Mutation and Modification Identification of Targeted Genes Associated with Type 1 Diabetes and the effects of Imatinib Mesylate.

Background and Significance
Type 1 diabetes, also known as juvenile diabetes, is a result of an autoimmune disorder where a loss of insulin production occurs because of pancreatic beta cells destruction. Among all people diagnosed with diabetes, 5-10% of them are diagnosed with type 1 diabetes (T1D) (3). It is clear that there is a genetic factor for T1D that involves many different genes on many different chromosomes (13). It is also clear that genetics, as well as environmental factors that may trigger and interact with susceptibility genes, are important for developing T1D (9). What is not clear from these studies are specific mutations and modifications of sites on these genes associated with T1D. It is important to us, then, to find out these mutations and modifications within genes that are associated with T1D. We hypothesize that the identification of specific modifications in key gene loci associated T1D will allow for a better understanding of the genetic bases for T1D, therefore, allowing drug companies to pursue a drug target to cure T1D.

We are interested in the major gene loci that have surfaced as being possible genetic bases for causing T1D as well as discovering new gene loci. Through previous research, the major known loci associated with T1D are the insulin locus (INS) on chromosome 11p15, histocompatibility complex (MHC) on chromosome 6p21, tyrosine phosphatase-22 protein (PTPN22) on chromosome 1p13 (KIAA0350) (6). In a recent study, another region of the genome on chromosome 16p13, which contains KIAA0350, has been identified as a strong candidate for being associated with T1D. This region is thought to contain a sugar binding C-type lectin and have three non-coding regions (rs2903692, rs725613 and rs17673553) which have been isolated with strong disequilibrium (8). Non-coding regions of genes have particular interest for us because a gene’s regulation and expression is controlled by these regions, thus, this experiment will pay particular attention to the three non-coding regions of KIAA0350.

Drugs and TID: Drugs have also shown to be a significant tool in fighting T1D. A recent study on non-obese diabetic (NOD) mice have shown that a 10 week treatment with the drug imatinib Mesylate (Gleevec) stops, and suggest to reverse, symptoms of diabetes (13). NOD mice share many phenotypic and genetic characteristics with type 1 diabetes in humans. No research, however, was done on the genotypic level of the effects of imatinib on a NOD mouse. Instead, the research focused on the phenotypic level of the improvement in the NOD mice symptoms, which include the independence form insulin. It is our interest, therefore, to study the genotypic effect of Imatinib on the genome of a person diagnosed with T1D.
Imatinib is a drug that is used against cancer. Instead of the traditional approach to fighting cancer, which is interrupting the division of rapidly dividing cells, imatinib targets Bcr-Abl tyrosine-kinase receptors by competitive inhibition. This results in an inhibition of any signaling transduction that might have otherwise occurred (5). Imatinib’s common use is to treat people diagnosed with chronic myelogenous leukaemia (CML) which affects the chromosome 9 and 22, resulting in a Philadelphia chromosome. Common side effects seen as a result to Imatinib are vomiting, fluid retention, fatigue, rash, diarrhea, headaches and muscle cramps, which are not severe and easily manageable. More severe side effects are usually only seen on long term Imatinib use. This study, however, will not be using a long term time frame in order to perform our assays.

Specific Aims
We propose two aims that deal with the identification of specific mutations and modifications in the key gene loci 11p15, 1p13, 6p21, and KIAA0350, thus, helping to diagnose and characterize a genetic source of T1D.

- **Aim 1: In what gene(s), and where in the gene(s), are the majority of these contributing factors of T1D confined to?** This will be performed with a human mapping array set. We hypothesize this will help confine the region of the genes that will be further analyzed in this study. A resequencing of the confined regions will then identify, on a large scale, the common mutations within and between individuals diagnosed with and without T1D. We hypothesize that a majority of mutation will occur in the regulatory regions of the genes.

- **Aim 2: What are the epigenetics of T1D, and how does the drug imatinib (Gleevec) affect the targeted genes?** An analysis of the methylation and acetylation of the DNA and histones will be performed before the initial treatment of the drug in order to create a baseline. The same analysis will be preformed after the treatment and then compared to the baseline analysis. We hypothesize that there will be a significant change in methylation and acetylation due to the imatinib treatment.

The main rational for our study is that the characterization of targeted genes associated with T1D will greatly advance our knowledge of this genetic disease. If this study shows the non-coding regions rs2903692, rs725613 and rs17673553 have a significant role in causing T1D, a definitive genetic basis will be established, thus allowing a specific target for drug developers to concentrate on. We realize that our targeted genes are based on previous studies who restricted there study population to only white European descent. Due to this, careful analysis will be made when genotyping our population. In addition to characterizing targeted genes before and after imatinib treatment, this study will serve as a large stepping stone in current and future research on other genetic diseases. It will illuminate our knowledge of the role which genes play in immune and chronic genetic diseases like T1D.
Research Plan

Specific Aim 1: In what gene(s), and where in the gene(s), are the majority of these contributing factors of T1D confined to?

This assay will allow us to confine our study to specific genes associated with T1D. It is essential to locate and identify specific genes associated with T1D because it will allow us to establish a genetic basis for the disease. Our sample population will include siblings and individuals from a variety of ethnic backgrounds consisting of Asian, African, European and Native American descent. This will ensure that any universal mutations we see in the genes of interest are not due to ethnic backgrounds. A total of 1,000 clinically diagnosed T1D participants will act as the experimental group while 1,000 non-T1D precipitants will act as the negative control group. The genes that are targeted to be isolated and analyzed from the DNA samples of the 2,000 participants are INS on chromosome 11p15, MHC on chromosome 6p21, PTPN22 on chromosome 1p13, and chromosome 16p13 which contains KIAA0350 and three non-coding regions of interest.

Once the DNA samples are obtained, an efficient way of amplifying them is by using polymerase chain reaction (PCR) in conjunction with Taq polymerase (7). This will reduce the amount of material needed for this portion of the experiment due to the thermostable properties of Taq polymerase. Affymetrix Genechip© Human Mapping 500K Array Set will be adequate for whole-genome association analysis across two populations. This is done with two sets of arrays that are each capable of genotyping 250,000 SNPs. The software employed to analyze all of the data from the Affymetrix Genechip© is Genchip© Genotypes Analysis Software (1). In order to target genotyping, custom SNP panels can be used with the 500K Array Set. The results of this analysis will isolate the genes of interest specified above as well as confine the area of KIAA0350 on chromosome 6p13 to about 120KB. It will also allow us to identify any addition gene loci that are unexpectedly differentially expressed between the experimental and control sample groups.

We will study the sequential differences within and between the experimental and control populations by using a resequencing chip. Affymetrix Costumseq® Resequencing Arrays can be used carry out this assay (1)(2). Each of the targeted genes will be resequenced and compared to each other within the T1D group, non-T1D group and between the two different groups. Bayesian statistical analysis will be employed on our findings to measure the likely hood of a mutation being either common mutation throughout a populations or a random error in the DNA polymerase proof reading. Mutations that occur in a common region of base pairs within a gene will be looked for among the T1D group in order to characterize and group together any mutation with T1D. However, it is clear from previous research that one mutation in one gene is not what ultimately leads to an individual developing T1D (13)(9). Because of this fact, all
of the targeted genes will be compared to each other to see if any mutations in one gene coincide with mutations in one or more other genes. This is a critical step that we believe will show results. It will further understanding exactly what genes are affected throughout a variety of individuals with T1D and help give insight into what genes function together.

If two genes are statistically shown to have strong likelihood of mutating together, further research will be put into exploring the functions and products of those genes to give insight on how they interact with each other. We will particularly be looking for mutations in regulator, non-coding, regions including rs725613, rs17673553 and re2903692 of KIAA0350. If a mutation is discovered to reside in a non-coding region, the regulator properties of this region will be assayed. If a mutation should reside on a protein-coding ORF, the gene will be explored for important transcripts and/or regulator properties if none is presently known. Discoveries involving gene-gene interactions would also help future studies because it will deepen our understanding of how genes work and dysfunction arises.

**Expected results and pitfalls.** The techniques used in this specific aim are very capable to do. We expect to find additional gene loci associated with T1D that were not previously discovered because our study does encompass a larger variety of ethnic backgrounds. We also anticipate a majority of the mutations associated with T1D will be found in the non-coding regions of a gene. It is possible that we will not find additional genes associated with T1D or any mutations in the already known genes to be associated with T1D. This is not very likely, however, due out sample size and previous studies suggesting multiple unknown genes are involved in developing T1D.

**Specific Aim 2: What are the epigenetics of T1D and how does the drug imatinib (Gleevec) affect the targeted genes?**

Assays of our targeted genes must go beyond the nucleic acid level because regulatory properties of genes are not confined to the sequence of nucleotides. To carry out this analysis, an assay of the epigenetic modifications of these genes will be done, specifically the methylation and acetylation of histones and DNA. This will account for any differences between the experimental and control group that might have been missed at the sequential level. Methylation and acetylation of histones and DNA are very important aspects of chromatin that should not be overlooked in considering differential gene expressions within a genetic disease. In general, the methylation of DNA means that the gene expression is inhibited where hypomethylated DNA, a CpG island, is expressed more readily (4). This is the opposite for the acetylation of histones; the more acetylation means the more that the DNA associated with the histones is being transcribed. Because this does not affect the sequence of the DNA, the whole gene must be analyzed for epigenetic modifications and not just the already identified mutated regions of the target genes. Non-coding regions rs2903692, rs725613 and rs17673553 of
KIAA0350 will, again, be looked at closely. These non-coding regions is where we hypothesize are going to have the greatest difference of methylated DNA and acetylated histones when comparing KIAA0350 between individuals diagnosed with and without T1D.

In order to carry out the epigenetic modification analysis, bisulfite modifications of DNA can be used. This allows nonmethylated cytosines to uracil. Amplification of the DNA using PCR then converts the uracil to thymine whereas the methylated cytosines are protected against bisulfate modifications. We can then analyze the bisulfate modified DNA by using methylation-sensitive primers (MSPs) to assess the number of CpG islands (14). To verify the results from the bisulfate assay, a digest of the target genes with an enzyme will be done. An enzyme that could be used to digest the different genes would be SacII CCGCGG (4), an enzyme with CG in the recognition site which would be inhibited if methylated. The DNA would then be run on a gel. If multiple bands appear, the enzyme has cut the gene which therefore means the gene is not methylated and thus expressed. For an analysis of the acetylation of the histones, a native ChIP (nChIP) will be used. As opposed to the conventional ChIP, a micrococcal nuclease digestion is used to prepare the chromatin for analysis with the nChIP (14). Employing nChIP we adequately allow us to assess modifications of histones.

At this point, 200 of the initial 1,000 T1D participants will be chosen based on their health and rise factors that may interfere with imatinib. To choose the participants, a screening process will occur that will ensure all individuals taking imatinib will be void of heart, neurological or pulmonary problems, nursing, pregnant, and hypersensitivity to imatinib. The group of 200 patients will be subject to a short 10 week trial of 400mg once daily imatinib doses (13). Subjects will be closely monitored for heart, liver, neurological and pulmonary problems along with hypertension and weight gain or loss. At the end of the 10 weeks, the epigenetic modification of methylation and acetylation will be, again, analyzed and compared to the previous epigenetic modification analysis.

**Expected results and pitfall.** We expect to find epigenetic modifications in the DNA and histones due methylation and acetylation between the control and experimental group. We also expect a different epigenetic profile before and after the imatinib trail in the selected experimental group. It is not very likely that no differential methylation or acetylation is observed between our two groups. This would suggest that the phenotypic symptoms of T1D are purely based on the sequence of the regulatory factors of the targeted genes. This is not what was suggested in the earlier study on NOD mice. This previous study suggested that there is an epigenetic change to the genome as opposed to a sequential change because of the NOD mice were T1D symptom free with no intervening DNA treatment.
Summary: This experiment is aimed at finding the underlying genetic causes of T1D in order to expose possible drug targets for companies to be able develop medicine in order to cure T1D. These advancements will relieve stress put on families of T1D suffers due to high medical and health care expenses. With the identification of a genetic source for T1D, several other medical problems will also be assessed since T1D can lead to kidney and heart disease, foot complications, blindness and many other complications. In addition to the obvious benefit to the diabetic community, this research will allow others to follow in the footsteps of T1D research in the hopes of finding a genetic source for other harmful diseases.

Works Cited


11. Louvet, Cédric et al. (2008) Tyrosine kinase inhibitors reverse type 1 diabetes

