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Emergence of TCF7L2 as a Most Promising Gene in Predisposition of Diabetes Type II

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"*Subject never gives you anything, you have to extract from it, and thus making it alive again.*" Udai G

KEYWORDS T2D (type 2 diabetes); BMI (body mass index; LD (linkage disequilibrium); SNP (single nucleotide polymorphism); WNT pathway

ABSTRACT The genetics of the complex disorder like Diabetes Type II, which is clinically diagnosed as disease of insulin resistance and impaired insulin secretion leading to impaired glucose homeostasis in body, remains a nightmare for geneticists. But the recent progress in identification of a most promising marker in predisposition of diabetes Type II, namely, TCF7L2 with its large effect size and its global presence in various ethnically and geographically different populations offers some hope as the robust genetic approach like genome-wide association studies seem to corroborate the evidence in favour of association of this gene with predisposition to the disease. This paper presents a comprehensive review of studies on the association of this gene with type II diabetes.

INTRODUCTION

Diabetes type II (T2D) is a non-autoimmune, complex, heterogeneous and polygenic metabolic disease condition in which body fails to produce enough insulin. Diabetes type II is characterized by abnormal glucose homeostasis, and its pathogenesis appears to involve complex interactions between genetic and environmental factors. There are two hypotheses regarding the pathophysiology of T2D. According to one hypothesis the primary defect is represented by insulin resistance, which is already present at very early stage of the prediabetic state. While initially the beta cells are able to compensate for this resistance, overt diabetes occurs when the beta cells become exhausted. The alternative hypothesis proposes that the primary defect in T2D is due to mild dysregulation in insulin secretory mechanisms that leads to overt diabetes following the secondary superimposition of insulin resistance (Korc 2003). Therefore, T2D (formerly known as adult onset diabetes) occurs when impaired insulin effectiveness (insulin resistance) is accompanied by the failure to produce sufficient β cell insulin. The burden of diabetes is to a large extent the consequence of macrovascular and microvascular complications of the disease (Permutt et al. 2005). The peripheral symptoms of diabetes type 2 include variable inability of the liver to properly suppress hepatic glucose release and, production of adipose tissue derived hormones and cytokines that antagonize insulin action.

GENETICS OF DIABETES TYPE II

The genetic status of T2D is appreciated by twin, family and admixture studies, which suggest that the risk varies widely across populations, from 5% or less in White and Asian populations to 50% or more among Pima Indians and South sea Island populations (Elbein et al. 2002). The genetic differences in disease predisposition in different ethnic groups have also been demonstrated by some studies in admixed populations (Barroso 2005). Lifetime concordance rates among identical twins approach 100%. Concordance rate is found to increase with the duration of follow up. Most conservative estimates place long term concordance at about 60%, which is at least double to that of dizygotic twins. Such

figures are although consistent with a hypothesis of major gene effect, yet no such gene was identified (Elbein et al. 2002). The familial aggregation of the disease is evidence for genetic contribution to disease. These studies compared the disease prevalence within family members of a proband to that expected in the general population, a higher prevalence in the family members is probed because of higher proportion of shared genes expected among the family members (Barroso 2005).

Despite considerable evidence for a strong genetic component in T2D, the high prevalence of this disease in the general population suggests that susceptibility genes may be common, of low penetrance, and thus challenging to identify (Elbein et al. 2002). The pertinent question is whether genetic influences are exerted by few "major genes", each with relatively large effect, or by a large number of "polygenes", each with relatively minor effect (Stern 2000). Two models have been proposed for understanding the allelic architecture of complex disorders like diabetes type II: The first, Common Disease/ Common Variant (CD/CV) hypothesis, which predicts that the genetic risk for common diseases will often be due to disease-predisposing alleles with relatively high frequencies – that is, there will be one or a few predominating disease alleles at each of the major underlying disease loci. There is currently not enough empirical evidence to either prove or disprove this hypothesis. There are a few stereotyped examples of such common variants like APOE allele in Alzheimer's disease, Factor V^{Leiden} in deep venous thrombosis, and PPAR Pro12Ala in type II diabetes (Reich et al. 2001). Theoretical rationality behind this hypothesis is based on several reasons. "First, common variants tend to be of high frequency in all populations, facilitating cross-population comparisons. Second, LD around common alleles represents a 'worst case' scenario; LD around rare alleles is expected to extend further because such alleles are generally young and there has been less historical opportunity for recombination to break down ancestral haplotypes. Third, LD around common alleles can be measured with a modest sample size of 80-100 chromosomes to a precision within 10-20% of the asymptotic limit. Last, LD around common alleles will probably be particularly relevant to the search for genes predisposing to common disease" (Reich et al. 2001).

The second model i.e. "Mosaic Model" (proposed by Diabetes Unit, Institute of Genomics and Integrative Biology, New Delhi), suggests that a small number of common variants and a large number of rare variants interact to form the allelic architecture of diabetes type II; this model is criticized because of the overemphasis of the importance of rare variants by its supporters on the basis of heterogeneous data. Due to the complex polygenetic nature of this disorder, many genes are thought to be involved. "Diabetes genes" may show only a subtle variation in the gene sequence, and these variations may be extremely common. The difficulty lies in linking such common gene variations, known as polymorphisms, with an increased risk of developing diabetes. Nevertheless, the identification of diabetes genes would have huge impact on disease management. In the long run, it may be more beneficial to develop treatments based on these genetic mechanisms than to rely on the use of will power to modify lifestyle (Permutt et al. 2005).

Biochemistry of TCF7L2

Transcription factor 7-like 2 also known as TCF-4, is a nuclear receptor for CTNNBI (=β catenin), which in turn mediates the canonical WNT signaling pathway. WNTs are ligands secreted by different cells; about 19 WNTs haven been identified illustrating the complexity of this signaling pathway (Smith 2007), which is one of the key developmental and growth regulatory mechanisms of the cell (Vliet-Ostaptchou et al. 2006). WNT signaling is critical for glucagon-like peptide-1 (GLP-1) secretion by the intestinal endocrine L-cells. Thus, alteration in this pathway could lead to reduced secretion of GLP-1 which, in turn, could have effect on both insulin secretion after a meal and the generation of new β cells from the ductal precursor cells. WNT pathway is important for:

1. Normal embryogenesis and cell proliferation,
2. Causation of several cancers,
3. Normal self renewal of stem cells,
4. Regulating myogenesis and adipogenesis and adipose cell differentiation and
5. Normal development of pancreas

Unless WNT signaling is inhibited, committed preadipocytes will not differentiate into mature adipose cells. The potential increase in WNT signaling in carriers of TCF7L2 risk variants could be expected to influence adipose tissue growth

and development and, thus BMI (Smith 2007). TCF7L2 might play some role in regulation of adipogenesis by altering transcriptional regulation of the genes encoding CCAAT/enhancer-binding protein-á (CEBPA) and PPARG (Cauchi et al. 2006). Recent work suggests that the Calpain system is involved in the constitutive regulation of β-catenin signaling functions, which raises the hypothesis of a potential interaction between TCF7L2 and Calpain-10 (Benneti et al. 2005). It has been shown that TCF7L2 is involved in the activation of mRNA expression of the proglucagon and the glucagon-like peptide-1 genes in gut endocrine cells. Glucagon-like peptide-1, produced in the gut and brain, lowers blood glucose levels through (Yi et al. 2005):

1. Stimulation of insulin secretion and biosynthesis,
2. Inhibition of glucagon release and gastric emptying,
3. Enhancement of peripheral insulin sensitivity, and
4. Induction of satiety.

The studies from Lyssenko et al. (2007) confirm earlier findings by establishing that the predisposition to type-2 diabetes is the result of reduced insulin secretion rather than reduced insulin action, making the pancreatic β cell the most likely primary cell target of altered TCF7L2 activity. However, this was contrary to an initial, much repeated**,** hypothesis suggesting that the genotype at risk was altering insulin secretion indirectly by reducing intestinal TCF7L2 activity, which in turn reduced the secretion, of incretins, glucagon intestinal peptide (GIP), and GLP-1 (Grant et al. 2006). They also show that insulin secretion in the subjects bearing at-risk genotype was reduced in response to a variety of stimuli including intra venous (i.v.) glucose and arginine and not just oral glucose. In addition, GIP levels were not reduced, suggesting that even though GLP-1 levels were not measured, there was a reduced β cell response to incretin secretion, rather than reduced incretin secretion. WNT signaling has recently been shown to regulate pancreatic β cell proliferation. Addition of WNT3a to cultured b cells and conditional overexpression of β-catenin led to increased β cell proliferation in vitro. Since interaction between TCF7L2 and β-catenin is a prerequisite for transcription of target genes, it will be important to examine whether TCF7L2 also affects β cell proliferation (Lyssenko et al. 2007).

Review of TCF7L2 Association Studies

TCF7L2 had not previously been implicated in type 2 diabetes and was not an obvious diabetes gene. A PubMed search for the keywords "TCF7L2" or "TCF4" reveals 218 articles but none of that shared the term "diabetes" before the Grant et al. (2006) publication (Saxena et al. 2006). Grant et al. (2006) previously reported a suggestive evidence of linkage to 10q. They genotyped high density microsatellite markers (i.e. 228 markers, to an average density of one marker every 46 kb) across 10.5 Mb region in Icelandic population (1185 cases and 931 controls). They identified association of DG10S478 marker (tetranucleotide repeat having six alleles) with T2D. When they genotyped this in HapMap samples, it became clear that allele G of SNP rs12255372 is nearly perfectly correlated with allele 0 of DG10S478; and allele T of rs12255372 with other alleles of DG10S478. The phylogenetic analysis of haplotype variation within LD block where DG10S478 resides demonstrates that all nonzero alleles of DG10S478 and rs12255372 T belong to a single monophylectic lineage. Hence, it is reasonable for them to collapse all nonzero alleles of DG10S478 into one composite allele X.

DG10S478 is located in intron 3 of TCF7L2 gene on chromosome 10q25.2. This marker is within a well defined LD block of 92.1 kb that encompasses part of intron 3, the whole of exon 4 and part of intron 4; no other known gene resides within this LD block. They sequenced exon 4 of TCF7L2 in a subset of Icelandic cohort (cases/controls: 331/320), Danish cohort (cases/controls: 282/449), and US cohort (cases/controls: 226/210). In addition they screened all other TCF7L2 exons in Icelandic population (cases/controls: 93/91) and no variation was found. To further investigate the possibility of association of other marker alleles in LD block demonstrating a higher correlation with T2D than allele X, they used HapMap samples. The five SNPs with strongest correlation to DG10S478 in HapMap samples are rs12255372; rs7903146; rs7901695; rs11196205; rs7895340. They genotyped these snips in three cohorts and found similar results. All five SNPs also showed association with T2D. The strength of association for rs12255372 (relative risk-1.52), rs7903146 (relative risk-1.54) with T2D was higher than other SNPs, and comparable to each other. Hence they also recommend that these two SNPs

be included in any replication effort. The mode of inheritance suggested by them is multiplicative model. They have shown that heterozygous carriers and homozygous carriers have relative risk of 1.45 and 2.41, respectively, compared with non-carriers. They suggested the corresponding population attributable risk (PAR) of 21 % for TCF7L2 gene. In summary, they identified previously unknown candidate gene, TCF7L2, for T2D within previously reported linkage region 10q. They observed association of composite at-risk allele of microsatellite marker DG10S478 within intro 3 of TCF7L2 gene to T2D in Iceland, which was subsequently also found in Denmark and US with similar frequency and relative risk.

Enthusiasm generated by Grant et al. (2006) lead other researchers working on Diabetes Type 2 to explore this gene region with the help of tag SNPs in different European and US populations. The salient features of these studies and their findings are summarized in Table 1.

Majority of these studies (mentioned in Table1) are based on association approach followed by case-control research design, and/

Table 1: Outline of different association studies and salient features of the findings

S. No.	*Samples and Population*	*Aim of the study and SNPs explored*	*Design*	*Salient features of the findings*	*Source*
1.	8310 individuals from Scandinavia, Poland, and the US.	TCF7L2 might influence insulin secretion for T2D patients. And 13 tag SNPs that capture 32 of 44 TCF7L2 variants.	Association study. Family based case-control design.	rs7903146 was most significantly (OR .40)associated with T2D.TCF7L2 may act through insulin secretion to increase T2D risk.	Saxena et al. 2006
2.	Case/control (=2158/ 2574) and388 parent-offspring trios from U.K.	Aim of the study was replication on different population. And Four TCF7L2 SNPs (rs4506565, rs12243326, rs12255372, and rs7903146)	Association study. Both Case/ Control, and family based designs	All four SNPs were significantly associated with T2D. SNP rs7903146 showed strongest association. With Relative risk of 1.36. No evidence of mutation within the coding regions.Population attributable Risk (PAR) is 16%(approx).	Groves et al. (2006)
3.	1. cases/control: 697/ 10702^2. cases/ control:920/922of U.S. Population.	Aim of the study was replication on two well established cohorts. Meta-analysis of five populations. And Genotyped rs12255372 SNP of TCF7L2 gene.	Association study. Case-control cohort design. Limitation: Some controls were not checked for diabetes.	Strong association of rs12255372 SNP (OR-1.86; 2.15 for women and men respectively) with an increased risk of T2D.They could not determine whether this SNP is a causative variant or a surrogate for an underlying causal variant.PAR for rs12255372 18.7%.Meta-analysis suggests 1.48 fold increase risk for cases.	Zhang et al. (2006)

Table 1: Contd....

S. No.	*Samples and Population*	*Aim of the study and SNPs explored*	*Design*	*Salient features of the findings*	*Source*
4.	256 non diabetic female subjects fromEuropean American and African American populations.	Aim was whether variation in the TCF7L2 gene was associated with insulin resistance, impaired insulin secretion. And SNPs rs12255372 and rs7903146 of the TCF7L2 gene.	Association study. General population based design	Demonstrated first time that SNP rs12255372 in the TCF7L2 gene was significantly associated with beta-cell function. Suggested TCF7L2 gene important factor for regulating insulin secretion.	Munoz et al. (2006)
5.	944 subjects representing general population and127 diabetic cases of older people from Italy (rather than selected cases and controls)	Aim was to Comparison of general population with diabetes patients. And Genotyped rs7903146 (C/T) polymorphism.	Association study	In general population sample, shows significant trend towards higher fasting glucose levels with increasing number of T alleles. In group of patients with diabetes and IFG, T allele carriers were less likely to have metabolic syndrome features.	Melzer et al. (2006)
6.	Amish subjects WithT2D (n=137); With impaired glucose tolerance (n=139); and With normal glucose tolerance (n=342).Non Amish subjects (n=48)	Aim of the study was replication. And Genotyped four SNPs (rs7901695, rs7903146, rs11196205, rs12255372) and DG10S478.	Association study. Case-control design	Demonstrated first time a role for *TCF7L2* in regulating both insulin sensitivity and glucose-stimulated insulin release. rs7903146 showed strongest association with OR matching with original study.	Damcott et al. (2006)
7.	2,367 French T2D and 2,499 control subjects.And 1,346 Nonobese T2D subjects.	Aim was Replication and to understand the regulation of TCF7L2. And Two SNPs rs7903146 and rs12255372 were genotyped.	Association and Linkage Study. Case control design and Familial transmission disequilibrium test also performed.	Risk allele of rs7903146 and rs12255372 significantly increased T2D risk with allelic OR of 1.69 and 1.60 respectively.Non-obese T2D subjects increased the allelic OR to 1.89 and 1.79, respectively. Thus suggest interaction between BMI and TCF7L2. TDT determined that TCF7L2 gene	Cauchi et al. (2006)

Table 1: Contd....

S. No.	*Samples and Population*	*Aim of the study and SNPs explored*	*Design*	*Salient features of the findings*	*Source*
				variant does not explain the linkage signal by testing the correlation between familial at-risk allele frequency and familial linkage score at different locations.	
8.	3548 subjects with impaired glucose tolerance from U.S.	Aim was to predict the progression to diabetes in persons with impaired glucose tolerance (without T2D).To measure the effect of Lifestyle intervention and treatment with metformin. And Two SNPs (rs12255372 and rs7903146) were genotyped.	Association study (Prospective research). Drug Intervention based design.	Homozygous T allele at rs7903146 (hazard ratio: 1.55) were more likely to develop T2D than homozygous for the C allele.The effect of the risk-genotype was greatest in the placebo group compared to metformin and lifestyle intervention group and they found similar results for rs12255372 (hazard ratio: 1.53). This study indirectly supports that TCF7L2 variants affects insulin secretion and insulin resistance.	Florez et al. (2006)
9.	2369 case/controls (=678/ 1691).Extended their analyses to the metabolic syndrome and its components in another population based study comprising 1404 KORA participants.	Aim was Replication and understanding of pathogenic mechanism.And Two TCF7L2 polymorphisms rs12255372 and rs7903146 were genotyped.	Association study (Prospective research). Case-control design.	Strongly confirmed the T alleles at both SNPs as risk variants for T2DM. Neither of the two SNPs was significantly associated with the metabolic syndrome. TCF7L2 may primarily affect pancreatic beta cells.	Marzi et al. (2007)
10.	Case/controls (577/596) from African American population.	Aim was just replication. And Genotyped 13 SNPs in six known T2D genes. Plus they also genotyped	Association study. Case control design.	Strongest association for rs7901695 (OR-1.30), and	Sale et al. (2007)

Table 1: Contd....

S. No.	*Samples and Population*	*Aim of the study and SNPs explored*	*Design*	*Salient features of the findings*	*Source*
		70 ancestry-informative markers to take into the account the impact of admixture.		rs7903146 (OR-1.51) with T2D	
11.	545 subjects (178 diabetic) of Mexican-American population.	Aim was replication. And Genotyped two recommended SNPs (rs7903146 and rs12255372) Plus Nine other SNPs from nine haplotype blocks across the gene.	Association and Linkage analysis.	Nominal association between four SNPs (rs3814573, rs7903146, rs12255372, rs10885390) with T2D Identified a common protective haplotype defined by these four SNPs that was significantly associated with type 2 diabetes.	Lehman et al. (2007)
12.	Cases/controls: 3225/3291 from Scotland, UK.	Aim was to provide large scale, population based evidence for TCF7L2. And Genotyped Two SNPs rs12255372 and rs7903146.	Association study. Case-control design.	Both SNPs were found to be in tight LD in Scottish population. Susceptibility for risk alleles was clearly codominant. Cases with the T allele of rs7903146 were slimmer by BMI and waist circumference measures. PAR for rs7903146 18.9%.	Kimber et al. (2007)
13.	Cases/controls: 1151/953 from Finnish population	Aim was replication. And Genotyped Five SNPs rs12255372; rs7903146; rs7901695; rs11196205; rs7895340). Further studied 12 more SNPs that tagged all 63 SNPs in LD with rs12255372	Association study. Case-control design.	Confirmed the association with the same risk allele (*P* value <0.05) for all five SNPs and strongest results for rs12255372	Scott et al. (2007)
14.	Study I -507 individuals with IGT. Study II - 1766 men of Type 2 diabetes and impaired glucose regulation from FinlandStudy III-	Aim was to investigate role of TCF7L2 in Study I- T2D prevention study. Study II- Population based cross-sectional study. Study III- Measuring insulin secretion, and sensitivity	Association study.	**Study I** -SNP rs7903146 was not significantly associated with the risk of diabetes. **Studies II, III**-T allele of rs12255372 was	Wang et al. (2007)

Table 1: Contd....

S. No.	*Samples and Population*	*Aim of the study and SNPs explored*	*Design*	*Salient features of the findings*	*Source*
	Study on Offspring of T2D Parents in 238 nondiabetic offspring of T2D probands	and adipose tissue expression of TCF7L2. And Genotyped two SNPs rs12255372 and rs7903146.		significantly associated with decreased insulin secretion. No significant interaction between BMI and rs12255372	
15.	Cases/controls: 1630/1064 from Japanese population.	Aim was replication. Analyzed four SNPs (rs12255372, rs7903146, rs7901695 and rs11196205 and one tetranucleotide repeat polymorphism (DG10S478).	Association study. Case-control design.	SNP rs12255372 showed the strongest association (OR= 1.70).The microsatellite polymorphism showed an almost complete LD with rs1255372. Showed differences exist in allelic frequencies and LD pattern in different ethnic populations.	Hayashi et al. (2007)
16.	Genotyped 995 T2D patients, and ethnically matched control 399 subjects from South Indian population.	Replication was their aim. And Sequencing three SNPs (rs7903146, rs12255372 and rs4506565).	Association study. Case-control design.	All three variants showed increased relative risk when homozygous rather than heterozygous, most marked in risk for rs12255372. All three SNPs showed strong linkage disequilibrium	Chandak et al. (2007)
17.	Cases/control: 1205/824 from Japanese population	Aim was only replication. And Five SNPs rs7901695, rs7895340, rs11196205, rs12255372 and rs7903146.	Association study. Case-control design.	SNP rs7903146 showed a significant association with T2D (OR= 1.69).	Horikoshi et al. (2007
18.	1520 unrelated subjects from the largest ethnic group of Taiwan (i.e. Han Chinese)	Aim was only replication with the help of additional SNPs. And Genotyped 20 SNPs across the TCF7L2..	Association study. General population based study.	They found risk-conferring alleles in exon 4 LD block Identified. Detected a novel association of SNP rs290487 located in LD block spanning over intron 7 to intron 13 with T2D.	Chang et al. (2007)
19.	Case/control: 502/920 of Dutch Breda cohort	Aim was only replication.And Genotyped two intronic SNPs (rs7903146, rs12255372) in TCF7L2 gene.	Association study. Case-control design.	The two SNPs were in LD with each other in their control population. PAR from these variants in Dutch population is 10%.	Vliet-Ostaptchouk et. al. (2007)

or occasionally using linkage approach to discover genetic variants responsible for T2D. Both prospective and retrospective studies, are equally and well successful in the replication of TCF7L2 gene around the globe in different populations and thus strengthened its association with T2D. These studies confirmed the *number one* status (in terms of effect) and *universal* presence of TCF7L2 for T2D.

In the light of "Out of Africa" hypothesis, the association of TCF7L2, and hope for finding ancestor LD regions makes this part of the world extremely important. It would be a matter of prejudice if researchers look for the role of TCF7L2 in all parts of the world except Africa. With this thing in mind perhaps, Helgason et al. (2007) tried to replicate and most importantly refine the definition of TCF7L2 T2D risk variant, $HapB_{T2D,}$ to the ancestral allele T of rs7903146, through replication in West Africa (case/control: 621/448) and Danish T2D (case/control: 1149/2400) studies and an expanded Icelandic study. In both regions association for rs7903146 T was stronger than rs12255372 and DG10S478 X, hence their overall results rule out these latter markers as causal variants. These results also support the notion that relatively diverse populations provide the means to refine association signals detected in relatively homogenous populations characterized by larger regions of strong LD. On the basis of phylogenetic reconstruction of haplotypes within TCF7L2 exon 4 LD block they identified two major lineages:

1. More diverse lineage, HapB, contains all but one of the haplotypes carrying rs7903146 T, a subset they refer to as $HapB_{T2D.}$
2. HapA consist of relatively homogenous cluster of haplotypes (most frequent (95%) in East Asians HapMap group because of tested strong positive selection sweep, which is also observed to some lower extent in European, and West African populations and proved by greater F_{ST} value.

Hence, $HapB_{T2D}$ cannot account for large fraction of T2D risk in East Asian countries because its frequency is only 2% in HapMap group. The positive selection causes near fixation in East Asians and that the weaker or shorter selective episodes may also have increased the frequency of HapA in European, and West African populations. But what was the phenotypic effect of HapA that increased reproductive success in the past? To answer this they tested the association of HapA (carry rs7903146 A and rs7903146 C) and $HapB_{T2D}$ (carry rs7903146 T) to BMI (case/control: 5/6) and they found Hap A is associated with increased BMI, whereas $HapB_{T2D}$ associated with decreased BMI (effects are stronger for males). But it does not mean that $HapB_{T2D}$ leads to lower BMI, as it could simply reflect the joint independent risk. Both of these haplotypes are not independent because 80% of haplotypes in populations of European ancestry has either HapA or $HapB_{T2D,}$ They also examined 14 metabolic traits in a subset of Icelandic controls, two (in fasting plasma concentration of Ghrelin and Leptin) of these traits showed significant differences in HapA (decrease in level of Ghrelin and increase in the level of Leptin). They explained this in the light of the role of Ghrelin and Leptin in neuroendocrine regulation of appetite and long-term regulation of fat storage and energy metabolism. Now the selective advantage of HapA through the effects on energy metabolism contradicts the key prediction of "Thrifty genotype" hypothesis, where as $HapB_{T2D}$ a major risk factor for T2D is negatively associated with BMI and is not the variant that contributed to adaptive evolution in recent past.

For the systematic review of almost all these above mentioned studies to assess the strength and genuineness of association via meta-analysis and to replicate the study in some new populations Cauchi et al. (2007) explored North African region for the first time. They investigated the association between the TCF7L2 rs7903146 polymorphism and T2D in Moroccans (cases/controls: 504/406) and in white Austrians (cases/controls: 486/1075). The allelic odds ratio for T2D were 1.56, 1.52 in Moroccans and Austrians, respectively. They found no heterogeneity between these populations. They further systematically reviewed the association of the SNP with T2D risk in meta-analysis, combining their data with 27 previous studies, adding up to a total of 29195 controls and 17202 cases. Calculated global OR was 1.46, suggesting that in any tested human population the effect of TCF7L2 is very similar. No publication bias was detected. The absence of heterogeneity between populations is also indicative of universal contribution of this gene to T2D. The TCF7L2 gene can be distinguished by its tremendous reproducibility of association with T2D and its risk OR being twice as high when compared to other gene variants previously confirmed by meta-analysis.

Confirmation of TCF7L2 via Genome-Wide Association Studies

The outcome of much promising genome-wide association studies also points towards the high magnitude of the role of TCF7L2 in the context of diabetes type 2. Sladek et al. (2007) conducted two-stage, genome-wide association study to identify T2D susceptibility loci. At the first stage, they tested 392,935 SNPs in 1363 French case control cohort, initially studied diabetic subjects with BMI < 30 kg m^{-2} (subjects have at least one affected first degree, and in order to decrease phenotypic heterogeneity and to enrich variants determining insulin resistance beta cell dysfunction through mechanism other than obesity). Because of unequal male/female ratio in their case controls they genotyped 12666 additional sex chromosome snips, separately for each gender. They also conducted population stratification analysis (using component analysis) to find out any spurious association and they found spurious association for only one SNP. None of the other previously identified T2D genes (like PPARG, KCNJ11) found to be significant, because of limited power of stage 1 to detect their modest effect. From their stage 1 results they prioritized 57 SNPs showing significant association in stage 2 analysis (case/control: 2617/2894). In total 8 SNPs representing five unique loci (TCF7L2; SLC30A8; HHEX; LOC387761; EXT2) showed significant association after Bonferroni correction was applied for the 57 SNPs. The population attributable risk (PAR) of four novel loci with TCF7L2 is 70%. It is worth noting that for three of four novel loci, the risk allele is the major one, it also suggests that allelic heterogeneity does not seems to be large (Sladek et al. 2007). This study is followed by further application of genome-wide association studies by Scott et al. (2007), Zeggini et al. (2007), and Wellcome Trust case control consortium (2007), which also strengthened the relevance of TCF7L2 in type 2 diabetes.

The accelerator hypothesis, which suggests that the Type 1 diabetes is nothing but an extreme form of type 2, was remained to be tested, because researchers were looking for polymorphic molecular markers with large effect size. TCF7L2 gene provided the researchers an opportunity to test this yet untested hypothesis at population level. Field et al. (2007) investigated the accelerator hypothesis which suggests that type1 and type 2 diabetes are the same disease of hyperglycaemia induced beta cell damage but that type 1 diabetes has the added effect of autoimmunity. One way of testing this hypothesis is to check for common causal pathway between type 1 and type 2 diabetes genes with large effect in type 1 diabetes sample. They analyzed the two SNPs, rs12255372 and rs7903146, in 6199 white UK type 1 diabetic subjects and 7596 control subjects. Their study had 80% power to detect an effect with an OR as low as 1.12. They found no evidence for association between TCF7L2 and type 1 diabetes. Their results did not support a model of shared major causal pathway in type 2 and type 1 diabetes. On the same lines Shaat et al. (2007) investigated whether common genetic variants that have been found associated with T2D or related phenotype would also confer risk for gestational diabetes mellitus. They genotyped SNP rs7903146 of TCF7L2 in 1881 Scandinavian women (cases/control: 649/1232), and found significant difference between cases and control in their genotype frequencies.

DISCUSSION AND CONCLUSION

The above studies on TCF7L2 suggest the robust nature of association between the common risk alleles of TCF7L2 and T2D, which could predict the risk of T2D prospectively. The two most frequently studied and promising SNPs rs12255372 (G and T allele), rs7903146 (C and T allele) have shown their ubiquitous presence in different human populations.

Which is the Causal Allele among the Important Variants of TCF7L2?

As most early association studies over-estimate genetic effects, validation not only of reported genetic associations, but also of their effect sizes is essential (Marzi et al. 2007). This first reported association of 5 snips of TCF7L2 with T2D (Grant et al. 2006) led to the storm of papers in the form of replication, or other extended type of studies, in almost all major human populations of the world. The research related to genetics of complex disorders is generally plagued with inconsistent reproducibility and became a vexing problem for genetic association studies. False positive reports of association, false negative attempts at replication, and genetic heterogeneity often complicate the picture, and thus a true genetic association usually emerges only after carefully conducted, large-scale association studies which confirm the original report (Florez

et al. 2006). But the consistent replication of association of markers on TCF7L2 gene around the globe has strengthened its position as a strong candidate gene for type 2 diabetes. The speed of confirmation and replication of the findings has certainly been unprecedented (Zeggini and McCarthy 2007). Grant and coworkers exhaustively assessed coding variation in Whites in the region, suggesting that other functional variants in this gene are unlikely to have been missed. The other single-nucleotide polymorphisms in linkage disequilibrium with them were also strongly associated with diabetes. But, which of these SNPs is responsible for the observed association requires further study in adequately powered samples (Permutt et al. 2005). Therefore, more extensive genotyping and sequencing is clearly warranted, as are functional studies of the most associated alleles to document that they function through TCF7L2 rather than some adjacent gene (Saxena et al. 2006). On the basis of pattern of LD around rs7903146 in HapMap groups, Helgason et al. 2007, concludes that T allele is not itself the risk variant, and the unidentified causal variant it tags is unlikely to lie outside the sequenced region. The magnitude of the TCF7L2 effect is much higher than any other confirmed T2D candidate. The individual effects of the other variants are modest, ranging from 10 to 30%. Interestingly, no major interactions with the T allele have been found to strongly modulate T2D susceptibility, even if BMI, gender, drugs, lifestyle interventions may modulate TCF7L2 effects (Cauchi et al. 2007). Undoubtedly, the addition of *TCF7L2* grows the list of bonafide type 2 diabetes susceptibility genes that includes P12A *PPARG*, E23K *KCNJ11*, and SNPs in *CAPN10* (Damcott et al. 2006). Interestingly, there is no evidence that rare mutations in TCF7L2 contribute to the burden of monogenic diabetes as in case of other potential candidate genes (Groves et al. 2006; Cauchi et al. 2007).

Therefore, to find out the causal allele, there is a need of resequencing TCF7L2 gene and its nearby region, and it is also essential to sequence other functionally related genomic regions of TCF7L2 gene, so that, a complete and more comprehensive picture will emerge.

TCF7L2 Presents Essentially a Multiple or Additive Model

Grant et al. (2006) rejected dominant and recessive model because heterozygous had greater risk than non-carriers, and lower risk than homozygous carriers. They suggested multiplicative model, but risk of homozygous carriers relative to the heterozygous carriers is greater than that of the risk of the heterozygous carriers relative to the non-carriers. Scott et al. (2006) found additive effects on the risk of type 2 diabetes for each additional risk allele from *TCF7L2*, *PPARG* (P12A), and *KCNJ11* (E23K). These SNPs are likely to represent only a small proportion of the total set of genetic variants associated with the risk of type 2 diabetes; thus, an individual with multiple risk alleles from this particular set of three variants could still have a low risk of type 2 diabetes compared with others in the population. Cauchi et al. (2006) also suggested additive model of inheritance which triples the risk of developing type II diabetes for homozygous cases. Marzi et al. (2007) confirmed a good fit for multiplicative model, as clear copy number effect was found. Other studies also suggest additive model of inheritance for this gene (Vliet-Ostaptchouk et al. 2006; Saxena et al. 2006; Scott et al. 2006; Zhang et al. 2006; Groves et al. 2006).

TCF7L2 Risk Variants and Metabolic Syndrome (especially BMI) association

It has been found that when WNT signaling is inhibited, the committed preadipocytes will not differentiate into mature adipose cells. In contrast**,** loss of functional mutations in WNT signaling leads to rapid recruitment and growth of preadipocytes. Thus, potentially increased WNT signaling in carriers of TCF7L2 risk variants could be expected to influence adipose tissue growth and development, and thus BMI. When committed preadipocytes exposed to cytokines such as IL-6, TNF-alpha at very early stage of development i.e. before WNT secretion and signaling is inhibited, these cytokines promote WNT activation to different degrees, thus allowing the development of partially differentiated (IL-6) or completely undifferentiated (TNF-alpha) cells (Smith 2007). Most of the studies on TCF7L2 variants have not shown any association with most of the metabolic syndromes (Saxena et al. 2006; Florez et al. 2006; Marzi et al. 2007; Zhang et al. 2006; Chandak et al. 2007; Munoz et al. 2006), but one intriguing observation reported in some studies is that there is a negative relation of BMI with TCF7L2

variants, which in some studies is found to be significant (Florez et al. 2006; Cauchi et al. 2006; Kimber et al. 2007; Horikoshi et al. 2007; Vliet-Ostaptchouk et al. 2006; Wang et al. 2007; Helgason et. al. 2007). This indicates that the risk conferred by the at-risk variant is not operating through increased BMI, however carriers of the risk variant may have an earlier age of onset than noncarriers (Grant et al. 2006). Lower waist circumference is also found to be significantly associated with risk allele (Melzar et al. 2006). The lack of an association of risk alleles with metabolic syndrome could be due to several reasons: First, not all subjects with the metabolic syndrome will eventually develop type 2 diabetes. It has been found that less than 50 % of the risk of T2D was attributable to the presence of the metabolic syndrome. A quite possible explanation for this finding might lie in the substantial heterogeneity of the metabolic syndrome allowing for many combinations of metabolic disturbances with different implications for diabetes risk, but resulting in the same diagnosis. Second, it is possible that there are distinct polygenic determinants of the metabolic syndrome with small effect sizes, which could not be observed in various studies and would require an even larger sample (Marzi et al. 2007). Wang et al. (2007) suggests higher lipid profile is unlikely to be an artifact of TCF7L2 polymorphism. Association of metabolic syndrome like BMI with TCF7L2 may help in providing insight in obesity research like PPARG. Body mass index data and some preliminary associations with leptin and ghrelin levels, however, could point towards a central mechanism. Such uncertainty highlights a key attraction of the 'reverse genetics' approach—that is, the opportunity to uncover novel cellular and physiological mechanisms (Owen, McCarthy 2005)

Which Comes First: Insulin Secretion or Insulin Resistance?

Studies have shown that both impaired insulin secretion and, insulin action seem to be inherited and could represent the primary defects in glucose metabolism in the offspring of T2D probands (Vauhkonen et al. 1997). As discussed before diabetes is a disease of insulin resistance and impaired insulin secretion, but which comes first is a matter of controversy. Most of the reliable candidate genes like PPARG, KCNJ11 favour impaired insulin secretion and most importantly, the case of TCF7L2 also gives indication for impaired insulin secretion via beta cell dysfunction (Damcott et al. 2006; Florez et al. 2006; Saxena et al. 2006; Scott et al. 2006; Chandak et al. 2007). Thus current evidence strongly supports the idea that the predominant effect of TCF7L2 dysfunction on T2D development is mediated through impairment of insulin secretion (Zeggini, McCarthy, 2007). One study on Amish population (Damcott et al. 2006) for the first time suggested evidence for Insulin resistance in T_2D manifestation. The evidence also indicates that beta-cell function may be more highly determined by heritable factors compared with other known pathogenic traits involved in T2D (Munoz et al. 2006). Therefore, the results of studies on TCF7L2 strongly suggest that these variants do not cause insulin resistance in persons with impaired glucose tolerance and support the notion that the primary cause of diabetes is impaired insulin secretion. Wang et al. (2007) showed that insulin response to an intravenous glucose was impaired, while that to an oral glucose load was not significantly impaired, suggesting that a deficient 'incretin effect' is unlikely to be the only explanation why rs12255372 is associated with impaired insulin secretion. Very recently, Lyssenko et al. (2007) in multiple sub-studies observed impairment in both glucose and arginine-stimulated insulin secretion in carriers of risk alleles of TCF7L2. The CT/TT risk genotypes were also associated with a consistent impairment in insulin response to oral glucose over time in individuals who converted to diabetes (prospective study). They further explored the mechanisms by which variants in TCF7L2 could impair insulin secretion, and studied genotype-phenotype correlations in human islets. Expression of TCF7L2 was 5-fold higher in islets from patients with T2D than in islets from nondiabetic donors. More importantly, nondiabetic carriers of the TT genotype had the highest expression of TCF7L2 in islets.

Therefore, as is the case with other well known T2D genes, the well established candidate gene, TCF7L2, also supports the hypothesis that insulin secretion leads to insulin resistance.

Improvement in Possibilities of Diabetes Management and Proof for CDCV Model

Grant et al. (2006) suggested population attributable risk for risk variants was 21%, which

was substantial from public health point of view. The identification of a common allele in the *TCF7L2* gene that increases the risk of type 2 diabetes by approximately 1.45 in heterozygotes and 2.41 in homozygotes is quite provocative (Florez et al. 2006). Theoretical studies also have emphasized that as few as 20 susceptibility variants on the scale of TCF7L2, PPARG, KCNJ11 may suffice to explain as much 50% of the disease (Yang et al. 2005). The odds ratio for successful translation of genetic information into clinical management have shortened considerably because of large effect size of TCF7L2 (Zeggini, McCarthy, 2007). Kimber et al. (2007) found that the T allele of rs7903146 was associated with a slightly earlier age of diagnosis, it does provide replication support for earlier observations (Cauchi et al. 2006; Lehman et al. 2007*)* and is consistent with the observation that the risk alleles of TCF7L2 are enriched further in groups with early-onset type 2 diabetes. Such a high replication of TCF7L2 variants around the world strengthens the CDCV model which emphasizes that common variants across the population are responsible for the complex disorder like T2D. This also motivates the researchers around the world to find out more variants of TCF7L2 quality, so that a clear and confined allelic architecture of T2D will come up. Prospective studies have demonstrated the role of TCF7L2 variants in the rate of progression from impaired glucose tolerance to diabetes. The lack of gene dosage effects seen in prospective studies is likely to have been due to lack of power.

Kimber et al. (2007) observed a clear gene dose effect for TCF7L2, since the homozygote rare variant conferring an odds ratio of about 2. This is meant, for the first time, that studies containing hundreds, rather than thousands, of cases have had sufficient statistical power to detect this effect reliably. It is hoped that careful dissection of the performance of current therapies in patients with different TCF7L2 genotypes may lead to specific treatment strategies based on TCF7L2 genotype. Wang et al. (2007) has shown that lifestyle-intervention can efficiently reduce the risk conferred by genetic factors, even when risk genotypes are related to impaired insulin secretion. It may be because the lifestyle intervention improves insulin sensitivity, making less insulin secretion from beta cells necessary to maintain non-diabetic glucose tolerance. Further, there is a possibility that lifestyle intervention, in addition to improving insulin sensitivity, could also improve insulin secretion capacity. Evidence of lowering the diabetigenic effect of risk genotype (esp. rs 12255372) by lifestyle intervention has also been suggested.

Undoubtedly the knowledge on the role of TCF7L2 gene can play a very significant role in diabetes management, by identifying the prospective diabetes patients who carry the diabetes related genetic load in a population so that preventive and/or treatment measures can be initiated.

Ethnicity is Important or not – Odds Ratio/ PAR Varies among Populations

It is well known that there are significant differences in the frequencies of certain genetic variations among different ethnic groups and this should be true for all disease genes. The risk variants of TCF7L2 have been tested by different scholars on different ethnic groups. The meta-analysis of all association studies by Cauchi et al. (2007) clearly suggests that OR varies among different populations, which correspondingly leads to variation in population attributable risk. The clear distribution of the risk variants again emphasizes the need of ethnicity based association studies in population genetic research. However, the effect of TCF7L2 is found to be almost similar in various human populations in terms of their allele frequencies, and is indicative of universal and uniform nature of contribution of this gene to T2D. The population attributable risk being only driven by the prevalence of T allele in a specific ethnic group. This is a unique situation as the previously deciphered candidate genes for T2D have always shown some degree of discrepancy between populations in terms of their allele frequencies. In contrast to this, the consistent replication across European populations confirms that the causal *TCF7L2* variant influences disease risk reproducibly, without the need to yet invoke population-specific effects (Saxena et al. 2006). The absence of linkage disequilibrium between rs7903146 and rs12255372 variants of TCF7L2 in African Americans may help distinguish whether one of the two SNPs (or the haplotype formed by the risk alleles at both loci) is the sole source of the association signal and reflects the importance of ethnicity in association studies (Florez et al. 2006). Almost all association studies based on TCF7L2 were able to replicate the risk nature of

the gene in different popu-lations, except those in which ethnicity of the subjects was not clear and leads to lower effect than originally reported (Saxena et al. 2006, Humphries et al. 2006). Munoz et al. (2006) found that it is important to consider the low degree of LD between the two SNPs rs7903146 and rs12255372 in African Americans and Yorubans. Because they found only rs12255372 association with impaired insulin secretion, so they predicted rs12255372 might be associated with T2D in African derived populations (Munoz et al. 2006). Hayashi et al. (2007) also suggested differences in LD pattern in the risk locus in White and Japanese population (Hayashi et al. 2007). Recently a study on Chinese population supported the importance of ethnicity in diabetes research by finding significant association with novel SNP (rs290487), rather than with the SNPs found to be associated in European populations (Chang et al. 2007). Further, Grant et al. (2006) also suggested that there are underlying differences in the population allele frequencies rather than differences due to case or control sampling criteria.

The foregoing studies very categorically stress on the importance of ethnicity in research designs of genetic studies related to complex disorders.

Pathophysiology

TCF7L2 gene product is high mobility group box containing transcription factors that have role in WNT signaling pathway (Grant et al. 2006). Grant et al. (2006) put forth the hypothesis that variants in *TCF7L2* influence type-2 diabetes susceptibility through altered transcriptional regulation of the insulinotropic hormone glucagon-like peptide-1 (GLP1), a peptide encoded by *GCG* and expressed in the brain and gut. This hypothesis was driven by observations of intestine-specific roles for TCF7L2, including a role in the development of colon cancer and the observation that TCF7L2-null mice, which die shortly after birth, lack epithelial stem-cell compartments in the small intestine. An alternative hypothesis is that variants in TCF7L2 disrupt adipogenesis and/or adipocyte function by altering transcriptional regulation of CEBPA and PPARG, leading to deposition of triglycerides in peripheral tissues and resulting in insulin resistance (Damcott et al. 2006). No functional significance has been attributed to the TCF7L2 T allele so far, which is unlike the most T2D SNPs. Therefore, the binding of transcription factors and alternative splicing events should be studied in the intronic region where the T allele is located. TCF7L2 gene is significantly expressed in human tissues that are vital to glucose homeostasis, including visceral and subcutaneous fat, but its physiological functions still requires evaluation (Cauchi et al. 2007). Recently, Loos et al. (2007) suggests that TCF7L2 may impair β-cell proinsulin processing by increases proinsulin level. In conclusion, it will be a great interest to learn molecular mechanisms of action of the TCF7L2 gene in the pathogenesis of diabetes type 2. There is high degree of evolutional conservation in the 3' end of human TCF7L2 gene, which was found to be associated with T2D. Functional studies will be required to determine the relationship between sequence variations, gene expression, protein product and function in human tissues (Chang et al. 2007). Lyssenko et al. (2007) found a paradoxical relationship i.e. the expression of *TCF7L2* correlated strongly and positively with expression of the insulin gene but negatively with glucose-stimulated insulin secretion. One possible explanation would be that the increase in insulin gene expression is compensatory to a posttranscriptional defect in insulin secretion, which could involve multiple steps in glucose and ATP-stimulated insulin secretion including exocytosis.

To sum up, we emphasize that finding the etiological variants of TCF7L2 should be a major and immediate goal, because most risk SNPs like rs12255372 and rs7903146 are on intronic regions of the TCF7L2 gene. Very high density SNP mapping in population association studies of this gene region may help in finding the main causal variant(s) of TCF7L2. Further, to explore the biochemical pathway of impaired insulin secretion through β cell will definitely prove to be insightful in exploiting the genetic causation of type 2 diabetes. Finally, recent trend of high resolution cytogenetic exploration of sub-microscopic variations (i.e. copy number variations) in the vicinity of well replicated regions (like TCF7L2) of human genome can also be expected to help in designing powerful genetic studies on type II diabetes.

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