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Research Article
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The Rhesus Monkey: A Nonhuman Primate Model For T2DM- Associated Gene Screening

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Abstract

To examine the genetic basis of the spontaneous development of Type 2 diabetes mellitus (T2DM), we have conducted an initial genome wide association study (GWAS) using Affymetrix 6.0 gene chip arrays on 8 normal (Controls), and 14 insulin resistant(IR)/prediabetic(preDM)/T2DM rhesus monkeys (*Macaca mulatta*). Both IR/preDM and T2DM were classified as cases as they could be clearly defined in this model as either in the early progression toward overt DM or already overtly diabetic. The successful call rate on all chip assays averaged 91.9% with high heterozygosity (42%), demonstrating the project applicability for genotyping the rhesus monkey using the human platform. Principal component analysis classified the groups accurately according to their source – i.e. case or control. Five SNPs (Chr 18, and 1 of the human and rhesus genomes) passed the tests for multiple comparison correction and were of complete homology between the rhesus monkey and human SNPs. We identified on our Affymetrix 6.0 array 9 out of the reported 18 SNPs associated with T2DM by prior GWAS in humans (candidate human genome sequence polymorphisms with T2DM), that were present on the 6.0 array. Eight were of the same genotype, and in one SNP representing the WFS1 gene all monkeys except for one control, carried the non-ancestral allele at this position. Resequencing of the 4 most significant SNPs in 51 additional NPH subjects revealed a SNP in the same location as in human that was of significantly higher prevalence in T2DM animals and two others that were at a different location then the homolog human SNPs and were not differently distributed among the NHPs T2D patients and controls. Expression levels (measured in heart, liver and muscle) of these four candidate genes demonstrated reduced expression of *CBLN2* compared to normal animals following adjustments. In addition, *CBLN2* SNPs were significantly associated with HOMA-IR in patients from a Finland and Sweden Type 2 diabetes GWAS. These data demonstrate the successful use of human SNP platforms to identify genetic variants associated with T2DM in rhesus monkeys.

Keywords: Rhesus monkey; GWAS; T2DM; CBLN2

Introduction

Type 2 diabetes mellitus (T2DM) currently affects 210 million people and is recognized as an epidemic in most developed countries. T2DM results from a combination of genetic susceptibility, environment, behavior (calorie intake and physical activity), and as yet unexplained risk factors [1]. Although the prevalence of T2DM rises with age, considerable individual and ethnic differences (controlled for economic status) in the age of onset and prevalence of T2DM in older people have been reported [2-5], suggesting that genetic factors may also influence the timing of onset of T2DM.

The genetic contributions to the increased risk of T2DM with age remain unclear [6]. Candidate gene and positional cloning efforts have revealed many putative susceptibility variants that can have differential effects on insulin secretion and action in different age groups. For instance, the TCF7L2 (Transcription factor 7-like 2) gene polymorphism has been associated with reduced hepatic insulin sensitivity in young human subjects, whereas it has been associated with peripheral increased insulin sensitivity in older human individuals [7]. This observation, aided by advances in genotyping technology, has led to genome-wide searches for common T2DM-associated variants [8-12]. However, it is still extremely difficult to map genes related to T2DM in humans, in part because the genetic effects are apparently significantly confounded by environmental factors such as dietary intake and life style, which greatly reduce the power for dissecting the genetic components of T2DM. The advantages provided by a constant well controlled environment and a shorter life span, have provided many insights into the human health and disease conditions through the study of nonhuman primates (NHP) [1]. In addition, the control of a stable and consistent environment should permit the identification of genetic loci responsible for early pre-disposition to T2DM (pre-diabetes (preDM)) prior to progression to overt T2DM, a period difficult to identify in humans, and a period highly represented in many human "control" groups.

The rhesus monkey (*Macaca mulatta*) provides a unique opportunity to search for and identify genetic variants that confer susceptibility to T2DM for the following reasons: a) the rhesus monkey and the human aligned genomic sequences are 90.76% similar [13]; b) For high-confidence orthologs there is 97.5% identity at both the

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nucleotide and amino acid levels; c) 89 percent of the rhesus monkey genes are syngenically aligned with the corresponding human genes; and d) an extraordinary amount of physiological similarity exists between rhesus monkeys and humans [13]. *Ad libitum* fed animals in a sedentary environment (despite a low fat high fiber diet) frequently develop obesity that is often followed in middle and old age by the onset of T2DM [1,14-17]. This type of central middle-age onset obesity is highly similar to humans [18-20]. Moreover, the rhesus monkey has been shown to develop spontaneous naturally-occurring T2DM and the pattern of insulin secretion and insulin resistance are highly similar [14,21-23]

Several fundamental discoveries that are relevant to human health have also been made using the rhesus monkey model. For example, the rapid periodicity of insulin secretion under basal conditions was first discovered in rhesus monkeys and later identified in humans. In addition, such a strategy (i.e. hybridization of rhesus monkey RNA to a human expression array) was successfully applied to create the DNA sequence for the rhesus gene chip [24,25]. These marked similarities [13], both genetic and phenotypic, led us to hypothesize that the rhesus would serve as an excellent model to discover and to study the potential role of genes associated with the development of T2DM.

Materials and Methods

Population

Monkeys: Fourteen rhesus monkeys (11 male, 3 female) with insulin resistance, impaired glucose tolerance (IGT) and T2DM (21.7 ± 5.5 years old) were defined by clinical features of T2DM/preDM (repeated fasting hyperglycemia ≥126 mg/dl; glucose intolerance by intravenous glucose tolerance test (IVGTT) with Kgluc ≤1.5) and compared to 8 healthy (3 male, 5 female) control monkeys (17 ± 3.6 years old) for the initial genome screening (Table 1). Second phase (1st phase cohorts were not included) analyses were performed on a cohort of 36 rhesus subjects (22.4 ± 4.6 years old) with preDM and T2DM (23 males, 13 females) compared to 15 similar aged (22.7 ± 6.8 years old) healthy control monkeys (7 males, 8 females) (Table 2). All monkeys were of Indian origin and all had been born in breeding colonies in the USA and had been continuously maintained on primate chow (with occasional fruits and treats as enrichment). The chow composition was by % calories 18.2% protein, 13.1% fat, and 68.7% carbohydrates, with negligible cholesterol (75 parts per million). The animals were single housed throughout adult life in order to assure *ad libitum* food intake. Among control and the T2DM/preDM monkeys each had one monkey over the age of 40 at the time of death, the diabetic monkey having received insulin for >10 years. Each had been characterized longitudinally for clinical traits including anthropometric measures, glucose tolerance and insulin secretion (Table 3a). All metabolic conditions were naturally occurring, and not experimentally induced. All protocols were reviewed and approved by the Institutional Animal Care and Use Committee.

Humans: For translation purposes we used 1449 patients with T2DM and 1482 controls matched for gender, age, body mass index, and region of origin (all from Finland and Sweden) [8], and each had been characterized for clinical traits such as, anthropometric measures, glucose tolerance and insulin secretion (Table 3b).

DNA preparation and genotyping

DNA of the NPH was prepared and subjected to genome wide scanning using the Affy. v6 Human microarrays**.** Briefly, each DNA sample was cleaved with restriction enzyme (NSP I and STY I), and the NSP I/STY I fragments were ligated with adaptors. Following a Poly Chain Reaction (PCR) to preferentially amplify smaller NSP I/STY I fragments, the products were end labeled. The use of small NSP I/STY I fragments allows for a 95% reduction in the complexity of the genomic DNA, providing efficient hybridization to the Affymetrix chip. Hybridization was followed by washing and staining with the Affymetrix GeneChip Fluidics Station 450, and scanning with the Affymetrix GeneChip Scanner 3000. Initial analysis of the raw data which involved calculation of signal intensities was performed with the Affymetrix GeneChip Operating Software (GCOS), and subsequent genotype determination across 906,000 SNPs was performed with the Affymetrix Genotyping Console 3.0.1. The Affymetrix Birdseed algorithm provided indications of the Signal Detection Rate and SNP Call Rate for every chip, which are reliable indicators of the success of sample processing, avoidance of contamination, efficiency of hybridization, and performance of the scanning.

Quality control

Samples were prepared in parallel with a process control (genomic DNA from supplier). The SNP Call Rate of 91% that passed the confidence test indicated adherence to protocol and the purity of the

N	Age	Wt. (Kg)	Sex F/M	Met Status	Diabetes Duration (Y)
8	23.4 ±6.2	15 ±3.5	0/8	DM	6.1 ±2.6
6	19.4 ±3.7	12.7 ±3.1	3/3	IR/PreDM	None
8	17 ±3.6	9.5 ±1.6	5/3	Normal	None

Table 1: Group characterizations for initial GWAS screening in rhesus.

N	Age	Wt. (Kg)	Sex F/M	Met Status	Diabetes Duration (Y)
18	23.7 ±4.8	13.2 ±4	6/12	DM	5.6 ±2.6
18	20.7 ±3.9	14.9 ±4.1	7/11	IR/PreDM	None
15	22.7 ±6.8	9.5 ±2.2	7/8	Normal	None

Table 2: Group characterization for validation and gene expression in rhesus.

	Controls		Type 2 diabetics		All	
Variable	N	Mean (SD)	N	Mean (SD)	N	**Mean (SD)**
N (M / F)	11/12		34/16		45/28	
Age (yrs)	23	22.2(6.82)	50	22.6(4.74)	73	22.4(5.47)
Weight (kg)	23	9.74(2.03)	50	14.1(4.17)	73	12.6(4.14)
Fast. glucose (mmol/l)	23	62.5(8.94)	50	179(115)	73	123(101)
Fast. insulin (µU/l)	23	65.6(38.2)	50	69(47)	73	67.5(42.7)
HOMA-IR	23	0.04(0.17)	50	1.78(2.91)	73	0.93(2.26)
HOMA-β	23	5.34(6.9)	50	9.8(18)	73	7.62(14.2)

Table 3a: Characteristics of the rhesus group.

	Controls		Type 2 diabetics		All	
Variable	N	Mean (SD)	N	Mean (SD)	N	**Mean (SD)**
N (M / F)	707 / 760		742 / 722		1449 / 1482	
Age (yrs)	1467	58.8 (10.1)	1464	64.5 (10.2)	2931	**61.6 (10.5)**
BMI (kg/m2)	1452	26.7 (3.7)	1450	28.6 (4.5)	2902	**27.6 (4.2)**
Fast. glucose (mmol/l)	1346	5.3 (0.6)	1341	9.3 (3.6)	2687	**7.3 (3.2)**
Fast. insulin (µU/l)	1421	5.8 (4.8)	1371	15.2 (48.2)	2792	**10.4 (34.2)**
HOMA-IR	1302	1.36 (1.26)	1261	5.25 (8.74)	2563	**3.27 (6.50)**
HOMA-β	1301	68.2 (64.1)	1261	32.1 (295.9)	2562	**50.4 (213.3)**

Table 3b: Characteristics of Human study populations, data extracted from the Finland and Sweden study.

DNA sample. In addition we applied three steps of quality control (QC). A. Following the genomic sheering, adaptor ligation and PCR amplification (assays were based on the adaptors) we ran a agarose gel to test if the DNA products smear distributed as suggested by the manufacture (molecular weight 200-1100 bp). B. Product concentration was measured using the Nanodrop 1000 Spectrophotometer (Thermo Scientific, Waltham, MA). Those samples which yielded more than 200µg continued to the next stage of the protocol, Fragmentation and Labeling. C. After Fragmentation with DNAse I, 1.5µl of each sample was run on a 4% TBE agarose gel to confirm successful amplification of the expected molecular weight, <180 bp. After hybridization and scanning of the gene chip, the image was checked for the correct pattern of control oligos. The image was processed and QC metrics assessed. The Contrast QC was required to be greater than 0.4 for any single chip and the average Contrast QC for the batch was required to be greater than 1.7. Oligonucleotide controls (implemented by the manufacturer in each DNA chip) helped to evaluate hybridization, fluidics and scanning steps by comparing to standards recommended by Affymetrix.

Tissue collection

The RNA is obtained from the vastus lateralis muscle and the fat was from the abdominal subcutaneous area. All tissues were immediately flash frozen and stored at -80°. All samples were obtained after an overnight fast between 8 and 10 am. The light dark cycle of 12 hours on/off was constant throughout the year, and constant temperature and humidity were maintained. Thus, no circadian or seasonal effects would be expected. All samples were obtained ante mortem under anesthesia for both biopsy and necropsy samples.

Total RNA isolation and cDNA synthesis

RNA was isolated using a commercially available reagent, TRIzol (Invitrogen), following the instructions of the manufacturer. For synthesis of single strand cDNA, we employed materials provided in the kit Superscript III (Invitrogen). Briefly, 10 µl containing 5 µg of total RNA, 0.5 µg of oligo-dT primer, and 1 µl of dNTPs (deoxynucleotide triphosphate mix 10 mM each) were heated at 65°C for 10 min. Samples were then chilled on ice, and a 9 µl mixture containing the following components was added: 2 µl of 10xRT buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl), 4 µl of 25 mM $MgCl_2$, 2 µl of 0.1 M DTT, 1 µl of recombinant RNAse inhibitor. The samples were gently mixed and collected by a brief centrifugation. After incubation at 42°C for 2 min, 1 µl of Superscript III reverse transcriptase was added, and samples were mixed and incubated at 42°C for 50 min. The reactions were terminated by chilling on ice and template RNA was removed by adding 1 µl of RNAse H to each sample and incubating at 37°C for 20min.

Quantitative Real Time-Poly Chain Reaction (qRT-PCR)

Quantitative real-time PCR experiments used the LightCycler 480 systems (Roche), including a PCR instrument with capacity for 96 and 384 simultaneous samples. All primers were synthesized by Invitrogen and were designed with *PRIMER 3* software (http://frodo.wi.mit.edu/primer3/input.htm). The default parameters of the program were: product size 150-600, primer size 18-25, Tm 55-62 with max difference of 2°C. Within the proposed optimal primers, those spanning introns were selected (Table 4). The mRNA copy number for each unknown sample was calculated from the standard curve by the instrument software. For each standard curve point (we used at least 5 standards to minimize errors), the LightCycler software determined a threshold cycle (Ct), which represented the lowest number of cycles necessary to measure a fluorescent signal statistically significantly above background. Samples were run in duplicate. The Ct was calculated using an arithmetic Fit Points Method provided by the LightCycler software, which first calculates the five lowest measured data points from each sample, then subtracts it from each data point. Since the Ct will occur always during the exponential phase of amplification, the only limiting component of the reaction is the template. After building a standard curve for each PCR product, we can express the concentration of unknown samples in absolute copy numbers (done automatically by the software) or in copy numbers calculated after normalization against a housekeeping gene (e.g., GAPDH).

Principal component analysis

Principal Component Analysis (PCA) was performed using JMP software (JMP Genomics Version 5. SAS Institute Inc., Cary, NC).

Set 1-Primer assay for SNP conformation

Gene	Forward	Reverse	Product length	Tm
CBLN2	TGTCTTTTCCAAGGGGATGA	TTTGATGCATAACAGGCAACT	178	60
TTLL7	TGTAAGGTGGAGGCTTAGAGAAA	ACACTCATGCACACACACACA	310	60
WDR40C (DCAF12L2)	GTGGGAAGCAGTGGTAGGG	AGCCCCAAAGTGGGTTTTAC	226	60
ATP2B3	AAGTGGTTGAGTGGGTGTCC	AGAAGCCCCAAATGGACTTT	280	60
RAB39B	CAAGGTCATTTTGGGGTGAG	GGACAAAGGTAAAGCCCATTC	205	60

Set 2-Primer assay for qRT-PCR

Gene	Forward	Reverse	Product length	Tm
CBLN2	GCAAGTGCTTGGTGGTGTG	AAATCCCTTTTCTCGGTGCT	243	60
TTLL7	ATGATGGACTTGTGCGAATG	ACGTTTGCTGCCTTTGTTCT	152	60
WDR40C (DCAF12L2)	CTGCGATGAGTTGTCCCTCT	GGCTCTCTCCTCCAGGAACT	220	60
ATP2B3	TGACCTCACCTGCATAGCTG	GGTTGAACTCCTTCCCTTCC	206	60

Set 3-Primer assay for coding variants

Gene	Forward	Reverse	Product length	Tm
CBLN2				
Exon 1	AACGACCCTGGCCTTGAC	GAACTCCACGTCCAAGAAGC	596	60
Exon 2	ACTGAGGAGGGGCTTGCTAT	CCAAACCTGAAAGGATCCAG	205	60
Exon 3	TGCTGAGCTCTACTCTCTGCAT	CCACCATCTAGGGTGCTCTG	248	60

Table 4: Primer assays for 5 candidate genes in rhesus.

Resequencing

PCR for DNA resequencing has been applied using the primer assays (Table 4) and the standard protocol. In a 5µl reaction, 25µl of AmpliTaq Gold PCR Master Mix (Applied Biosystems), 2 µl of primer assay (1µl of 10pM/µl each), 1µl DNA (50ng/µl) and 22 µl of double distilled water were added. The rhesus DNA samples were then subjected to a PCR reaction of 10min 95°C follow by 35 cycles of 30 sec. 95°C, 30 sec. 60°C and 30 sec. 72°C the reaction ended with 7 min. 72°C. Product was than submitted to resequencing both forward and reverse, using the ABI 3730 system according to the manufacture's protocol.

Results

We conducted an initial genome wide association study (GWAS) of 14 T2DM/PreDM monkeys (11 males, 3 females) with an average age of 21.7 ± 5.5 years compared to 8 healthy controls (3 males, 5 females) with an average age of 17 ± 3.6 years (Table 1) using the Affy v6 gene chip array.

Affymetrix 6.0 Human genome-wide platform has 906600 available SNPs. Only SNPs that were present across 19 or more rhesus subjects of the initial genome screened rhesus population (321,292 SNPs) have been used for further analysis, 42% percent of which were heterozygous. Of the remainder after the first filtration, 185,215 SNPs (58%) were not polymorphic and only 131,740 (41%) followed the Hardy-Weinberg equilibrium (HWE) rule, of which 42,679 (13.3%) and 52,040 (16.2%) passed the significance threshold ($p<0.05$) for allele and genotype frequency respectively. The successful call rate on all chip arrays averaged 91.9% with high heterozygosity 42%, confirming the applicability of genotyping the rhesus monkey using the human platform. Manhattan plots of all SNP significant differences between cases and control are presented in (Figure 1). Neither gene distribution nor allele distribution passed the multiple comparison Bonferroni correction for significance. Thus, we set the barrier threshold at 10^{-6} allowing one false positive per million parallel tests. Q-Q plot analysis confirmed the normal distribution of our result demonstrated by the Manhattan plot shown in (Figure 3).

To explore the possibility that genomic information can discriminate the two groups we then applied principal component analysis (PCA) to classify the groups accurately according to their source – i.e. case or control. This classification was based solely on the results obtained using the human SNP variants within the two groups of rhesus (Figure 2). Comparison of the healthy control group to the combined insulin resistant (preDM) plus the T2DM group, resulted in five SNPs (genes *CBLN2, TTLL7, WDR40C, ATP2B3, RAB39B*) that passed the tests for multiple comparison correction, and these were located on chromosomes 18, 1 and X of the human genome (Table 5). Although the results were adjusted for sex, the X chromosome results should be interpreted with caution at the present time as the result may have been overestimated due to an imbalanced sex distribution among the groups. We then applied BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) on the highly significant sequences (the resulting probes from our comparison i.e. cases vs. controls) from the human chip (Affy. v6) to the rhesus reference sequence (Table 5). The length of the blast sequence was a couple of hundred bp. Interestingly, there was complete homology between the rhesus and human SNPs, which validated our approach to finding genes associated with diabetes in rhesus monkeys using the human Affy. v6 platform. For further confirmation we used the entire cohort (i.e. 73 samples) using the assay of primer set 1 (Table 4a). These results support the hypothesis that more genetic variance can be detected among the extreme phenotypes [26].

To establish the translation of human to rhesus and vice versa, we identified on our array 9 out of the reported 18 SNPs associated with T2DM by prior GWAS in humans (Table 6). Interestingly, all of the animals had a defined genotype, although non polymorphic (all but one were homozygote), one SNP, representing the WFS1 gene, was heterozygous only in one of the control monkeys. Resequencing of the four most significant SNP regions in the 73 rhesus subject cohort demonstrated completely conserved sequences in two SNP regions (compared to the human sequences). One sequence demonstrated a SNP in the same location as in humans and one SNP clearly defined the T2DM animals and controls. These results establish our ability to detect DNA variants using the human genotyping platform applied to a rhesus model in order to discover T2DM associated SNPs.

We have then determined the association of one of our candidate genes (i.e. *CBLN2)* with clinical characteristics of diabetes in a patient

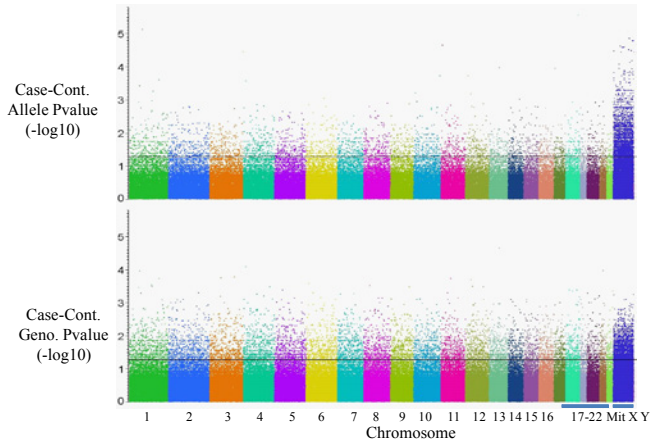


Figure 1: Manhattan plot of p values (-log) of allele/genotype frequency differences following case-control analysis in rhesus.

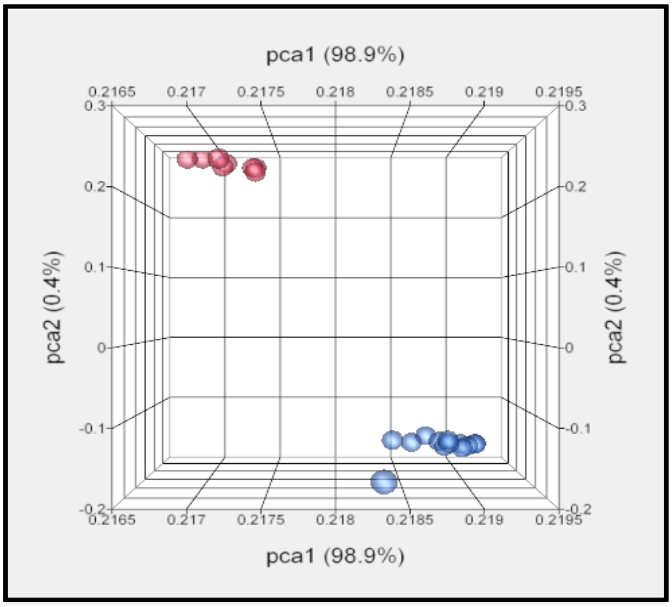


Figure 2: Principal component analysis among the rhesus cohort classified the two original groups (Normal Vs. IR and T2DM) into two distinct separate groups adjusted for gender.

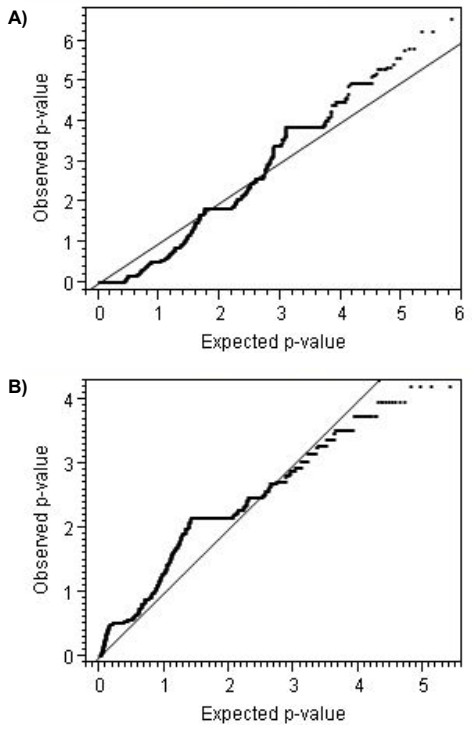
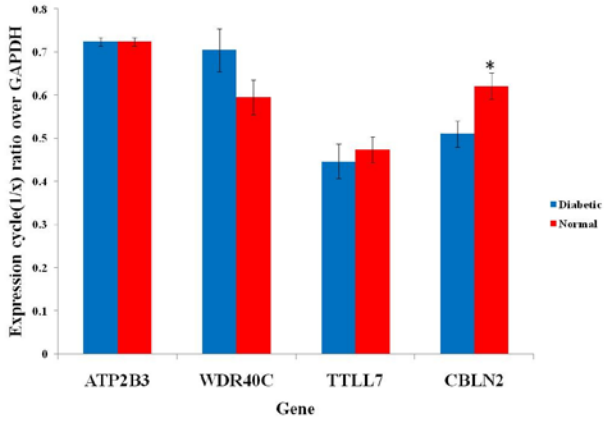


Figure 3: Q-Q plot of observed vs. expected p-value of the allele (A)/genotype (B) frequency following Case-Control (rhesus sample) analysis. Even distribution across the expected line represents normal distribution.



*p value<0.05 compare to normal

Figure 4: Candidate gene expression in rhesus adjusted for gender and tissues and normalized by GAPDH. The results are presented as a ratio between the experimental gene and the GAPDH.

sample from Finland and Sweden that had been previously analyzed for GWAS for diabetes [8]. In the *CBLN2* one SNP was significantly associated with and 3 were borderline significantly associated with insulin resistance defined by HOMA (Table 7). None was associated with diabetes although one SNP (rs608970) in the *CBLN2* had a p value =0.11 (n.s.).

Expression levels of four candidate genes (genes *CBLN2, TTLL7, WDR40C, ATP2B3*) were then measured in 51 rhesus subjects (Table 2). Total RNA was extracted from the liver (18 rhesus samples), heart (5 rhesus samples) and skeletal muscle (28 rhesus samples), converted to cDNA, and subjected to the qRT-PCR using *GAPDH* as an internal control for normalization (primer assays are listed in Table 4b). The T2DM rhesus monkeys demonstrated a significant reduction of the *CBLN2* mRNA expression (precursor for CER protein which controls plasma insulin levels [27]) compared to normal animals following adjustments for tissue and gender (Figure 4). None of the other three tested candidate genes (*TTLL7, WDR40C, and ATP2B3*) demonstrated significant differential expression.

We extended our efforts to find functional variants that defined the two populations (case and control) in the *CBLN2* gene (Table 4c). A couple of variants have been detected, however, only one demonstrated a significantly higher prevalence among cases.

Discussion

Recently GWAS has been highly criticized due to the fact that despite countless genome-wide association studies, most of the genetic variance in risk for most common diseases remains undiscovered. For example, only a few have identified causative variants, and in aggregate, these common variants likely explain <10% of the genetic variance of T2DM regardless of the application of GWAS to groups containing tens of thousands of cases and controls [28].

Based upon the apparent limitations of human GWAS, we hypothesized that an analysis of a more defined and environmentally controlled population that develops insulin resistance/preDM and T2DM may provide a more tractable approach to identifying genetic susceptibility loci. In this regard, analysis of gene expression in rhesus monkeys has been successfully carried out using human expression arrays [29-32]. Although the rhesus monkey whole genome SNP array is not yet available (a rhesus array is available only for transcription not for SNPs), our data indicated that the Affy. V6 can be successfully used due to the high degree of similarity between the human and rhesus monkey genomes.

Using this approach we have identified a new gene that is a part of a gene family that serves as a precursor for a protein expressed in the pancreas and that controls plasma insulin levels [27].

The results reported here are of *CBLN2* and not the other 3 gene family members (*CBLN1, CBLN3* and *CBLN4*, which are expressed in the central nervous system (CNS) and in peripheral tissues [27]). *CBLN1* and *CBLN3* are expressed in the rat endocrine pancreas [27]. This family of genes acts together and therefore we believe that this family member, that we have recognized and that was demonstrated to be differentially expressed between T2DM and healthy controls, has the same effect (controls plasma insulin levels) in monkeys and is likely to translate to humans as this has been demonstrated with the patient samples from Finland and Sweden.

The small sample size of the initial genome screening, suffered from lack of power to detect genome wide significance (approximately 5%). However, the present report is an initial study that shows the successful translation of the human genome array to rhesus. Importantly, despite the lack of power, we were able to identify a gene that may have an impact in diabetes susceptibility and is plausibly related to diabetes. In addition the expression analysis has been done on a much larger cohort that emphasizes the robustness of the study despite the lack of power. This is probably due to the strict and constant environmental conditions and the accurate definition and detailed longitudinal phenotyping of the animals.

Full name	Human (Hg18)			rhesus				
	Chr.	location	SNP	Chr.	SNP	Normal (%)	T2DM(%)	P value
Cerebellin 2 precursor (CBLN2)	18	67153509	A/G	18	T/C	0(T)	100(T)	2.73E-06
Tubulin tyrosine ligase-like family, member 7 (TTLL7)	1	84278365	C/T	1	A/G	12(A)	82(A)	7.25E-06
WD repeat domain 40C (WDR40C)	X	125363403	A/G	X	T/C	36(T)	96(T)	1.33E-05
ATPase, Ca++ transporting, plasma membrane 3 (ATP2B3)	X	152458554	C/T	X	G/A	12(G)	81(G)	1.58E-05
RAB39B, member RAS oncogene family (RAB39B)	X	154126766	C/T	X	C/T	12(T)	92(T)	1.61E-05

Table 5: Gene homology between Human and rhesus (Normal vs. IR/T2DM).

dbSNP_RS_ID	Gene	Chromosome	Physical Position	Genotype
rs4402960	IGF2BP2	3	187000000	GT
rs7754840	CDKAL1	6	20769229	CG
rs10811661	CDKN2B	9	22124094	CC
rs8050136	FTO	16	52373776	AA
rs1801282	PPARG	3	12368125	CC
rs10012946	WFS1	4	6344251	CC(1CT)
rs1326663	SLC30A8	10	122200000	AG
rs1111875	HHEX	10	94452862	CT
rs4411878	ADAMTS9	3	64678705	AG

Table 6: Human T2DM GWAS candidate genes and their genotype among the rhesus monkey.

CHR	POS build 36	SNP	MAF	P (T2D)	P (HOMA-IR)	Monkey GWAS
18	68326429	rs11151771	0.2038	0.1508	0.5721	
18	68344464	rs1432074	0.07419	0.7027	0.4973	
18	68355046	rs2298726	0.06355	0.8076	0.05348	0.03256
18	68358320	rs658995	0.012	0.1958	0.003404	-
18	68369906	rs609782	0.07579	0.4929	0.6201	
18	68370063	rs608970	0.1486	0.111	0.05663	
18	68375431	rs17086171	0.1391	0.2141	0.2413	
18	68390056	rs4892015	0.1496	0.1591	0.06025	0.080512

Table 7: CBLN2 association with T2DM characteristics in GWAS of Human patients in Finland and Sweden.

In summary, we have demonstrated that GWAS analysis using the human Affy. V6 chip can be successfully used to identify potential candidate genetic loci that may contribute to the genetic susceptibility of insulin resistance/preDM and T2DM. Surprisingly, this was accomplished by genome screening of a very small sample size and has led to the identification of *CBLN2*, a functional gene with reported expression in pancreatic islets. Although these data now provide a proof-of-principle, future studies are needed to establish the relevance of the discovered gene in the pathophysiology of diabetes.

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