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RESEARCH ARTICLE

ASSOCIATION OF BANK1 GENE POLYMORPHISM WITH TYPE 1 DIABETES IN A SAMPLE OF EGYPTIAN CHILDREN

[1]Elsayed Amer, [1]Soha Abd El-Hady Ibrahim, *,[1]Ola Galal Ali Behairy, [1]Seham Elsayed Ahmad Ali, and [2]Shuzan Ali Mohammed

[1]Department of Pediatrics, Faculty of Medicine, Benha University, Egypt
[2]Department of Medical Biochemistry Faculty of Medicine, Benha University, Egypt

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ABSTRACT

Background: The etiology of human type 1 diabetes is still largely obscure, but it is recognized that both genetic and environmental factors are implicated in its risk.
The aim of the study: To assess the genetic association of BANK1 rs3733197 polymorphisms in a sample of Egyptian children with T1DM and compare it to healthy controls.
Methods: We enrolled 100 children;75 children with T1DM and 25 healthy control children matched for age and sex. Children were subjected to: history taking, clinical examination, measuring fasting serum glucose, glycosylated hemoglobin (HbA_1C), serum total cholesterol and genotyping of BANK1 Alanine383Thereonine SNP (rs3733197) with real- time PCR allele discrimination.
Results: Patients had statistically significant difference regarding allele frequency of BANK 1 (rs3733197) SNP as and it exhibited an increased G allele frequency in diabetic group compared with controls. The allelic association analysis confirmed a significant association with T1DM (OR=2.15, 95% CI = 1.12-4.15), while A allele was decreased in T1DM compared to controls (OR= 0.46, 95% CI = 0.24-0.89). Only the AA genotype of the additive model in the diabetic group was statistically significant decreased as compared with the control group (p=0.022, OR=0.26 and 95% CI = 0.08-0.86). No statistically significant difference regarding other genotype distribution could be detected. Also, there was no statistically significant association between genotypes and alleles of BANK1 rs3733197 SNP with any of the clinical or laboratory data of the study.

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INTRODUCTION

Type 1 diabetes (T1DM) which is a polygenic disease resulting from the interaction between environmental (viral, toxic, nutritional, socioeconomic) and genetic factors (Dorman, 1997). It is more common in children and young adults (Aribi *et al*., 2004). Among Eastern Mediterranean and Middle Eastern countries, Egypt accounts for about a quarter of the region's total; the incidence in Egypt is about 8/100000 per year in children under 15 years (Soltèsz *et al*., 2006). Susceptibility to the inheritance of T1DM occurs through a series of genes, mostly related to HLA class II-locus on chromosome 6. Susceptibility genes are essential, but not sufficient in explaining the immune-pathogenesis of T1DM (Akerblom *et al*., 2002). B-cell scaffold protein with ankyrin repeats 1 (BANK1) gene is located on chromosome 4q24 and codes for BANK1 which is an adaptor protein containing 785 amino acids. BANK1 is a Lyn tyrosine kinase substrate which promotes tyrosine phosphorylation of inositol 1,4,5-trisphosphate receptors. It induces calcium mobilization from intracellular stores upon β-cell receptor activation (Yokoyama *et al*., 2002). The role of β cells as antigen-presenting cells in T1DM is widely accepted. BANK1 is highly expressed in β cells. It activates β cells through the β cell receptor signaling cascade. Therefore, impairment of the function of this gene would lead to a disease-susceptible state (Orozco *et al*., 2009). Functional variants in BANK1 gene has been implicated in genetic predisposition to autoimmune phenotypes as; systemic lupus erythematosus and systemic sclerosis (Rueda *et al*., 2010). rs3733197 (A383T) is located in exon 7 of the BANK1 coding region, its minor allele (A) resulted in alanine to threonine replacement at position 383 (Kozyrev *et al*., 2008).We aimed to assess the genetic association of BANK1 polymorphisms in a sample of Egyptian children with T1DM.

Corresponding author: Ola Galal Ali Behairy,
Department of Pediatrics, Faculty of Medicine, Benha University, Egypt.

MATERIALS

This is a cross- sectional controlled study conducted at the Pediatric Endocrinology Clinic at Benha University Hospital during the period from May 2014 to May 2015. Two groups of subjects were enrolled; group 1 included 75 children with T1DM. Their diagnosis was based on the American Diabetes Association Criteria for T1DM (Genuth *et al*., 2003). Obesity and other forms of diabetes were excluded. Group 2 included 25 clinically healthy children without personal or family history of DM.The study gained approval from the ethical committee of the Faculty of Medicine, Benha University. Informed written consent was obtained from parents or caregivers of enrolled children.

METHODS

All children were subjected to full history taking and physical examination including body mass index (BMI) that was calculated as weight/height^2 (kg/m^2) (height measurements were placed in Frankfurt plane). Peripheral blood samples (2ml) were collected for routine workup, including complete blood count (CBC), glycosylated hemoglobin (HbA1c) (%), blood glucose level (fasting and 2 hours post prandial) (mg/dl) and serum cholesterol (mg/dl). Urine was tested for ketonuria.Abdominal ultrasound was performed to determine liver span and presence of any complication.

Molecular study

DNA was extracted from 200µl EDTA blood; using Purelink® Genomic DNA minikit Cat No. K1820-01 *(Invitrogen, Life Technologies)*. DNA purity and concentration were measured by nanodrop 2000 *(Thermo Fisher Scientific, Wilmington, USA)*. Readings of optical density were taken at wave lengths 260 and 280 nm (Ramadan *et al*., 2014). The ratio of readings at 260 nm and 280 nm provides an estimate of purity of DNA. Pure preparationhad a ratio of 1.7 - 2.0. Real- time PCR detection of BANK1 rs3733197was performed using Taqman 5′ allele discrimination assay *(Applied Biosystem, Foster City, California, USA)*.The PCR mix contained; 2X Taqman Universal PCR master mix, No AmpErase UNG (10µl), 20X Working Taqman SNP assay (1µl), DNA Template (20ng) and up to 20µl Nuclease-free H_2O. Amplification was done in Stepone Real-Time PCR System *(Applied Biosystem, Singapore)*.

The thermal cycling conditions were; pre-PCR Read (1cycle, 15s, 60^{o}C), Amplitaq Gold Enzyme activation (1cycle, 10m, 95^{o}C), cycling (40 cycles: denaturation, 15s, 92^{o}C and anneal/extend, 1m, 60^{o}C). Two no template controls using DNase-free water were run in each analysis. The success rate for genotyping was 100%. There were no deviations from Hardy-Weinberg equilibrium using the Online Encyclopedia for Genetic Epidemiology software (http://www.oege.org/software/hardy-weinberg.html).

Statistical analysis

Data were tabulated, coded then analyzed using the STATA version 11 (STATA corporation, College Station, Texas). Quantitative data were presented as Mean±SD. Student t-test was used to compare between two groups. Pearson's correlation coefficient was used to test the correlation between variables and Chi-square test was used to compare the frequency of qualitative variables among the different groups. The Odd's Ratio (OR) and 95% Confidence Interval (95%CI) were for risk estimation. The level of significance was set at $p<0.05$.

RESULTS

Clinical and demographic data of studied children were shown in Table 1. There was no significant difference between studied groups except for significant increased positive family history of diseasein T1DM ($p<0.001$). Table 2 showed that statistical significant increasein FBS, PPS & serum cholesterol in T1DM ($p<0.001$ for all). Table 3 showed the minor allele frequency (MAF) in T1DM and controls (0.48, 0.30 respectively). Patients had statistical significant increased G allele (p=0.02, OR=2.15, 95%CI=1.12-4.15) but statistical significant decreased A allele (p=0.02, OR=0.46, 95% CI=0.24-0.89) in diabetic children compared to controls. There was statistical significant increase in GG+A Ggenotype distribution of the dominant model (p=0.021, OR=0.29, 95% CI=0.1-0.86) and statistical significant decrease in AA genotype (p=0.022, OR=0.26, 95%CI=0.08-0.86) in T1DM compared to controls. T1DM showed no statistically significant association between rs3733197 genotypes and other variables as shown in Table 4 and 5, except for statistical significant increased diabetic complications in AG genotype (p=0.03, OR=3.5, 95%CI=0.93-13.65).

Table 1. Socio-demographic characteristics of studied children

Variable	T1DM (n.=75)	Controls (n.=25)	Test	P
Age (years)	9.3±2.12	9±1.46	t= 0.56	0.58
Sex (female/male)	33(44)/42(56)	13(52)/12(48)	χ^2= 0.48	0.49
Consanguinity N/Y	46(61.3)/29(38.7)	19(76)/6(24.0)	χ^2= 1.77	0.18
Family history N/Y	31(41.3)/44(58.7)	25(100) /0(0)	χ^2= 26.2	<0.001**

Mean ± SD, n. (%), N: no, Y: yes, **high significant

Table 2. Clinical and laboratory findings of studied children

Variable	T1DM (n.=75)	Controls (n.=25)	t Test	P
BMI (kg/m^2)	21.6±1.63	21.3±1.25	1.07	0.29
Liver span (cm)	10.3±0.74	10.4±0.73	0.43	0.67
FBS (mg/dl)	115±19.23	88.7±11.44	8.24	<0.001**
PPS (mg/dl)	181.3±35.59	105.6±9.09	16.85	<0.001**
Cholesterol (mg/dl)	173.2±40.98	145.2±24.16	4.14	<0.001**
Glycated HbA1c (%)	6.73±0.74	4.88±0.44	6.68	<0.05*
Ketone bodies in urine	75(100)/0(0)	-	-	-
Duration of disease (years)	2.7±1.52	-	-	-
Complications[a]	15(25%)/45(75%)			

Mean ± SD, n. (%),[a]: (fatty liver, muscle spasm, Persistent abdominal pain and Psychiatric depression), **: high significant

Table 3. Genotype and allele frequencies of BANK 1 rs3733197 in studied children

Variable		Diabetics (n.=75)	Controls (n.=25)	χ^2 Test	P	OR(95% CI)
Allelic Model	G (major)	105(70)	26(52)	5.38	0.02*	2.15(1.12-4.15)[c]
	A (minor)	45(30[a])	24(48[b])			0.46(0.24-0.89)[d]
Additive Model	GG	39(52)	9(36)	R	-	1
	AA	9(12)	8(32)	5.21	0.022*	0.26(0.08-0.86)
	AG	27(36)	8(32)	0.21	0.65	0.78(0.27-2.27)
Recessive Model	GG	39(52)	9(36)	R	-	1
	AA+AG	36(48)	16(64)	1.92	0.17	0.52(0.2-1.32)
Dominant Model	AA	9(12)	8(32)	R	-	1
	GG+AG	66(88)	17(68)	5.32	0.021*	0.29(0.1-0.86)

n.(%), [a:] minor allele frequency in patients, [b:] minor allele frequency in controls,R: reference,[c:]: A is the reference, [d:]: G is the reference

Table 4. Association of BANK 1 rs3733197 genotypes with sex, consanguinity and family historyin T1DM children

Genotype	Sex		Test	p	OR(95% CI)
	Females (n.=33)	Males (n.=42)			
AA n.(%)	5(15.2)	4(9.5)	FET	0.49	1.7(0.33-9.30)
AG n.(%)	15(45.5)	12(28.6)	χ2= 2.29	0.13	2.08(0.72-6.07)
GG n.(%)	13(39.3)	26	χ2= 3.75	0.05	0.4(0.14-1.12)
	Consanguinity				
	Positive (n.=29)	Negative (n.=46)			
AA n.(%)	4(13.8)	5(10.9)	FET	0.73	1.31(0.24-6.72)
AG n.(%)	9(31)	18(39.1)	χ2= 0.51	0.48	0.7(0.23-2.07)
GG n.(%)	16(55.2)	23(50)	χ2= 0.19	0.66	1.23(0.44-3.48)
	Family history				
	Positive (n.=44)	Negative (n.=31)			
AA n.(%)	7(15.9)	2(6.5)	FET	0.29	2.74(0.47-28.66)
AG n.(%)	16(36.4)	11(35.5)	χ2= 0.01	0.94	1.04(0.36-3.05)
GG n.(%)	21(47.7)	18(58)	χ2= 0.78	0.38	0.66(0.23-1.83)
	Complications				
	Positive (n.=15)	Negative (n.=60)			
AA n.(%)	1(6.7)	8(13.3)	FET	0.68	0.46(0.01-4.03)
AG n.(%)	9(60)	18(30)	χ^2= 4.69	0.03*	3.5(0.93-13.65)
GG n.(%)	5(33.3)	34(56.7)	χ^2= 2.62	0.11	0.38(0.09-1.43)

Table 5. Association of BANK 1 rs3733197 genotypes with age, disease duration, BMI, liver span, FBS, PPS, Hb and cholesterol in diabetic children

Variable	AA (n.=9)	AG (n.=27)	GG (n.=39)	Test (FET)	p
Age (years)	8.22±2.11	9.37±2.15	9.41±2.09	1.22	0.30
Disease duration (years)	2.17±1.14	3.18±1.94	2.51±1.17	2.31	0.11
BMI (kg/m^2)	21.09±1.29	21.8±1.46	21.63±1.78	0.66	0.52
Liver span (cm)	10.44±0.73	10.39±0.86	10.18±0.66	0.86	0.43
FBS (mg/dl)	114±15.93	116.92±18.13	113.9±20.92	0.21	0.81
PPS (mg/dl)	181.4±28.59	183.8±31.33	179.56±40.2	0.11	0.89
Controlled HbA1c	6.26±0.05	6.4±0.26	6.33±0.26	0.86	0.43
Uncontrolled HbA1c	8.07±0.36	8.1±0.27	7.95±0.12	0.77	0.68
Cholesterol (mg/dl)	168.1±40.5	182.11±52.96	168.2±30.28	1.00	0.37

Mean±SD

DISCUSSION

The genes that encode BANK1 have been associated with a number of autoimmune phenotypes (Chang *et al*., 2009). We assessed the genetic association of BANK1 rs3733197 with T1DM in Egyptian children. The current study was conducted on 100 children; 75 with T1D Mand 25 apparently healthy control children. As regard the socio-demographic characteristics, there was only statistical significant difference ($p<0.001$) between studied groups regarding their family history of T1DM. Sipetic *et al*. found that the risk of T1DM is increased in children and adolescents with positive family history for T1DM or other autoimmune diseases in relatives (Sipetić *et al*., 2002). Bonficaio *et al*. in performing HLA genetic typing, they concluded that the risk of diabetes in children of affected families could be due to a combination of genetic markers and family history in very early life (Bonifacio *et al*., 2004). There was statistical significant difference between the studied groups regarding fasting and postprandial blood sugar and serum total cholesterol, being higher in T1DM.Our finding concerning total cholesterol agree with Emma-Okon *et al*. and Bayır *et al*., who stated that T1DM had higher levels of total cholesterol, LDLc and HDLc compared to controls, although it was statistically non-significant in Emma-Okon *et al*., that may be due to well-monitored diabetic patients (Emma-Okon *et al*., 2010 and Bayır*et al*., 2014). The increased glycated HbA1c in T1DM in our study was in agreement with Ismail *et al*. (2016). In the present study, there was statistically significant difference regarding the allele distribution with increased G allele frequency in T1DM (70%) compared to controls (52%); G allele is a possible risk factor for T1DM in children increasing the risk of T1DM by 2.15 folds. While the A allele showed a statistically significant decrease in T1DM (30%) compared to controls (48%), suggesting its protective role against T1D Mdecreasing T1DM risk by 0.46 fold. As regard genotype of BANK1 rs3733197 SNP, there was statistical significant decrease in AA genotype of the additive model but increased GG+AG genotype in the

dominant model in T1DM compared to controls (p=0.022, 0.021), respectively. Our findings agreed with Zouidi *et al*. as regard G allele and GG genotype distribution; they studied polymorphisms in specific autoimmune- associated genes in T1DM patients from Southern Tunisia. They found that G allele and GG genotype of rs3733197 were significantly increased in T1DM patients compared to controls. They established that BANK1 rs3733197 is a new T1DM genetic susceptibility factor and is an independent signal with T1DM (Zouidi *et al*., 2014). Guan *et al*. and Bobbala *et al*. suggested that individuals with BANK1 functional mutations lead to BANK1 phosphorylation and signaling and might lead to the production of auto-antibodies (Guan *et al*., 2011 and Bobbala *et al*., 2012). BANK1 controls Toll-like receptor 7-mediated-type I interferon production and IgG production, nuclear translocation of interferon regulatory factor 7, activation of signal transducer and activator of transcription 1 during the development of autoimmune disease (Wu *et al*., 2016).

BANK1 rs3733197 was associated with significantly increased complications in T1DM of the AG genotype, but no statistical association between BANK1 rs3733197 genotypes with other demographic, clinical or laboratory finding in diabetic patients. In contrast to our result, Hong *et al*. found that individuals with the minor allele of rs3733197 had significantly increased cholesterol levels (Hong *et al*., 2015). Our results support the finding that G allele may be an independent risk factor for T1DM, on the other hand, the A allele (minor allele) resulting from Ala→Thrat position 383 of BANK1 (rs3733197) appears to be protective for T1DM among Egyptian children. This may participate in explaining the genetic predisposition to T1DM in Egypt but the study is limited by the small sample size.The inclusion of multiple BANK1 polymorphisms on wider scale studies are needed for better assessment and understanding of the possible role of BANK1 gene in the pathogenesis of T1DM in children.

Conclusion

The current study concluded that the A allele of BANK1 rs3733197 Ala383Thr is protective but the G allele is an independent risk factor of T1DM among Egyptian children with significant increased diabetic complications among T1DM of AG genotype.

Conflicting interests

The authors declare that they have no conflicting interests.

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