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Pima Indian Diabetes susceptibility differs significantly from European susceptibility.

## **Introduction**

Diabetes is classified as an epidemic by the CDC<sup>1</sup>. It is a chronic impairment in a person's ability to react properly to glucose dissolved in the blood. As of the end of 2007, it affects 23.6 million people of the United States. The majority of these people are diagnosed with Type 2 Diabetes Mellitus (T2DM). This variety makes up 95% of those 24 million. The Center for Disease control estimates that the total cost of diabetes in the United States is \$174 billion. The impact of diabetes on a person's lifestyle is progressive over time, beginning with the body's cells being resistant to insulin-mediated glucose uptake. Eventually, the pancreas cannot sustain the high insulin production level needed for glucose uptake and insulin levels drop.

Complications linked to Diabetes is the sixth leading cause of death in the US. A person's risk for developing type 2 diabetes has been shown to be highly linked to obesity and any family history of diabetes.<sup>2</sup>

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<sup>1</sup> Diabetes At a Glance. <http://www.cdc.gov/ncccdphp/publications/aag/ddt.htm>

<sup>2</sup> National Diabetes Fact Sheet, p. 5., 2007, <http://apps.nccd.cdc.gov/DDTSTRS/FactSheet.aspx>

Diabetes is a complex disease that is not well-understood. Unlike other simpler inheritable diseases, diabetes has consistently been shown to be not linked to any single gene or genetic marker. Current hypotheses are that diabetes is caused by a large number of genes working in tandem to generate the phenotype of insulin resistance. Therefore, just as a known mechanism for diabetes is missing, so is any definite way to permanently cure the symptoms.

As can be expected, several studies have been made to discover the mechanism of T2DM. There is strong evidence that there exist multiple mechanisms through which diabetes can function. These differences have been shown to exist by Genome-Wide Association Studies performed on assays of genetic markers called Single-Nucleotide Polymorphisms (SNPs). It is possible through a GWAS to link certain alleles of these SNPs to the presence of versions of a gene that can cause observable disease in a person. Multiple GWAS studies have been performed using thousands of SNPs on Caucasian, Asian, and African populations. This has led to the identification of several genes such as CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and others as being statistically correlated with a person's susceptibility towards developing type 2 diabetes. However, when these same genes are tested for statistical significance in a population that is non-Caucasian (such as Africans, Asians, or Native Americans) many of these genes are not shown to be significantly associated with diabetes. Therefore, it is highly probable that the disease known as type 2 diabetes develops differently among different populations.

This paper will examine the efforts made from 2007 onward in a long-term study on the Pima Indian tribe by the intramural research program of the National Institute of Diabetes and Digestive and Kidney Diseases. Located in southern Arizona, this group of Native Americans has one of the highest incidences of diabetes in any population of the United States. 29.3% of Native American adults located in this area of Arizona and are served by the Indian Health Service have been diagnosed with diabetes.<sup>3</sup>

### **Pima Indian Study**

In an article published in December of 2007, Robert L. Hanson lead a project to identify SNPs associated with type 2 diabetes among 300 diagnosed diabetics. The number of SNPs analyzed in the study was 80,044. 334 non-diabetics were used as controls. Population stratification in this GWAS was not considered significant due to the fact that only full-heritage (and therefore genetically similar) Pima Indians were included in the study. The SNP analysis pointed to only 10 genomic regions being statistically significant. The lowest P value found was for rs686989 with a value of  $2.7 \times 10^{-6}$ . However, “no single marker achieved experiment-wide statistical significance”. In other words, the study was unable to clearly identify any single or group of genes strongly linked to whether a given Pima Indian had diabetes or not. Only the combined distribution of low P-values among various markers was able to reject the null hypothesis that no association exists between any SNP and the presence of the disease.

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<sup>3</sup> National Diabetes Fact Sheet, p. 1., 2007, <http://apps.nccd.cdc.gov/DDTSTRS/FactSheet.aspx>

The study by Hanson to associate possible genetic variants to a person's susceptibility towards developing diabetes is best described as preliminary work. The study was meant to serve as a starting point in identifying regions of the human chromosome linked to diabetes. Regarding SNPs identified in other GWA Studies of T2DM involving several European populations, Hanson points out that "None of the SNPs most strongly associated in the present study was in any of these regions."<sup>4</sup> The SNPs that were found to be statistically significant were used as the starting point for examining smaller more specific regions of DNA near these SNPs. The most significant SNP was located in a non-coding region, and therefore functional analysis was impossible. The next two significant SNPs however were studied.

### **CACNA1E**

The second most strongly linked SNP was surrounding the gene CACNA1E on chromosome 1q21-25. This gene codes for a protein subunit that makes up voltage-gated calcium ion channels that are highly expressed within neurons and pancreatic b-cells. In a paper published in December 2007 with Hanson, this gene was sequenced for 24 Pima Indians not directly related. The original SNP was shown to be in high linkage disequilibrium with a statistically significant (P-value:  $6 \times 10^{-4}$ ) SNP labelled +8130 G/A. Functional analysis suggested that this variant affected mRNA expression for the protein. However, when +8130 G/A was tested for association towards diabetes with 2,427 members of the general Pima Indian population, the significance of this variant dropped to only  $P=8 \times 10^{-2}$ . This result arose because the majority of the

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<sup>4</sup> Hanson, et. al., p. 3051, Diabetes, vol 56, December 2007

participants recruited for the study came from families with “a high prevalence of young-onset diabetes”.<sup>5</sup>

### **ARHGEF11**

The third most strongly linked SNP was located within the gene ARHGEF11 on chromosome 1q21-q23 which had its findings published in May 2007.<sup>6</sup> However, after a study similar to that of CACNA1E (24 participants), the lowest SNP P-value among 75 SNP variants was only  $P=0.03$ . This is to say that no statistically significant association can be proven between diabetes and ARHGEF11 among the Pima Indians.

In summary, these studies failed to show any clear association between any single gene and type 2 diabetes. This can be partially explained by the lack of statistical power that this study was able to use as a result of the small population available to be tested.

Comparable GWAS studies into type 2 diabetes have genotyped as many as 1,363<sup>7</sup> diabetic subjects while studies by Hanson’s group genotyped only a maximum of 300 diabetic subjects. This is likely due to the relatively small size of Native American groups in comparison to the number of caucasians. In addition, the fact that Genome-Wide Association Studies are being performed mainly upon majority populations means that minority groups that have a known high genetic predisposition towards the disease are not likely to fully benefit from understanding how caucasian diabetes works. This is because a more recent study by Hanson’s group officially rules out any major similarity

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<sup>5</sup> Muller, et al., p. 3093. Diabetes Vol 56, Dec 2007.

<sup>6</sup> Ma, et al., p. 1454. Diabetes , Vol 56, May 2007

<sup>7</sup> Sladek, et al., p. 881, Nature, vol 445

between genes known to make caucasians susceptible to T2DM and Pima Indian genes.

### **No Native American-Caucasian T2DM Association**

In February 2009, Rong Rong, Robert L. Hanson, et. al., published the results of a comprehensive round of tests that genotyped 3,501 Pima Indians against 47 SNPs centered in and around the genes FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761, and CDKN2B. These genes were among associations identified in multiple other GWA Studies of T2DM.<sup>8</sup> The Body Mass Index of each subject was also compared to the SNPs present. 370 of the subjects had quantitative traits such as Acute Insulin Response (AIR) and plasma insulin concentrations over time measured.<sup>9</sup>

The study found that not a single one of the SNPs associated among the other GWAS populations provided any evidence that the previously mentioned genes were “significantly associated with type 2 diabetes or obesity among full-heritage Pima Indians living in the Gila River Indian Community in Arizona”. Minor correlations were found between most gene alleles and measured BMI as well as glucose levels in response to AIR tests and Oral Glucose Tolerance Tests (OGTT). For the most part, however, these genes contributed very little towards determining whether a person developed T2DM or not. Only FTO, a gene implicated in obesity showed a modest correlation with T2DM ( $P = 0.03$ )<sup>10</sup>. Subjects homozygous for the risk allele of FTO were 1.6 kg/m<sup>2</sup> heavier than those homozygous for the non-risk allele.

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<sup>8</sup> See references 2-7 found on: Rong Rong, et al., p. 487, Diabetes, Vol 58, February 2009

<sup>9</sup> Rong Rong, et al., p. 478, Diabetes, vol 58, Feb 2009

<sup>10</sup> Rong Rong, et al., 485, Diabetes, vol 58, Feb 2009

## **Implications**

The fact that no SNP alleles highlighted as associated with T2DM in other GWA Studies appeared among the Pima Indians is very important. It suggests that a different set of genes yet to be identified is responsible for causing the genetic susceptibility. It also suggests that if any progress is made in linking the caucasian GWAS genes to a T2DM mechanism, this will not benefit the Pima Indians since their version of T2DM is not statistically associated with those genes. This is to be expected of any other disease caused by multiple genes (polygenic diseases) since different ethnic groups have dissimilar genetic make-ups. Also, because GWA Studies require a large number of subjects and controls to produce meaningful results, smaller minority populations with distinct genetic characteristics will suffer from low-power SNP statistical analyses.

## **Conclusion**

The epidemic of Type 2 Diabetes Mellitus among the Pima Indian population will require continued investigation into statistically significant portions of the human genome if T2DM is to be fully understood. If initial studies mentioned in this paper are any indication, the complex mechanism of T2DM in Pima Indians (and quite possibly other distinct ethnic groups) will involve a different set of genes. Progress in understanding the mechanism of T2DM in populations such as the Pima will likely require continued independent research even if progress is made in understanding T2DM mechanisms for other ethnic populations. The low populations of minority ethnic groups also will hinder future studies statistically as it has for Hanson and his colleagues if GWA Studies remain the primary method for identifying genes causative of disease.

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