International Journal of Risk & Safety in Medicine 23 (2011) 119–123
DOI 10.3233/JRS-2011-0522
IOS Press

Genetic factors in risk assessment for the development of type 2 diabetes mellitus in a small case series

S. Abou-Hussein[a,*], C. Savona-Ventura[b], S. Grima[b] and A. Felice[a]
[a]*Department of Genetics, Faculty of Medicine and Surgery, University of Malta, Msida, Malta*
[b]*Department of Obstetrics and Gynaecology, Faculty of Medicine and Surgery, Mater Dei University Hospital, Msida, Malta*

Abstract. *Objective*: This study aimed to investigate the role of genetic biomarkers in assessing risk for the eventual development of type 2 diabetes mellitus (T2DM).

Methods: Three Maltese women with a history of previous severe GDM and with apparent similar clinical risk factors underwent anthropomorphic and metabolic reassessment 4–7 years post-partum. They were further genotyped for four specific genetic single nucleotide polymorphisms (SNPs) using the qPCR technique for the alleles of SLC2A2 (rs5393A/C), FTO (rs9939609A/T), PCK (rs2071023C/G) and CDKAL1 (rs10946398A/C).

Results: While the previous obstetric history of all the cases was similar, the biological status was characterized by an increasing degree of obesity correlating to increasing severity of current carbohydrate intolerance. Genotyping showed that all the tested SNPs were homozygous mutant in the T2DM woman and heterozygous in the impaired glucose tolerance woman. The woman with normal glucose tolerance was shown to be wild type for SLC2A2 (rs5393A/C).

Conclusions: There appeared to be an interrelationship between eventual severity of carbohydrate metabolism abnormalities and the genetic allele status. It would appear that the specific allele-scoring can be used to identify further the potential risk of developing T2DM.

Keywords: Type 2 diabetes mellitus, risk factors, previous gestational diabetes mellitus, single nucleotide polymorphism, specific allele-scoring

1. Introduction

In every society there are communities, families and individuals whose chance of future illness, accident or untimely death are greater than those of others. Special vulnerability to illness results from the possession of a number of interacting characteristics that range from genetic to biosocial factors. The ability to measure some of these risks provides a means with which to allow health providers to apply a preventive aspect of health care especially in medical conditions that initially have an insidious onset before full-blown clinical presentation. One such condition is the metabolic syndrome with its wide spec-

*Address for correspondence: Dr. Samir Abou-Hussein, Laboratory of Molecular Genetics, Faculty of Medicine and Surgery, University of Malta, Msida, MSD 2090, Malta. Tel.: +356 25551021; E-mail: samir2001ly@gmail.com.

trum of clinical features that include Type 2 DM [T2DM], hypertension, and lipid profile abnormalities. Unfortunately, assumed risk factors based on "historic" and biological characteristics have been shown to have poor sensitivity and specificity [1].

The Maltese population has been repeatedly shown to have a high prevalence of T2DM that appears to be contributed to in part by environmental [2] and in part by genetic factors [3]. The present study attempts to investigate the possible role of genetic factors being used as supplementary markers for risk assessment in the development of T2DM. The identification of effective supplementary markers would hopefully strengthen the sensitivity and specificity of the currently identified risk factors and enable targeting of dedicated health services to those individuals who really need them.

2. Methods

Three women aged 39–40 years identified from their obstetric history as being at an increased risk of their eventually developing T2DM were investigated 4–7 years after their pregnancy with a 75-gram oral glucose tolerance test and lipid profile to identify their current metabolic status. The metabolic profile of these three women varied from T2DM to normal glucose tolerance [NGT]. The women were further assessed to identify the presence of other current risk factors such as obesity and hypertension. In addition, blood was collected to determine the presence of variations in a series of alleles of genes, in terms of single nucleotide polymorphism (SNPs) that have previously been identified from the published literature and past studies carried out in the present population to be relevant in the involvement in various biological pathways of glucose homeostasis and inflammatory responses, conferring a strong susceptibility to T2DM [3]. The selected specific protein coding genes included: SLC2A2 (Solute carrier family 2-facilitated glucose transporter-member 2) gene, FTO (Fat mass and obesity associated) gene, PCK (phosphoenolpyruvate carboxykinase) gene and CDKAL1 (CDK5 regulatory subunit associated protein 1-like 1). The tested specific correspondent SNPs of these genes included: SLC2A2 (rs5393 A/C), FTO (rs9939609 A/T), PCK (rs2071023 C/G) and CDKAL1 (rs10946398 A/C). The latter methodology as been previously detailed [3]. Ethical approval for the study was obtained from the Ethical Committee of the University of Malta. The women gave their signed consent for the study.

3. Results

The previous obstetric history and the current biological status of the three cases are summarized in Table 1. It would appear that the previous obstetric history is similar in all the three individuals, with the less severe cases actually delivering macrosomic infants in contrast to the individual with T2DM. The anthropomorphic characteristics are markedly different, these differences dating to the gestational period. The biological status of the individuals is characterized by an increasing degree of obesity, as defined by the Body Mass Index (BMI) and waist circumference, with increasing severity of carbohydrate intolerance.

In contrast to the obstetric history and biological factors, the genotyping characteristics of the selected SNPs were however markedly different between the three probands. The case with T2DM has been noted to have simultaneous homozygous mutations in four of the tested SNP genes. In contrast, the case with IGT only showed heterozygosity for the four SNP genes; while the case with normal glucose tolerance only showed heterozygosity for two SNP genes, but had in addition one mutant and one wild type SNP gene (Table 2).

Table 1
Clinical risk factors in the three women with previous GDM

Risk factor Current carbohydrate metabolic status	Case 1 Normal glucose tolerance	Case 2 Impaired glucose tolerance	Case 3 Type 2 diabetes mellitus
Pregnancy data			
Past history of severe GDM [defined as a			
2-hour post-glucose load of ≥11.0 mmol/l]	Yes	Yes	Yes
1. age at diagnosis (years)	31	34	36
Age now (duration after first diagnosis in years)	38 (7)	40 (6)	40 (4)
H/O gestational hypertension	No	No	No
Family history of			
Diabetes	No	Yes	Yes
Obesity	No	Yes	Yes
BMI during last pregnancy [kg/m^2]	20	31	36
Gestational age at delivery [weeks]	39	40	38
Infant birth weight [gm]	4220	4440	3020
Infant head circumference [cm]	36	37	33
Neonatal complications	Hypoglycaemia	Nil	Nil
Current biological data			
Current lipid profile			
Total Cholesterol mmol/l	6.20	5.02	5.70
LDL mmol/l	4.15	2.89	4.04
HDL mmol/l	1.46	1.58	1.07
S.TG mmol/l	1.30	1.20	1.30
Current age [years]	39	41	40
Parity + miscarriages	3 + 0	2 + 0	2 + 1
Current BMI [kg/m^2]	23.7	26.3	37.3
Current waist circumference [cm]	81	98	112
Waist hip ratio	0.87	0.94	0.98
Current blood pressure reading [mmHg]	110/70	120/80	130/90

Table 2
Identified SNP genes in the three women with previous GDM

SNP genes Current carbohydrate metabolic status	Case 1 Normal Glucose Tolerance	Case 2 Impaired Glucose Tolerance	Case 3 Type 2 Diabetes Mellitus
SLC2A2 (rs5393 A/C)	Wild type	Heterozygous	Homozygous Mutant
PCK (rs2071023 C/G)	Heterozygous	Heterozygous	Homozygous Mutant
FTO (rs9939609 A/T)	Heterozygous	Heterozygous	Homozygous Mutant
CDKAL1 (rs10946398 A/C)	Homozygous Mutant	Heterozygous	Homozygous Mutant

4. Discussion

The ability to identify individuals at high risk of developing significant disease conditions has an important public health import since this would provide a means of introducing lifestyle and medical interventions early enough to prevent significant disease progression. Ideally a screening test should be simple, inexpensive, non-invasive, detects all or most of the patients having the disease in question and does not mistakenly detect too high a proportion of normal subjects, subjecting them to intervention unnecessarily. The use of clinical risk factors to determine risk of developing gestational and T2DM in the Maltese as well as in other populations has been previously investigated [1, 4–8]. In the Maltese female population, a relationship to increased risk of developing severe gestational diabetes has been noted for a maternal age greater than 35 years [RR 2.8; specificity 86.4%; sensitivity 38.7%], obesity [RR 3.0; specificity 93.6%; sensitivity 18.9%], previous multiparity [RR 4.1; specificity 97.0%; sensitivity 12.4%] with a history of macrosomia [RR 1.7; specificity 86.7%; sensitivity 25.5%] [1, 4–6]. Observed predictive factors related to an increased risk of developing IGT/T2DM in the Maltese population included a previous history of GDM [RR 5.1; specificity 78.5%; sensitivity 69.6%], age greater than 40 years [RR 2.3; specificity 76.3%; sensitivity 47.8%], obesity [RR 3.1; specificity 77.4%; sensitivity 56.5%], and the association of hypertension [RR 3.1; specificity 93.5%; sensitivity 26.1%] [5, 7]. These clinically-based risk factors have thus been shown to be useful in identifying those patients who may be truly disease free, but are not sufficiently useful when used in isolation to reliably identify those patients who eventually develop diabetes.

The cases described herein illustrate that whereas the clinical picture of all three cases seems approximately identical, except for the degree of obesity, the rate and severity of development of carbohydrate intolerance had varied considerably. It thus appears that the presence of clinical risk factors alone is not sufficiently sensitive to predict the eventual development of diabetes or its rate of development. While all three cases may eventually develop a diabetic state, the rate of development has obviously progressed differently. A more accurate method of risk determination would ensure that restricted health services are timely targeted to those really at risk.

The observations based on the cases described herein confirm that testing for identified alleles can be an added resource to help those individuals at high risk of developing diabetes early. The woman with a high profile of homozygous mutations of specific SNPS appeared to be a greater risk of developing T2DM than her counterparts who were shown to have either; heterozygous or wild genotypes only. Alternatively, the woman with homozygous mutations of these specific SNPs of specific genes may have developed the disease at a faster rate than her counterparts. The true relevance of these reported observations needs to be investigated by formal directed population studies that aim to assess the specificity and sensitivity of using the SNP markers alone and/or in combination with the known clinically-based risk factors. The interplay between the genetic variations also is important factor to trace, as in this study; SLC2A2 (rs5393 A/C) was the most influential when correlated with the development of diabetes, especially when it is associated with a positive family history and obesity, whereas CDKAL1 (rs10946398 A/C) was the least influential. Glucose transporter 2 isoform is an integral plasma membrane glycoprotein of the liver, islet beta cells, intestine, and kidney epithelium. SLC2A2 is a Facilitative Glucose Transporter that is likely to mediate the bidirectional transfer of glucose across the plasma membrane of hepatocytes and is responsible for uptake of glucose by the beta cells because of its low affinity for glucose. It has been suggested that it may comprise part of the glucose-sensing mechanism of the pancreatic beta cell [3]. Since the influence of genetic contributors appears to vary from one ethnic population to another, the relevance of specific SNPs used as risk factors needs to be investigated on a broader scale before being assumed to be of general rather than local usefulness.

5. Conclusions

It thus appears that the added use of the specific allele-scoring in women identified at risk on the basis of their clinical status can be used to further quantify the risk of developing T2DM. This supports other studies which have suggested that knowledge of common genetic variation appropriately reclassifies younger but not older people for type 2 diabetes risk beyond clinical risk factors [9]. Further studies are however necessary on a larger set of the population to confirm the significance of the reported observation. The effective identification of patients at risk of eventually developing diabetes allows for directed resource availability to individuals who really will benefit from the intervention.

Conflict of interest

The authors report no conflict of interest.

References

[1] C. Savona-Ventura, J. Azzopardi and R. Sant, Risk factors for gestational diabetes mellitus in the Maltese population: a population based study, *International Journal Risk and Safety in Medicine* **13** (2000), 1–7.
[2] C. Savona-Ventura, The thrifty diet phenotype – a case for endogenous physiological teratogenesis, In: *Birth Defects*, Engels JV, ed., New Research, Hauppauge, New York Nova Science Publishers, USA, 2006, pp. 183–200.
[3] S.Y. Abou-Hussein, Allele scoring of genetic risk in previous gestational diabetes mellitus. Thesis – Master of Science, University of Malta, Malta, 2009.
[4] C. Savona-Ventura, A.G. Schranz and M. Chircop, Risk factors for gestational impaired glucose tolerance in the Maltese population: a cross-sectional study, *Journal of Obstetrics and Gynaecology* **21** (2001), 591–594.
[5] C. Savona-Ventura, The clinical significance of gestational impaired glucose tolerance in the Maltese population. Thesis – Doctor of Medical Science, National Research Institute of Mother and Child, Poland, 1997.
[6] C. Savona-Ventura, A.G. Schranz and B. Chazan, The clinical significance of gestational impaired glucose tolerance in the Maltese population, *Archives of Perinatal Medicine* **3** (1997), 55–64.
[7] A.G. Schranz and C. Savona-Ventura, Long-term significance of mild gestational diabetes mellitus – a longitudinal study, *Experimental and Clinical Endocrinology and Diabetes* **110** (2002), 219–222.
[8] C. Kim, K.M. Newton and R.H. Knopp, Gestational diabetes and the incidence of type 2 diabetes – a systematic review, *Diabetes Care* **25** (2002), 1862–1868.
[9] J.M. de Miguel-Yanes, P. Shrader, M.J. Pencina, C.S. Fox, A.K. Manning, R.W. Grant, et al., MAGIC investigators, DIAGRAM+ investigators. Genetic risk reclassification for type 2 diabetes by age below or above 50 years using 40 type 2 diabetes risk single nucleotide polymorphisms, *Diabetes Care* **34** (2011), 121–125.