Genetic Variability Within ADA Gene and Susceptibility to Type 2 Diabetes in Obese Subjects

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Abstract: The complex relationship between adenosine, obesity and Type 2 Diabetes (T2D) prompted us to investigate a possible role of genetic variability within adenosine deaminase (ADA) gene in the susceptibility to T2D in obese subjects. With enzymatic activity ADA contributes to the regulation of adenosine concentration in body fluids and as ecto- enzyme modulates signaling events involving adenosine receptors on cell surface.

Three polymorphic sites within the ADA gene, ADA_1 (exon 1), ADA_2 (intron 2) and ADA_6 (exon6) were examined in 299 subjects from the White population of Rome admitted to the Hospital for cardiovascular diseases. Each site shows two alleles: the alleles with higher frequency are ADA_1*1, ADA_2*1 and ADA_6*2.

Informed consent was obtained by the patients to participate to the study that was approved by the Council of Department.

For the three sites examined the relative risk of T2D in obese subjects (vs non obese) is higher in carriers of the homozygous genotype for the allele with higher frequency. The three loci cooperate to the risk that is low in subjects carrying no homozygous genotype (O.R. 1.25) but it is very high (O.R. 13.240) in subjects carrying the three homozygous genotypes.

From a practical point of view, the study of ADA gene may help to detect obese subjects at high risk of T2D who need active preventive measures. The results point to the importance of further studies on the role of genetic variability within ADA gene on the relationship within obesity and T2D.

Keywords: Obesity, T2D, ADA gene, Enzyme polymorphism, Heterozygote advantage.

INTRODUCTION

Several experimental observations suggest a complex relationship among adenosine, obesity and type 2 diabetes. An increase of adenosine A1 receptor activity is associated with obesity in T2D [1-3] and we have found an association between adenosine deaminase locus 1 (ADA_1) genetic polymorphism and Body Mass Index (BMI) in T2D [4]. Adenosine may increase the sensitivity to insulin in adipose tissue [5-7] and in obese subjects a decrease of A1 receptors number has been reported [8]. Adenosine deaminase (ADA) controls the irreversible deamination of adenosine to inosine contributing to the regulation of adenosine concentration in body fluids.

The biochemical genetic polymorphism of ADA was discovered in 1968 by Harris group [9] who identified by starch gel electrophoresis three common genotypes determined by the presence of two codominant alleles ADA_1*1 and ADA_1*2 at an autosomal locus. ADA_1*1 is more enzymatically active as compared to ADA_1*2.

The structure of ADA gene has been elucidated by Viginton *et al.* in 1986 [10]. The gene is localized on chromosome 20q13.11 and is composed by 12 exons spanning about 32 Kb of DNA. Several polymorphic loci have been identified within exonic and intronic regions of the gene [11]. The possible relationship between this genetic variability and human diseases is at present unknown. Besides the enzymatic function in deamination of adenosine, ADA acts also as ecto-enzyme contributing to the degradation of extra cellular adenosine and interacting with the anchoring proteins CD26 and A_1R facilitates signaling events [12, 13].

In the present note we have studied the role of ADA genetic variability in the relationship between obesity and T2D in a sample of subjects admitted to the Hospital for cardiovascular diseases. The high susceptibility to T2D in obese subjects is well known: many obese subjects, however, do not become diabetic while non obese subjects may become diabetic suggesting that genetic factors have an important role in the relationship between obesity and diabetes. Indeed we have recently reported an effect of p53 codon 72 polymorphism on the susceptibility to Type 2 Diabetes in overweight subjects [14].

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We have examined three single nucleotide polymorphisms (SNPs) within the ADA gene: ADA_1 (Taq 1 site nt 4050-4053) on exon 1 corresponding to the biochemical genetic polymorphism described by the group of Harris in 1968; ADA_2 (Pst 1 site nt 19465-19470) localized on intron 2 and ADA_6 (MluNI site nt 31230-31235) localized on exon 6.

MATERIAL AND METHODS

We have studied 299 subjects from the White population of Rome admitted consecutively to the Hospital for cardiovascular diseases. These patients have been considered also in previous studies [14]. Verbal consent was obtained by the patients to participate to the study that was approved by the council of department. The data have been collected a few years ago, before the establishment of an Ethical Committee. All procedures followed were in accordance with the ethical standards and with the Helsinki Declaration of 1964 and its later amendments.

Genotypes of ADA_1, ADA_2 and ADA_6 were determined by DNA analysis as previously described [15]. Statistical analyses were performed by commercial software (SPSS) [16]. The number of subjects is not exactly the same in all tables because of random loss of some genotype determination among the loci considered. Eta(η) is a measure of the strength of association between two variables. Eta squared (η^2) represents the proportion of variance of dependent variable explained by the independent variable.

RESULTS

Table **1** shows some demographic data on the subjects studied.

Table 1: Demographic Data on the Subjects Studied

	Proportion	Mean ±S.D.
Females	47.4%	
Age (years)		66.7±11.6
BMI		27.4±5.2

Table **2** shows the relative risk to become diabetic in obese subjects vs non obese subjects in relation to homozygosity for ADA_1, ADA_2 and ADA_6 polymorphisms. For all ADA sites the relative risk to become diabetics is higher in homozygous subjects for the more common allele than in carriers of the less frequent allele.

Table 2: The Effect of ADA_1, ADA_2 and ADA_6 Genotypes on the Relative Risk of Diabetes in Obese Subjects vs Non Obese Subjects. Odds Ratio: Diabetes vs non Diabetes / BMI >30 vs BMI ≤ 30

ADA_1 genotypes		
	*1/*1	Carriers of ADA_1*2 allele
N° of subjects	264	35
O.R.	5.81	3.50
95% C.I.	2.93 – 11.56	0.51 – 25.32
η^2	0.125	0.066
ADA_2 genotypes		
	*1/*1	Carriers of ADA_2*2 allele
N° of subjects	187	110
O.R.	8.52	3.28
95% C.I.	3.66 – 20.06	1.13 – 9.59
η^2	0.184	0.056
ADA_6 genotypes		
	*2/*2	Carriers of ADA_6*1 allele
N° of subjects	192	106
O.R.	5.91	4.23
95% C.I.	2.72 – 12.93	1.25 – 14.43
η^2	0.137	0.069

Table **3** shows the relationship between the number of homozygous genotypes for the more frequent allele in the three ADA loci and the relative risk to become diabetics in obese subjects vs non obese subjects. Odds ratio and strength of association between obesity and T2D increases with the number of homozygous genotypes. Three-way contingency table analysis by a log linear model shows a statistically significant interaction among diabetes, BMI and ADA suggesting that the association between obesity and diabetes depends on ADA genotype. The analysis also shows a significant cooperative effect of ADA and obesity on the susceptibility to diabetes: the strength of association between ADA and diabetes, however, is lower as compared to the strength of association between obesity and diabetes.

We have analyzed the effect of sex and age (≤65 years' vs > 65 years) on the relationship between genetic variability of ADA gene and susceptibility to diabetes in obese subjects Odds Ratio diabetics vs non diabetics/ BMI>30 vs BMI ≤ 30 is always higher in carriers of homozygous genotypes in the three loci than in other subjects.

Table 3: The Relationship between Genetic Variability within the ADA Gene and the Relative Risk of Diabetes in Obese vs Non Obese Subjects. For ADA_1, ADA_2 and ADA_6 the More Frequent Alleles are Respectively ADA_1*1, ADA_2*1 and ADA_6*2 and the Homozygous Genotypes are ADA_1*1/*1, ADA_2*1/*1 and ADA_6*2/*2

Number of Homozygous Genotypes for the More Frequent Allele at ADA_1, ADA_2 and ADA_6 Loci				
		0	1 and 2	3
BMI≤ 30	% proportion of diabetics	16.7%	21.1%	8.9%
	Total n°	6	128	89
BMI > 30	% proportion of diabetics	20.0%	47.4%	56.7%
	Total n°	5	38	30
Odds Ratio Analysis				
O.R.		1.25	3.37	13.24
95% C. I.		0.033 - 2.470	1.464 – 7.770	4.284 – 42.438
Chi square test of independence				
X^2		0.412	8.951	27.928
df		1	1	1
P		0.777	0.004	$<10^{-6}$
η^2		0.04	0.07	0.26
Three-way contingency table analysis by a log linear model (for this analysis class 0 has been joined with class 1+2)				
x=ADA; y=diabetes ; z=BMI	G	df	p	
xyz interaction	5.575	1	0.023	
xy independence	7.083	2	0.030	
yz independence	35.977	2	<<0.0001	
Independence of y from x and z	37.180	3	<<0.0001	

DISCUSSION AND CONCLUSION

The data suggest a relationship between genetic variability within the ADA gene and susceptibility to T2D in obese subjects. The susceptibility appears negatively correlated with the number of loci in which is present the variant with low frequency suggesting an heterozygote advantage in the protection of obese subjects from diabetes. The mechanism underlying this advantage has to be elucidated. If confirmed in other clinical settings, our observation could allow to assign a higher risk of diabetes to obese subjects who are homozygous for the major allele in all the three loci of ADA gene.

ADA_1 is an exonic polymorphism associated with quantitative variation of enzymatic activity. ADA_2 is an intronic polymorphism and ADA_6 is an exonic polymorphism characterized by a synonymous substitution, which does not change the protein sequence but could influence tissue specific expression. ADA_2 and ADA_6 could be in linkage disequilibrium with ADA sites directly responsible for the association observed. Indeed the analysis of linkage disequilibrium within the ADA gene performed by Cruciani *et al.* [17] has shown areas of weak association and areas of strong association between pairs of internal markers.

The correlation between obesity and diabetes is stronger in subjects homozygous for the more frequent allele in all the three loci examined than in other subjects. It is possible that the three loci directly or indirectly control different mechanisms involved in the susceptibility to diabetes in obese subjects: the maximum risk is attained when the three mechanisms operate simultaneously.

Further investigations on the role of ADA in the susceptibility to T2D in obese subjects would be rewarding. The limitations of our study are represented by the relatively small number of subjects examined and by the fact that it has been carried out on a sample of subjects admitted to the hospital for cardiovascular diseases and this could have introduced some non-identified bias.

CONFICLT OF INTEREST

All authors declare that they have no conflict of interest.

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