Journal of Genetic Syndromes & Gene Therapy
ISSN: 2157-7412

Gloria-Bottini et al., J Genet Syndr Gene Ther 2013, 4:7
DOI: 10.4172/2157-7412.1000160

Research Article Open Access

Genetic Variability within ADA Gene and Endometrium Cancer

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Abstract

Adenosine Deaminase (ADA) is a polymorphic enzyme that degrades irreversibly adenosine to inosine and is present in cell cytoplasm and in interstitial fluid was it acts as ecto-ADA. Ecto-ADA shows also extra-enzymatic activity acting as co- stimulatory molecule of adenosine receptors .Adenosine is a purine nucleoside that hasan important role in cancer development.In solid tumors high level of this substance is determined by hypoxia resulting in inhibition of T cell killer activation .

We have recently found an association between colon cancer and ADA genetic polymorphism. We studied three polymorphic sites ADA_1, ADA_2 and ADA_6 and found that ADA_1 *2/ ADA_2 *1 haplotype is more represented, while ADA_1 *2/ ADA_2 *2 is less represented in cancer than in controls. ADA_2 *2/ ADA_6 *2 is less represented in patients than in controls. The present note reports a study in endometrium cancer.

We have studied 70 women with endometrium cancer from the White population of Rome.Data on 109 subjects with colon cancer and on 246 blood donors reported in a previous paper are also shown.

The three polymorphisc sites of ADA gene (ADA1, ADA_2 and ADA_6) were analyzed. Genotypes were determined by RFLP-PCR.

Statistical analyses were carried out by SPSS package. Haplotype frequencies are maximum likelihood estimates.

No statistically significant difference is observed in the distribution of ADA haplotypes between the two cancers. In both cancers ADA_1 *2/ ADA_2 *1 haplotype is more represented while ADA_1 *2/ ADA_2 *2 is less represented than in controls. ADA_2 *2/ ADA_6 *2 haplotype is less represented in both cancers than in controls. A border line difference between the two classes of cancers is observed in the distribution of ADA_1/ADA_6 haplotypes.

The present study was suggested by the following: i) High levels of adenosine in cancer inhibits T cell killer activation, ii) ADA contributes to control level of adenosine, iii) Polymorphic sites of ADA may influence extra-enzymatic function of ecto-ADA. Our data confirm the association observed between ADA and colon cancer making unlikely the possibility of a mere sampling chance artifact and suggest that genetic polymorphisms within the ADA gene may have an important role in susceptibility to cancer.

Keywords: Cancer genetics; ADA gene; Endometrium cancer

Introduction

Adenosine is a purine nucleoside that hasan important role in cancerdevelopment of. High level of adenosine is determined by hypoxia in solid tumors resulting in inhibition of T cell killer activation [1]. The intra and extra cellular concentration (about 100 nM) of adenosine is controlled by adenosine deaminase (ADA) and adenosine kinase activities.

Adenosine deaminase (ADA) is a polymorphic enzyme that degrades irreversibly adenosine to inosine and is present in cell cytoplasm and in interstitial fluid was it acts as ecto-ADA. Ecto-ADA shows also extra-enzymatic activity acting as co- stimulatory molecule of adenosine receptors [2]. The polymorphism of ADA was discovered by Spencer et al. [3] and is due to the presence of two codominant alleles ADA_1*1 and ADA_1*2 at an autosomal locus. Correspondingly there are three phenotypes $ADA_1$1, $ADA_1$21, $ADA_1$2 with enzymatic activity decreasing in the order $ADA_1$1>$ADA_1$21>$ADA_1$2.

With the introduction of DNA analysis the structure of ADA gene (chromosome 20) has been elucidated and several intragenic single nucleotide polymorphism have been found [4]. The polymorphism associated to functional variation discovered by Spencer et al. is due to G>A transition at nt 40-52 (exon 1). The role of this extensive variability within the ADA gene has not been yet elucidated. Both enzymatic and extra-enzymatic activities could be influenced by this variability with important effect on susceptibility and clinical course of diseases.

We have recently found an association between colon cancer and ADA genetic polymorphism [5]. We studied three polymorphic sites ADA_1, ADA_2 and ADA_6. The three polymorphic sites spanning approximately 28 Kb can be genotyped using known RFLP-PCR protocols based on the presence/absence of a Taq I site (ADA_1) (nt 4050-4053, exon 1), a Pst I site (ADA_2) (nt 19465-19470, intron 2), and a Mlu NI site (ADA6) (nt 31230-31235, exon 6) respectively. Only the ADA_1 site at exon 1 leads to a functional variation, as the G>A transition at nt 4052 results both in the loss of the Taq I site (TCGA/TCAA) and in the substitution of asparagine for aspartic acid at codon 8. The Asp8/Asn

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Received July 02, 2013; **Accepted** July 11, 2013; **Published** July 13, 2013

Citation: Gloria-Bottini F, Nicotra M, Benedetti-Panici PL, Pietropolli A, Saccucci P, et al. (2013) Genetic Variability within ADA Gene and Endometrium Cancer. J Genet Syndr Gene Ther 4: 160. doi:10.4172/2157-7412.1000160

mutation is the molecular genetic basis for the common biochemical polymorphism at the ADA_1 locus reported by Spencer et al. [3].

In colon cancer we have analyzed ADA haplotypes and found that ADA_1 *2/ ADA_2 *1 haplotype is more represented, while ADA_1 *2/ ADA_2 *2 is less represented than in controls. ADA_2 *2/ ADA_6 *2 is less represented in patients than in controls [1]. The present note reports a study in endometrium cancer that confirms the association observed in colon cancer.

Material and Methods

Subjects

We have studied 70 women with endometrium cancer from the White population of Rome. Clinical and demographic characteristics are described in Table 1. Verbal informed consent was obtained from these women to participate to the study that was approved by I.R.B. Data on 109 subjects with colon cancer and on 246 blood donors reported in a previous paper [5] are also shown.

Determination of ADA genotypes

Three polymorphisc sites of ADA gene: ADA_1, ADA_2 and ADA_6 were analyzed. Genotypes were determined by RFLP-PCR as previously described [5]. Genomic DNA was extracted from venous blood samples collected in NaEDTA. PCR amplification was carried out as described by Hirschhorn et al. [4] with slight modifications. Briefly, the PCR volume was 25 µl containing 100 ng of DNA, 1.5 mM $MgCl_2$, 2,5x reaction buffer, 10 pmols of each primer, 50 mM each dNTP, 2 U of Supertherm DNA polymerase (LPI FISCHER). Thirty cycles (1 min. at 95°C, 1 min and 30 sec. at 65°C, 2 min. at 72°C) were performed using a DNA Thermal Cycler (Perkin Elmer). Sense and antisense primers for the Taq I polymorphism (ADA_1) was respectively:

5'- ACCGAGCCGGCAGAGACCCAC-3'

5'-ACTTGACAGACAGCGAAACTGAGACCCAGA-3'

Sense and antisense primers for the Pst I polymorphism (ADA2) were respectively:

5'- GAGCACAAGCTTTGGAATTGGGCTTGGGTT-3'

5'- ACACCAGGAGGACAAGACTCAGAGGCCCAGAA-3'

Sense and antisense primers for the MluNI polymorphism (ADA6) were respectively:

5'- CATAGCAGTTAGGATTTGAAGACACTGAGCCC-3'

5'- AGGAGACACCATGGTCCCTGGTTCTTGTGAT.

7 µl of each reaction was digested with 2U of the specific enzyme according to manufacturer's instructions. Each digestion was resolved on 3% agarose gel in TAE (Tris/acetate/EDTA) buffer 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 the restriction sites have been signed as allele *1 and as allele *2 respectively. Figures 1-6 show a representative RFLP-PCR picture of the polymorphic sites studied.

Statistical analysis

Statistical analyses were carried out by SPSS package [6,7]. Haplotype frequencies are maximum likelihood estimates (MENDEL program, Dept. of Biostatistics, University of Michigan, Ann Harbor, MI).

Variable	Proportion (%)	Mean ± S.E.
Pregnancies	78.8%	
Abortions	52.9%	
Smoking	23.5%	
Age (years)		61.7 ± 1.09
Menopause (years)		49.9 ±1.15
BMI		28.65 ± 0.63
Duration of menstrual cycle		29.18 ± 0.18

Table 1: Clinical and demographic data on patients with endometrial cancer.

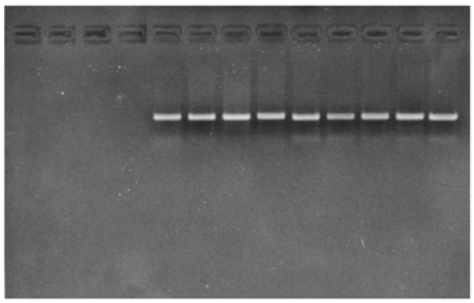

Figure 1: Amplification by PCR from genomic DNA of exons 1 of ADA gene. The fragment of amplification in all subjects is 365 bp.

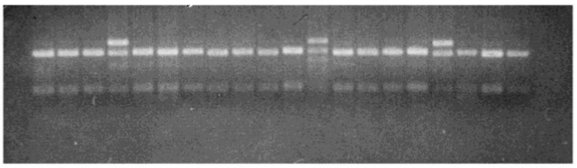

Figure 2: Fragments of amplified ADA1 after digestion with Taq I. Are visible from left to right, respectively, fourth, twelfth and seventeenth line, three heterozygotes ADA1 1-2, others are homozygous ADA1 1-1. In this picture there isn't the homozygous ADA1 2-2. The dimension of bands is 365 bp, 278 bp and 78 bp but the latter is not visible.

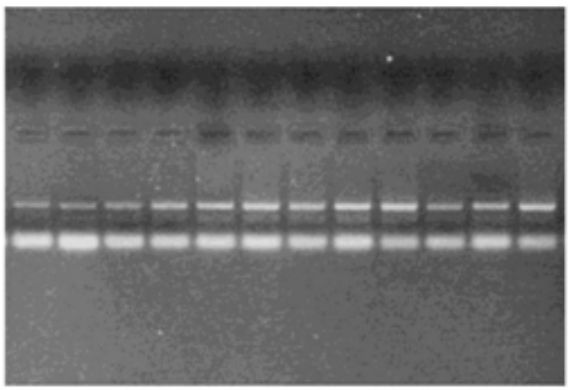

Figure 3: Amplification by PCR from genomic DNA of intron 2 of ADA gene. The fragment of amplification in all subjects is 690 bp.

Results

Table 1 shows demographic and clinical characteristics of the sample study. There is a tendency of overweight and a moderate propensity to smoking in women with endometrium cancer.

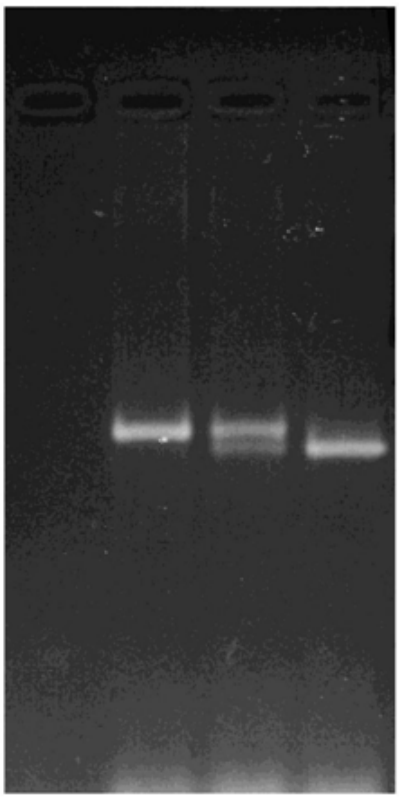

Figure 4: Fragments of amplified ADA2 after digestion with Pst I. Are visible from left to right, respectively, three subjects ADA2 2-2, ADA2 1-2 and ADA2 1-1. The dimension of bands is 690 bp, 635 bp and 65 bp but the latter is not visible.

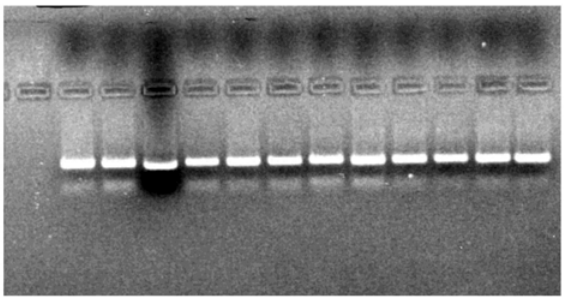

Figure 5: Amplification by PCR from genomic DNA of intron 6 of ADA gene. The fragment ofamplification in all subjects is 371 bp.

Table 2 shows the distribution of ADA alleles in patients with cancer and in controls. In all ADA sites no statistically significant difference is observed between colon and endometrium cancer. No difference is observed in ADA_1 site between cancers and controls. ADA*2 allele of ADA2 site is less represented in cancers than in controls (p=0.053), while ADA*1 allele of ADA_6 site is more represented in cases that in controls (p=0.043).

Table 3 shows the distribution of ADA haplotypes in cancer and in controls. No statistically significant difference is observed in the distribution of ADA_1-ADA_2 and ADA_2-ADA_6 haplotypes between the two cancers. In both cancers ADA_1 *2/ ADA_2 *1 haplotype is more represented than in controls (p=0.03, O.R. 2.25; 95% C.I. 1.07-4.80). On the contrary, in both cancers ADA1 *2/ ADA_2 *2 haplotype is less represented than in controls (p=0.024, O.R. 0.26; 95% C.I. 0.072-0.868). ADA_2 *2/ ADA_6 *2 haplotype is less represented in both cancers than in controls (p=0.013, O.R. 0.56; 95% C.I. 0.354-0.891). A border line difference between the two classes of cancers is observed in the distribution of ADA_1/ADA_6 haplotypes (p=0.062).

Discussion

The present observation confirms the association observed between ADA and colon cancer making unlikely the possibility of a mere sampling chance artifact.

The rationale of the present observation was suggested by: i) High levels of adenosine in cancer inhibits T cell killer activation, ii) ADA contributes to control level of adenosine, iii) Polymorphic sites of ADA may influence extra-enzymatic function of ecto-ADA.

ADA_2 is an intronic polymorphism and ADA_6 is a synonymous substitution so these alterations do not change the protein sequence, but could influence its tissue-specific expression in various cells. Moreover, these sites may not be causal but only markers of DNA sequences responsible for the observed associations.

The degradation of extracellular adenosine could contribute to make killer T cell resistant to inhibitory effect of adenosine. Polymorphic sites of ADA could be involved in the susceptibility to cancer influencing ADA activity and in turn adenosine concentration. A role of genetic variability of ADA gene in the control of activity as ecto-enzyme is also possible: polymorphic sites of ADA gene could influence amino acid sequences involved in the binding of ecto-ADA

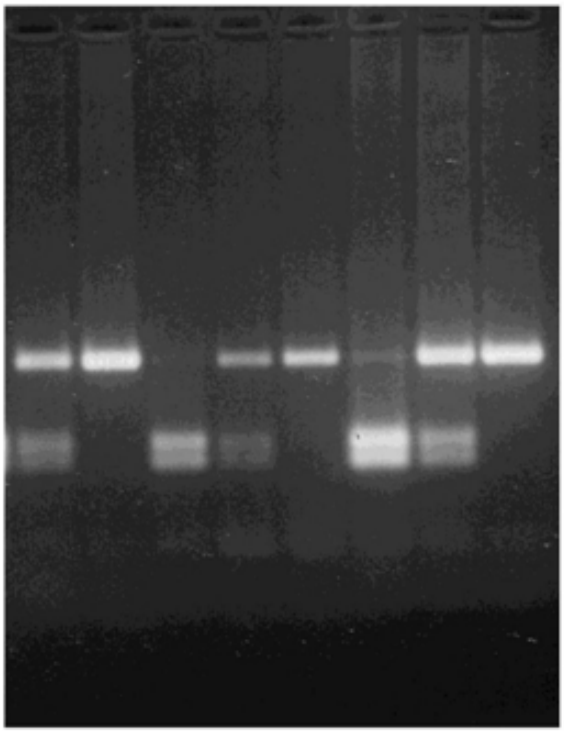

Figure 6: Fragments of amplified ADA6 after digestion with Mlu NI. Are visible from left to right, respectively, three subjects ADA6 1-2, ADA6 2-2 and ADA6 1-1. The dimension of bands is 371 bp, 196 bp and 175 bp.

		Endometrium cancer	Colon cancer	Controls
Loci	Alleles	% Proportion	% Proportion	% Proportion
ADA_1				
	*1	88	92	89
	*2	12	8	11
	Total n°	138	218	492
ADA_2				
	*1	76	78	71
	*2	24	22	29
	Total n°	136	206	492
ADA_6				
	*1	22	25	18
	*2	78	75	82
	Total n°	140	198	490

Table 2: Distribution of ADA alleles in patients with endometrial cancer in patients with colon cancer and in controls.

Loci (Haplotypes)		Haplotypes % proportion				Total n°
		***1/*1**	***1/*2**	***2/*1**	***2/*2**	
ADA_1/ADA_2	Colon cancer	71.7%	20.7%	7.1%	0.5%	198
	Endometrium cancer	66.4%	21.4%	10.1%	2.1%	132
	Controls	69.7%	22.0%	3.9%	4.3%	316
	Chi square test of independence χ^2 df p Colon cancer vs endometrium cancer 3.088 3 0.378 ODDS RATIO ANALYSIS *2/*1 vs other haplotypes/all patients vs controls OR=2.25 95% CI 1.07-4.80 (p=0.030) *2/*2 vs other haplotypes/all patients vs controls OR=0.26 95% CI 0.072-0.868 (p=0.024)					
ADA_1/ADA_6	Colon cancer	20.0%	72.6%	4.7%	2.7%	190
	Endometrium cancer	19.5%	68.7%	2.6%	9.2%	136
	Controls	17.1%	74.2%	3.4%	5.2%	312
	Chi square test of independence χ^2 df p Colon cancer vs endometrium cancer All haplotypes 7.346 3 0.062					
ADA_2/ADA_6	Colon cancer	18.9%	63.5%	7.0%	10.6%	176
	Endometrium cancer	13.3%	62.8%	7.6%	16.3%	134
	Controls	14.0%	58.3%	6.4%	21.5%	272
	Chi square test of independence χ^2 df p Colon cancer vs endometrium cancer 3.181 3 0.364 ODDS RATIO ANALYSIS *2/*2 vs other haplotypes/all patients vs controls OR=0.56 95% CI 0.354-0.891 (p=0.013)					

Table 3: Distribution of ADA haplotypes in patients and controls.

on the surface of the cell with important effect on susceptibility to cancer. The immune suppressive activity of adenosine represents an impediment to immunotherapy, therefore, an increased activity of ecto-ADA could have protective effect against cancer development and evolution. More extensive studies on the relationship between ADA gene variability and cancer are warranted.

A limitation of the present study is represented by the small number of women with endometrium cancer studied.

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