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SYMPOSIUM

A Genomics Study of Type 2 Diabetes Mellitus in U.S. Air Force Personnel

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Abstract

The military community is at high risk for type 2 diabetes (T2D), especially as it relates to military beneficiaries, although preventive measures can be implemented to reduce disease onset. This study evaluates the prevalence of risk-associated single nucleotide polymorphisms in patients diagnosed with T2D within active duty, retired military, and military-dependent populations on Lackland Air Force Base compared to nondiabetic controls. Results will be used as a basis of comparison to analyze risk-conferring genotypes in the young, healthy active duty population to generate the prevalence of T2D risk-associated factors in our current and future war fighters. Identifying genetic markers of T2D prior to abnormal glucose control and insulin resistance may ultimately adjust future risk through early detection, healthy lifestyle modifications, and disease management programs.

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Introduction

This presentation discusses a translational research study on the prevalence of type 2 diabetes (T2D) risk-associated markers in the United States Air Force (USAF). A panel of established variant single nucleotide polymorphisms (SNPs) in patients diagnosed with T2D is being evaluated within Air Force active duty (AFAD), retired military, and military-dependent populations on Lackland Air Force Base compared to a nondiabetic control population. These results will be used as a basis of comparison to analyze risk-conferring genotypes in the young, healthy active duty population, including basic military trainees, to generate the prevalence of T2D risk-associated factors in our current and future war fighters. The frequency of normal and risk alleles is being evaluated by allelic discrimination and DNA sequence confirmation to validate ambiguous SNP genotypes and elucidate new SNPs of medical relevance. The results of this study will provide a better overall understanding of the genetic epidemiology of T2D in the U.S. Air Force. Identifying genetic markers of T2D prior to abnormal glucose control and insulin resistance is critical in reducing disease onset and may ultimately adjust future risk to the war fighter through early detection and healthy lifestyle modifications.

Impact of Diabetes to the U.S. Military

The military community is at high risk for T2D despite requirements to maintain weight/body fat criteria and strict adherence to physical fitness standards.[1] The USAF Clinical Information's Branch (AF/SGRKH, Brooks City Base, personal communications) reports the incidence of T2D at over 38,000 personnel with fewer cases in active duty (1222) than retired military (18,954) and military-dependent populations (18,192). Nonetheless, the incidence of diabetes in military personnel is actually similar to

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Abbreviations: (AFAD) Air Force active duty, (BMI) body mass index, (PCR) polymerase chain reaction, (SNPs) single nucleotide polymorphisms, (T2D) type 2 diabetes, (USAF) United States Air Force

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that of the civilian population (1.9 cases vs 1.6 cases per 1000 persons per year),[1] as diabetes of any type is cause for rejection from military service. Several obstacles prevent members of the U.S. military from implementing regular healthy lifestyle practices, including the extreme demands of tours of duty in remote locations and the stress of continuous changes in environment and routine with irregular and challenging schedules. Most active duty military personnel that develop diabetes are referred for medical discharge or retirement,[1,2] but the consequences to military readiness are far and above the cost of health care. As military personnel confront the difficulties of weight reduction and are selected for separation from the military, there is a significant cost to train new war fighters and a decline in overall workforce skills, knowledge, and experience. Early detection of risk factors that contribute to the physiological development of T2D in this population of relatively young and presumably fit individuals is critical for the prevention of diabetes and significant to both military and civilian populations.

Clinical practice allows for prediction of the risk of diabetes in patients manifesting symptoms of disease and exhibiting physical indications for measured testing. In combination with clinical symptoms, predictors of T2D are also family history and recent smoking history. Knowledge of risk factors for T2D specific to the U.S. military population is limited. Paris and colleagues[1] studied differences in common demographic and clinical determinants of diabetes in the military population and reported that risk factors for T2D in U.S. military personnel are similar to the general U.S. population. In this case-control study, risk factors for T2D in the U.S. military include increased body mass index (BMI), African American and Hispanic origin compared with white race, and junior enlisted vs officers.[1] African American origin is also a risk factor within the USAF specifically. The self-identified Black/African American race group comprises only 14.7% of the total active duty population, but 29.3% of the AFAD T2D population, while the self-identified white race group comprises 73.9% of the total active duty population, but 57.4% of the AFAD T2D population (AF/SGRKH, Brooks City Base, personal communications).

Genetics of Type 2 Diabetes Mellitus

The genomic prevalence of T2D risk-associated markers in the U.S. military is not known. Genome-wide association studies analyzing common SNPs have cataloged several loci of human DNA sequence variation conferring risk for T2D.[4–22] Deciphering the sequences of the human genome that are preferentially associated with T2D has uncovered disease-associated SNPs identifying variants involved in disease-causing mechanistic pathways.[24–37] For the purpose of this study, 16 SNPs associated with T2D identified in previous studies are targeted for analysis[4–6] (**Table 1**).

Table 1.
Sixteen T2D-Associated SNPs Being Genotyped and Respective Published *P* Values from Meta-Analyses

SNP	Gene locus	Chromosome	Published *P* value	Source
rs10923931	NOTCH2	1	4.1×10^{-8}	Zeggini and colleagues[4]
rs7903146 / rs7901695	TCF7L2	10	1.0×10^{-48}	Saxena and colleagues[5]
rs13266634	SLC308A	8	5.3×10^{-8}	Saxena and colleagues[5]
rs4402960 / rs1470579	IGF2BP2	3	8.9×10^{-16}	Saxena and colleagues[5]
rs1801282	PPARg	3	1.7×10^{-6}	Saxena and colleagues[5]
rs5015480 / rs1111875	HHEX	10	5.7×10^{-10}	Saxena and colleagues[5]
rs5215 / rs5219	KCNJ11	11	6.7×10^{-11}	Saxena and colleagues[5]
rs8050136 / rs9939609	FTO	16	9.2×10^{-5}	Lyssenko and colleagues[6]
rs7754840	CDKAL1	6	4.1×10^{-11}	Saxena and colleagues[5]
rs10811661	CDKN2A/2B	9	7.8×10^{-15}	Saxena and colleagues[5]
rs7578597	THADA	2	1.1×10^{-9}	Zeggini and colleagues[4]
rs4607103	ADAMTS9	3	1.2×10^{-8}	Zeggini and colleagues[4]
rs864745	JAZF1	7	5.0×10^{-14}	Zeggini and colleagues[4]
rs12779790	CDC123 / CAMK1D	10	1.2×10^{-10}	Zeggini and colleagues[4]
rs10010131	WFS1	4	0.001	Lyssenko and colleagues[6]
rs7961581	TSPAN8 / LGR5	12	1.1×10^{-9}	Zeggini and colleagues[4]

Methods

Study Populations

Patients 18 years or older that are active duty, retired military, or military dependents will be included in the study if they have been diagnosed previously with T2D. Population controls will be selected from a cohort of Air Force retired and dependent military patients who do not carry the diagnosis of diabetes and from the generally healthy (nonchronically ill), over-40 Air Force active duty population. Genotyping will also be performed in the 18- to 32-year-old, active duty population (mean age 24), including basic military trainees, to generate the prevalence of T2D risk-associated factors in our current healthy population prior to the manifestation of clinical risk factors. This study was approved by the Wilford Hall Medical Center Institutional Review Board for enrollment of 3000 patients, and written informed consent is being obtained from all participants.

Measurements

Standardized questionnaires of clinical symptoms and patient history are administered at entry. Personal information obtained for the study at time of enrollment includes age, gender, race/ethnicity, smoking history, family history of disease, T2D medical complications, age at T2D diagnosis, and medications. Laboratory tests and measurements obtained include blood pressure, BMI, hemoglobin A1c, fasting glucose, total cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, albumin, creatinine, potassium, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin.

Genotyping

Whole blood is collected into BD Vacutainer™ acid citrate dextrose tubes. Purification from buffy coat is performed on the automated DNA purification Maxwell 16 system. SNP genotyping is performed by allelic discrimination assays utilizing fluorogenic 5′ nuclease chemistry. Oligoribonucleotide primers are predesigned specifically to regions flanking single nucleotide polymorphisms in the genomic DNA template and are included in the TaqMan Universal PCR Master Mix (Applied Biosystems) used in this study by design. Each genetic locus is evaluated by performing a polymerase chain reaction (PCR) on purified genomic DNA. Allelic discrimination assays classify unknown samples for normal or risk allele. In SNP genotyping, a single mismatch between probe and target sequences is discriminated with competing probes designed to support strong and specific binding. Each probe is labeled with a fluorescent dye that provides fluorescent signals at different wavelengths, and each dye is assigned a specific allele corresponding to the common or variant SNP. Heterozygosity for common and variant alleles will fluoresce at both signals. Initial SNP allelic discrimination curves observed by real-time PCR results are confirmed by DNA sequencing of each gene.

Ambiguous SNP genotypes generated from PCR are calculated using raw fluorescent data of the allelic discrimination curves. Based on the kinetic properties of PCR amplification, PCR amplification efficiency is addressed by determining the net fluorescent difference between maximal and minimal fluorescent signals and the beginning of the exponential growth phase of the reaction. For resequencing confirmation, new primers were designed for each target region, and the specific region containing the SNP is amplified by PCR. Sequences are then obtained using ABI 3730.xls capillary sequencers, and data are analyzed using Applied Biosystems SeqScape® software v2.6 for reference-based analysis, including mutation detection and analysis, SNP discovery and validation, allele identification, and sequence confirmation. Sequence analysis will be performed for the validation of ambiguous genotypes and evaluation of new SNPs.

Statistical Analysis

Each SNP association with T2D will be evaluated by comparing the allele frequencies in diabetic patients versus nondiabetic controls. This analysis is based on the case-control design, and logistic regression will be used to estimate the change in log odds (and the odds ratio) of diabetes associated with a change in genotype. Initially, each SNP will be considered individually as a predictor along with covariates such as age and BMI. The SNP genotype will be coded as the count of the copy number of the minor allele (0,1,2) so that the additive mode of inheritance is assumed. Other modes of inheritance will be considered secondarily. For each SNP, subjects will be stratified by ethnicity and analyzed separately in order to reduce the confounding effects of population substructure. Next, subjects of all ethnicities will be analyzed together with ethnicity introduced as a categorical covariate. The Benjamini Hochberg method will be used to adjust for the multiple hypotheses testing due to the number of SNPs.

Discussion

Population-based whole genome association studies in patients with T2D have identified single nucleotide genetic variants that regulate complex traits and explain a substantial portion of disease risk. This study evaluates

the prevalence of T2D risk-conferring markers within the USAF population. Although the mechanistic pathways of a majority of identified variants remain to be elucidated, many of the SNPs evaluated for the purpose of this study influence β-cell function and have been associated with an enhanced risk of future diabetes and prediction of future disease, especially in young adults.[3,6]

The inheritance of susceptibility genes in combination with pathophysiological pressures from environmental factors contribute to the multifactorial development of T2D. Previous studies analyzed the predictive value of genotyping risk-associated markers for T2D compared to common phenotypic risk factors alone. Lyssenko and colleagues[6] analyzed 16 known DNA variants and reported that genotyping risk-associated SNPs had a minimal but statistically significant effect on the prediction of future T2D and that assessment of genetic risk factors is more meaningful when measured earlier in life. Meigs and colleagues[3] genotyped SNPs at 18 loci associated with diabetes in participants of the Framingham Offspring Study, essentially all of European ancestry, and calculated the genotypic score in relation to phenotypic and clinical risk factors of diabetes.[3] Results indicate that when limited to adults, genetic risk factors provide only a slightly better prediction rate of new cases of diabetes over selected clinical risk factors alone.[3] However, Meigs and colleagues[3] reported that a combination of genomic risk alleles is a strong risk factor for T2D in younger persons and suggested that this information is useful for genetic screening in youth before obvious risk factors have developed.

Conclusion

Both genetic background and lifestyle choices contribute to the underlying causative physiology of type 2 diabetes. Behavior modifications can prevent or delay the onset of T2D; unfortunately, individuals that already carry the diagnosis of T2D cannot always bring blood glucose levels down to the normal range through lifestyle changes. In these cases, blood glucose-lowering diabetic medications are a treatment option; however, diabetes medications do not always lower blood glucose levels near the normal range. The timely identification of candidates for T2D is ultimately the best option for prevention, delay, and successful treatment of disease.

Early detection of genetic risk factors that contribute to the physiological development of T2D has been made possible by genomic advances. Identifying genetic markers of type 2 diabetes is critical in reducing disease onset and in delaying complications that add significant cost to military health care. Results of the Diabetes Prevention Program raised the possibility that behavior modification resulting from lifestyle intervention can mitigate the risk conferred by genetic variants and suggest that persons that implement more healthy lifestyles can better overcome susceptibility to genetic risk than those with less healthy behaviors.[38] Providing evidence-based medicine to the war fighter through genetic screening would afford the opportunity for early prevention and control strategies by altering lifestyle and environmental factors that contribute to disease or by medication-based treatment. A better understanding of the genetic architecture of T2D is essential for evaluating the future risk of disease.

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Disclosure:

The opinions expressed in this document are solely those of the author and do not represent an endorsement by or the views of the United States Air Force, the Department of Defense, or the United States Government.

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