

Relationship between CYP2A6 Genetic Polymorphism, as a Marker of Nicotine Metabolism, and Ulcerative Colitis

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ABSTRACT: **Background:** Ulcerative colitis (UC) is a common and difficult-to-treat disease. In non-smokers the relative risk of developing UC is 2.9 compared with smokers, who tend to have a later onset and a milder disease. Nicotine is the component of cigarette smoke responsible for the favorable effects in UC. Nicotine is metabolized by the enzyme CYP2A6. Subjects who are homozygotes for CYP2A6*4 gene polymorphism are poor nicotine metabolizers, while homozygotes for CYP2A6*1A polymorphism are extensive metabolizers.

Objectives: To compare the frequency of CYP2A6 and CHRNA3 polymorphisms among smokers and non-smokers with UC, and their effect on disease severity.

Methods: Data on the age at onset of disease, disease activity, and treatment were obtained from questionnaires completed by the 69 subjects in our study group. CYP2A6 *1A,*4A and CHRNA3 polymorphisms were determined by polymerase chain reaction and restriction enzyme analysis.

Results: Nine percent of the patients were current smokers, 30% were former smokers and 61% non-smokers. Among smokers and former smokers 63% were homozygotes for CYP2A6*1A and 4% were homozygotes for CYP2A6*4A, whereas among non-smokers 66% were homozygotes for CYP2A6*4A ($P < 0.0001$). There was no significant effect of CYP2A6 or CHRNA3 genotype on UC activity.

Conclusions: We found a very high proportion of poor nicotine metabolizers among non-smoking patients with UC and a very low proportion among current and former smokers, making it difficult to determine the effect of poor metabolizer genotype on disease activity in smokers with UC. However, it may be possible to identify UC patients who are poor metabolizers of nicotine and who may benefit from nicotine or nicotine-like pharmacological treatment.

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degree of protection against UC. Since then numerous studies have confirmed this observation [2]. Non-smokers were found to have a relative risk of 2.9 of developing ulcerative colitis compared to smokers, whereas former smokers had a risk of 1.64 compared to non-smokers [3]. Smokers had a later age at onset of UC and a milder form of the disease [2]. Smoking cessation was associated with a relapse of UC, whereas patients with UC who started smoking after the diagnosis had a reduced relapse rate [4]. The mechanism involved in the risk reduction of UC and disease modulation by smoking is not clear. Cigarette smoking has been associated with alterations in immunoregulatory T cells as well as cytokine profiles [5]. However, no effect of smoking was found in patients with systemic lupus erythematosus; in patients with Crohn's disease cigarette smoking is associated with worse outcome [6,7]. Another possible mechanism for UC disease modulation by smoking is the effect of cigarette smoke on gut motility and colonic mucus production [8,9].

Cigarette smoke is composed of volatile and particulate phases that contain more than 3500 different compounds. Nicotine is the major compound in the particulate phase. Nicotine is responsible for the psychoactive effects of cigarette smoking and has many cardiovascular effects, but not all the effects of cigarette smoking are caused by nicotine. However, there is evidence that nicotine is the compound responsible for the effects of cigarette smoking on UC. Several studies compared transdermal nicotine to placebo in active disease and found that 39–50% of the patients treated with nicotine had remissions compared to about 9% on placebo [10]. Another study compared transdermal nicotine to oral prednisolone in active disease and found no significant differences between the two treatments [11]; nicotine was also found to be effective as an alternative to prednisolone when added to sulfasalazine [12].

Although the exact mechanism of action of nicotine in UC is not clear, there are several effects of nicotine that may contribute to the amelioration of symptoms in these patients. Transdermal nicotine was associated with reduced interleukin-8 production in patients with UC [13]. Nicotine also affects motility of the intestine and reduces smooth muscle activity; this effect is probably mediated by nitric oxide [9]. Nicotine acts on nicotinic cholinergic receptors. Nicotinic subtype alpha-3 receptors have been found in colonic mucosa

Ulcerative colitis is a common gastrointestinal disease that is difficult to treat effectively. The relationship between cigarette smoking and UC was first reported in 1982 by Harries et al. [1]. They found that smoking confers some

[14] and polymorphisms in the untranslated region of the CHRNA3 gene have been described [15].

In mammals nicotine is primarily metabolized to cotinine [16]. The enzyme responsible for the conversion of nicotine to cotinine is CYP2A6 [17]. In humans large inter-individual differences in nicotine metabolism have been shown, and there are reports of individuals who are totally deficient in cotinine formation [18]. Several polymorphisms in CYP2A6 resulting in deficient enzyme activity have been described and the relationship between nicotine metabolism and CYP2A6 genetic polymorphisms has been demonstrated [19,20]. Subjects who are homozygotes for CYP2A6*4 are completely deficient in cotinine formation; the heterozygotes for the CYP2A6*4 allele tend to show a lower capacity for cotinine formation in contrast to homozygotes for CYP2A6*1A who have a higher capacity to produce cotinine [19].

So far, several clinical implications of CYP2A6 polymorphisms have been described. Individuals with reduced or deficient activity of CYP2A6 are less prone to smoking, and if they smoke they smoke fewer cigarettes and are less addicted to nicotine than individuals with the wild-type enzyme [20].

The suggested mechanism is reduced clearance of nicotine with the subsequent reduction of nicotine intake. Since cigarette smoking, and in particular nicotine, has been shown to modify the disease in UC, it is possible that 2A6 polymorphisms may play a role in disease modulation in smokers with UC, and it may also help identify patients who may benefit from nicotine-based therapy. Polymorphisms in the CHRNA3 gene may also have a role in the response to nicotine treatment. The aim of the present study was to compare the frequency of CYP2A6 and CHRNA3 polymorphisms among smokers and non-smokers with UC, and to examine their effect on disease severity.

PATIENTS AND METHODS

We recruited 69 patients with UC from the Inflammatory Bowel Disease clinic at Shaare Zedek Medical Center in Jerusalem. Information regarding the age at onset, disease activity and treatment was obtained from questionnaires completed by the subjects. The smokers in the study group completed the Fagerstrom Test for Nicotine Dependence questionnaire [21]. Disease activity was defined by a yes/no answer to the question "is your disease currently active," the number of stools per day, and the presence of bloody stools. We recorded the use of immunosuppressants and steroid drugs currently (at the time of the blood sampling) and within the previous year.

GENOTYPING

DNA was isolated from peripheral lymphocytes by a salt extraction method [22]. All the samples were analyzed for the polymorphisms CYP2A6*1 and CYP2A6*4 [Table 1] and CHRNA3.

Table 1. Demographic data of the patients

	Smokers (N=6)	Former smokers (N=21)	Non-smokers (N=43)
Age (yrs)	29 ± 5	49 ± 17	38 ± 13
Male (N)	3	16	18
Ashkenazi origin (N)	3	6	18

For CYP2A6, polymerase chain reaction was applied to all the samples using the following primers: forward 5'-CACCGAAGTGTACCCTATGCTG-3' and reverse 5'-AAAATGGGCATGAACGCCC-3'. The PCR product was double digested with Eco8I (BSTU1, Fermentas, UK) and ACCII (BSH12361, Fermentas) restriction enzymes [19]. The CYP2A*1A yielded 800,433 and 104 base pair fragments and the CYP2A6*4 yielded 759, 285,149,104 and 41 base pair fragments.

CHRNA3 IVS3-5 T>C polymorphism was determined by performing Taq1 (Fermentas) digestion [23] of the PCR product using the following primers: forward GGAAGTAAAACCGGCTGATTC and reverse AAGGCCAGGTTTTAAGCACAGT.

STATISTICAL ANALYSIS

Data were expressed as mean ± standard deviation. ANOVA was used for statistical comparison between groups. Differences were considered significant at $P < 0.05$. For the genotype analysis smokers and former smokers were analyzed as one group.

RESULTS

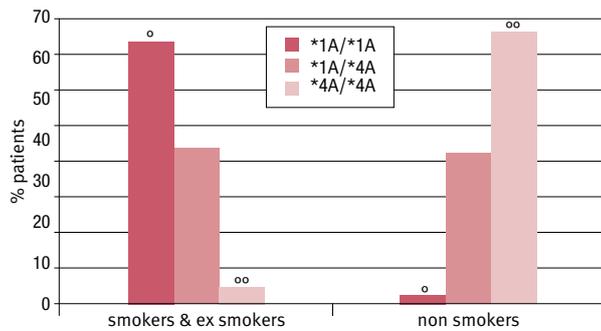
Demographic data are presented in Table 1. Of the 69 patients 9% were current smokers and 30% were former smokers. The smokers and former smokers had a significantly later age of disease onset compared to non-smokers (35 ± 15 vs. 28 ± 11 , $P = 0.03$). No statistically significant differences were found in current disease activity and steroid or immunosuppressive drug use among smokers, former smokers and non-smokers [Table 2]. However, the number of smokers was very small.

There was a striking difference in the prevalence of CYP2A6 genotypes between smokers and former smokers compared to non-smokers: 63% of smokers and former smokers were homozygotes for the *1A allele (extensive metabolizers of

Table 2. Clinical parameters of the patients

	Active disease (%)	Steroid use (%)	Immunosuppressive drug use (%)
Smokers	30	30	60
Former smokers	57	52	28
Non-smokers	68	36	36

Figure 1. CYP2A6 genotype in smokers and non-smokers



*1A/*1A = extensive nicotine metabolizers
 *1A/*4A = intermediate nicotine metabolizers
 *4A/*4A = poor nicotine metabolizers
 ° $P < 0.0001$
 °° $P < 0.0001$

Table 3. CYP2A6 genotypes in relation to clinical parameters

Genotype	Active disease (%)	Steroid use (%)	Immunosuppressive drug use (%)
*1A/*1A	58	66	66
*1A/*4A	73	45	36
*4A/*4A	58	34	34

*1A/*1A = extensive metabolizers of nicotine
 *1A/*4A = intermediate metabolizers of nicotine
 *4A/*4A = poor metabolizers of nicotine

nicotine) versus only 2% of non-smokers ($P < 0.0001$). Only 4% of the smokers and former smokers were homozygotes for the *4A allele (poor metabolizers of nicotine) compared to 66% of non-smokers ($P < 0.0001$). No significant difference in the prevalence of heterozygotes (intermediate metabolizers of nicotine) was found between the two groups [Figure 1].

There was no correlation between the CYP2A6 genotypes and the age at disease onset, current disease activity or treatment with steroids or immunosuppressive drugs [Table 3].

There was no significant difference in the CHRNA3 genotypes between smokers and former smokers compared to non-smokers. There was no correlation between CHRNA3 genotypes and any of the clinical parameters of UC. There was also no significant correlation between the CYP2A6 and CHRNA3 genotypes and the ethnic origin of the patients in our group.

DISCUSSION

The aim of our study was to compare the frequency of CYP2A6 and CHRNA3 polymorphisms among smokers and non-smokers with UC, and to examine their effect on disease severity. We demonstrated a striking difference in the prevalence of CYP2A6 genotypes between smokers (current

and past) and non-smokers with UC: 63% of smokers were homozygotes for *1A allele (i.e., extensive metabolizers of nicotine) and only 4% were homozygotes for the *4A allele or were poor metabolizers. The reverse was found for the non-smokers. Because of the very small number of poor metabolizers of nicotine among the smokers it was impossible to determine whether this phenotype confers an additional benefit from smoking on disease activity by allowing higher nicotine plasma levels derived from smoking.

Ethnic differences in allele frequencies of CYP2A6 have been described [24,25]. Nakajima et al. [25] reported that 9% of Caucasians had alleles associated with reduced enzymatic capacity for nicotine metabolism compared to 21.9% of Blacks and 50% of Japanese. Among Caucasians, only 7% of current smokers were poor metabolizers of nicotine compared to 12% among non-smokers [24]. Our study group included Caucasians of different ethnic origins (46% Ashkenazi Jews and 54% Sephardic Jews*). Although genetic differences among these two groups are well known, the differences in CYP2A6 allele frequencies have not been investigated. We found a similar frequency of poor metabolizers among smokers compared to the finding by Schoedel et al. [24] (4% vs. 7%, respectively). The striking difference in the frequency of poor metabolizers among non-smokers (66% vs. 12%) could be explained by the relatively small group or the different ethnic origin. This difference is even more significant since we investigated only the CYP2A6*4A allele, whereas Schoedel's team investigated four different alleles resulting in a poor nicotine metabolizer phenotype.

We found that smokers and former smokers had a significantly later age of disease onset compared to non-smokers, as previously described [2,4]. However, in our group there was no significant difference between smokers and non-smokers in current disease activity, or in steroid and immunosuppressant drug use. This finding may be explained by the very small number of current smokers in our group.

On examining the effect of CYP2A6 genotypes on disease activity in the whole group of UC patients, we did not find a correlation. CYP2A6 is responsible for the metabolism of nicotine, which is thought to affect the disease activity in UC by altering cytokine production and intestinal motility. Since the majority of our patients were not current smokers and thus were not exposed to a significant amount of nicotine, we could not demonstrate an independent effect of CYP2A6 genotypes on ulcerative colitis activity.

Altered intestinal motility may have a role in UC pathogenesis. Acetylcholine is the main neurotransmitter in the gut, and cholinergic nicotinic receptors of alpha-3 subtype were identified in the colonic mucosa. Nicotine, as well as acetylcholine, is an agonist of the CHRNA3 receptors. Thus,

*Ashkenazi refers to Jews of European descent, and Sephardic to Jews of North African or Middle Eastern origin

CHRNA3 polymorphisms may be expected to affect disease activity not only through nicotine, but also directly. However, in the present study no correlation between CHRNA3 genotypes and disease activity was found.

We noted a very high proportion of poor nicotine metabolizers among non-smoking patients with UC and a very low proportion among current and former smokers. Therefore, we could not determine the effect of poor metabolizer genotype (CYP2A6*4A homozygotes) on disease activity in smokers with UC. However, it may be possible to identify UC patients who are poor metabolizers of nicotine and who may benefit from nicotine or nicotine-like pharmacological treatment. Further studies of CYP2A6 genotypes among different ethnic groups are warranted.

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“The happiness of the bee and the dolphin is to exist. For man it is to know that and to wonder at it”

Jacques Cousteau (1910-1997), French naval officer, explorer, ecologist, filmmaker, innovator, scientist, photographer, author and researcher who studied the sea and all forms of life in water. He co-developed the aqua-lung, pioneered marine conservation and was a member of the Académie française

“Education costs money, but then so does ignorance”

Sir Claus Moser (born 1922), Garman-born British statistician

“Human beings, who are almost unique in having the ability to learn from the experience of others, are also remarkable for their apparent disinclination to do so”

Douglas Adams (1952-2001), English writer and dramatist. He is best known as the author of *The Hitchhiker's Guide to the Galaxy*, which started life in 1978 as a BBC radio comedy before developing into five books that sold over 15 million copies in his lifetime, a television series, several stage plays, comics, a computer game, and in 2005 a feature film