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IMMUNE RELATED DISEASES AND THEIR RELATIONSHIP WITH THE GENETIC VARIABILITY WITHIN THE ADENOSINE DEAMINASE GENE

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Research

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CONFLICTS OF INTEREST
There are no conflicts of interest for any of the authors.

ABSTRACT

Background: Adenosine deaminase (*ADA*) structural gene consists of 12 exons. A number of common variants have been found within the coding and intronic region of the gene: the possible role of this variability on *ADA* function are unknown. In the present study we reexamined our data on a set of immune related diseases [Type 1 Diabetes (T1D), Rheumatoid Arthritis (RA), Endometriosis (ENDO) and Non Diabetic Coronary Artery Diseases (NDCAD)] investigating with a new approach the relevance of *ADA* genetic variability on susceptibility to these disorders.

Methods: Three single nucleotide polymorphisms were considered, namely Taq1 site (ADA_1, nt 4050-4053, exon 1), Pst1 site (ADA_2, nt 19465-19470, intron 2) and MLu NI (ADA_6, nt 31230-31235, exon 6). We reanalyzed the data on 199 children with T1D, on 79 subjects with RA, on 98 women with ENDO, on 136 NDCAD and on 246 healthy blood donors. *ADA* genotype was determined by restriction fragment length polymorphism (RFLP) analysis. The subjects were classified according to the number of homozygous genotypes for the more frequent alleles at the three loci. All subjects were from White population of Rome.

Results: All diseases show a strong excess of subjects with the more frequent genotype in all loci as compared to controls suggesting a protective effect of heterozygosity within the *ADA* gene.

Conclusions: The data suggest that the combination of polymorphic sites within the *ADA* gene influences the susceptibility to some immune related disorders and calls for further studies on the relationship between *ADA* gene structure and immune functions.

KEYWORDS: *ADA* gene ; Coronary Artery Disease; Endometriosis; Rheumatoid Arthritis ; Type 1 Diabetes

INTRODUCTION

The *ADA* gene is composed of 12 exons and is localized on 20q13.11 [1]. Differences among normal sequences have been found both within the coding and intronic regions of gene and a study on linkage disequilibrium has shown areas of weak associations and those of strong associations within pairs of internal markers [2,3].

The presence of a Taq1 site (ADA_1) (nt 4050-4053) at exon 1 corresponds to a functional variation due to a substitution of asparagine for aspartic acid at codon 8.

This mutation represents the basis of the biochemical polymorphism described by Spencer et al [4] with two common alleles ADA_1*1 and ADA_1*2.

Adenosine deaminase catalyzes the irreversible deamination of adenosine to inosine, contributing to the regulation of adenosine concentration in body fluids. Adenosine is a local hormone that influences blood flow and platelet aggregation, increases insulin sensitivity in adipocytes [5-7] and decreases insulin sensitivity in muscle fibers [8,9]. Decreased concentration of adenosine weakens T cell activation while its high concentration increase the activity of adenosine receptors strengthening T cell activation [10,11]. Adenosine is a cardio protective agent [12] and impairment of adenosine related signal transduction contributes to chronic heart failure [13].

ADA acts also as an ecto-enzyme [14,15] contributing to degradation of extracellular adenosine [11] and to the signal transduction through interaction with CD26 and A1R [16].

In the present study we investigated the possible influence of allelic combination within the *ADA* gene on the susceptibility to a set of immune related disorders. Three single nucleotide polymorphisms ADA_1, ADA_2 and ADA_6 were examined [17]. The position of polymorphic sites within *ADA* gene and the restriction enzymes are presented in table 1. ADA_1 is an exonic site involved in the regulation of enzymatic activity. ADA_2 is an intronic polymorphism and ADA_6 is a synonymous substitution, therefore they do not change the protein sequence but could influence tissue specific expression. Moreover, ADA_2 and ADA_6 polymorphisms may be markers of DNA sequences responsible for functional variation in the enzymatic activity and ecto enzymatic functions.

Table 1. Polymorphic sites studied within the *ADA* gene

Name	Position within the gene	Restriction Enzyme
ADA_1	Exon 1 nt 4050-4053	Taq1
ADA_2	Intron 2 nt 19465-19470	Pst1
ADA_6	Exon 6 nt 31230-31235	MluN1

MATERIAL AND METHODS

We studied 199 children with Type 1 Diabetes (T1D), 79 subjects with Rheumatoid Arthritis (RA), 98 women with endometriosis, 136 non diabetic subjects with Coronary Artery Disease (CAD) and 246 healthy blood donors (Controls).All subjects were from the White population of Rome. Data on these subjects have been previously reported [18-22]. An analysis on the role of combination of ADA_1,ADA_2 and ADA_6 loci ,however, has not been previously performed. Informed verbal consent was obtained from the patients or from their mothers to participate in the study that was approved by the Council of our Department. The collection of data was performed 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 ita later amendments. *ADA* genotypes were determined by PCR. Genomic DNA was extracted from venous blood samples collected in NaEDTA using the procedure of Kunkel [23]. PCR amplification was carried out according to Hirschorn [17] as previously described [18].

The three intragenic polymorphisms spanning over about 28 Kb have a known molecular basis and include the presence/absence of a Taq1 site (ADA_1, nt 4050-4053, exon 1), of a PstI site (ADA_2, nt 19465-19470, intron 2) and a MluNI site (ADA_6, nt 31230-31235, exon 6), The PCR volume was 25 µl containing 100ng of DNA, 1.5mM di MgCl2, 2.5 reaction buffer, 5pmoles of prymer, 50 mM dNTP, 2U of Supertherm DNA polymerase. Thirthy cycles were prformed using a DNA Thermal Cycler. 7µl of each reaction was digested in 2U of the specific enzyme according to the manufacturer's direction. Each digestion was resolved in 3% agarose gel in Tris acetate / EDTA buffer at Ph 8.0. Following electrophoresis the gel was stained with ethidium bromide and the fragments were visualized by U.V..

The alleles corresponding to the presence and absence of restriction site were assigned as allele *1 and allele *2 respectively.

Table 2 shows the scheme by which we classified the subjects according to the combination of ADA_1, ADA_2 and ADA_6 genotypes. Subjects carrying three common genotypes were ranked 3 while those carrying only genotypes with the rare alleles were ranked 0.

Determination of genotypes was made in groups of twenty including cases and controls to eliminate errors due to incomplete enzymatic digestion.

Chi-square test of independence was performed using the SPSS package [24].

TABLE 2. SCHEME SHOWING THE CLASSIFICATION OF SUBJECTS ACCORDING TO THE COMBINATIONS OF *ADA1, ADA_2* AND ADA_6 GENOTYPES.

ADA_1	ADA_2	ADA_6	Classification
C	C	C	3
C	C	R	2
R	C	C	
C	R	C	
C	R	R	1
R	C	R	
R	R	C	
R	R	R	0

C= Homozygous for the common allele;
R=Homozygous or heterozygous for the rare allele

Fig 1. The relationship between *ADA* gene heterozygosity and relative risk of immune related diseases.

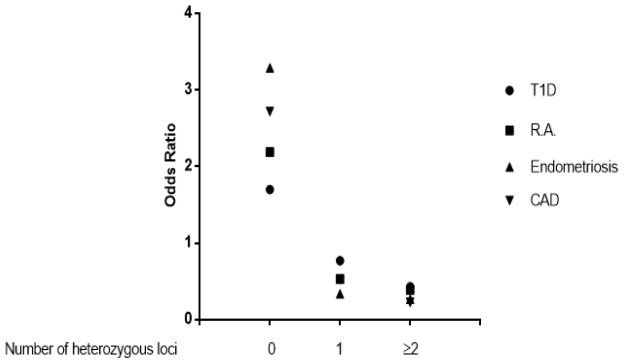

Fig 1.

Table 3. Distribution of total number of common ADA_1, ADA_2 and ADA_6 genotypes in T1D, in Rheumatoid Arthritis, in Endometriosis, in CAD non diabetic and in blood donors (controls).

	Controls (A)	T1D (B)	Rheumatoid Arthritis (C)	Endometriosis (D)	CAD non diabetic (E)
3 common genotypes	20.7%	30.8%	36.4%	46.2%	41.5%
2 common genotypes	36.2%	41.5%	33.8%	27.7%	38.6%
1 common genotype	37.0%	22.1%	28.6%	26.2%	17.0%
No common genotypes	6.1%	5.5%	1.3%	0.0%	3.0%
Total n°	246	195	77	65	135

Chi square test of independence	χ^2	df	p
A vs B	13.324	3	0.004
A vs C	10.413	3	0.015
A vs D	19.847	3	0.0002
A vs E	28.010	3	0.00003
B vs C vs D vs E	17.190	9	0.046

RESULTS

Table 3 shows the distribution of the number of homozygous genotypes for the more frequent ADA_1, ADA_2 and ADA_6 alleles in controls and in the immune related diseases considered. All diseases show a statistically significant difference as compared to controls with a strong tendency towards an excess of homozygotes for the more frequent allele (rank 3). A border line difference (P=0.046) is observed among the diseases studied. Figure 1 shows the Odds Ratio for the diseases considered in relation to the number of homozygous genotypes for the more frequent allele at the three *ADA* loci studied. The odds ratio for all diseases is very high in subjects who were homozygous for the more common allele in all *ADA* loci and decreases with the number of heterozygous loci.

DISCUSSION

The parameter proposed in the present study is an indicator of the number of mutation within the *ADA* gene and of the degree of heterozygosity. This new analysis is explorative: however, the results suggest that further studies in this area could be rewarding.

T1D and RA are autoimmune diseases. Immunological factors may have an important role in endometriosis [25-26] and in CAD [27-28]; furthermore the role of adenosine deaminase in immune function is well known.

We have previously performed association studies of *ADA* gene in T1D, in RA and in CAD. In T1D haplotype differences were observed between T1D children and controls [19]. In CAD differences between patients and controls were also observed [21,22]. Only a slight border line association was observed in RA [18].

The present approach is different from that of our previous studies and seems more productive suggesting that the combination of polymorphic sites within *ADA* gene influences the susceptibility to immune related diseases. The loci investigated could have a role on *ADA* activity and in turn on adenosine concentration. These loci could also influence the function of *ADA* as ecto-enzyme, thereby modulating the interaction of *ADA* with adenosine receptors on the surface of T cells. The association, however, could be due to other loci in linkage disequilibrium with the loci studied.

CONCLUSION

The result suggests that the heterozygosity within the *ADA* gene is protective against immune related diseases

AUTHOR CONTRIBUTION

Gloria-Bottini, Magrini, Bottini have conceived the paper
Manca Bitti and Rapini have collected and analyzed the samples and have contributed to statistical analyses
Saccucci and Neri have carried out laboratoty analyses
Gloria-Bottini and Bottini have directed the statistical analyses
All Authors have approved the final revision of the paper

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