure, mmHg (([2xDBP]+SBP) / 3)	1.01	0,283
BMI, kg/m^2	1.11	<.0001
Smoking, %	1.23	0,226
HDL cholesterol, mg/dl	0.98	0,003
Triglycerides, mg/dl	1.00	0,0005
Alcohol, ounces/week	0.98	0,254
Treated hypertensive	6.20	0,441
Mean (CI) T2D 33-SNP Genetic Risk Score	1.19	0,013
ACE-inhibitors/ARB_treated x Mean (CI) T2D 33-SNP GR Score	0.92	0,531

11. SCIENTIFIC PRODUCTION RELATED TO THIS WORK AND TO THE APPLICATION OF KNOWLEDGE OF GENETIC VARIATION FOR TYPE-2 DIABETES PREDICTION BY THE AUTHOR

PUBLICATIONS

1. de Miguel-Yanes JM, Porneala B, Pencina MJ, Fox CS, Florez JC, Siscovick DS, Dupuis J, Meigs JB. Interaction of common beta-cell-associated variants with hypertension on change over time in fasting glucose and incident type 2 diabetes: the Framingham Offspring Study. Submitted to Journal of Hypertension. Under review.
2. de Miguel-Yanes JM, Shrader P, Pencina MJ, Fox CS, Manning AK, Grant RW, Dupuis J, Florez JC, D'Agostino RB Sr, Cupples LA; the MAGIC Investigators; the DIAGRAM Investigators, Meigs JB. Genetic risk reclassification for type 2 diabetes by age below or above 50 years using 40 type 2 diabetes risk single nucleotide polymorphisms. Diabetes Care. 2011; 34:121-125.
3. de Miguel-Yanes JM, Manning A, Shrader P, McAteer J, Goel A, Hamsten A, Fox C, Florez JC, Dupuis J, Meigs JB. Variants at the endocannabinoid receptor CB1 gene (*CNR1*) and insulin sensitivity, type 2 diabetes and coronary heart disease. Obesity (Silver Spring). 2011; 19:2031-2037.

POSTERS IN CONGRESSES

1. de Miguel-Yanes JM, Porneala B, Pencina MJ, Fox CS, Florez JC, Siscovick DS, Dupuis J, Meigs JB. Common beta-cell–associated genetic variants do not interact with hypertension or hypertension treatment in their associations with change over time in fasting glucose or risk of new onset type 2 diabetes. European Federation of Internal Medicine Congress. Madrid, October 24-27, 2012. In press.
2. de Miguel-Yanes JM, Porneala B, Pencina MJ, Fox CS, Florez JC, Siscovick DS, Dupuis J, Meigs JB. Common beta-cell-associated genetic variants and hypertension or anti-hypertensive drugs do not interact in their positive associations with risk for rising fasting glucose or incident type 2 diabetes in Framingham. AHA Epidemiology and Prevention / Nutrition, Physical Activity and Metabolism Scientific Sessions. San Diego CA, March 12-15, 2012.

3. de Miguel-Yanes JM, Shrader P, Sullivan LS, Fox CS, Dupuis J, Manning AK, Florez JC, Wilson PWF, D'Agostino RB Sr, Cupples LA; the MAGIC Investigators; the DIAGRAM Investigators, Meigs JB. A type 2 diabetes (T2D) genetic risk score using 37 single nucleotide polymorphisms (SNPs) significantly reclassifies T2D risk beyond clinical T2D risk factors. American Diabetes Association (ADA), Orlando FA, June 25-29, 2010.
4. de Miguel-Yanes JM, Manning AK, McAteer JB, Florez JC, Fox CS, Cupples LA, Dupuis J, Meigs JB. Association of genotype at a SNP near the endocannabinoid receptor CB1 gene (*CNR1*) with insulin sensitivity and risk of diabetes or coronary heart disease in Framingham. AHA Epidemiology and Prevention / Nutrition, Physical Activity and Metabolism Scientific Sessions. San Francisco, CA, March 2-5, 2010.