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14. ABSTRACT
Type 1 diabetes is considered an autoimmune disease characterized by the presence of inflammatory cells in the islets of Langerhans. These cells are T lymphocytes, considered responsible for the destruction of the insulin producing beta-cells present in the islets. When the majority of the beta cells are dead, the disease presents, frequently with an abrupt and clinically serious onset. The aim of this program is to determine whom among the Army personnel is at high risk to develop the disease in order to prevent the unexpected onset of the disease that may be associated with tragic consequences, and to initiate an educational program aimed at reducing practical and psychological hurdles. Furthermore, different individuals develop disease complications (i.e., retinopathy, nephropathy, neuropathy) at different time-points after the onset. The susceptibility to complications could also be genetic. The human genome will be scanned systematically to characterize these susceptibility genes. Proteomic analysis will be performed in tandem to confirm the genetic associations.

15. SUBJECT TERMS
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INTRODUCTION:

Genetic factors contribute to risk for developing nephropathy in patients with Type 1 Diabetes (T1D). Cigarette smoking is deleterious to kidney function and is a risk factor for Diabetic-Nephropathy (DN) as well as end-stage renal disease (ESRD) in patients with T1D. ***Hypothesis****: Genetic variants and smoking interact to amplify risk for T1DN and substantially increase incidence of T1D-ESRD.* In order to test the hypothesis, we assembled a repository of DNA samples (N=2,881) obtained from case (T1DN) and control T1D individuals whose extensive health history and lifestyle choices were recorded at the time of recruitment. Comparison of T1DN and T1D participants will allow us to confirm the association between genes and T1DN.

BODY:

The prevalence and course of DN are similar in T1D and T2D patients when matched for duration of the disease (Hasslacher et al., 1989). Diabetic nephropathy follows an established natural history. The cardinal clinical feature of the syndrome is the progressive increase in urine protein excretion rate (Remuzzi et al., 2006). The clinical course starting with microalbuminuria through proteinuria and azotemia culminates with ESRD. Before the onset of overt nephropathy a prolonged period of clinical silence hides various changes in renal function such as hyperfiltration, hyperinfusion, and increasing capillary permeability to macromolecules.

A progressive rise in arterial blood pressure and albuminuria accompanies glomerular filtration rate (GFR) decline. Microalbuminuria remains the best predictor of DN in both T1D and T2D patients (Mogensen, 2003). Risk factors correlated with disease progression are poor glycemic control, hypertension, dyslipidemia, elevated serum cholesterol, and smoking (Orth et al., 1998; Chaturvedi et al., 2001; Hovind et al., 2001). ESRD is the major cause of mortality in T1D patients and is the dominant indicator of fatality due to cardiovascular disease (Gall et al., 1995; Ritz and Stefanski, 1996).

The evidence for a dominant genetic role in determining susceptibility to kidney disease in T1D patients is primarily the result of epidemiological studies indicating that prevalence of DN increased during the first 15 years after onset of T1D. After 20 years duration of diabetes the incidence of new cases of nephropathy among T1D patients plateaus and in fact may decrease (Krolewski et al., 1985; DRWG, 1999; Pambianco et al., 2006). These observations have frequently been interpreted as indicating that there exists a subset of patients susceptible to development of kidney disease. Additional evidence for genetic risk has been obtained from family studies showing the clustering of DN among T1D siblings (Seaquist et al., 1989; Borch-Johnsen et al., 1992; Quinn et al., 1996; Hurjutsalo et al., 2004). Siblings experiencing T1D have a significantly increased risk for DN when the T1D proband experiences the disease. Interpretation of the data generated from genetic analysis of T1DN is complicated by the possibility that signals are related to coincident diseases (e.g., hypertension) as well as environmental exposures such as smoking. In order to interpret these results it is necessary to compare the results of genetic testing of control populations as well as replication of the results in independently recruited case cohorts.

The risk associated with onset of kidney disease in T1D patients is influenced by the action of individual genes, interaction between genes, as well as the interplay between genes and environment; these factors become manifest in the familial clustering of T1DN incidence. Environmental influences and genetic variants interact to amplify risk for T1DN and substantially increase incidence of ESRD. Poor glycemic control and hypertension are examples of deleterious effects on kidney function and are independent risk factors for nephropathy in patients with diabetes. Characterization of risk factors influencing the incidence of T1DN will improve our understanding of the molecular mechanism(s) underlying this chronic disease and permit appropriate intervention to reduce its consequences.

Aim 1: *To identify genetic variants conferring risk to T1DN perform a Stage 2 follow-up of results from a Genome-Wide Association Scan (GWAS).* The experimental plan accomplished the goal of Aim 1 in three steps: a) selection of candidate SNPs and genes from the Stage 1 GWAS for follow-up; b) genotyping of SNPs using a cohort of case family trios and an independent cohort of case and control singletons; and c) joint analysis of the genotype data from the GWAS and the follow-up in order to determine which genes and/or genomic regions contain genetic variation affecting risk for T1DN. The research design enabled the analysis of allelic association between genetic marker and the disease while minimizing statistical anomalies linked to population stratification and admixture.

Genome-Wide Association Scan: A genome wide scan using DNA samples obtained from the Genetics of Kidneys in Diabetes (GoKinD) study was performed in order to identify susceptibility genes associated with T1DN. Stage 1 of the study employed a human gene chip microarray to evaluate genetic variants using DNA samples obtained from case (N=197) and control (N=197) participants (Table 1). All participants were of Caucasian ancestry and reported having never smoked cigarettes. Cases had T1D-ESRD and controls exhibited T1D for at least 15 years with evidence of normal kidney function.

Table 1. Characteristics of the T1D and T1DN Cohort.

	Genome-Wide Association Scan			Confirmation Phase			Confirmation Phase		
	Stage 1 Case/Control Cohort			TDT Cohort			Stage 2 Case/Control Cohort		
	Case 1 Singleton	Control 1 Singleton	p-value[2]	Father	Mother	Proband	Case 2 Singleton	Control 2 Singleton	p-value[2]
Demographic Characteristics:									
Number of Singletons	197	197	----	121	121	121	220	645	----
Caucasian Ancestry (%)	100	100	----	100	100	100	100	100	----
Male Gender (%)	47	47	----	100	0	39	45	38	----
Body Mass Index (kg/m2)[1]	26(6)	26(4)	0.8	28(4)	26(5)	25(5)	26(5)	26(4)	0.3
Ever Smoked Cigarettes (%)	0	0	----	----	----	0	0	36	----
History of Diabetes:									
Type 1 Diabetes (%)	100	100	----	0.8	2.5	100	100	100	
Age at T1D Diagnosis (yr)[1]	12(7)	12(8)	0.8	----	----	11(6)	11(6)	13(7)	< 0.001
Duration of T1D (yr)[1]	33(5)	30(9)	< 0.001	----	----	31(6)	30(8)	25(7)	< 0.001
Pancreas Transplant (%)	54	0	----	----	----	32	18	0	----
HbA1C (%) with Pancreas Transplant[1]	5.8(1.7)	----	----	----	----	5.6(1.4)	5.6(1.4)	----	----
HbA1C (%) without Pancreas Transplant[1]	7.9(1.5)	7.3(1.1)	< 0.001	----	----	7.9(1.6)	8.2(1.5)	7.5(1.2)	< 0.001
History of Diabetic Nephropathy:									
Diabetic Nephropathy (%)	100	0	----	----	----	100	100	0	----
End-Stage Renal Disease (%)	100	0	----	----	----	65	36	0	----
Time to Onset of T1D-ESRD (yr)[1]	26(8)	----	----	----	----	26(7)	26(7)	----	----

1. Values in parentheses indicate standard deviation from the mean.

2. p-values were calculated using Student's T-Test.

Suggestive signals for association were observed within a number of genetic regions and overlapped a variety of candidate genes including those previously associated with susceptibility to essential hypertension. During stage 2 analysis, individual genetic variants were evaluated using 121 case family trios followed by genotyping 220 case and 645 control singletons (Table 1). Staged analysis of genetic variants associated with T1DN indicated high probability that a 42kb region located downstream of *IRS2* on chromosome 13q34 as causal for nephropathy in T1D populations.

Study Design: A staged study design was employed to evaluate SNPs for their association with T1DN. During stage 1 analysis T1D-ESRD (case) and T1D (control) participants were compared using the Affymetrix 500K SNP genotyping array in order to gather data from a GWAS. DNA samples were selected from the GoKinD study collection and consisted of 197 case and 197 control singletons. Participants were chosen for stage 1 analysis based on self-reported shared ancestry and history of never smoking cigarettes. Stage 1 participants also shared similar BMI and age at onset of T1D (Table 1). Case and control samples differed, however, in the presence of ESRD, duration of T1D, and HbA1C levels measured at the time of recruitment. The participants chosen for confirmation phase analyses employed 121 case family trios along with a cohort of 220 case and 645 control singletons. DNA samples used in the confirmation phase differed from the stage 1 cohort primarily in that case participants included T1DN in addition to participants with T1D-ESRD (Table 1). Moreover, control participants included individuals with a positive history of smoking cigarettes. Cigarette smoking has been implicated as an influential factor leading to increased incidence of kidney disease (Christiansen, 1978; Couper et al., 1994; Orth, 2002). Exclusion of smokers from the case cohorts was mandated by the study protocol, however, inclusion in the control cohorts was allowed as long as control participants maintained healthy kidney function at the time of recruitment.

500K Affymetrix Genome-Wide Microarray Analysis: Material from the GoKinD cohort was provided as solutions of DNA originating from family trios and singleton probands and was purified from lymphoblastoid cell lines or from whole blood. DNA solutions were provided at 0.05 ml aliquots containing roughly 0.05 mg DNA per aliquot dissolved in TE buffer, pH 7.5. DNA samples (100 ng/ul) were genotyped using GeneChip 500K arrays by the Affymetrix Services Laboratory (Affymetrix Inc., Santa Clara, CA). Data was provided as cell intensity files (raw data) and as BRLMM algorithm called genotypes (Rabbee and Speed, 2006). Because of the known confounding associated with BRLMM called genotypes (Luca et al., 2007; WTCCC, 2007) the raw data were analyzed by the University of Pittsburgh Computational Genetics Laboratory using the Bayesian calling algorithm (CHIAMO) (www.statx.ox.ac.uk/~marchini/software/gwas/). In a related project the CHIAMO algorithm had been observed to lead to marked improvement for genotyping calls (Luca et al., 2007). However, the BRLMM algorithm provided greater confidence in calls for the X chromosome. Thus, CHIAMO was used for genotype calls of the 22 autosomal chromosomes and the BRLMM algorithm was used for X linked markers.

Statistical Quality Control and Exclusion of Participants with Mixed Ancestry: Data was received on 500,447 SNP genotypes from 394 individuals (Table 2). Multiple features of the data were evaluated during the quality control phase of the analysis, e.g., missingness, minor allele frequency, and deviation from Hardy Weinberg Equilibrium (HWE). Individuals and loci with substantial missing data were excluded from analysis of the GWAS because these features usually indicate poor DNA quality, and problems with genotype calls lead to poor quality results.

Evaluation of population structure was conducted by estimating continuous, multidimensional ancestry and relationships from among the genotyping results from tag-SNPs using principal component analyses (Price et al., 2006). Interpretation of HWE tests can be highly influenced by population structure. Therefore, HWE tests to evaluate genotyping quality of loci used those markers that by HWE were within homogeneous populations identified by this clustering (Figure 1). For example, high quality data used during principal component analyses implied that participants were of European ancestry; however, data from one individual was removed due to evidence of possibly mixed ancestry. Final quality control analyses included the following requirements: Maximum missingness frequency <0.05, 90.1% of the SNPs passed; Minor allele frequency >0.05, 67.7% of the SNPs passed; and p-value >0.005 for failure of HWE over all samples >0.005, 64.4% of the SNPs passed (Table 2). After quality control analysis the study retained 322,347 SNPs from 386 participants, specifically 193 cases (T1D-ESRD) and 193 controls (T1D).

Stage 1 GWAS Results: Figure 2 summarizes results from the GWAS by plotting the transformed p-values against location along each chromosome. The 3 panels indicate the association for individual SNPs with the T1D-ESRD phenotype for each of the 3 major modes of inheritance, i.e., additive, dominant, and recessive. A few observations about the data are worth mentioning. As expected, results from the GWAS are highly correlated, making the effective number of tests smaller. Likewise, clusters of SNPs are in tight linkage disequilibrium (LD), again making the effective number of tests smaller. For example, data simulations showed that the effective number of tests is approximately 322,347/2.3=140,151. Using Bonferroni methods, this means that a p-value of less than 3.6×10^{-7} is genome significant for alpha=0.05. The smallest observed p-value in the data occurred on chromosome 19 near the ZNF71 locus and is 5.7×10^{-7}, almost but not quite, significant for Stage 1. As shown in Table 2, the fraction of SNPs with p-values exceeding significance varies proportionally with the p-value cutoff. For example, an uncorrected p-value <0.0001 resulted in 290, 258, and 258 for additive, dominant and recessive modes of inheritance, respectively (Table 2).

Table 2. Summary of the Genome-Wide Association Scan.

	Quality Control Summary	
Quality Control Step:[1]	Failed SNPs	Remaining SNPs
Affymetrix 500K Array	----	500,477 (100%)
Missingness (freq < 0.05)	49,359	451,088 (90.1%)
Minor allele frequency (freq > 0.05)	112,097	338,991 (67.7%)
Exact HW test (p > 0.005)	16,644	322,347 (64.4%)

	Mode of Inheritance		
Distribution of p-values:[2]	Additive	Dominant	Recessive
p-value < 0.05	0.0539	0.048	0.0489
p-value < 0.01	0.0104	0.0087	0.0092
p-value < 0.001	0.0009	0.0008	0.0008

1. Number of SNPs removed and remaining after each quality control step. Values in parenthesizes are % of SNP assayed on the Affymetrix 500K array remaining after each quality control step.
2. Fraction of SNPs that are significant at each p-value cutoff.

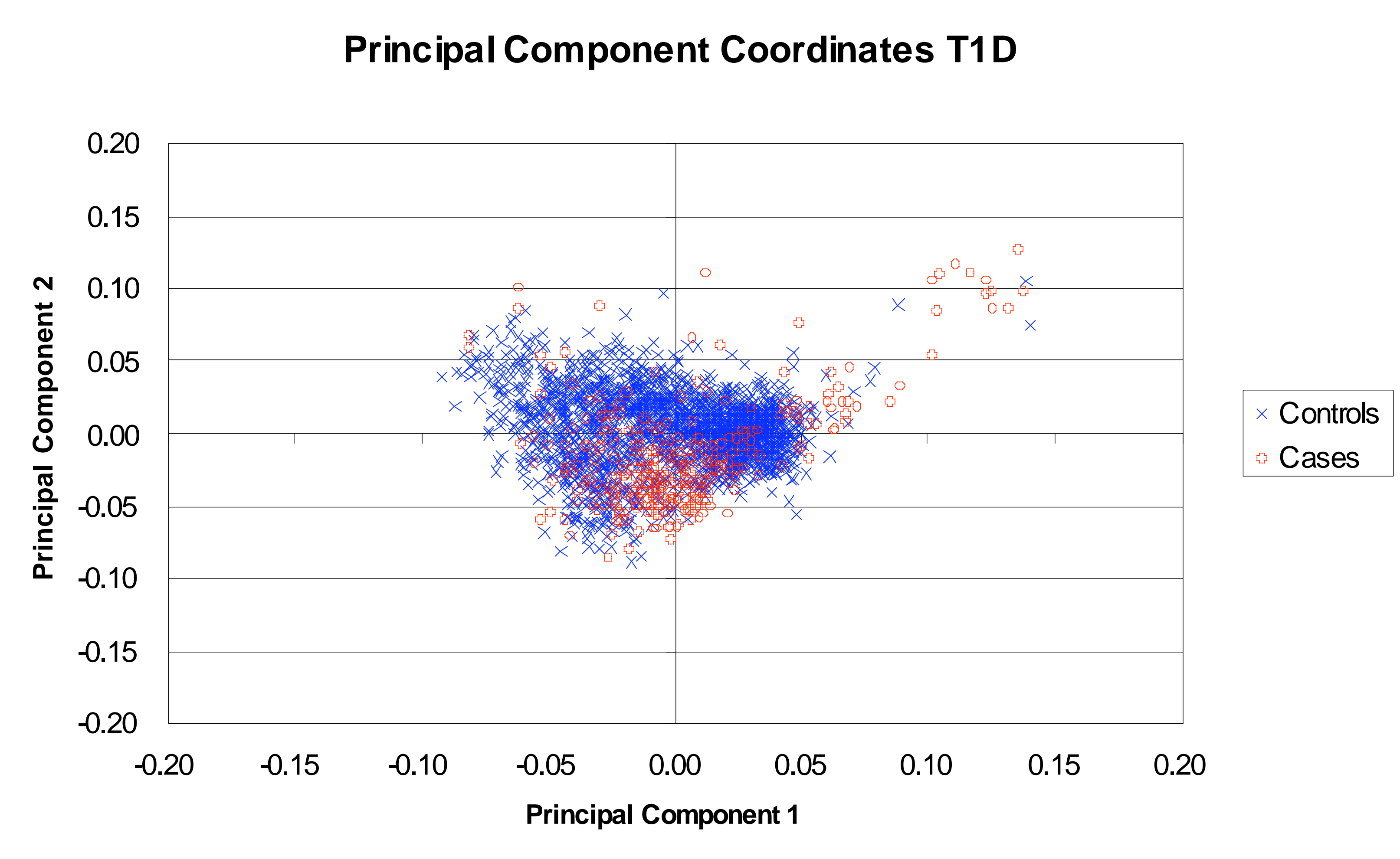


Figure 1

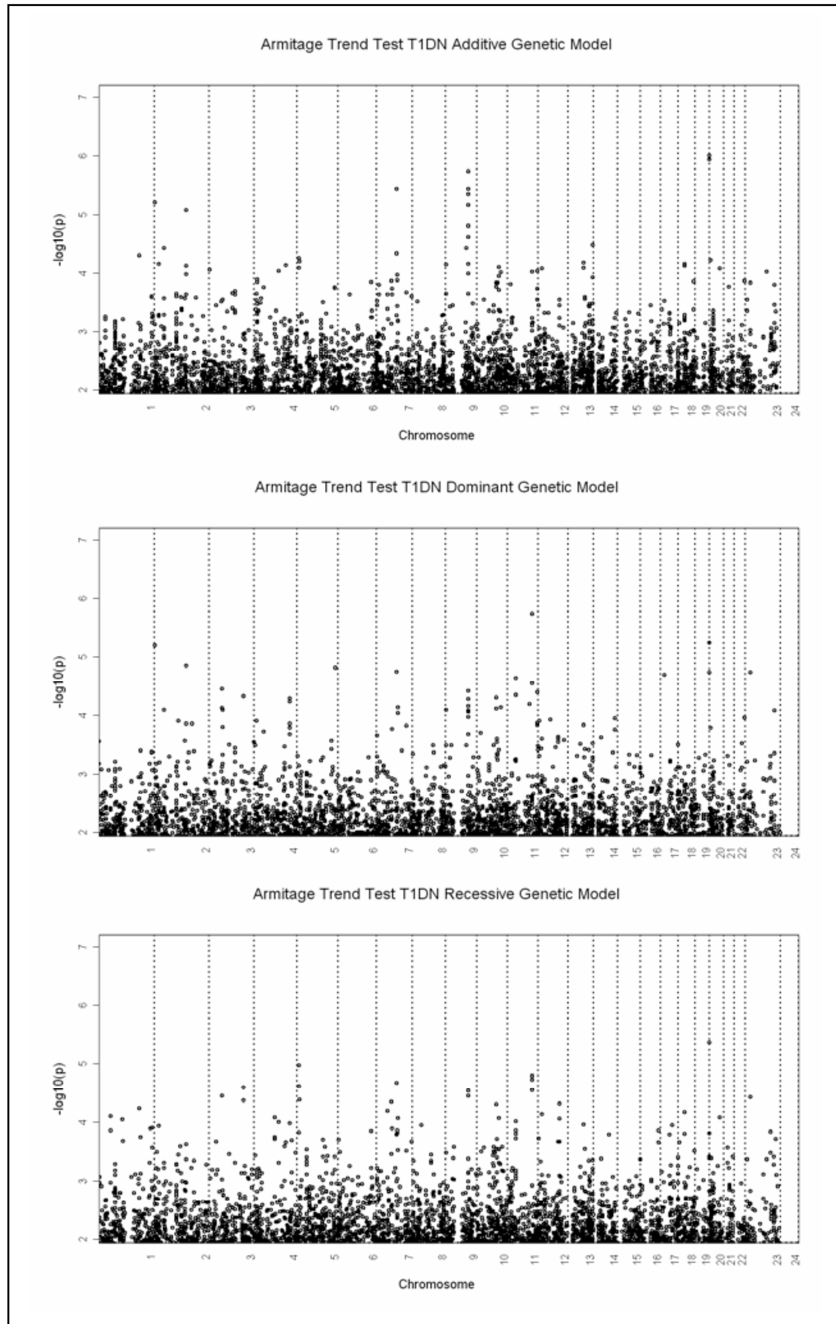


Figure 2

Overall, the SNPs remaining after quality control analysis provided a well behaved collection of data that can be used to identify high profile genetic markers for analysis during stage 2 in order to reproduce the results observed during the stage 1 GWAS.

Replication of GWAS Data: Select markers were chosen in order to replicate the association signal of SNPs measured during the stage 1 GWAS (Figure 2). The replication phase of the study assayed 166 markers for association with the phenotype. SNPs were chosen based on 3 criteria: 1) SNPs approaching genome-wide significance, uncorrected p-value <0.00001; 2) association with potential candidate genes for T1DN or hypertension and p-value <0.001; and 3) clusters of 3 or more SNPs with p-values < 0.001. Thus, selection of confirmation phase SNPs involved combining results from genotypes with biological data (Pasquali et al., 2007; Roeder et al., 2007). The study design was found to have roughly 80% power to identify a gene containing a risk locus for the following combinations of minor allele frequency (MAF) and odds ratio (OR) under a dominant, additive or multiplicative model: MAF ≥ 0.05 & OR ≥ 2.5; MAF ≥ 0.10 & OR ≥ 2.0; MAF ≥ 0.15 & OR ≥ 1.75. For the same genetic models and (MAF = 0.4 & OR = 1.5), power is roughly 75%.

SNP Genotyping Assays: Individual genotyping assays were performed using TaqMan assays (Applied Biosystems Inc. Foster City, CA). TaqMan analysis used 384-well trays in which 2 ng of DNA was placed in each well and allowed to dry overnight followed by storage at -20ºC. Samples are dissolved with 10 ul TaqMan master mix containing AmpliTaq Gold DNA polymerase, AmpErase UNG, dNTPs with UTP and $MgCl_2$. Thermocycler conditions are 95ºC for 10 minutes followed by 92ºC for 15 sec and 60ºC for 1 minute for 40 cycles. The amplification process was followed by incubation at 60ºC for 7 min and rapid cooling of the reaction mixtures to 4ºC. Fluorescent signals from each sample well were obtained using an Applied Biosystems Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). The TaqMan assay analysis software SDS version 2.2.2 (Applied Biosystems, Foster City, CA) was used to determine the genotype of each participant.

Confirmation phase genotyping results are summarized in Table 3. Of the 166 SNPs selected for analysis there were 43 SNPs (26%) resulting in suggestive allele transmission when examined by TDT using 121 family trios. Indicated in bold font, Table 3 summarized the results for those alleles that were inherited along with the T1DN phenotype greater than 56.9% of the time, exceeding the 75th percentile of transmission disequilibrium for all SNPs measured during TDT analyses. Moreover, 10 SNPs exhibited p-values <0.05 and are indicated in red font.

There were 2 SNPs (rs6492208 and rs2391777) that when assayed using the stage 2 case (N=220) and control (N=645) cohort resulted in p-values exceeding significance at p-value <0.005 for a recessive mode of inheritance (Table 4). Figure 3 illustrates the GWAS results for chromosome 13q34, indicating the location of peak signal for association at roughly 150kb downstream of the *IRS2* locus. The location of other nearby loci MYO16, COL4A1, and COL4A2 are also shown.

An LD Structure Model for the Region of Peak Signals Along Chromosome 13q34: Linkage disequilibrium (LD) in the region on chromosome 13q34 identified by the stage 1 GWAS and stage 2 analyses was investigated using H-clust (Figure 4) in order to select tag-SNPs to represent clusters of genetic polymorphisms that are in substantial LD (r2 > 0.8) for GWAS data (Rinaldo et al., 2005). To maximize power of GWAS, tag-SNPs were chosen to have large correlation with the other SNPs in the cluster, on the basis of their minor allele frequency (MAF) and on the number of SNPs they tag (de Bakker et al., 2005). To apply 'optimal' tests for a set of SNPs, Roeder et al. (2005) showed that it is useful to know how well genotyped or 'measured' SNPs predict proximate 'unmeasured' SNPs. To choose tag-SNPs, the method analyzed fully-sequenced stretches of DNA in a sample of individuals (ENCODE regions http://hapmap.org/; SeattleSNPs http://pga.mbt.washington.edu/; & NIEHS SNPs http://egp.gs.washington.edu/). The results indicate that SNPs found in the region of peak signal on chromosome 13q34 can be divided into 13 statistically reconstructed haplotypes as well as 10 outlier SNPs. Genotyping of the stage 1 cohort by Affymetrix 500K GeneChip or of selected tag-SNPs using TaqMan assays indicated that tag-SNPs in clusters 5 and 13 are in substantial LD with the T1D-ESRD phenotype, i.e., p-value <0.005 (Figure 4). Likewise, the remaining clusters and outlier SNPs, with the exception of rs2150479, did not deviate significantly from the null hypothesis.

An additional step performed during fine mapping of the peak region has been to characterize the variation of the nearby target gene *IRS2*. SNPs within the *IRS2* locus were examined by TDT (Table 3) as well as with the stage 2 case and control cohort (Table 4). The results for SNPs rs1865434, rs11618950, rs4773092, and rs3742210 located within the 3' untranslated region as well as intron 1 and exon 2 showed no evidence for association with the phenotype. In contrast to mapping of the *IRS2* locus, fine mapping of the peak region on chromosome 13q34 indicated that a roughly 42kb region delimited by SNPs rs1411766 and rs1547241, located in H-clusters 5 and 13, are in LD with a causal polymorphism for T1D-ESRD.

Genome Functional Analysis the T1DN Associated Haplotype: The location of the peak region as well as nearby and overlapping genetic elements are illustrated in Figure 5. The region of peak association is bordered by a recombination hotspot potentially separating it from the *IRS2* locus. However, a copy number variant (CNV) is located downstream of the peak region and, in fact, may be in linkage disequilibrium with the genetic element identified during the GWAS and replication phase of the study. We are currently genotyping SNPs for covariance between the peak region and the CNV in order to test this model for how genes influence risk of T1DN. Another element located within the haplotype associated with the peak region is an expressed sequence tag (EST) DW445755 likely to generate a non-coding RNA (Figure 6). Comparative genome analysis indicated a region of evolutionary conservation occurring in the promoter region of the EST. Non-coding RNAs have been implicated in gene regulation and expression of the EST located in the peak region has been observed in model for liver regeneration in humans.

Table 3. TDT Analysis of T1DN Family Trios (N=121).

Family Trios: T1DN and T1D-ESRD

dbSNP ID	Chr	Location	Locus Name	Allele 1	Allele 2	Total Trans	%Trans	Chi Sq	p-value
rs1774817	1	57,015,674	C1orf168	42	45	87	48.3	0.1	7.5E-01
rs12742885	1	236,581,043	CHRM3	41	40	81	50.6	0.0	9.1E-01
rs12760396	1	236,581,078	CHRM3	40	40	80	50.0	0.0	1.0E+00
rs2689127	**1**	**237,005,643**	**CHRM3**	**63**	**45**	**108**	**58.3**	**3.0**	**8.3E-02**
rs2841347	**1**	**237,019,769**	**CHRM3**	**50**	**73**	**123**	**40.7**	**4.3**	**3.8E-02**
rs10197310	**2**	**102,386,462**	**IL18RAP**	**33**	**25**	**58**	**56.9**	**1.1**	**2.9E-01**
rs11687768	2	102,392,170	IL18RAP	27	33	60	45.0	0.6	4.4E-01
rs10210176	2	102,445,948	IL18RAP	32	27	59	54.2	0.4	5.2E-01
rs7586146	**2**	**140,489,344**	**LRP1B**	**22**	**16**	**38**	**57.9**	**0.9**	**3.3E-01**
rs167770	3	115,362,252	DRD3	40	38	78	51.3	0.1	8.2E-01
rs226082	3	115,363,703	DRD3	43	42	85	50.6	0.0	9.1E-01
rs7638876	3	115,376,990	DRD3	41	43	84	48.8	0.0	8.3E-01
rs1531139	3	144,300,614	CHST2	51	50	101	50.5	0.0	9.2E-01
rs989769	3	153,163,708	SUCNR1	56	55	111	50.5	0.0	9.2E-01
rs842523	3	153,242,605	SUCNR1	59	61	120	49.2	0.0	8.6E-01
rs1351267	3	153,246,391	SUCNR1	61	60	121	50.4	0.0	9.3E-01
rs76705595	4	14,375,041	CPEB2	44	48	92	47.8	0.2	6.8E-01
rs4342182	4	14,380,447	CPEB2	46	46	92	50.0	0.0	1.0E+00
rs11735198	4	14,387,574	CPEB2	43	47	90	47.8	0.2	6.7E-01
rs4507355	4	14,391,155	CPEB2	46	47	93	49.5	0.0	9.2E-01
rs4502675	4	14,391,557	CPEB2	43	45	88	48.9	0.0	8.3E-01
rs4383613	4	14,394,118	CPEB2	42	45	87	48.3	0.1	7.5E-01
rs4698089	4	14,394,722	CPEB2	46	47	93	49.5	0.0	9.2E-01
rs615604	4	23,597,866	PPARGC1A	33	29	62	53.2	0.3	6.1E-01
rs582804	4	23,601,571	PPARGC1A	29	33	62	46.8	0.3	6.1E-01
rs10026136	4	23,609,578	PPARGC1A	31	30	61	50.8	0.0	9.0E-01
rs4235093	4	72,565,116	SLC4A4	44	43	87	50.6	0.0	9.1E-01
rs6846301	4	72,566,355	SLC4A4	16	21	37	43.2	0.7	4.1E-01
rs10155123	4	96,304,247	BMPR1B	59	54	113	52.2	0.2	6.4E-01
rs17007046	4	124,662,749	SPRY1	19	17	36	52.8	0.1	7.4E-01
rs1403142	4	149,261,743	NR3C2	40	44	84	47.6	0.2	6.6E-01
rs1403143	4	149,261,784	NR3C2	59	58	117	50.4	0.0	9.3E-01
rs1040288	4	149,267,567	NR3C2	57	51	108	52.8	0.3	5.6E-01
rs7687754	4	149,268,094	NR3C2	59	57	116	50.9	0.0	8.5E-01
rs4835128	4	149,275,166	NR3C2	59	56	115	51.3	0.1	7.8E-01
rs4835488	4	149,281,643	NR3C2	59	60	119	49.6	0.0	9.3E-01

rs6850722	**4**	**156,692,216**	**GUCY1A3**	**66**	**47**	**113**	**58.4**	**3.2**	**7.4E-02**
rs1976041	4	156,705,490	GUCY1A3	67	51	118	56.8	2.2	1.4E-01
rs2625274	4	156,707,590	GUCY1A3	64	50	114	56.1	1.7	1.9E-01
rs716428	**4**	**156,726,319**	**GUCY1A3**	**50**	**70**	**120**	**41.7**	**3.3**	**6.8E-02**
rs990619	**4**	**156,727,128**	**GUCY1A3**	**74**	**52**	**126**	**58.7**	**3.8**	**5.0E-02**
rs1123037	**4**	**156,734,175**	**GUCY1A3**	**70**	**52**	**122**	**57.4**	**2.7**	**1.0E-01**
rs6835414	**4**	**156,734,392**	**GUCY1A3**	**48**	**66**	**114**	**42.1**	**2.8**	**9.2E-02**
rs11100647	4	167,568,282	TLL1	39	40	79	49.4	0.0	9.1E-01
rs11740668	5	457,623	AHRR	56	57	113	49.6	0.0	9.3E-01
rs10805643	5	39,618,799	DAB2	53	45	98	54.1	0.7	4.2E-01
rs2086634	5	42,173,224	GHR	47	50	97	48.5	0.1	7.6E-01
rs3913459	5	42,185,500	GHR	49	51	100	49.0	0.0	8.4E-01
rs4957204	5	42,193,406	GHR	47	50	97	48.5	0.1	7.6E-01
rs4866836	5	42,357,524	GHR	43	43	86	50.0	0.0	1.0E+00
rs4866753	5	42,359,887	GHR	43	40	83	51.8	0.1	7.4E-01
rs1396775	5	42,417,663	GHR	55	47	102	53.9	0.6	4.3E-01
rs6887976	**5**	**113,996,634**	**KCNN2**	**43**	**26**	**69**	**62.3**	**4.2**	**4.1E-02**
rs6595812	5	127,603,502	SLC12A2	28	24	52	53.8	0.3	5.8E-01
rs2060210	5	151,069,895	SPARC	62	56	118	52.5	0.3	5.8E-01
rs265992	5	174,779,261	DRD1	58	53	111	52.3	0.2	6.4E-01
rs6458094	**6**	**39,177,246**	**KCNK5**	**52**	**39**	**91**	**57.1**	**1.9**	**1.7E-01**
rs9640583	7	88,647,585	FLJ32110	31	36	67	46.3	0.4	5.4E-01
rs2214339	**7**	**88,651,225**	**ZNF804B**	**30**	**40**	**70**	**42.9**	**1.4**	**2.3E-01**
rs13229065	**7**	**93,012,567**	**CALCR**	**44**	**64**	**108**	**40.7**	**3.7**	**5.4E-02**
rs12704683	7	93,017,906	CALCR	0	0	0			
rs5014937	**7**	**93,017,926**	**CALCR**	**46**	**25**	**71**	**64.8**	**6.2**	**1.3E-02**
rs1541433	7	93,063,054	CALCR	43	50	93	46.2	0.5	4.7E-01
rs7341502	7	93,067,389	CALCR	55	58	113	48.7	0.1	7.8E-01
rs7799704	7	93,067,476	CALCR	47	58	105	44.8	1.2	2.8E-01
rs2528533	7	93,077,670	CALCR	46	58	104	44.2	1.4	2.4E-01
rs2677079	7	93,094,337	CALCR	55	54	109	50.5	0.0	9.2E-01
rs2690289	7	95,287,919	DYNC1I1	62	56	118	52.5	0.3	5.8E-01
rs2618974	7	95,288,199	DYNC1I1	60	54	114	52.6	0.3	5.7E-01
rs1227517	7	95,295,096	DYNC1I1	58	54	112	51.8	0.1	7.1E-01
rs4077793	8	21,616,512	GFRA2	46	51	97	47.4	0.3	6.1E-01
rs4237094	8	82,182,650	PAG1	43	39	82	52.4	0.2	6.6E-01
rs10504731	8	82,196,082	PAG1	39	39	78	50.0	0.0	1.0E+00
rs10957999	8	82,196,866	PAG1	39	49	88	44.3	1.1	2.9E-01
rs11776886	8	92,393,000	SLC26A7	57	56	113	50.4	0.0	9.3E-01
rs10505338	8	119,824,671	TNFRSF11B	46	38	84	54.8	0.8	3.8E-01
rs178907	9	10,119,179	NR4A3	42	39	81	51.9	0.1	7.4E-01

rs920771	9	101,203,134	NR4A3	50	45	95	52.6	0.3	6.1E-01
rs7032399	9	101,211,288	TGFBR1	49	56	105	46.7	0.5	4.9E-01
rs7040144	9	101,216,224	NR4A3	47	52	99	47.5	0.3	6.2E-01
rs7042668	9	101,216,521	TGFBR1	45	42	87	51.7	0.1	7.5E-01
rs4743350	9	101,217,469	TGFBR1	45	44	89	50.6	0.0	9.2E-01
rs9792459	9	101,231,335	TGFBR1	50	54	104	48.1	0.2	6.9E-01
rs10115446	9	101,256,076	NR4A3	56	47	103	54.4	0.8	3.8E-01
rs6478979	9	101,256,702	NR4A3	49	58	107	45.8	0.8	3.8E-01
rs10760689	9	101,311,483	NR4A3	56	53	109	51.4	0.1	7.7E-01
rs1907720	10	78,422,658	KCNMA1	25	22	47	53.2	0.2	6.6E-01
rs10509690	10	97,236,026	SORBS1	39	36	75	52.0	0.1	7.3E-01
rs17458865	10	97,286,244	SORBS1	29	37	66	43.9	1.0	3.2E-01
rs17537576	10	97,290,052	SORBS1	31	29	60	51.7	0.1	8.0E-01
rs10786586	10	102,077,952	PKD2L1	28	25	53	52.8	0.2	6.8E-01
rs11022423	11	12,552,583	PARVA	43	35	78	55.1	0.8	3.7E-01
rs3794102	11	35,250,762	SLC1A2	57	65	122	46.7	0.5	4.7E-01
rs7105690	11	35,253,348	SLC1A2	64	58	122	52.5	0.3	5.9E-01
rs3794098	11	35,255,184	SLC1A2	62	55	117	53.0	0.4	5.2E-01
rs4756212	11	35,318,887	SLC1A2	48	60	108	44.4	1.3	2.5E-01
rs12365943	11	108,608,422	LOC399947	53	52	105	50.5	0.0	9.2E-01
rs 7315599	**12**	**4,193,511**	**PARP11**	**51**	**36**	**87**	**58.6**	**2.6**	**1.1E-01**
rs1493759	12	90,615,063	DCN	60	58	118	50.8	0.0	8.5E-01
rs1387763	12	90,631,744	DCN	60	60	120	50.0	0.0	1.0E+00
rs9599694	13	70,036,873	DACH1	45	40	85	52.9	0.3	5.9E-01
rs314690	13	78,906,958	NDFIP2	48	60	108	44.4	1.3	2.5E-01
rs2149315	13	95,726,768	HS6ST3	57	59	116	49.1	0.0	8.5E-01
rs8000148	13	95,773,569	HS6ST3	59	54	113	52.2	0.2	6.4E-01
rs4771934	13	95,791,757	HS6ST3	60	58	118	50.8	0.0	8.5E-01
rs7323332	13	95,792,869	HS6ST3	62	57	119	52.1	0.2	6.5E-01
rs1410266	13	95,848,907	HS6ST3	53	67	120	44.2	1.6	2.0E-01
rs4771946	13	95,860,425	HS6ST3	55	68	123	44.7	1.4	2.4E-01
rs16951472	**13**	**95,871,978**	**HS6ST3**	**40**	**21**	**61**	**65.6**	**5.9**	**1.5E-02**
rs2150479	13	109,041,870	IRS2	54	58	112	48.2	0.1	7.1E-01
rs1411766	13	109,050,161	IRS2	61	57	118	51.7	0.1	7.1E-01
rs17412858	13	109,050,609	IRS2	56	59	115	48.7	0.1	7.8E-01
rs6492208	13	109,055,727	IRS2	43	56	99	43.4	1.7	1.9E-01
rs2391777	**13**	**109,056,244**	**IRS2**	**44**	**59**	**103**	**42.7**	**2.2**	**1.4E-01**
rs2391778	13	109,056,554	IRS2	52	60	112	46.4	0.6	4.5E-01
rs1411765	13	109,057,069	IRS2	64	51	115	55.7	1.5	2.3E-01
rs7320497	13	109,058,000	IRS2	53	64	117	45.3	1.0	3.1E-01
rs1001680	13	109,060,557	IRS2	57	45	102	55.9	1.4	2.3E-01

rs1547241	13	109,071,606	IRS2	45	58	103	43.7	1.6	2.0E-01
rs167952	13	109,072,232	IRS2	55	52	107	51.4	0.1	7.7E-01
rs7984924	13	109,081,246	IRS2	52	66	118	44.1	1.7	2.0E-01
rs7986237	13	109,081,322	IRS2	58	50	108	53.7	0.6	4.4E-01
rs7989418	13	109,081,443	IRS2	49	62	111	44.1	1.5	2.2E-01
rs7989848	13	109,081,469	IRS2	65	51	116	56.0	1.7	1.9E-01
rs1929210	13	109,083,479	IRS2	47	60	107	43.9	1.6	2.1E-01
rs9521445	**13**	**109,083,535**	**IRS2**	**62**	**47**	**109**	**56.9**	**2.1**	**1.5E-01**
rs9515090	13	109,084,543	IRS2	44	53	97	45.4	0.8	3.6E-01
rs870059	**13**	**109,092,251**	**IRS2**	**47**	**64**	**111**	**42.3**	**2.6**	**1.1E-01**
rs1865434	**13**	**109,206,609**	**IRS2**	**24**	**34**	**58**	**41.4**	**1.7**	**1.9E-01**
rs11618950	**13**	**109,232,311**	**IRS2**	**37**	**26**	**63**	**58.7**	**1.9**	**1.7E-01**
rs4773092	**13**	**109,233,954**	**IRS2**	**44**	**64**	**108**	**40.7**	**3.7**	**5.4E-02**
rs3742210	**13**	**109,234,233**	**IRS2**	**64**	**41**	**105**	**61.0**	**5.0**	**2.5E-02**
rs2069599	14	95,782,530	BDKRB2	33	29	62	53.2	0.3	6.1E-01
rs4842999	15	83,247,357	SLC28A1	53	46	99	53.5	0.5	4.8E-01
rs8055868	16	23,255,752	SCNN1B	15	19	34	44.1	0.5	4.9E-01
rs9302755	**16**	**49,667,140**	**SALL1**	**50**	**69**	**119**	**42.0**	**3.0**	**8.2E-02**
rs2017684	16	50,026,393	SALL1	51	67	118	43.2	2.2	1.4E-01
rs10852513	**16**	**50,046,878**	**SALL1**	**73**	**51**	**124**	**58.9**	**3.9**	**4.8E-02**
rs11656096	17	19,563,235	SLC47A2	57	53	110	51.8	0.1	7.0E-01
rs7214841	17	19,572,507	SLC47A2	55	56	111	49.5	0.0	9.2E-01
rs12952746	17	42,298,412	WNT9B	28	24	52	53.8	0.3	5.8E-01
rs 1052586	17	42,373,462	GOSR2	61	59	120	50.8	0.0	8.6E-01
rs11079742	17	42,375,127	GOSR2	66	55	121	54.5	1.0	3.2E-01
rs9303532	17	42,389,001	RPRML	59	65	124	47.6	0.3	5.9E-01
rs4939662	18	47,072,630	SMAD4	53	50	103	51.5	0.1	7.7E-01
rs4260160	18	47,097,495	SMAD4	53	55	108	49.1	0.0	8.5E-01
rs1563411	18	47,115,726	SMAD4	53	51	104	51.0	0.0	8.4E-01
rs11673097	**19**	**61,811,246**	**ZNF71**	**46**	**62**	**108**	**42.6**	**2.4**	**1.2E-01**
rs887955	**19**	**61,812,181**	**ZNF71**	**42**	**61**	**103**	**40.8**	**3.5**	**6.1E-02**
rs2215930	19	61,814,858	ZNF71	57	65	122	46.7	0.5	4.7E-01
rs6018169	**20**	**44,979,735**	**EYA2**	**21**	**15**	**36**	**58.3**	**1.0**	**3.2E-01**
rs16992555	**20**	**45,477,451**	**PRKCBP1**	**12**	**17**	**29**	**41.4**	**0.9**	**3.5E-01**
rs17314640	**23**	**22,064,024**	**PHEX**	**29**	**12**	**41**	**70.7**	**7.0**	**7.9E-03**
rs2269466	**23**	**22,065,345**	**PHEX**	**12**	**29**	**41**	**29.3**	**7.0**	**7.9E-03**
rs5951426	**23**	**22,201,527**	**ZNF645**	**3**	**9**	**12**	**25.0**	**3.0**	**8.3E-02**
rs17280555	**23**	**41,869,139**	**CASK**	**11**	**16**	**27**	**40.7**	**0.9**	**3.4E-01**
rs5905222	**23**	**115,304,929**	**AGTR2**	**30**	**16**	**46**	**65.2**	**4.3**	**3.9E-02**
rs4824344	**23**	**115,341,305**	**AGTR2**	**17**	**26**	**43**	**39.5**	**1.9**	**1.7E-01**
rs5905239	**23**	**115,343,125**	**AGTR2**	**27**	**17**	**44**	**61.4**	**2.3**	**1.3E-01**

rs3131425	**23**	**125,804,772**	**ACTRT1**	**8**	**5**	**13**	**61.5**	**0.7**	**4.1E-01**
rs3131446	**23**	**125,856,790**	**ACTRT1**	**7**	**11**	**18**	**38.9**	**0.9**	**3.5E-01**
rs35850911	**23**	**127,797,090**	**ACTRT1**	**4**	**1**	**5**	**80.0**	**1.8**	**1.8E-01**
rs2179787	**23**	**127,828,021**	**ACTRT1**	**5**	**3**	**8**	**62.5**	**0.5**	**4.8E-01**
rs2858147	23	127,840,842	ACTRT1	10	13	23	43.5	0.4	5.3E-01
rs5976926	**23**	**127,868,281**	**ACTRT1**	**9**	**14**	**23**	**39.1**	**1.1**	**3.0E-01**
rs5932540	**23**	**127,875,098**	**ACTRT1**	**9**	**14**	**23**	**39.1**	**1.1**	**3.0E-01**

Bold Font: p-value less than 0.05 and/or % transmission exceeded the 75th percentile. Red Font: p-value less than 0.05.

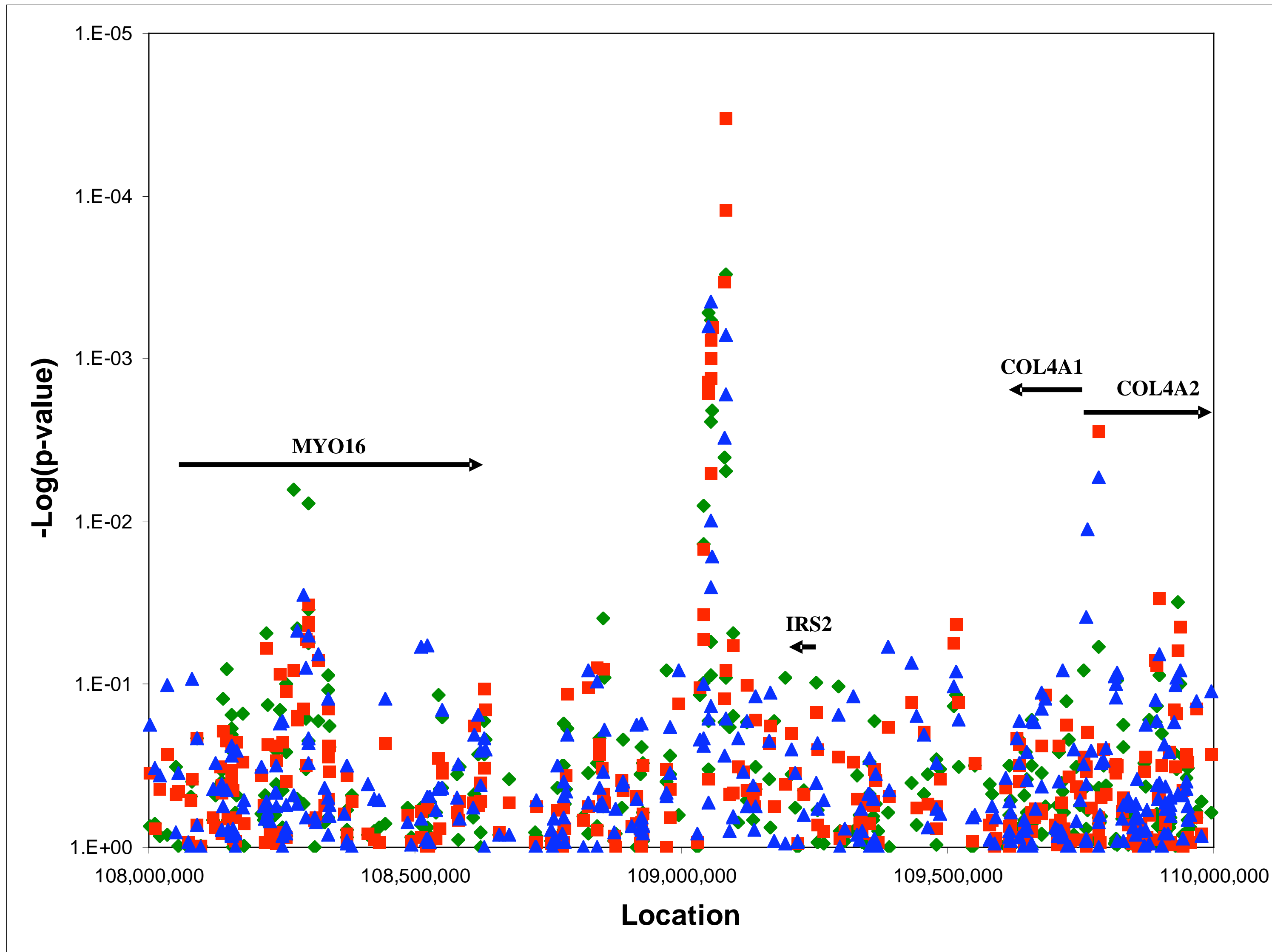


Figure 3

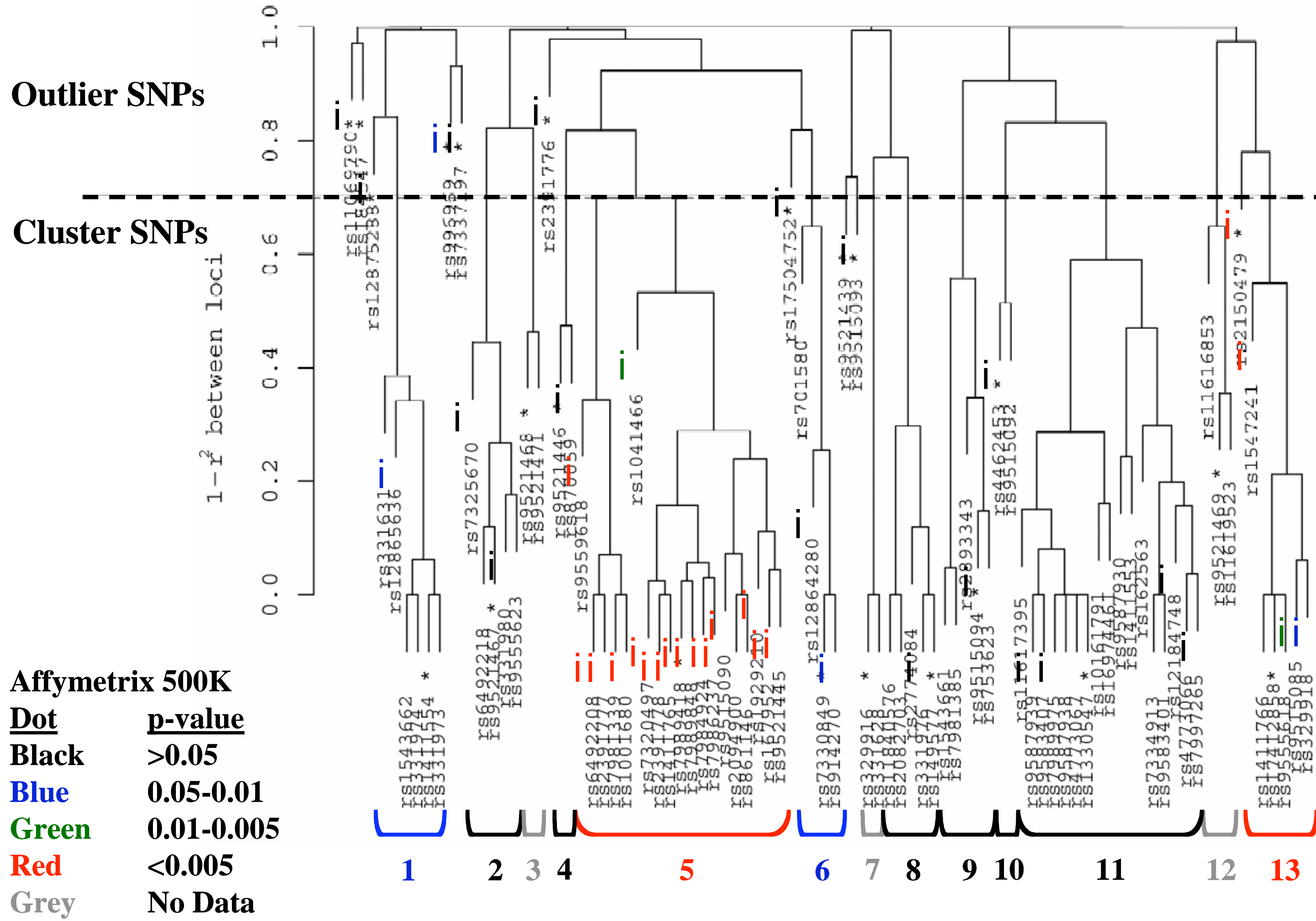
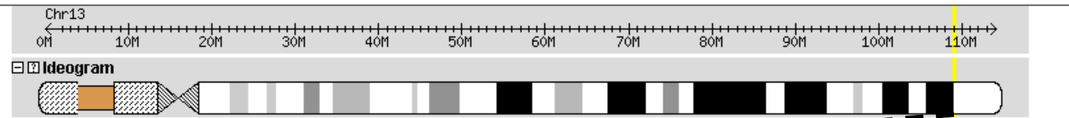
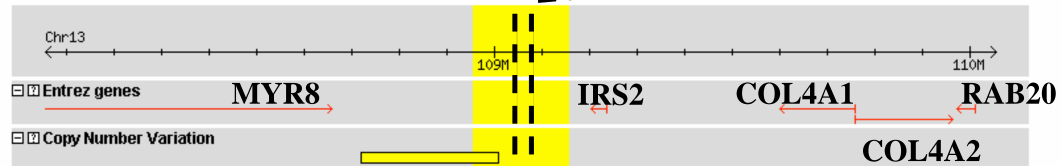


Figure 4

Chromosome 13 Ideogram



Chromosome 13 2Mb Region



Chromosome 13 Peak Region

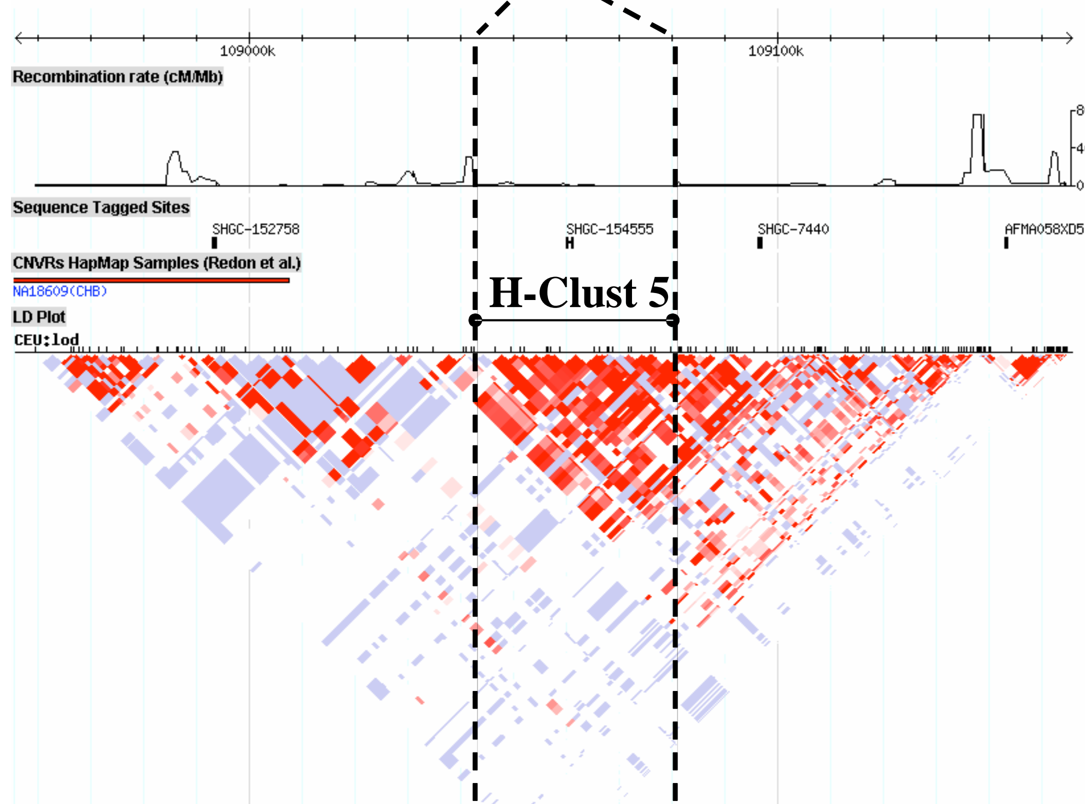


Figure 5

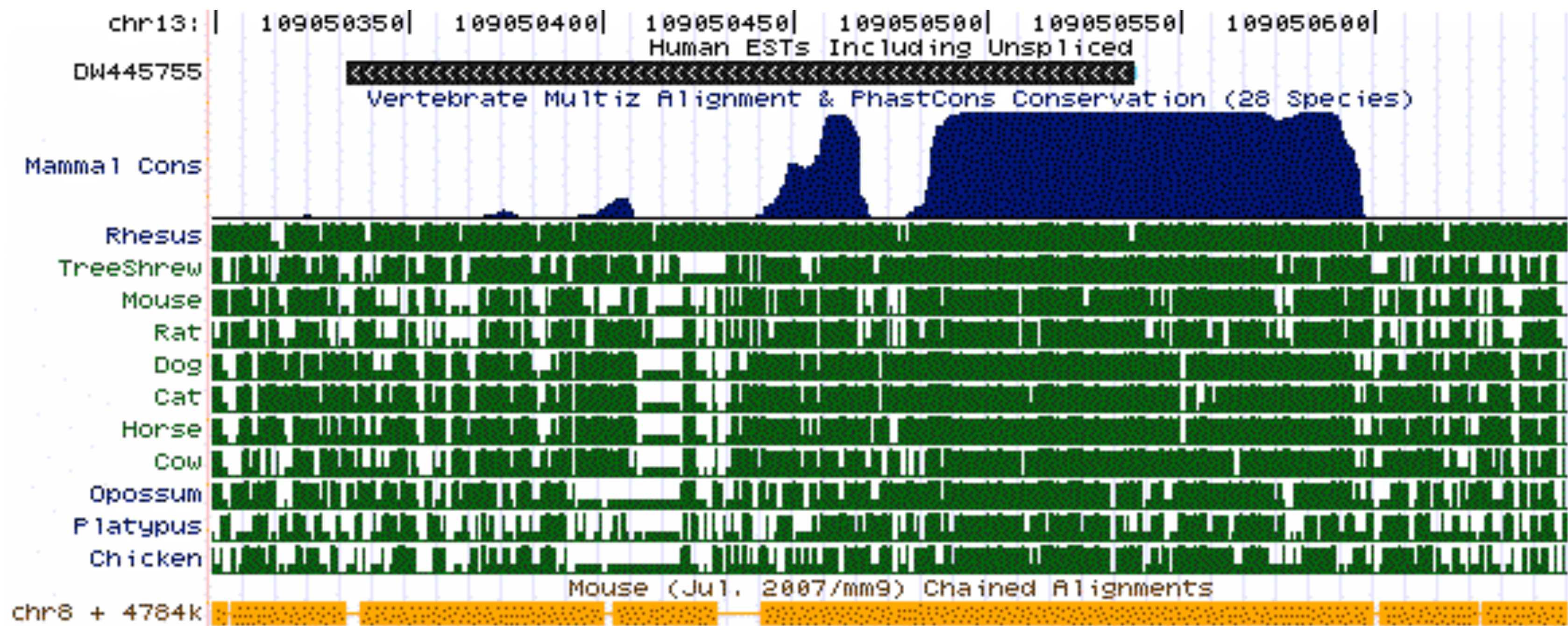


Figure 6

Table 4. Case (N=220) and Control (N=645) Analysis of Select SNPs for Association with T1DN.

				Genotype Based Typing			Allele Based Typing	
dbSNP ID	Chr	Location	Locus Name	Additive	Dominant	Recessive	Allelic	OR (95% CI)
rs2841347	1	237,019,769	CHRM3	1.2E-01	7.1E-02	5.3E-01	1.1E-01	1.2 (1.0-1.6)
rs10197310	2	102,386,462	IL18RAP	4.7E-01	5.5E-01	5.4E-01	4.6E-01	1.1 (0.8-1.6)
rs7586146	2	140,789,344	LRP1B	6.9E-01	8.1E-01	6.2E-01	6.9E-01	1.1 (0.7-1.6)
rs6846301	**4**	**72,566,355**	**SLC4A4**	**5.9E-01**	**4.7E-02**	**9.6E-01**	**5.9E-01**	**1.1 (0.7-1.7)**
rs6850722	4	156,692,216	GUCY1A3	7.7E-01	4.8E-01	8.2E-01	7.7E-01	1.0 (0.8-1.3)
rs1976041	4	156,705,690	GUCY1A3	9.3E-01	4.7E-01	5.8E-01	9.3E-01	1.0 (0.8-1.3)
rs716428	4	156,726,319	GUCY1A3	8.7E-01	3.7E-01	5.0E-01	8.7E-01	1.0 (0.8-1.3)
rs990619	4	156,727,128	GUCY1A3	7.6E-01	4.4E-01	7.9E-01	7.6E-01	1.0 (0.8-1.3)
rs1123037	4	156,734,175	GUCY1A3	7.5E-01	4.7E-01	8.4E-01	7.6E-01	1.0 (0.8-1.3)
rs6835414	4	156,734,392	GUCY1A3	9.6E-01	4.5E-01	5.2E-01	9.6E-01	1.0 (0.8-1.3)
rs6887976	5	113,996,634	KCNN2	1.6E-01	9.6E-02	8.3E-01	1.6E-01	1.3 (0.9-1.8)
rs6595812	5	127,603,502	SLC12A2	9.1E-02	9.4E-02	5.0E-01	8.7E-02	1.4 (0.9-2.1)
rs13229065	7	93,012,567	CALCR	9.0E-01	6.8E-01	5.4E-01	9.0E-01	1.0 (0.8-1.3)
rs16951472	13	95,871,978	HS6ST3	3.8E-01	3.3E-01	5.2E-01	3.8E-01	1.2 (0.8-1.6)
rs2150479	**13**	**109,041,870**	**IRS2**	**2.0E-02**	**7.5E-02**	**3.0E-02**	**1.6E-02**	**1.4 (1.1-1.8)**
rs6492208	**13**	**109,055,727**	**IRS2**	**5.4E-03**	**2.0E-01**	**2.2E-03**	**4.7E-03**	**1.4 (1.1-1.7)**
rs2391777	**13**	**109,056,244**	**IRS2**	**7.9E-03**	**1.9E-01**	**4.3E-03**	**7.1E-03**	**1.4 (1.1-1.7)**
rs7320497	**13**	**109,058,000**	**IRS2**	**2.2E-02**	**3.2E-01**	**7.6E-03**	**1.8E-02**	**1.4 (1.1-1.8)**
rs1001680	**13**	**109,060,557**	**IRS2**	**2.0E-02**	**1.3E-01**	**2.7E-02**	**1.8E-02**	**1.4 (1.1-1.8)**
rs1547241	13	109,071,606	IRS2	3.7E-01	7.4E-01	2.0E-01	3.5E-01	1.1 (0.9-1.5)
rs167952	**13**	**109,072,232**	**IRS2**	**2.2E-02**	**2.4E-01**	**1.2E-02**	**2.0E-02**	**1.4 (1.0-1.8)**
rs7984924	**13**	**109,081,246**	**IRS2**	**1.0E-02**	**9.1E-02**	**1.3E-02**	**8.7E-03**	**1.4 (1.1-1.8)**
rs7986237	**13**	**109,081,322**	**IRS2**	**1.7E-02**	**1.4E-01**	**1.6E-02**	**1.4E-02**	**1.4 (1.1-1.8)**
rs7989418	**13**	**109,081,443**	**IRS2**	**1.5E-02**	**1.6E-01**	**1.1E-02**	**1.3E-02**	**1.4 (1.1-1.8)**
rs9515090	**13**	**109,084,543**	**IRS2**	**1.4E-02**	**1.5E-01**	**1.2E-02**	**1.1E-02**	**1.4 (1.1-1.8)**
rs870059	13	109,092,251	IRS2	6.2E-02	8.8E-02	1.7E-01	5.5E-02	1.2 (1.0-1.5)
rs1865434	13	109,206,609	IRS2	7.3E-01	7.8E-01	7.3E-01	7.3E-01	1.1 (0.7-1.6)
rs11618950	13	109,232,311	IRS2	5.5E-01	9.5E-01	4.5E-01	5.3E-01	1.1 (0.8-1.6)
rs4773092	13	109,233,954	IRS2	7.4E-01	6.0E-01	3.8E-01	7.3E-01	1.0 (0.8-1.4)
rs9302755	16	49,667,140	SALL1	6.2E-01	8.8E-01	4.0E-01	6.2E-01	1.1 (0.8-1.4)
rs2017684	16	50,026,393	SALL1	6.1E-01	7.9E-01	5.5E-01	6.1E-01	1.1 (0.8-1.4)
rs10852513	16	50,046,878	SALL1	8.7E-01	7.7E-01	6.1E-01	8.7E-01	1.0 (0.8-1.3)
rs11673097	19	61,811,246	ZNF71	6.6E-01	5.4E-01	9.6E-01	6.5E-01	1.1 (0.8-1.4)
rs887955	19	61,812,181	ZNF71	7.5E-01	6.3E-01	9.2E-01	7.4E-01	1.0 (0.8-1.4)
rs6018169	**20**	**44,979,735**	**EYA2**	**8.3E-02**	**3.7E-02**	**5.8E-01**	**7.6E-02**	**1.5 (1.0-2.3)**
rs16992555	20	45,477,451	PRKCBP1	7.7E-01	8.8E-01	4.4E-01	7.7E-01	1.1 (0.7-1.7)

rs17314640	23	22,064,024	PHEX	9.6E-01	9.9E-01	9.4E-01	7.1E-01	1.1 (0.8-1.4)
rs2269466	23	22,065,345	PHEX	9.9E-01	9.1E-01	9.5E-01	7.4E-01	1.1 (0.8-1.4)
rs17280555	23	41,869,139	CASK	2.7E-01	9.7E-01	1.1E-01	2.6E-01	1.2 (0.9-1.7)
rs5905222	23	115,304,929	AGTR2	1.7E-01	6.5E-01	7.7E-02	1.1E-01	1.3 (0.9-1.8)
rs4824344	23	115,341,305	AGTR2	2.2E-01	7.3E-01	9.2E-02	2.1E-01	1.2 (0.9-1.6)
rs5905239	23	115,343,125	AGTR2	1.7E-01	7.0E-01	5.8E-02	1.6E-01	1.2 (0.9-1.7)
rs3131446	23	125,856,790	ACTRT1	5.1E-01	3.6E-01	7.2E-01	7.3E-01	1.1 (0.7-1.5)
rs35850911	23	127,797,090	ACTRT1	4.4E-01	7.5E-01	1.8E-01	6.2E-01	1.2 (0.6-2.2)
rs2179787	23	127,828,021	ACTRT1	2.9E-01	4.5E-01	2.0E-01	3.7E-01	1.3 (0.7-2.4)
rs2858147	23	127,840,842	ACTRT1	6.6E-01	2.5E-01	4.0E-01	3.6E-01	1.2 (0.8-1.9)
rs5976926	23	127,868,281	ACTRT1	9.3E-01	5.9E-01	2.8E-01	6.4E-01	1.1 (0.7-1.7)
rs5932540	23	127,875,098	ACTRT1	8.4E-01	7.0E-01	2.9E-01	7.4E-01	1.1 (0.7-1.6)

Blue Font: p-value between 0.05 and 0.01
Green Font: p-value between 0.01 and 0.005
Red Font: p-value less than 0.005
Yellow Highlight: Odds Ratio and 95% CI greater than 1

Summary of the Results from Aim 1: The principal goal of our study has been to exploit the results from a GWAS to assess the contribution of specific loci on susceptibility for developing T1D-ESRD. We genotyped 500,477 SNPs in 197 cases and 197 controls followed by second stage analysis of selected SNPs using a cohort of 121 family trios as well as a stage 2 case and control cohort of 220 T1DN and 645 T1D participants. DNA samples were derived from the GoKinD collection. As the sequencing of the entire human genome is now completed and recently updated the results allow disposal of a large number of single nucleotide polymorphisms frequently occurring in our genome as being unrelated to the phenotype. Moreover data from the HapMap project allowed definition of statistically determined haplotypes such as groups of variations able to segregate together (Figures 4 and 5). Comparison of co-segregating region of DNA can be used to mark the genome and demonstrate that the genetic variation segregates with the trait (T1DN in this case) (Risch and Merikangas 1996).

Impaired kidney function is a principal component underlying the physiology of essential hypertension (MCSG, 1999; Stern et al., 2004; Pasquali et al., 2007). Physiological responses to mutations in *IRS2* have been linked to activation of the principal sodium reuptake channel expressed in renal tubular cells, *NHE3*. Family-based studies have reported increased incidence of hypertension, 30% to 40% higher in patients who develop T1DN (Viberti et al., 1987; Krolewski et al., 1988) while patients with uncomplicated T1D exhibit relatively low blood pressures (Stern et al., 2004). Evidence for genetic susceptibility to hypertension in T1DN patients included increased incidence of parental hypertension (Krolewski et al., 1988). Parents of T1D-ESRD patients frequently exhibit increased blood pressure when compared with parents of unaffected T1D patients, suggesting a familial basis linking hypertension with impaired kidney function. Studies designed to deconstruct the role of genetics on hypertension have indicated that different genes may contribute to increased incidence of hypertension in different ethnic groups (DeWan et al., 2001; Mullins et al., 2006). Genome-wide scans have implicated different regions of chromosome 3 in Caucasian and African American populations as well as genetic markers on chromosome 1 and 6 among Caucasians (DeWan et al., 2001; Freedman et al., 2003; Mullins et al., 2006). Improved knowledge of the inherited basis of T1D-ESRD may lead to deeper understanding of the molecular mechanisms underlying the disease, leading to improved therapeutic intervention, as well as improved understanding of why the majority of T1D patients maintain kidney function while a substantial minority go on to end-stage disease.

Successful mapping of genes influencing common causes of ESRD, including non-diabetic ESRD, has been reported for a genome-wide linkage scan using 483 African American families (Freedman et al., 2005). This study provided evidence for linkage occurring at chromosome 13q33.3 near the D13S796 microsatellite, a marker in linkage disequilibrium with the peak region identified in the GWAS and replication phase of our study. In a complementary study on the genetic causes underlying congenital anomalies of the kidney a 7Mb deletion was mapped to the region containing the D13S796 microsatellite (Vats et al., 2006). Expression studies examining genes in this region of chromosome 13 indicated strong renal expression for several genes and including *IRS2*. The *IRS2* gene encodes Insulin Receptor Substrate 2, a protein integrally involved during insulin signal transduction (Thirone et al., 2006). Multiple studies have implicated *IRS2* allelic variants with hepatic glucose homeostasis in obese T2D patients (Mammarella et al., 2000; Lautier et al., 2003). Animal models indicate that defects in hepatic *IRS2* activity result in upregulation of lipogenic enzymes, fatty acid synthase, and hepatic lipid accumulation (Taniguchi et al., 2005). Increased serum triglycerides are among the principal physiological risk factors correlated with T1DN providing a plausible mechanism by which dysregulation of *IRS2* may increase risk for nephropathy (Chaturvedi et al., 2001).

The GWAS has thoroughly evaluated the genome for causal gene variants influencing T1DN. At this point the analysis has identified a region downstream of the *IRS2* locus as being in LD with the phenotype. The *IRS2* locus, if in fact it is causal for risk for developing T1DN, has been implicated in regulation of sodium homeostasis though regulation of *NHE3* activity as well as in mediating pancreatic beta-islet cell growth and function. Either or both mechanisms are attractive models for linking the function of the *IRS2* gene product to susceptibility of developing T1DN. For example, over expression of *IRS2* in isolated pancreatic islets has been shown to cause beta cell proliferation as well as protection of beta cells from hyperglycemic induced apoptosis. In T1D patients with low residual levels of insulin derived C-peptide there has been observed an increased incidence of poor glycemic control and subsequent increased incidence of diabetic complications, including nephropathy. In a parallel model changes in renal tubular function controlled through the *IRS2* dependent insulin-signaling pathway have been shown to regulate *NHE3* activity. Alterations in the renal tubular compartment have been implicated as an early physiological event, preceding onset of T1DN. Results of the GWAS have indicated association of a roughly 42kb genetic element with susceptibility for T1DN. Functional studies in transgenic animal models will be required to address the link, if any, between the H-Clusters 5 and 13 region and kidney disease in diabetes.

Aims 2 and 3: Experiments designed to complete the final 2 Aims of the proposal are ongoing. *Aims 2 and 3 are to: ensure that SNPs identified by Aim 1 affect risk of T1DN as opposed to risk for T1D; and identify genetic variants that interact with smoking status in conferring risk for T1DN.* The approach to complete Aim 2 is to genotype a cohort of T1D control family trios and to perform joint statistical analysis of this data combined with those obtained during Aim 1 genetic studies. In Aim 3 we will finalize the replication stage of the project by statistical analysis of the entire cohort. The

analyses will allow smoking to be a variable in our calculations enabling the measurement of smoking as an environmental factor impacting genes that influence T1DN. Completion of the initial 2 years study will provide the foundation for a new research proposal to confirm the reproducible associations that have been identified. We will add new T1D and T1DN participants to that investigation in order to achieve the necessary statistical power that will confirm as well as quantify the effect of genes and gene-environment interaction on risk for T1DN.

KEY RESEARCH ACCOMPLISHMENTS:

Research Accomplishments Emanating from the Research Project:

Greater than 569 Family trios (257 cases and 312 controls) and 1,174 singletons (578 cases and 596 controls) have been obtained to allow identification of genetic markers linked to diabetic complications, including diabetic-nephropathy.

Using 197 T1DN (case) and 197 T1D (control) extensive genotyping data (500,477 genotypes per sample) was obtained and used to conclude a genome-wide association scan for T1DN markers.

Extensive genotyping data has been obtained (500,477 genotypes per sample) from 2,144 non-T1D participants and can be used to expand the genetic analyses to screen for markers of T1D.

Additional recruitment of T1DN (N=150) and T1D (N=150) singletons and T1DN family trios (N=150) has been completed.

Allele transmission data identified a reproducible locus on chromosome 13q34 with highly significant association with diabetic nephropathy.

Nominally significant results have been found with SNPs in linkage disequilibrium and identify a 42kb haplotype (chromosome 13:109,042,000-109,084,000) with significant association ($p<0.0000008$).

The T1DN associated haplotype overlapped at least 1 expressed sequence (DW445755; chromosome 13:109,050,335-109,050,538) and comparative genome analysis identified a highly conserved element in the promoter region.

Although the results shown above are nominally significant, they must be considered preliminary in view of the small sample size. In order to confirm and expand these results additional families will be recruited and additional SNPs in the regions of suggestive associations will be genotyped.

REPORTABLE OUTCOMES:

Dr. Trucco's laboratory has published 19 manuscripts in 2007 and 93 manuscripts in peer reviewed journals during the 5 year period beginning in 2002 to present (selected publications are listed below):

Luca D, Ringquist S, Klei L, Lee AB, Gieger C, Wichmann HE, Schreiber S, Krawczak M, Lu Y, Styche A, Devlin B, Roeder K, Trucco M. On the use of general control samples for genome-wide association studies: genetic matching highlights causal variants. Am J Hum Genet 2007 (in press).

Pasquali L, Trucco M, Ringquist S. Navigating pathways affecting type 1 diabetic kidney disease. Pediatr Diabetes 8:307-322, 2007.

Koike C, Uddin M, Wildman DE, Gray EA, Trucco M, Starzl TE, Goodman M. Functionally important glycosyltransferase gain and loss during catarrhine primate emergence. Proc Natl Acad Sci U S A 104:559-564, 2007.

Pasquali L, Fan Y, Trucco M, Ringquist S. Rehabilitation of adaptive immunity and regeneration of beta cells. Trends Biotechnol 24:516-522, 2006.

Balamurugan AN, Chang Y, Bertera S, Sands A, Shankar V, Trucco M, Bottino R. Suitability of human juvenile pancreatic islets for clinical use. Diabetologia 49:1845-1854, 2006.

Wang Z, Zhu T, Rehman KK, Bertera S, Zhang J, Chen C, Papworth G, Watkins S, Trucco M, Robbins PD, Li J, Xiao X. Widespread and stable pancreatic gene transfer by adeno-associated virus vectors via different routes. Diabetes 55:875-884, 2006.

Harnaha J, Machen J, Wright M, Lakomy R, Styche A, Trucco M, Makaroun S, Giannoukakis N. Interleukin-7 is a survival factor for CD4+ CD25+ T-cells and is expressed by diabetes-suppressive dendritic cells. Diabetes 55:158-170, 2006.

Rood PP, Bottino R, Balamurugan AN, Fan Y, Cooper DK, Trucco M. Facilitating physiologic self-regeneration: a step beyond islet cell replacement. Pharm Res 23:227-242, 2006.

Bottino R, Trucco M. Multifaceted therapeutic approaches for a multigenic disease. Diabetes 54:S79-S86, 2005.

Casu A, Trucco M, Pietropaolo M. A look to the future: prediction, prevention, and cure including islet transplantation and stem cell therapy. Pediatr Clin North Am 52:1779-1804, 2006.

Ringquist S and Trucco M. Regenerative medicine for diabetes treatment. Discovery Medicine 5:142-147, 2005.

Ringquist S, Pecoraro C, Gilchrist CM, Styche A, Rudert WA, Benos PV, Trucco M. SOP3v2: web-based selection of oligonucleotide primer trios for genotyping of human and mouse polymorphisms. Nucleic Acids Research 33:W548-552, 2005.

Trucco M. Regeneration of the pancreatic beta cell. Journal Clinical Investigation 115:5-12, 2005.

Phelps CJ, Koike C, Vaught TD, Boone J, Wells KD, Chen SH, Ball S, Specht SM, Polejaeva IA, Monahan JA, Jobst PM, Sharma SB, Lamborn AE, Garst AS, Moore M, Demetris AJ, Rudert WA, Bottino R, Bertera S, Trucco M, Starzl TE, Dai Y, Ayares DL. Production of alpha 1,3-galactosyltransferase-deficient pigs. Science 299:411-414, 2003.

Friday RP, Profozich J, Pietropaolo S, Trucco M, Pietropaolo M: Alternative core promoters regulate tissue-specific transcription from the autoimmune diabetes-related *ICA1* (ICA69) gene locus. Journal of Biological Chemistry 278:853, 2003.

Bottino R, Lemarchand P, Trucco M, Giannoukakis N: Gene and cell-based therapeutics for type 1 diabetes mellitus. Gene Therapy 10:875, 2003.

Dorman JS, Charron-Prochownik D, Siminerio L, Ryan C, Poole C, Becker D, Trucco M: Need for genetic education for type 1 diabetes (letter). Archives of Pediatric Adolescent Medicine 157:935, 2003.

Dr. Trucco's laboratory has published 4 invited book chapters during 2006 and 2007 (publications are listed below):

Lu Y, Boehm J, Nichol L, Trucco M, and Ringquist S. Multiplex HLA-Typing by Pyrosequencing. *In* Bugert P (ed): *Methods Mol Biol* (in press).

Ringquist S, Styche A, Rudert WA, and Trucco M. Pyrosequencing-based strategies for improved allele typing of human leukocyte antigen loci. *In* Marsh S (ed): *Methods Mol Biol* 373:115-134 (2007).

Ringquist S, Pecoraro C, Lu Y, Styche A, Rudert WA, Benos PV, and Trucco M. Web-based primer design software for genome-scale genotyping by pyrosequencing. *In* Marsh S (ed): *Methods Mol Biol* 373:25-38 (2007).

Ringquist S, Nichol L, and Trucco M. Transplantation genetics. *In* Rimoin D, Connor JM, Pyeritz R, Korf B. Emery A (eds): *Emery and Rimoin's Principles and Practice of Medical Genetics 5th Edition*. Churchill Livingstone (2007).

Ringquist S and Trucco M. Regenerating pancreatic islet function in juvenile diabetes. *Progress in Stem Cell Research*. Nova Science Publishers (2006).

Dr. Trucco's laboratory has presented 10 invited lectures and meeting abstract in 2004-2007 (publications are listed below):

Ringquist S and Trucco M. A proteomics approach necessary to confirm molecularly determined associations between genes and nephropathy. Department of Defense Symposium on Advanced Technology to Improve Prediction and Prevention of Type 1 Diabetes Mellitus, 2004.

Ringquist S, Ge X, Zhang L, Styche L, Balamurugan AN, Bottino R, Rudert WA, Trucco M. Proteomic scanning for markers associated with successful islet isolation and maintenance. Keystone Symposia Diabetes Mellitus, 2004.

Ringquist S, Rudert WA, and Trucco M. New advanced technology to improve prediction and prevention of type 1 diabetes. Department of Defense Symposium on Telemedicine and Advanced Technology Research Center, 2004.

Pecoraro C, Styche A, Rudert WA, Benos PV, Ringquist S, Trucco M. SOP^3: A web based tool for selection of

oligonucleotide primers for SNP analysis. University of Pittsburgh Science 2004 Symposia, 2004.

Ringquist S, Pecoraro C, Styche A, Lu Y, Rudert WA, and Trucco M. Genetic analysis of complex disease: web-base management tool for DNA polymorphisms. Windber Research Institute Showcase for Biotechnology, 2005.

Ringquist S and Trucco M. Genetic analysis of complex disease. Windber Research Institute Showcase for Biotechnology, 2005.

Ringquist S, Pecoraro C, Styche A, Lu Y, Rudert WA, and Trucco M. Genetic analysis of complex disease: web-base management tool for DNA polymorphisms. University of Pittsburgh Symposium Science, 2005.

Ringquist S and Trucco M. SOP^3: web-based selection of oligonucleotide primer trios for genotyping of human and mouse polymorphisms. University of Pittsburgh Symposium Science, 2005.

Trucco M. New Advanced Technology to Improve Prediction and Prevention of Type 1 Diabetes. Department of Defense Symposia on Distance Medicine, Fredrick, MD (Jan. 2006).

Pecoraro C, Lu Y, Pasquali L, Styche A, Rudert WA, Trucco M and Ringquist S. MAESTRA software in genome-wide and candidate gene analysis: a case study. Windber Research Institute Symposium Translational Health, August 13-15, 2006.

Patents and Licenses (applied for and/or issued)

Copyrighted Material:

SOP3 software for designing primers for PCR and pyrosequencing.

Degrees Obtained that are Supported by this Award

N/A

Development of Cell Lines

Lymphoblastoid cell lines from the Children's Hospital of Pittsburgh collection of diabetic families.

Tissue or Serum Repositories

Repository of 2,881 DNA samples from family trios and singletons in which the proband exhibited T1D, T1DN, or T1D-ESRD have been obtained.

Informatics and Databases

Development, testing, and publication of software application for designing assays for genotyping of human DNA samples.

Warehousing of databases of the human genome, human genetic polymorphisms, and human haplotype maps.

Extensive genotyping data (500,477 SNPs per sample) obtained for 197 T1DN and 197 T1D as well as 2,144 non-T1D participants.

Animal Models

None

Funding Applied for Based on Work Supported by this Award

None

Employment or Research Opportunities Applied for and/or Received Based on this Award

None

CONCLUSIONS:

Summary of the Importance and/or Implications of the Research

Healthcare costs associated with diabetes and diabetic complications, such as nephropathy, account for 10% of monies spent on healthcare. Early identification of individuals at risk for this chronic disease will aid in improved management, decreased severity, and reduced healthcare costs. Potential benefits of the project are: (1) improved forecasting of the genetic risk of developing diabetes and diabetic complications; and (2) the opportunity to apply preventative treatment focused on at-risk individuals.

Recommended Changes on Future Work

Challenges to successful completion of the project are as follows:

1. Obtain additional samples in order to improve on the statistical significance of the results:
In the interval since the last progress report we have obtained genotyping data on an additional 2,144 non-T1D participants, have made substantial progress in gaining access to another 3,000 non-T1D participants, and anticipant gaining access to the genotype results for an additional 2,000 T1D participants. The 3 cohorts have been recruited independently of one another and have been extensively genotyped using the Affymetrix microarray for analysis of 500,477 SNPs. Moreover, we anticipate receiving DNA samples from T1DN cases (N=300), T1D controls (N=300), and T1D family trios (N=150).

2. The need to validate alternative methods for monitoring the accuracy of the genotyping data:
We have completed a genome-wide association study for T1DN. The analysis used 197 case (T1DN) and 197 control (T1D) participants. Genotypes were obtained from 500,477 SNPs using a microarray manufactured by Affymetrix Corporation. Validation of the accuracy of the genotypes was confirmed by using TaqMan methodology for select SNPs. Experiments establishing reproducibility of the data were accomplished using independent cohorts of case (N=220) and control (N=645) singletons as well as case family trios (N=121). Analysis of the results obtained from combined cohorts provided compelling evidence for the accuracy of the genotyping data.

3. Development and testing of software for analysis of genome-wide association data:
Genome-wide association studies are dependent on the availability of very large and well characterized control populations. Large-scale efforts are underway to amass these resources (i.e., dbGAP in the U.S., the Wellcome Trust Case-Control Consortium in the U.K., as well as the POPGEN and KORA projects in Germany). However, how to use these databases effectively is an open question. To solve this problem we have developed novel GEnetic Matching (GEM) algorithms that enable optimal matching of case and control samples resulting in greatly improve statistical power during analysis of association studies (Lucas et al., in press).

Evaluation of the Knowledge as a Scientific or Medical Product

Diabetes affects 16 million Americans (greater than 5% of the population), and 800,000 new cases annually.

Diabetes occurs in men, women, children and the elderly. African, Hispanic, Native and Asian Americans are particularly susceptible to its most severe complications.

In the U.S. the costs associated with diabetes in terms of medical expenditure and lost productivity may be as high as $132 billion and the direct medical expenditure for chronic complications attributable to diabetes exceeds $24.6 billion.

Diabetic nephropathy accounts for 42% of new cases of end-stage renal disease, with over 100,000 cases per year at an average cost of $55,000 per patient annually.

Economic impact of diabetes is over $100 billion annually accounting for more than $1 in every $10 healthcare dollars and $1 of every $4 Medicare dollars spent.

As the military is a reflection of the US population, improved prediction of risk for developing diabetes and diabetic complications among active duty members of the military, their families and retired military personnel will potentially allow focused preventative treatment of at-risk individuals, providing significant healthcare savings and improved patient well being.

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Genetic Screening in Diabetes
Walter Read Health Care System
Robert A. Vigersky, M.D., COL MC

Introduction

Although deaths today from the acute effects of diabetes are rare, the associated vascular, retinal, neurological and renal complications are responsible for high levels of morbidity and mortality in diabetes. It has been observed that only a subset of diabetics appear to be susceptible to the development of the diabetic complications, i.e., nephropathy, autonomic neuropathy, and retinopathy and there is data to suggest that there is a genetic component to this increased susceptibility. The proposed investigation will test the hypothesis that there are allelic variations of some genes that make the development of diabetes-related microvascular complications (nephropathy, neuropathy, and retinopathy) more likely in patients who carry them than in those who do not. Initial emphasis will be examining candidate gene analysis in families for diabetic nephropathy, autonomic neuropathy, and retinopathy.

Body

The title of this study is "Genetic Screening in Diabetes." This is an observational study in which COL Vigersky and his research team will obtain DNA samples from the blood of patients at least one of three diabetic complications and from as many of their first-degree relatives as possible for genetic testing. The study will be performed at WRAMC for DEERS-eligible subjects and at the Uniformed Services University of Health Sciences (USUHS) for non-DEERS-eligible subjects.

After meeting eligibility requirements, all subjects complete a medical history, a quality of life questionnaire, a physical examination, blood and urine sampling and analysis, and additional procedures to rule out diabetes and the presence or absence of the three diabetes-related complications that are being studied. All blood samples will be typed and examined to evaluate if there are reasonable candidate genes that contribute to the genetic susceptibility and/or development of diabetic nephropathy, neuropathy, and retinopathy. It is expected that WRAMC will enroll up to 100 probands and 300 of their family members.

Key Research Accomplishments

- After extensive revisions, the study was approved by the Clinical Investigation and Human Use Committees at WRAMC in March 2006 and the Clinical Investigation Research Office in April 2006.
- After additional extensive revisions, the protocol was approved by the Institutional Review Board (IRB) at USUHS in April 2007 to conduct the study on non-DEERS eligible relatives. The non-DEERS eligible subjects are currently being seen in the White Flint Professional Building, Suite 303, 11119 Rockville Pike, Kensington, MD.
- Recruitment began on April 4, 2007.
- As of November 21, 2007, 18 probands and 15 family members have been enrolled into the study.
- Per protocol specifications, blood samples from enrolled subjects have been sent to the Rangos Research Center at the Children's Hospital of Pittsburgh, Pittsburgh, PA.

Reportable Outcomes

Funding in the amount of $159,000.00 was awarded to cover the costs associated with personnel and consumable supplies for this study under a Cooperative Research and Development Agreement between the T.R.U.E. Research Foundation and the Clinical Regulatory Office.

Conclusions

Not Applicable.

References

None.

Appendices

None.

Supporting Data

Not Applicable.

Genetic Screening in Diabetes: Candidate Gene Analysis for Diabetic Nephropathy
University of Hawaii
Lawrence Burgess, M.D. / Richard Arakaki, M.D. / Deborah Birkmire-Peters, PhD

INTRODUCTION:

The Genetic Screening in Diabetes: Candidate Gene Analysis for Diabetic Nephropathy project continues with an emphasis on enrollment of probands and their family members. With the assistance of collaborator nephrologist, recruitment effort through the largest dialysis unit of Fresenius Medical Care of Hawaii took place during the previous funding period. The effort at recruiting participants into this study, that is, subjects with physician-documented Diabetic Nephropathy (ESRD patients with dialysis or kidney transplant) with a first degree relative, still proved difficult despite having relatively easy access to approximately 170 potential participants at the dialysis center. Despite examination of 10% of our enrollment goal for this study 100 probands (individuals with Diabetic Nephropathy) and 300 family members (parents and siblings) the study team remains optimistic for the next funding period with new direction for recruitment as well as continuing current efforts. The logistical considerations in conducting this study were implemented and continued with web-based subject registration, computerized questionnaire data collection, computerized blood processing and DNA extraction, and participant and primary care physician follow-up post screening.

BODY:

The previous year's recruitment effort of eligible patients from the Diabetes Clinic run by Dr. Arakaki yielded four families with one complete set of both parents and the patient with ESRD. Of four participants with DM and ESRD, two patients required hemodialysis and the other two patients were maintained on peritoneal dialysis. Additional participants were enrolled over the last funding period through combined efforts of nephrologist, Dr. Ramona Wong and recruitment staff members. The primary location of recruitment was the main dialysis center of Fresenius Medical Care of Hawaii. This Center has 45 stations accommodating about 270 patients. Through the effort of Dr. Wong, who is the Medical Director of one of the center of Fresenius Medical Care and President of the National Kidney Foundation Hawaii Affiliate, access to these patients was facilitated. Shift nurses examined the Center's database and identified 178 patients with diabetes-related ESRD. The identified number of patients accounts for a prevalence of 66% of diabetic nephropathy as the primary cause of ESRD. This rate reflects the prevalence rate across Hawaii based on the Network 17 data set from the United States Renal Disease System. This rate is higher than 44% prevalence rate observed for the rest of the country (MMWR 54(43);1097-1100, 2005).

The recruitment staff members were introduced to the dialysis center and provided with the list of 178 patients based on dialysis shift schedule. Patients were then interviewed using the recruitment screening tool during the time they were at the dialysis center. The following information was obtained.

- 178 ESRD patients requiring dialysis with diabetic nephropathy
- 110 patients were screened; 68 refused or could not speak to our staff
- 42 patients were not eligible due to the lack of a 1st degree relative
- 33 patients were eligible but did not show interest and did not wish to be contacted for a number of reasons.
- 35 patients were eligible and interested, and provided contact information.
- Among the 35 patients, only 1 individual with his sister enrolled into the study. The remaining 34 failed to contact their 1st degree relative or their relative refused to participate or had logistic issues that kept them from participating.

The enrollment yield from this effort through the dialysis center of Fresenius Medical Care was quite low, considering a large potential participant pool. The factors for this low participation rate reflected similar reasons initially noted from patient recruitment effort through Dr. Arakaki's Clinic. The frequently voiced reasons are summarized below.

- First Degree relative; not keen on DNA research, living away from patient, too busy
- Participant having difficulty making time for the visit; preference for evaluation at the dialysis center, transportation
- Money not an incentive to participate
- Free examination and laboratory tests for patient and family not an incentive for participation

The recruitment experience in enrolling diabetic patients with ESRD and their family members into a clinical trial is of reportable interest. Despite a one time and less than one hour commitment, the barriers to participation are noteworthy among this patient population and may be of interest to others who are conducting clinical research activities in renal failure. We intend to examine the responses in more detail and write a descriptive paper for publication.

Despite the poor response from the Fresenius Medical Care Dialysis Center recruitment effort, additional patients were enrolled primarily through continued effort in Dr. Arakaki's Clinic. A total of 10 probands; 8 men and 2 women, and

14 1st degree relatives; 5 men and 9 women were studied. Among the family members, there was 1 mother, 2 fathers, 3 brothers and 8 sisters. All individuals seen for this study were Asian and Pacific Islanders.

The following table describes the clinical and metabolic characteristics of the family members.

	No DM (6)		IFG-IGT (2)		DM (6)	
Category	Average	Range	Average	Range	Average	Range
Age (yrs)	57.5	48-80	53.5	45-62	63.7	50-82
BMI (Kg/M2)	26.9	21-33.7	34.8	30-39.4	30.9	24-40
Wt (Kg)	72.4	62-90	92.5	67-118	75	50-95.7
A1c (%)	5.4	5.1-5.9	6.05	6.0-6.1	9.15	5.2-11.4
FBS (mg/dl)	74.7	67-78	108.5	104-113		
2 Hr BS (mg/dl)	110	69-137	134	111-158		
HTN Hx	2/6		1/2		4/6	
SysBP (mmHg)	122.7	112-134	140	136-144	134	107-159
DiaBP (mmHg)	74.3	70-82	82	71-93	74.2	68-80
Hyperlipidemia Hx	2/6		1/2		5/6	

Even though this characterization of a small number of individuals is far removed from statistical analysis, the comparison is informative and the profiles appear to reflect the traditional characteristics noted among relatives of diabetics. As noted above, family members without diabetes are leaner, have lower BS levels, and have less co-morbid conditions of HTN and hyperlipidemia than members with diabetes and pre-diabetes. This preliminary finding is encouraging for the similarity to well-characterized and larger population studies, and does not represent unique findings that may impact genomic associations. Still, more individuals are needed to show significant differences among the family members of patients with diabetic nephropathy. Analysis of additional collected data such as DM complications, lipid profiles, medication use, and the quality of life questionnaire would be informative and meaningful with more participants. To this consideration, the study team has come to the realization that recruiting participants with ESRD may not yield targeted enrollment goals. Thus, in the next funding period, individuals with diabetic nephropathy and chronic renal failure based on a serum creatinine level of >2.0 mg/dl (written in the protocol) will be targeted for recruitment and enrollment of family members. Examination of the Dr. Arakaki's database revealed 200 diabetic patients with nephropathy and approximately 50 individuals with a serum creatinine level greater than 2.0. mg/dl. Additional patient will be screened from Dr. Ramona Wong practice.

KEY RESEARCH ACCOMPLISHMENTS:

- Recruitment effort through a large dialysis center with assistance from collaborative nephrologist was initiated and completed.
- 10 probands and 14 family members were enrolled into the study.
- All individuals were characterized and data collected; quality of life questionnaire, anthropometric measurements; Michigan Neuropathy Sensory Index physical examination; clinical chemistry evaluation; and peripheral blood mononuclear cell DNA extracted and sent to the University of Pittsburgh.
- New direction in recruitment of diabetic patients with nephropathy and their family members are planned

REPORTABLE OUTCOMES: NONE

CONCLUSIONS:

This Genetic Screening in Diabetes: Candidate Gene Analysis for Diabetic Nephropathy project has been initiated. Although a small sample size has been examined to date, it is clear that the protocol can be readily implemented. The characterization of the participants reveal findings that are expected among the family members of this predominantly Asian and Pacific Islander population. Lessons have been learned from the experience in recruiting from the dialysis center and a new direction for increasing enrollment has been developed.

REFERENCES: Morbidity and Mortality Weekly Report 54(43); 1097-1100, 2005.

APPENDICES: NONE