Human genetics of diabetes mellitus in Taiwan

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1. ABSTRACT

Diabetes mellitus (DM) is a disease defined by biochemical hyperglycemia. Currently it is classified into four major categories: type 1 DM (T1DM), type 2 DM (T2DM), gestational DM (GDM) and other DM. Within each category, the etiology is still heterogeneous. The pathogenesis of most DM is multi-factorial, including many genetic and environmental factors. T1DM, T2DM and GDM are polygenic. In the category of other DM, there are at least six maturity onset diabetes of the young (MODY) and many other genetic syndromes associated with DM, which are monogenic in origin. In this review, we briefly summarized the current status of genetics in DM, described what has been done in this specific area in Taiwan and discuss what should be done after the era of genome-wide association study.

2. INTRODUCTION

Diabetes mellitus (DM) comprises a collection of clinically and genetically heterogeneous disorders characterized by inappropriate hyperglycemia. The underlying pathophysiology is related to deficiency of insulin secretion, a reduction in the biologic effectiveness of insulin, or a combination of these two. The most recent update of DM classification system and diagnostic criteria were developed by an international workgroup sponsored by the National Diabetes Data Group of the National Institute of Health of the US in 1997 (1) and by World Health Organization Expert Committee in 1999 (2). The diagnostic criteria include: (A) symptoms of diabetes (i.e. thirst, polyuria and unexplained weight loss) plus a random plasma glucose concentration higher than 200 mg/dL (11.1mmol/L); (B) fasting plasma glucose higher than 126
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mg/dL (7.0 mmol/L) after an overnight (at least 8-hour) fast; and (C) two-hour plasma glucose higher than 200 mg/dL (11.1 mmol/L) during a standard 75-gram oral glucose tolerance test. Currently, DM can be broadly categorized as 4 groups: type 1 DM (T1DM), type 2 DM (T2DM), other specific types of DM and gestational DM (GDM) (1). Any of these 4 types is still not a single homogeneous disease; for example, the “other specific types of DM” category contains tens of known disease entities, including mitochondrial diabetes, maturity-onset diabetes of the young (MODY), and other genetic or non-genetic causes.

Genetic factors play an important role in the pathogenesis of diabetes. T1DM and T2DM are complex traits with both genetic and environmental predisposition. Multiple lines of evidence, including family study and twin study, support the important role of genetic factors, most likely multiple genes, in the pathogenesis of T1DM or T2DM. Mitochondrial DM is maternally inherited and is caused by genetic variations, such as substitution or deletion, at the genes of mitochondrial genome or nuclear genes encoding mitochondrial proteins. All types of currently known MODY are single gene diseases inherited in an autosomal dominant pattern. In this article, we will briefly update the progress of human genetic study of DM worldwide and then review the work conducted in Taiwan.

3. GENETICS OF TYPE 1 DM

3.1. Summary of the current progress of type 1 DM genetics

T1DM is a chronic autoimmune disorder with the immune-mediated destruction of pancreatic beta cells (3). The disease incidence differs significantly among different geographic areas and populations, ranging from more than 20 cases/year/100,000 individuals in the Scandinavian population to around 1 case/year/100,000 individuals in Asians (4, 5). The recurrence risk is (lambda of siblings) for T1DM is ~15 in Caucasians (6), and twin study suggests that 88% of the phenotypic variance is due to genetic factors (7).

Before the era of genomewide association study, there have been a handful of susceptibility genes identified with reasonable statistical evidence, including the human leukocyte antigen (HLA) region (8-10), insulin (INS) (11), protein tyrosine phosphatase, non-receptor type 22 (PTPN22) (12, 13), and cytotoxic T-lymphocyte associated 4 (CTLA-4) (14). Genome-wide association (GWA) study with a large sample size is an exciting and powerful new approach. Since 2006, several genes/regions, such as the regions around interferon-induced helicase 1 (IFIH1/MDA5) and chromosomes 12q13, 12q24 and 16p13, have been implicated with GWA (15). With the rapid progress of genotyping technology and sample collection, more and more plausible susceptibility genes will be identified and vigorously tested.

3.2. Review of type 1 DM genetics in Taiwan

For the Han population in Taiwan, there have been several genetic studies showing the association between HLA and T1DM. As early as 1980, with just 39 cases and 57 controls, Maeda et al. found that the incidence of HLA-DRw3 was increased in cases (relative risk=5.8, P=0.0027) (16). Subsequently, the increased risk of DR3/DR4 heterozygotes was demonstrated (17). When DNA-based typing techniques were introduced, researchers could conduct studies with higher precision. Overall, DR3 and DR4 were found to increase, while DR2 and DR5 were decreased in T1DM individuals in Taiwan (18). HLA DR9, when combined with DR3, increased the risk for T1DM (19). DQB-57-non-Arg is positively associated, but not sufficient to explain all the risk of T1DM (20). With 31 simplex T1DM families, Chuang et al. deduced the extended HLA haplotypes without ambiguity and showed that DRB1*0301/DQA1*0501/DQB1*0201, DRB1*0405/DQA1*0301/DQB1*0302 and DRB1*0405/DQA1*0301/DQB1*0401 were susceptibility haplotypes in Taiwan (19), which was later replicated by another group (21). The allele frequency and haplotype composition of classical HLA loci can differ significantly between populations (22, 23), which might be one of the reasons why the incidence of T1DM varies across the world.

The allele frequencies of the variable number of tandem repeats (VNTR) located 5' upstream of the insulin gene are also different across populations (24, 25). In Taiwan, Chuang et al. (26) could not find association between T1DM and the class I allele of VNTR (27) or other restriction fragment length polymorphism (RFLP) markers within INS. Association studies of T1DM and INS from other non-Caucasian populations have been inconsistent (25, 28).

Vitamin D receptor (VDR) has been tested as a candidate susceptibility gene in Taiwan. Chang et al. (29) recruited 157 cases and 248 controls and found that one RFLP marker in the 7th intron of VDR, with allele frequency 7.6% in cases and 3.6% in controls, was association with T1DM even after correcting for multiple testing (corrected P = 0.045).

4. GENETICS OF TYPE 2 DM

4.1. Summary of the current progress of type 2 DM genetics

T2DM is a chronic metabolic disorder characterized by insulin resistance and/or abnormal insulin secretion (30, 31). We are witnessing a global epidemic of DM, with the total number of diabetes individuals projected to rise from 171 million in 2000 to 366 million in 2030 (32). Although lifestyle, behavior and environment are important for T2DM pathogenesis, genetic factors definitely play a critical role. The estimated λ for T2DM is ~3.5 in Caucasians (33), which reflects shared environment and genetic predisposition between family members. Heritability values derived from twin studies vary, with most estimates between 30 and 70% (31).

There have been three T2DM susceptibility genes identified and repeatedly replicated, which are transcription factor 7-like 2 (TCF7L2) (34), inwardly-rectifying Kir6.2 component of the pancreatic beta-cell KATP channel (KCNJ11) (35) and peroxisomal
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proliferative activated receptor gamma (PPARG) (36). Other genetic studies also support the roles of the following genes: calpain 10 (CAPN10) (37), hepatocyte nuclear factor 4-alpha (HNF4A) (38), ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1) (40), insulin receptor substrate 1 (IRS1) (41), ATP-binding cassette, subfamily C, member 8 (ABCC8) (42), solute carrier family 2A, member 1 (SLC2A1) (42) and insulin (INS) (41). Recent genome-wide scans further implicate some other signals, such as fat-mass and obesity-associated (FTO), CDK5 regulatory subunit associated protein 1-like 1 (CDKAL1), insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2), solute carrier family 30, member 8 (SLC30A8), hematopoietically expressed homeobox (HHEX), and the region near cyclin-dependent kinase inhibitor 2A and 2B (CDKN2A and CDKN2B) (43-45).

4.2. Review of type 2 DM genetics in Taiwan

In Taiwan, there have been many candidate gene-based association reports for T2DM. Similar to the situation of the research field worldwide, the sample size of early studies tended to be small, mostly less then 500 cases. In some latest association studies conducted in Taiwan, the sample sizes could surpass 1000 cases and should have better power to detect susceptibility genes with small to moderate effect.

Glucokinase (GCK), the gene responsible for MODY2, was tested for association with T2DM in Taiwan by Wu et al. (46). Two short tandem repeat polymorphism (STRP) markers (GCK1 and GCK2) near GCK were genotyped, and one allele of GCK1 was found to have a lower frequency in cases than in controls (14.0% vs. 23.9%) although the P value (0.058) did not reach a statistically significant level caused by small sample size.

Chuang et al. collected 23 multiplex families, 89 unrelated T2DM individuals and 82 unrelated controls to test the role of IRS1 (47). The PCR-RFLP method was used to genotype the Gly971Arg variant. They did not find evidence of association.

A missense mutation, Gly40Ser, of glucagon receptor (GCG-R) was reported to be associated with T2DM in Caucasians with an allele frequency as high as 4.6% in France and 8.38% in Sardinia (48, 49). Huang et al. screened 213 T2DM subjects, 107 hypertension subjects and 121 normal controls and found none of them had this genetic variation (50). Their results demonstrated a strong genetic heterogeneity between different ethnic groups in some genomic regions.

The angiotensin-converting enzyme (ACE) gene has been tested for association with T2DM or other related phenotypes in Taiwan. Chuang et al. collected 107 subjects with T2DM, 67 with hypertension, 70 with both T2DM and coronary artery disease and 197 normal controls, and found no association of the insertion/deletion (I/D) polymorphism with any of the phenotypes they tested (51). On the other hand, Hsieh et al., using 336 T2DM patients and 263 matched normal controls, found an increased frequency of DD genotype in T2DM patients than in normal controls (18.2% vs. 9.1%, P<0.01) (52). Lee and Tsai showed that the ACE I/D polymorphism was significantly associated with the metabolic syndrome in 711 T2DM patients (P=0.001) (53). However, when Tseng and Tseng examined the relationship of this I/D polymorphism with the peripheral vascular disease complication of T2DM individuals, they did not find association (54).

A couple of immune-related genes were tested for the association with T2DM, including tumor necrosis factor-alpha (TNF-alpha) (55), interleukin-6 (IL-6) (56) and interleukin-10 (IL-10) (57). Up to now, none of them has been shown to be associated with T2DM in Taiwan.

The Arg16Gly polymorphism of beta2-adrenoreceptor (ADRB2) gene was examined by Chang et al. (58). With a collection of 130 patients and 130 age, gender and body mass index (BMI)-matched controls, they concluded that the homozygosity of Arg16 was associated with a higher frequency of T2DM, and also associated with an earlier onset of the disease.

Solute carrier family 2, facilitated glucose transporter, member 10 (SLC2A10) is another intriguing candidate gene for T2DM. Lin et al. performed the association study using 15 SNPs and 1 tandem repeat polymorphism in 375 cases and 377 controls, and found only a modest association signal from a haplotype of one of the four linkage disequilibrium blocks (59). They suggested that SLC2A10 does not appear to be a major determinant for T2DM in Taiwan.

Adiponectin is a plasma glycoprotein of adipose tissue origin, and is a promising candidate T2DM gene based on its biological relevance and other previous association studies. Yang et al. genotyped 1793 subjects of Chinese and Japanese descendants from 601 hypertensive families, and focused on variants in the adiponectin gene and PPARG (60). The phenotypes they were working on were insulin concentrations and insulin resistance index. They demonstrated that adiponectin is associated with insulin sensitivity, and they also showed that interaction with PPARG would modify this association. Later on, Yang et al., based on 1438 elderly individuals (> 65 years old) in Taiwan, found that genetic variants of adiponectin is associated with T2DM, obesity and metabolic syndrome (61). The effect of adiponectin genotype on the risk of T2DM was partially independent of BMI.

The discovery of TCF7L2 as a susceptibility gene is probably the biggest story in genetic study of T2DM (62). The association has been replicated in almost all the subsequent studies in the Caucasians, and there have been replication in other populations (63-65), although a Japanese group reported a much lower frequency of the risk allele (63). In Taiwan, Chang et al. genotyped 20 tagging SNPs across TCF7L2 in 760 T2DM cases and 760 unrelated controls (66). They demonstrated that rs7903146, the SNP with highest risk in previous studies, had a much lower allele frequency (~2.3%) in our population, and was not associated with T2DM. However, they found rs290487
and haplotypes containing it, which is ~150 kb downstream of rs7903146, were significantly associated with T2DM (odds ratio: 1.26, nominal P = 0.0021) and conferred a population attributable risk fraction of 18.7%. This population-specific risk SNP/region of TCFF1L2 in our population, if confirmed in subsequent studies, will provide valuable information for both basic research and clinical application.

5. GENETICS OF MITOCHONDRIAL DM

5.1. Summary of the current progress of mitochondrial DM genetics

DM can also be caused by mitochondrial diseases. Point mutation at mitochondrial DNA m.3243A>G, which is located at the tRNA (Leu, UUR) gene, was the first discovered causative variant (67, 68). Other point mutations, such as m.14709T>C (69, 70), and deletions (71, 72) can also be responsible for diabetes. Even mitochondrial protein encoded in nucleus has been implicated to cause diabetes (73). However, little is known about the factors determining the clinical phenotypes for any particular genotype. For example, the same m.3243A>G mutation can confer a spectrum of phenotypes in different individuals, including asymptomatic; maternally inherited diabetes and deafness; chronic progressive external ophthalmoplegia; or the MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) syndrome (74). Mitochondrial DM is likely under-diagnosed, because of the lack in awareness among medical doctors and the lack of proper diagnostic tools. There has been no good prevalence estimate of mitochondrial diabetes. The m.3243A>G is by far the most common disease-causing mutation (74), and can account for up to 3% of all diabetes individuals in some populations (75, 76).

5.2. Review of mitochondrial DM genetics in Taiwan

In Taiwan, there were sparse studies on mitochondrial diabetes. It is difficult to estimate the prevalence and relevant importance of different variants. Chuang et al. screened 23 T2DM pedigrees and found one of the 23 pedigrees carried the m.3243A>G mutation (77). With a similar approach, 2 out of 84 T1DM individuals were shown to have the m.3243A>G point mutation, although one of the two individuals also carried the susceptibility HLA haplotype (78). Pang et al. analyzed the genotypes of 77 patients with mitochondrial diseases, and found 32 patients with m.3243A>G mutation, 9 with m.8344A>G, 18 with m.11778G>A, 1 with m.8993T>C, 2 with m.8993G>T, and 21 with deletion (79).

It is an important question to ask if the clinical severity is related to the degree of heteroplasmy or not. Two studies in Taiwan touched this issue, and the researchers found no correlation between the clinical severity and the degree of heteroplasmy in various tissues, such as leukocytes, hair follicles and muscle tissues (77, 80).

A common variant (m.16189 T>C) has been reported in patients with MELAS (81) and might be associated with insulin resistance (82, 83). In a recent study enrolling 462 T2DM patients and 592 non-DM controls in Taiwan, Liou et al. (84) showed that a higher proportion of T2DM patients (39.2%) carry this variant compared to the proportion in non-DM controls (30.7%), with a multivariate odds ratio of 1.38. They also reported increased BMI as an aggravating factor for development of DM in subjects harboring the variant.

6. GENETICS OF MODY AND GENETIC SYNDROMES

6.1. Summary of the current progress of MODY genetics

MODY comprises a group of monogenic, autosomal dominant diabetes, characterized by an early age of onset (usually < 25 years old) (85). Up to now, 6 genes have been identified, including hepatocyte nuclear factor (HNF)-4α (MODY1) (86), glucokinase (GCK) (MODY2) (87), HNF-1α (MODY3) (88), insulin promoter factor-1 (IPF-1) (MODY4) (89), HNF-1β (MODY5) (90) and neurogenic differentiation factor 1 (NEUROD1) (MODY6) (91). In the Caucasian population, MODY3 and MODY2 accounts for more than 80% of all MODY patients, MODY1 is less common, and all the other forms are rare (92). In Japan, however, about 80% of MODY patients cannot be explained by known MODY genes (93).

6.2. Review of MODY genetics in Taiwan

In Taiwan, very few papers related to MODY have been published. Jap et al. sequenced the HNF-1α gene of 15 unrelated loosely defined MODY patients (94). None of previously described causal variants were found. Instead, a novel mis-sense mutation, Y218C, at the DNA binding domain of the HNF-1α gene was found in one patient. It seems that the most common causal variants in Caucasians do not account for most of the MODY patients in Taiwan.

In Chinese population in Hong Kong, Xu et al. collected 146 unrelated families fulfilling the minimum criteria for MODY and screened for mutations of MODY1, MODY2 and MODY3 by direct sequencing (95). The prevalence of MODY3, MODY2 and MODY1 was only 9%, 1% and 0% respectively. Among the 12 MODY3 mutations, 4 had not been reported before this study. They concluded that the majority of Chinese MODY patients are due to defects in unknown genes.

6.3. Summary of the current progress of genetic syndrome of DM

There are more than 50 genetic syndromes related to diabetes mellitus (http://www.ncbi.nlm.nih.gov/sites/entrez?db=omim). These syndromes are usually of Mendelian inheritance. Their phenotypes include not only DM but also many other phenotypes. Most of the time, the phenotype other than DM are much more prominent. Wolfram syndrome also referred to as DIDMOAD (diabetes insipidus, DM, optic atrophy, and deafness) is one of the examples. Mutations in WFS1 gene confers autosomal recessive Wolfram syndrome and autosomal dominant sensorineural hearing loss (96).
6.4. Review of genetic syndrome of DM in Taiwan

Only one report by Tsai et al. described a Taiwanese family with a Y669H (2005T>C) mutation in exon 8 of WFS1 gene (97). The carriers with the mutation had familial nonsyndromic low-frequency sensorineural hearing loss, but did not appear to have DM. Comprehensive review of any genetic syndrome associated with DM is beyond the scope of this article. Moreover, this area is rarely touched by scientists in Taiwan, mainly because they are relatively rare and probably under-diagnosed or under-reported by physicians.

7. PERSPECTIVES

With the advance of genotyping technology, statistic tools and large-size sample collection, genetic study of various forms of DM is, although still complicated, no longer a nightmare. The key point for future genetic study is to increase sample size and marker density. Besides, careful phenotyping to identify a more homogeneous subtype is always beneficial. Furthermore, genetic variants other than SNPs, such as copy number variation or inversion, should also be investigated. Last but not the least, population-specific susceptibility genes and/or alleles are likely to exist and should be vigorously looked for. A good example to justify the last statement is the recent discovery of different associated SNPs in TCF7L2 for T2DM in Taiwan (66) and in Hong Kong (98).

Genetics is exciting because it not only provides tools to test hypotheses, but actually can also generate new hypothesis. Both linkage analysis and genome-wide association (GWA) study can lead researchers to find susceptibility genes/pathways that surprise everybody. The identification of TCF7L2 as a susceptibility gene of T2DM is a good example, which can open a whole new basic research field and might guide treatment choices in the future. The next step ahead of us is to identify all the major genetic and environmental factors for diabetes. At that time, risk prediction will be possible and personalized medicine is no longer a dream.

In 2003, several leading diabetologists in Taiwan from several major medical centers, including National Taiwan University Hospital, Veteran General Hospitals at Taipei and Taichung, Chang-Gung Memorial Hospital at Taipei, and Chang-Hua Christian Hospital were summoned at Academia Sinica (AS) at Taipei for a GWA study of T2DM, a collaborative project of AS with the pharmaceutical giant GlaxoSmithKline. Unfortunately the project was not worked out as planned. The investigators in Taiwan missed a golden opportunity to participate in the international race of GWA study for T2DM. With several GWA studies published (43-45), it does not appear sensible to pursue a GWA study of T2DM in Taiwan. What we need to focus next should be population-based case-control studies of descent sample size to fully elucidate the proportion of genetic risk and the population-specific risk alleles of diabetes, respectively for T1DM, T2DM, MODY, mitochondrial DM. We also need to enhance the awareness of the genetic syndromes associated with DM among physicians and recruit more subjects to investigate the prevalence and mutation spectrum of these syndromes. We also need to investigate the genotype-phenotype correlation in our population. Furthermore, many T2DM genes discovered by GWA studies are of unknown function. Therefore, further molecular, biochemical and physiological investigation of gene functions should be performed.

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**Abbreviations:** DM: Diabetes mellitus; T1DM: type 1 DM; T2DM: type 2 DM; GDM: gestational DM; MODY: maturity onset diabetes of the young; GWA: genome-wide association; VNTR: variable number of tandem repeats; STRP: short tandem repeat polymorphism; RFLP: restriction fragment length polymorphism; DIDMOAD: diabetes insipidus, DM, optic atrophy, and deafness

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