Arg72Pro polymorphism of p53 may predict poor response to medical treatment in ulcerative colitis

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AIM: *Arg72Pro is a polymorphism commonly occurring in the proline-rich domain of Tp53. It can determine the development of different types of cancers, such as breast, lung, cervical, colorectal and hepatocellular carcinoma. Previous studies reported a correlation between Pro72 homozygosity and the clinical course of ulcerative colitis (UC). Our aim was to evaluate Arg72Pro genotype in patients who underwent proctocolectomy with ileo-pouch-anal anastomosis (IPAA) for UC compared with those who did not need surgery.*
MATERIAL OF STUDY: *The distribution of the different genotype of Arg72Pro was studied in 264 (234 medically treated [MT] and 30 IPAA) patients affected with UC observed between 2008 and 2011. IPAA patients underwent restorative proctocolectomy for refractory UC; MT ones were managed medically. Blood samples for genotyping were collected from all patients. Arg72Pro genotype analysis was carried out by polymerase chain reaction confronting two-pair primers (PCR-CTPP).*
RESULTS: In *MT patients (n=234) Arg/Arg, Arg/Pro and Pro/Pro frequencies were 51.28%, 41.02% and 7.7%, respectively, while in IPAA patients (n=30) were 53.4%, 23.3% and 23.3%, respectively. A statistically significant association was found between Pro/Pro and need for surgery (p=<0.0059, χ =7.59).*
CONCLUSION: *Our results showed that the Pro/Pro genotype was higher in IPAA (23.3%) than in MT (7.7%) patients. In UC patients the proline homozygosity identifies likeliness to resist to any standard pharmacologic therapy. It could potentially identify patients who would benefit from early surgical treatment, thereby reducing the rate of emergency colectomies and complications related to them.*

KEY WORDS: Arg72Pro, Arg72Pro polymorphism, Ileopouch-anal anastomosis, IPAA, p53 polymorphism, Ulcerative colitis

Introduction

The proline-rich domain is a region of p53 required for pro-apoptotic activity. Arg72Pro polymorphism commonly occurs within this domain. It is located in exon 4 of the Tp53 tumour suppressor gene, and encodes either arginine (CGC) or proline (CCC) at position 72 [1].
Several studies reported that p53 codon 72 polymorphism is widely and differently distributed throughout the human population and the genotype distribution depends on ethnicity and latitude [2-5]. Besides, the two polymorphic variants have different structural and functional properties, resulting in altered electrophoretic mobility and in a stronger capacity to induce apoptosis in the arginine compared to the proline allele [6,7].
Arg72Pro has been associated with several malignancies, such as breast, lung, cervical, colorectal and hepatocellular cancer [8]. A correlation between Pro72 homozygos-

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ity and the clinical course of ulcerative colitis (UC) has also been observed [9,10].
Our aim was to compare Arg72Pro genotypes in UC patients refractory to conventional medical treatment who underwent restorative proctocolectomy and ileo-pouch-anal anastomosis (IPAA) with patients who did not need surgery.

Material and Method

The distribution of the Arg72Pro genotypes was examined in 264 consecutive patients suffering from UC presenting at our Institution between January 2008 and November 2011. The patients were classified into two groups: 30 IPAA and 234 medically treated (MT) patients. The patients of the latter group were affected with intermittent chronic UC, successfully managed with medical treatment.

Medical Treatment

At diagnosis all patients were treated with mesalamine (1.6-4.8 g/day). Patients who did not respond to mesalamine received systemic steroids at the standard dose of 1mg/kg for 10 days, followed by progressive tapering (5mg/week). If patients did not get a sustained response, treatment with immunosuppressant agents (azathioprine, at the standard dose of 2 mg/kg) was introduced. Unresponsive patients suitable for treatment with biologic were administered induction therapy with Infliximab (Remicade®, Centocor Inc., Malvern, P.A. U.S.A.) 5 mg/kg (week 0, 2, 6). Patients who did not respond to azathioprine within 4 months or second Infliximab infusion and patients with sudden worsening of general health status were offered surgical intervention.

Surgery

All 30 IPAA patients underwent restorative proctocolectomy. Surgery was performed as 1-, 2- or 3-stage procedure depending on patient shape, general health status and drug intake at the time of surgery [11-13]. Ileostomy was closed after a mean of 2.5 and 6.3 months in 2-stage and 3-stage procedure patients, respectively; a pouchogram was performed in selected patients [14]. All 30 IPAA patients were refractory to medical treament. Patients were followed-up by means of clinical and serological examination, flexible endoscopy, and imaging [15,16].

Genotyping

Blood samples for genotyping were collected from all patients. Arg72Pro genotype analysis was carried out by polymerase chain reaction confronting two-pair primers (PCR-CTPP) [9].

Statistical Analysis

Data are expressed as mean ± standard deviation (SD), unless otherwise indicated. Comparisons between groups were performed using *t* test for unpaired data.
Frequencies were compared using Chi-square test with Fisher's correction, when appropriate. Logistic regression analysis was carried out to calculate the strength of association between the genotypes and each variable. Significance was assumed with p-values less then 0.05.

Results

The genotype distributions among cases and controls were in *Hardy-Weinberg* equilibrium ("agreement between observed and expected genotype frequencies", following Hardy-Weinberg law: "gene frequencies and genotype ratios in a randomly-breeding population remain constant from generation to generation") [17].
The p53 Arg72Pro genotype distributions in IPAA and in MT patients are summarized in Table I.
The frequencies of the three genotypes in MT patients (n=234) were distributed as follows: Arg homozygosity 51.28%, Arg/Pro 41.02% and Pro homozygosity 7.7%. In IPAA patients (n=30) genotypes were distributed as

Table I - *Genotype and allele frequencies of Arg72Pro in IPAA and MT patients.*

Genotype	MT (n=234) (%)	IPAA (n=30) (%)	P	X^2	Odds Ratio
Arg/Arg	120/234 (51.28%)	16/30 (53.4%)	ns	–	1.09
Arg/Pro	96/234 (41.02%)	7/30 (23.3%)	ns		0.44
Pro/Pro	18/234 (7.7%)	7/30 (23.3%)	0.0059	7.59	3.65
Allele frequencies			ns	–	0.73
Arg	71.8%	65%			
Pro	28.2%	35%			

IPAA: ileal pouch-anal anastomosis; MT: Medically Treated

follows: Arg homozygosity 53.4%, Arg/Pro 23.3%, Pro homozygosity 23.3%.
Pro/Pro genotype was significantly higher in IPAA (23.3%) compared with MT patients (7,7%) (p=<0,0059, χ^2=7,59).
The allele frequencies in IPAA and in MT patients were 65% and 71.8% for Arg allele, 35% and 28.2% for Pro allele, respectively. There were no statistically significant correlations in both groups concerning allele frequencies.

Discussion and Comments

UC is an inflammatory bowel disease characterised by a relapsing and remitting course of an acute inflammation of the colorectal mucosa [18].
Colectomy is a potentially life-saving procedure for patients with severe attacks of UC who fail medical therapy [19]. Medical management of UC is firstly aimed to reduce the symptoms and to achieve a remission, consequently improving the quality of life of affected people. Current medical therapy includes corticosteroides and immunosuppressives. More recently, biological drugs, monoclonal antibodies against TNF–α largely used in Crohn's disease also as salvage therapy after a failed colectomy with ileorectal anastomosis [20] and in complex perianal disease [21], has been approved by FDA for the treatment of UC refractory to conventional treatment [22]. Several studies reported that Infliximab does not abolish the need for surgery in patients affected with UC, rather allowing to delay colectomy in patients with severe UC presentation [23,24]. Avoiding a colectomy in emergency settings is important, but it should be considered that the use of biological drugs can be associated with serious adverse effects and drug-related complications [25-27]. Moreover, literature is contrasting concerning surgery and anti-TNF-α therapy, and some authors suggested that patients undergoing surgery for UC during treatment with biologic are at increased risk of post-operative complications [25].
In patients with steroid-refractory colitis, and who received immunomodulators induction with the intent to proceed to maintenance therapy, but who had no response, surgery is the treatment of choice. IPAA in experienced hands has excellent functional results, and in the long-term these seems to be stable [28,29], regardless of the pouch shape [30], while minor modifications are physiologically observed in ileal pouch mucosa [31,32]. Pro homozygosity is associated with refractoriness to therapy, suggesting that patients with Pro homozygosity have a different clinical course of disease, showing a higher trend toward a continuous disease than patients with Arg homozygosity or heterozigosity [9]. Starting with this concept, we wished to evaluate the polymorphism Arg72Pro in patients who did not respond to any drug therapy, undergoing surgical treatment. Predictably, Pro homozygosity was significantly higher in IPAA patients (p<0.0059).
These results confimed an association between Pro/Pro genotype and refractory UC. Other polymorphisms were found to be correlated with drug resistence in UC, i.e. I219V of hMLH1 polymorphism has been found in association with refractory UC [33] and with need for a surgical intervention [34], suggesting a synergistic role of multiple polymorphisms in resistance to therapy.
The G allele of hMLH1 and C allele of p 53 Arg72Pro have different biological activity. GG homozigosity of I219V could affect the immune response [35], while CC homozigosity could cause drug resistence because Pro allele induces apoptosis less effectively than p53 Arg allele [6]. As a result, the Pro homozygosity determines resistance to any standard pharmacologic therapy in patients affected with UC, with the consequent need for surgery.
Investigating further these aspects could be intriguing, aiming to clarify ideal treatment planning of UC patients.

Conclusions

Although preliminary, the present study offers a poorly described perspective. Timing of surgery represents a debated issue in UC. A study on 11204 patients affected with UC in the UK suggested that the threshold for surgery may be too high in this population, resulting in higher morbidity and mortality when emergency surgery is advocated [36]. Avoiding early postoperative complications in IPAA patients is essential as they negatively impact the long-term function and durability of the pouch [36-38]. It is our opinion that screening UC patients for the polymorphism Arg72Pro could be of use in selecting those who could benefit from an early surgical approach. This could result in fewer both drug- and surgery-related complications, potentially reducing the rate of emergency colectomies and increasing the number of patients suitable for 1-stage (avoidance of stoma) or 2-stage elective operations. This would plainly result in improved quality of care and better quality of life for patients affected with UC.

Riassunto

INTRODUZIONE: Il polimorfismo Arg72Pro si verifica nel dominio ricco di proline del gene Tp53. Tale polimorfismo può determinare lo sviluppo di diversi tipi di cancro, quali il tumore della mammella, del polmone, della cervice, del colon-retto e del fegato. Studi precedenti hanno riportato un'associazione tra omozigosi Pro72 ed il decorso clinico della Colite Ulcerosa (CU).
OBIETTIVI: Nostro obiettivo è stato valutare il genotipo Arg72Pro in pazienti sottoposti a proctocolectomia restaurativa (PR) con pouch ileale pelvica ed anastomosi ileopouch anale per CU e di confrontare tali dati con quelli di pazienti affetti da CU responsivi alla terapia medica.

MATERIALI E METODI: Le distribuzioni dei diversi genotipi di Arg72Pro sono state esaminate in 264 pazienti (234 trattati con terapia medica [TM] e 30 sottoposti a PR).osservati tra il 2008 ed il 2011. Nei pazienti operati la PR è stata effettuata per refrattarietà alla terapia medica. In tutti i pazienti sono stati prelevati campioni ematici ed è stata eseguita l'analisi del genotipo Arg72Pro mediante polymerase chain reaction con due primer appaiati (PCR-CTPP).
RISULTATI: Nei pazienti sottoposti con successo a TM (n=234) abbiamo riscontrato tali distribuzioni: Arg/Arg 51.28%; Arg/Pro 41.02%; Pro/Pro 7.7%. Nei pazienti che hanno richiesto PR (n=30) le distribuzioni erano le seguenti Arg/arg 53.4%; Arg/Pro 23.3%; Pro/Pro 23.3%. L'associazione tra Pro/Pro e necessità di chirurgia è risultata statisticamente significativa (p=<0.0059, χ^2=7.59).
DISCUSSIONE E CONCLUSIONI: I nostri risultati dimostrano che il genotipo Pro/Pro è maggiormente riscontrato in pazienti sottoposti a PR (23.3%) che in quelli responsivi a TM (7.7%). Nei pazienti affetti da CU tale omozigosi identifica verosimile resistenza alla TM, e potrebbe essere utilizzata per selezionare pazienti che potrebbero beneficiare di un approccio chirurgico precoce, riducendo – in ultima analisi – il tasso di colectomie d'urgenza e le relative complicanze.

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