TP53 codon 72 Arg/Arg polymorphism is associated with a higher risk for inflammatory bowel disease development

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AIM: To investigate the association between tumor protein 53 (TP53) codon 72 polymorphisms and the risk for inflammatory bowel disease (IBD) development.

METHODS: Numerous genetic and epigenetic drivers have been identified for IBD including the TP53 gene. Pathogenic mutations in TP53 gene have only been reported in 50% of colorectal cancer (CRC) patients. A single nucleotide polymorphism (SNP) in the TP53 gene resulting in the presence of either arginine (Arg)
or proline (Pro) or both at codon 72 was shown to alter TP53 tumor-suppressor properties. This SNP has been investigated as a risk factor for numerous cancers, including CRC. In this study we analyzed TP53 codon 72 polymorphism distribution in 461 IBD, 181 primary sclerosing cholangitis patients and 62 healthy controls. Genotyping of TP53 was performed by sequencing and restriction fragment length polymorphism analysis of genomic DNA extracted from peripheral blood.

RESULTS: The most frequent TP53 genotype in IBD patients was Arg/Arg occurring in 54%-64% of cases (and in only 32% of controls). Arg/Pro was the most prevalent genotype in controls (53%) and less common in patients (31%-40%). Pro/Pro frequency was not significantly different between controls and IBD patients.

CONCLUSION: The data suggests that the TP53 codon 72 Arg/Arg genotype is associated with increased risk for IBD development.

Key words: Inflammatory bowel disease; Colorectal cancer; 72 codon single nucleotide polymorphism; Tumor protein 53; rs1042522

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Core tip: Tumor protein 53 (TP53) codon 72 polymorphism distribution was analyzed by RFLP in 461 inflammatory bowel disease (IBD), 181 primary sclerosing cholangitis patents and in 62 healthy controls. The data suggests that the TP53 codon 72 Arg/Arg genotype is associated with increased risk for IBD development.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic intestinal disease characterized by gastrointestinal (GI) inflammation resulting in abdominal pain, chronic diarrhea and weight loss[1]. It is highly prevalent across the world with North America having the highest frequency of people suffering from Crohn’s disease[2] (CD, a subtype of IBD). Moreover, about 129000 Canadians suffer from Crohn’s disease[2] with Alberta having the highest per capita increase within Canada. Chronic inflammation is associated with malignancy and the early onset of IBD increases the risk of colorectal cancer (CRC) later in life. In fact, for every 10 years with IBD, colorectal cancer risk increases by 5%[3]. Studies have shown that the risk of IBD progressing to CRC in less than 20 years once IBD diagnosis is made is about 10%-20%[4,5]. The incidence of CRC in IBD patients is six times higher than that of the healthy population, with CRC accounting for about 15% of IBD related deaths[6]. IBD subtypes include ulcerative colitis (UC) and CD. The simultaneous occurrence of primary sclerosing cholangitis (PSC, defined as inflammation and subsequent obstruction of bile ducts both inside and outside of the liver) and UC was reported to increase chances of developing CRC by up to 25% in 10 years[7,8].

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in Canada with 24400 Canadians diagnosed with colorectal cancer and 9300 expected to die from it in 2014[9]. The sporadic form of CRC and IBD-related CRC have very few clinical differences, however a two fold higher rate of mortality is reported in the later form[10,11]. In the four step pathway to CRC tumorigenesis[12], TP53 inactivation usually occurs at a later stage leading to the transition into a carcinoma and represents a major driver event in CRC[12]. The TP53 tumor suppressor gene, located at 17p13, encodes a transcription factor that regulates expression of genes involved in numerous biological processes, such as cell cycle control, DNA repair, apoptosis[13]. Mutations in the TP53 gene have been reported in 50% of CRC cases that mainly lead to the loss of transcriptional control[14]. Mutations in the TP53 gene affect mainly five “hotspot” amino acid positions: 175, 245, 248, 273 and 282.

Over 200 single nucleotide polymorphism (SNP)s have been identified in the TP53 gene[15]. The most studied is codon 72 polymorphism (rs1042522) encoding either arginine (CGC, Arg) or proline (CCC, Pro). Codon 72 encodes an amino acid located in the proline rich region of TP53 (amino acids 64-92) whereby the proline constitutes one of the five PxxP motifs important for interactions with proteins involved in apoptosis and growth suppression[16]. Arg has never been found in primates other than humans[17] therefore Pro is considered the ancestral allele. The Pro allele was found to be more frequent in Africa as Arg is most prevalent in Europe suggesting that selection (most possibly UV and winter temperatures[18]) act on TP53 creating a latitudinal allele frequency gradient. Therefore, codon 72 polymorphisms will have an effect on TP53 function.

The two resulting TP53 polymorphisms (encoded by codon 72) differ in their biological and biochemical properties, such as electrophoretic mobility[16,19,20], subcellular localization[21-23], ability to induce apoptosis[16,24,25], ability to suppress transformed cell growth[16], susceptibility to HPV E6 degradation[26,27] and transcriptional activation[16,20,28,29]. Please see Table 1 for a summary of the biological properties of TP53 Arg vs Pro TP53. Furthermore, the frequency of either allele (Arg or Pro) is highly affected by ethnicity.
One study reported that the frequency of the proline allele increased from 17% in Swedish Saamis to 63% in Nigerian African Blacks[30]. This dictates the importance of comparing patients and controls of the same ethnic group in codon 72 polymorphism association studies.

The association between the 72 codon polymorphism and cancer risk has been studied extensively for many cancers, including CRC[31-40]. With only two papers studying association of 72 codon polymorphism and UC development[36,41], there has been no studies to associate codon 72 polymorphism with CD and PSC susceptibility. The purpose of this study is to investigate a possible link between the 72 codon polymorphism (rs1042522) and the risks of developing IBD and thus IBD-induced colorectal cancer.

MATERIALS AND METHODS

Cases and controls

Blood samples were collected at University of Alberta Adult and Pediatric Gastroenterology Clinics for IBD patients and at the University of Calgary for PSC and PSC/UC patients. The non-IBD control group was composed of 62 healthy blood donors from the same geographical region with no prior history of irritable bowel syndrome (IBS), IBD or cancer. The study was approved by the human ethics research board under the protocol No. Pro00001523 (study No. RES1598) and informed consent was obtained from all patients and controls before samples were analyzed. The diagnosis of IBD patients for this study was based on clinical, endoscopic and pathology findings as well as imaging findings on magnetic resonance or CT enterography for small bowel disease. Video capsule endoscopy was used to assess the small bowel in those with suspected IBD but have equivocal findings on endoscopy and imaging. Infectious causes were screen for and excluded. All pediatric patients were also screened for tuberculosis. In those under the age of 7, common immunodeficiency syndromes such as common immune variable deficiency, chronic granulomatous disease and mutations in IL-10 signalling pathway were screened for and excluded. The diagnosis of PSC was made on the basis of raised serum alkaline phosphatase levels and typical cholangiographic finding on ERC or MRC[18].

DNA extraction

Genomic DNA was extracted from peripheral blood collected by venous puncture. Blood was collected into PAXgene Blood DNA Tubes (QIAGEN, Country of Origin, Germany) and kept in a fridge at +4 °C until DNA extraction (1-2 d). DNA was extracted using PAXgene Blood DNA Kit (QIAGEN, Country of Origin, Germany) according to the manufacturer’s instructions. Polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) analysis of codon 72 was used to identify TP53 genotypes.

Polymerase chain reaction

The forward primer used was P329 5'-TGCCTCTTTCACCCATCTAC-3', and the reverse primer was P330 5'-ATACGGCCAGGCATTGAAGT-3' (IARK protocol, 2010 update)[43]. Each PCR reaction contained 1 x OneTaq® Standard Reaction Buffer (NEB), 0.3 µmol/L of each primer, 3 µmol/L of each dNTP, 100 ng genomic DNA, 0.1U OneTaq® Hot Start DNA Polymerase (NEB) in a final volume of 8 µL. The PCR conditions were as follows: denaturation at 94 °C for 4 min, followed by amplification for 40 cycles at 94 °C for 30 s, at 56 °C for 15 s, 68 °C for 40 s, with final extension at 68 °C for 10 min. The PCR products were checked on an agarose gel first 3 times and PCR appeared to work robustly, so for the rest of the analysis gel electrophoresis of PCR products was omitted.

RFLP analysis

Eight microlitres of PCR product was digested with 2 U of BstUI at 60 °C for at least 4 h. DNA fragments were run on a 2% agarose gel stained with ethidium bromide. The Arg allele was cleaved by BstUI, yielding two fragments 213 and 140 bp. The Pro allele was not cleaved by BstUI and had a single 353 bp band.
The allele and genotype frequencies of TP53 are summarized in Tables 2, 3 and Figure 2. The observed genotype frequencies did not deviate significantly from the Hardy-Weinberg equilibrium ($P = 0.6543$ for cases; $P = 0.2856$ for controls). We found that the allele distribution differed significantly between cases and controls. In particular, the frequencies of Arg and Pro alleles were 75.9% and 24.1% in IBD patients vs 58.9% and 41.1% in healthy controls ($P < 0.0001$, $\chi^2$ test, Table 2). This suggests that the TP53 codon 72 polymorphism may serve as a predisposing factor for IBD development.

The genotype for TP53 72 Arg/Arg tended to predominate in all patient groups compared to controls. In control samples utilized in this study, the genotype distribution for TP53 polymorphism revealed 32.3%, 53.2% and 14.5% for Arg/Arg, Arg/Pro and Pro/Pro genotypes, respectively. Allelic frequencies corresponded to 0.59 for the arginine and 0.41 for the proline allele (Tables 2 and 3). On the other hand, 56.9% of all cases were Arg/Arg, 38% were Arg/Pro
and 5.1% were Pro/Pro. The arginine allelic frequency was 0.76 and the proline allele frequency was 0.24. Significant differences between cases and controls were found for the Arg/Arg genotype compared with grouped Arg/Pro and Pro/Pro genotypes (Table 3). Pediatric and adult IBD patients did not show statistically significant differences for the presence of TP53 SNPs. To our knowledge, the 72 codon polymorphism distribution has never been studied in PSC patients. Our results indicate that PSC had higher Arg/Arg percentage compared to IBD groups, but it may be due to a small sample size effect ($n = 42$).

**DISCUSSION**

The healthy population utilized in this study was mainly composed of Caucasian individuals of Eastern European origin form the Edmonton area and Northern Alberta. Surprisingly, the TP53 72 codon polymorphism was different from the genotype distribution observed in controls in Europe (Slovac Republic, Sweden, Italy, Germany, for references please see Figure 3). Patients from Greece had TP53 72 codon polymorphism distribution most similar to our healthy controls. These data demonstrate the importance of careful selection of controls for genetic understanding of disease states. Although these differences were observed, we have used the controls within our geographic zone for the purpose of this study.

Several published articles have provided evidence that TP53 polymorphism at codon 72 may be associated with tumor development, although results are varied. Consistent with our results, it was shown that 72Arg is associated with higher CRC risk in Argentina[31], Greece[32], Germany[33] and Iran[34]. On the contrary, 72Pro has been detected as an indicator of higher risk of CRC in Malaysia[35], Turkey[36] and in one ethnic Kashmiri population[37]. At the same time, studies conducted in Japan[38], Korea[39] and United States[40] failed to link 72 polymorphism to CRC development. The varied nature of these findings could be explained by the differences in ethic distribution of 72 codon alleles through the world. It has been shown that 72Arg frequency increases from south to north and even then, the 72 codon polymorphism distribution can differ substantially between the neighboring countries (Figure 3).

**Table 3**  Genotype frequencies of TP53 polymorphism in cases and controls

| Patients/controls | Genotype, % ($n$) | OR $^1$ | 95%CI | $P$ value |
|-------------------|-------------------|---------|-------|-----------|
|                  | Arg/Arg           | Arg/Pro | Pro/Pro |           |
| Cases ($n = 642$): | 56.9 (365)        | 38.0 (244) | 5.1 (33) | 2.77 | 1.59-4.82 | 0.0001 |
| UC adult ($n = 151$) | 54.3 (82)        | 38.4 (58) | 7.3 (11) | 2.50 | 1.34-4.65 | 0.0102 |
| CD adult ($n = 138$) | 55.8 (77)        | 40.6 (56) | 3.6 (5) | 2.65 | 1.41-4.98 | 0.0011 |
| UC pediatric ($n = 78$) | 55.1 (43)     | 41.0 (32) | 3.8 (3) | 2.58 | 1.29-5.17 | 0.0078 |
| CD pediatric ($n = 94$) | 59.6 (56)     | 35.1 (33) | 5.3 (5) | 3.09 | 1.58-6.07 | 0.0023 |
| PSC ($n = 42$) | 64.3 (27)       | 31.0 (13) | 4.8 (2) | 3.78 | 1.66-8.63 | 0.0046 |
| PSC + UC ($n = 139$) | 57.6 (80)       | 37.4 (52) | 5.0 (7) | 2.85 | 1.52-5.35 | 0.0016 |
| Controls ($n = 62$) | 32.3 (20)       | 53.2 (33) | 14.5 (9) | | | |

$^1$OR is calculated for Arg/Arg vs Arg/Arg + Pro/Pro. CD: Crohn’s disease; UC: Ulcerative colitis; PSC: Primary sclerosing cholangitis.

**Figure 2**  TP53 72Arg/Pro polymorphism distribution in adult and pediatric inflammatory bowel disease, primary sclerosing cholangitis (PSC), PSC/ulcerative colitis (UC) and healthy non-inflammatory bowel disease (IBD) controls. TP53 72Arg homozygosity is strongly associated with the development of IBD. Please find $P$ values and ORs in Table 2.
To our knowledge, no studies have been done so far on 72 codon polymorphism distribution in the CD or PSC populations although two articles have explored 72 codon polymorphism in UC. The first, an Italian study, failed to reveal an association of 72 codon polymorphism with UC development; however, 72Pro homozygosity was associated with chronic UC and family history of CRC[41]. The other study was conducted in Turkey and 72Pro was found to be associated with moderate risk of UC[36].

Our results indicate that IBD and PSC patients have significantly different TP53 72 codon polymorphism distribution, suggesting that the 72Arg allele may predispose individuals to IBD development and an increased levels of apoptosis in the colonic area. This may lead to apoptotic degradation of the intestinal epithelial cell leading to the impairment of gut barrier - a prominent feature in IBD patients[44]. This increased ability of 72Arg allele to induce apoptosis (please see Table 1 for other isofrom’s differences) may partially explain why Arg/Arg genotype is more abundant in IBD group compared to controls. Additionally, it was shown that 72Arg genotype carriers have increased risk of TP53 mutations potentially leading to further imbalances and cancer development[33,45]. Thus, if differences between 72Arg and 72Pro carriers progress through IBD to cancer development are confirmed in long-term studies, it could be useful for IBD related cancer development prediction and better screening of 72Arg carriers.

In addition to other factors predisposing to IBD, there is accumulating evidence that epigenetic changes, such as DNA methylation and histone modifications also play a role in the pathogenesis of IBD (please see following reviews for references[36-40]). One of the histone acetyltransferase-containing domain transcription co-activators, p300/CBP1, acetylates histones making DNA more accessible to transcription machinery after being recruited by sequence-specific DNA binding transcription factors, such as TP53[30]. It was shown that proline-rich domain of TP53 influences the extent of interaction between TP53 and p300/CBP and, subsequently, histone acetylation and the promoter accessibility[31]. It is tempting to speculate that p300/CBP can discriminate TP53 isoforms and associate with them differently in a similar way as CD2AP/Cin85 adaptor protein family does[21]. This differential association could lead to differential epigenetic silencing of target genes. One of such candidate target genes is the tumor suppressor RASSF1A (Ras association domain family 1A). It was demonstrated that RASSF1A promoter has a TP53 binding site and TP53 is able to bind to the RASSF1A promoter and inhibit RASSF1A expression[32]. It would be interesting to study if 72 codon polymorphism can influence this TP53 function to repress RASSF1A expression. Interestingly, it was shown that RASSF1A epigenetic silencing has a role in cancer development (for a recent review please see[20]) and we demonstrated recently that RASSF1A plays a role in controlling inflammation in dextran sodium sulphate (DSS)-induced colitis in mice, a well-known model for IBD[34].

In conclusion, our data indicate 72 Arg/Arg TP53 genotype may predispose individuals to IBD development possibly through increased efficiency
of apoptosis induction. The increased risk of CRC development could also be explained by increased rate of TP53 mutations in patients carrying Arg<sup>72</sup> genotypes<sup>[33,45]</sup>. However, more studies are needed to confirm the clinical significance of this polymorphism and better understand the effect of 72 codon polymorphism on IBD development.

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