Journal of Crohn's and Colitis (2013) 7, 44–52

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TRAF3IP2 gene is associated with cutaneous extraintestinal manifestations in Inflammatory Bowel Disease

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Received 1 December 2011; received in revised form 28 February 2012; accepted 28 February 2012

KEYWORDS
TRAF3IP2 gene;
Crohn's disease;
Ulcerative colitis;
Genetic susceptibility;
Cutaneous extraintestinal manifestations

Abstract

Background and aims: Genome-wide association (GWA) studies recently identified a novel gene, *TRAF3IP2*, involved in the susceptibility to psoriasis. Common immune-mediated mechanisms involving the skin or the gut have been suggested. We therefore aimed to assess the role of *TRAF3IP2* gene in IBD, with particular regard to the development of cutaneous extraintestinal manifestations (pyoderma gangrenosum, erythema nodosum). The association with psoriasis was also assessed in a secondary analysis.
Methods: The analysis included 267 Crohn's disease (CD), 200 ulcerative colitis (UC) patients and 278 healthy controls. Three *TRAF3IP2* SNPs were genotyped by allelic discrimination assays. A case/control association study and a genotype/phenotype correlation analysis have been performed.
Results: All three SNPs conferred a high risk to develop cutaneous manifestations in IBD. A higher risk of pyoderma gangrenosum and erythema nodosum was observed in CD patients carrying the Rs33980500 variant (OR 3.03; P=0.026). In UC, a significantly increased risk was observed for both the Rs13190932 and the Rs13196377 SNPs (OR 5.05; P=0.02 and OR 4.1; P=0.049). Moreover, association of TRAF3IP2 variants with ileal (OR=1.92), fibrostricturing (OR=1.91) and perianal CD (OR=2.03) was observed.

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1873-9946/$ - see front matter
doi:10.1016/j.crohns.2012.02.020

Conclusions: This is the first preliminary report indicating that *TRAF3IP2* variants increase the risk of cutaneous extraintestinal manifestations in IBD suggesting that the analysis of the *TRAF3IP2* variants may be useful for identifying IBD patients at risk to develop these manifestations.

1. Introduction

Genome-wide association studies (GWA) indicate that Crohn's Disease (CD) and Ulcerative Colitis (UC) share many of the almost 100 loci associated with Inflammatory Bowel Diseases (IBD).[1–3] An association between CD and UC is also suggested by the observed 8- to 10-fold greater risk of IBD among patients' relatives, with a variable twin concordance.[1,4,5] A significantly higher risk of psoriasis is observed in patients with IBD, thus suggesting an association between these conditions.[6,7] Several alterations of the host immune response are shared by patients with IBD and with psoriasis, further supporting a possible association between IBD and psoriasis.[8–10] Supporting this concept, specific immunomodulatory treatments, including methotrexate and anti-TNFs, show a high efficacy both in psoriasis[11] and in IBD.[12,13] Moreover, recent preliminary evidences unexpectedly report the possible new development of psoriasis in patients treated with anti-TNFs for IBD.[14–16] Overall, these observations suggest that CD, UC and psoriasis are complex, multifactorial diseases which may share common pathogenetic mechanisms involving the gut or the skin. Current evidences therefore support the hypothesis that common genetic determinants may be involved in the susceptibility to develop both IBD and psoriasis. Overlap in loci associated with psoriasis and IBD has indeed been reported.[1] Several genes encoding factors in the IL-23 pathway have been associated with both psoriasis and IBD.[1,17,18] In particular, variants in the *IL23R* and *IL12B* and tyrosine kinase 2 (*TYK2*) genes have been associated with CD and psoriasis.[17,19] Identifying shared susceptibility loci for psoriasis, CD and UC may help to characterize common or disease-specific pathogenetic mechanisms. Moreover, this analysis may also help to identify patients at higher risk to develop psoriasis after anti-TNF treatments.

Recent genome-wide association (GWA) studies have identified a novel gene, *TRAF3IP2*, as involved in the susceptibility to psoriasis (PsV) and psoriatic arthritis (PsA).[20–22] A replication study including 6 European cohorts of PsA, identified a coding variant, rs33980500, as the most associated SNP (OR 1.95).[22] The gene *TRAF3IP2* encodes a protein, ACT1, modulating the humoral immunity through its inhibitory effect on CD40 and BAFFR mediated signaling.[23,24] This protein is also involved in the IL-17-mediated cellular immune responses,[23,24] by allowing the incorporation of the TNF-receptor-associated factors (TRAF) TRAF3 and TRAF6, followed by the activation of the MAPK and NF-κB pathway.[23–25] PsV and PsA share common inflammatory pathways. The comparable association between *TRAFIP2* and both PsA and PsV suggests that the *TRAF3IP2* gene locus represents a shared susceptibility locus for both conditions.[20–22] Recently, GWA studies led to the identification of several loci associated with disease susceptibility, some of them predisposing to multiple immune-related disorders.[26] *TRAF3IP2* may represent a candidate gene for susceptibility to the cutaneous manifestations related to IBD, due its association with psoriasis, sharing several features with IBD, including its role in the IL-17 mediated T-cell immune response.[24] Cutaneous extraintestinal manifestations of IBD including pyoderma gangrenosum and erythema nodosum, more frequently involve the lower limbs in patients with colonic CD or UC. As observed for intestinal symptoms related to IBD and also for psoriasis, both cutaneous extraintestinal manifestations show a marked responsiveness to anti-TNFs, with possible complete healing. This observation supports that common pathogenetic mechanisms may be involved in determining the development of chronic intestinal inflammation, cutaneous extraintestinal manifestations and psoriasis in patients with IBD.

These observations prompted us to assess the role of *TRAF3IP2* gene in IBD [(MIM266600)], with particular regard to patients with pyoderma gangrenosum and erythema nodosum. In a secondary analysis we also included patients with psoriasis. Both a case/control association and a genotype–phenotype correlation study were performed in an Italian cohort of IBD patients, subgrouped according to clinical characteristics. This analysis was focused on two coding and one intronic SNPs, previously identified by GWA studies as psoriasis susceptibility variants.[20–22]

2. Materials and methods

2.1. Case-control study cohorts

The study population included 467 patients with IBD, including 267 with CD and 200 with UC. Clinical characteristics of patients are summarized in Table 1. All patients were under regular follow up at the Tertiary Referral IBD Center of the University Tor Vergata of Rome, Italy. The diagnosis of CD or UC was established according to conventional clinical, radiological, endoscopical and histological criteria.[12] Disease duration (mean ± SD) was 16 ± 10 years for CD, 10.5 ± 8.6 years for UC (Table 1) and 13.4 ± 10.3 years for all IBD patients. The control population included 278 ethnically matched healthy unrelated blood donors. The study protocol was approved by the local ethics committee, and written informed consent was obtained from each patient.

2.2. Clinical characteristics of patients with IBD

Clinical characteristics of patients with CD and with UC were prospectively collected at each visit and data were retrospectively reviewed from clinical records. CD phenotype was classified according to the prevalent pattern of the

Table 1 Clinical characteristics of patients with Crohn's disease (CD) and ulcerative colitis (UC).

		CD	UC
Patients (n=)		267	200
Females (%)		52.7	42
IBD duration (mean ± SD years)		16 ± 10	10.5 ± 8.6
Age at diagnosis of IBD (mean ± SD, years)		30 ± 11	37.4 ± 15
Familial history of IBD (%)		21	13.5
CD localization	Ileum (%)	59.5	n.a.
	Colon (%)	9.1	n.a.
	Ileo-colon (%)	28.6	n.a.
	Upper GI (%)	2.8	n.a.
CD behaviour	Fibrostricturing (%)	46.1	n.a.
	Penetrating (%)	25.4	n.a.
	Inflammatory (%)	28.5	n.a.
UC extent	Proctitis (%)	n.a.	50.8
	Left-sided (%)	n.a.	15.4
	Pancolitis (%)	n.a.	32.8
Perianal disease (%)		31.1	0%
Extraintestinal manifestations	Total (%)	57.5	32.1
	Articular (%)	46.8	24
	Cutaneous (%) [a]	7.1 [b]	4.1 [c]
	Ocular (%)	6.4	3.6
	Hepatic (%)	8.3	5.1
Psoriasis (%)		5.1 [d]	4.1 [c]
Appendectomy (%)		37.7	5
Previous surgery (%)		42.1	6.5
Smoking history	Yes	31	13
	No	46	62.5
	Ex	23	24.5

n.a. = not applicable.
[a] Including only patients with erythema nodosum or pioderma gangrenosum.
[b] CD: 18/252 patients with a detailed history including cutaneous manifestations.
[c] UC: 8/195 patients with a detailed history including cutaneous manifestations.
[d] CD: 13/252 patients with a detailed history including cutaneous manifestations.

disease.[12] UC extent was defined according to current guidelines[12] (Table 1). A present or past history of cutaneous extraintestinal manifestations of IBD, including erythema nodosum and pyoderma gangrenosum was confirmed by a dermatologist and reported for each patient (excluding those with an uncertain diagnosis of the cutaneous manifestation). A present or past history of psoriasis (including either PsV or PsA) was considered in a separate analysis.

2.3. DNA extraction and genotyping

DNA was extracted using a Qiagen blood DNA mini kit. We studied two coding polymorphisms, rs33980500 and rs13190932, and one intronic, rs13196377, in the *TRAF3IP2* gene. Genotyping of the three SNPs was performed by allelic discrimination assay by TaqMan technology using MGB-specific allelic probes (coded C___2473124_10, C___2473123_20 and C___2475647_10 respectively) and ABI PRISM 7000. We always used samples with known genotypes (by direct sequencing) in each run of the allelic discrimination assay as genotype controls.

2.4. Haplotypes analysis

Haplotypes were inferred using Arlequin.[27]

2.5. Statistical analysis

The Hardy–Weinberg Equilibrium (HWE) was verified by the Pearson chi square test. Differences in allelic, genotypic and haplotypic frequencies between cases and controls and among different phenotypic classes were evaluated by the Pearson chi square test or by the Fisher Exact test, when required. Since the very low frequency of variant homozygous genotypes, the heterozygotes and variant homozygotes (1 degree of freedom [df]) were considered together. Odds ratios (OR) with 95% CI were calculated. In order to assess whether the association of *TRAF3IP2* is dependent or independent from *CARD15*/*NOD2*, a 3 way contingency table analysis by log-linear model was performed. (Small differences in the number of subjects reported in the tables depend on the fact that not for all subjects was it possible to perform the genotyping for all the three SNPs). In both CD and UC patients, a correlation was searched between the genetic variants and the following variables: CD localization, CD behaviour, UC extent, age at time of diagnosis of IBD, presence of extraintestinal manifestations in both CD and UC (arthropathic, cutaneous, ocular, hepatic). The presence of cutaneous extraintestinal manifestations in IBD was considered in patients with a present or past history of erythema nodosum, pyoderma gangrenosum. In a separate

analysis, patients with psoriasis were included. The cut off for statistical significance has been considered for p value <0.05. All statistical analyses were performed by SPSS program.

3. Results

3.1. Cutaneous extraintestinal manifestations of IBD

In our IBD population, a present or past history of cutaneous manifestations, including only pyoderma gangrenosum or erythema nodosum was observed in 26 out of the 447 (5.8%) patients with IBD. This IBD subgroup included only patients with a detailed history including possible cutaneous manifestations of the disease at any time during the natural history of the disease. As shown in Table 1, well defined cutaneous manifestations of the disease were observed in 8 out of the 195 (4.1%) UC patients considered in this analysis, including 7 patients showing erythema nodosum and 1 pyoderma gangrenosum. Among the 267 CD patients enrolled, 18 (7.1%) had a present or past history of cutaneous manifestations of the disease, including erythema nodosum in all 18 patients and pyoderma gangrenosum in one patient showing both cutaneous lesions at different phases of the disease. A present or past history of psoriasis was observed in 21 out of the 447 patients with IBD (4.69%), including 8 patients with UC (4.1%) and 13 patients with CD (5.1%) (Table 1). None of the 21 IBD patients with psoriasis had a history of erythema nodosum or pyoderma gangrenosum. Among the 21 IBD patients with psoriasis, 9 were treated with anti-TNFs (Infliximab n=7; Adalimumab n=1; Certolizumab n=1). In particular, a history of anti-TNF treatment was observed in 8 out of 13 CD patients with psoriasis (Infliximab n=6; Adalimumab n=1; Certolizumab n=1) and in 1 out of the 8 UC patients with psoriasis (1 Infliximab). In none of these 21 IBD patients psoriasis developed after anti-TNFs treatment.

3.2. Case/control association study

Deviations from Hardy–Weinberg equilibrium for the three SNPs were not observed. Table 2 shows the *TRAF3IP2* genotypes and alleles distribution in healthy controls and in patients with CD and UC. As shown, no statistically significant associations were observed between the variant alleles considered and CD or UC. The observed trend for an association between the variant alleles of rs33980500 and rs13196377 SNPs and susceptibility to CD did not reach statistical significance (OR 1.52, P=0.058 and OR 1.63, P=0.063, respectively). Accordingly, in UC the variant allele of rs13196377 SNP showed no statistically significant association (OR=1.6; P=0.09) (Table 2). When considering all IBD patients and combining together the allelic frequencies, the rs13196377 was the most associated SNP (OR=1.62; P=0.04). The variant homozygote genotype (for each SNP), although very rare, was observed only in IBD (both CD and UC) and never in healthy controls.

3.3. Genotype/phenotype correlation

A genotype/phenotype correlation analysis between the *TRAF3IP2* variants and clinical characteristics of IBD patients was performed. In both CD and UC patients, no correlation was observed between the genetic variants tested and the following variables: CD localization, CD behaviour, UC extent, age at diagnosis of IBD and extraintestinal manifestations (articular, ocular, cutaneous). Differently, when the correlation was searched between genetic variants and specific extraintestinal manifestations in IBD, a significant

Table 2 Case/control association analysis.

	Controls	CD			UC		
Rs33980500	N=278 (%)	N=259 (%)	P (1 df)	OR (95% CI)	N=200 (%)	P (1 df)	OR (95% CI)
CC	240 (86.3)	208 (80.3)	0.061	1.55 (0.98–2.45)	168 (84)	0.48	1.2 (0.72–2)
CT	38 (13.7)	50 (19.3)			29 (14.5)		
TT	0	1 (0.4)			3 (1.5)		
C	518 (93.2)	466 (90)	0.058	1.52 (0.98–2.35)	365 (91.3)	0.27	1.31 (0.8–2.1)
T	38 (6.8)	52 (10)			35 (8.7)		
Rs13190932	N=271 (%)	N=259 (%)	P (1 df)	OR (95% CI)	N=200 (%)	P (1 df)	OR (95% CI)
GG	243 (89.7)	228 (88)	0.55	1.18 (0.69–2.03)	176 (88)	0.57	1.18 (0.66–2.11)
GA	28 (10.3)	30 (11.6)			23 (11.5)		
AA	0	1 (0.4)			1 (0.5)		
G	514 (94.8)	486 (93.8)	0.48	1.21 (0.72–2.04)	375 (93.8)	0.48	1.22 (0.7–2.13)
A	28 (5.2)	32 (6.2)			25 (6.2)		
Rs13196377	N=269 (%)	N=258 (%)	P (1 df)	OR (95% CI)	N=200 (%)	P (1 df)	OR (95% CI)
GG	244 (90.7)	222 (86)	0.095	1.58 (0.92–2.72)	172 (86)	0.11	1.59 (0.9–2.82)
GA	25 (9.3)	34 (13.2)			27 (13.5)		
AA	0	2 (0.8)			1 (0.5)		
G	513 (95.4)	478 (92.6)	0.063	1.63 (0.97–2.74)	371 (92.8)	0.09	1.6 (0.92–2.78)
A	25 (4.6)	38 (7.4)			29 (7.2)		

Heterozygotes and variant homozygotes were considered together (1 df) in the comparisons between genotypes by Chi square test.

Table 3 Extraintestinal cutaneous manifestations analysis.

	CD				UC			
Rs33980500	Without N=213 (%)	With N=18 (%)	P (1 df)	OR (95% CI)	Without N=179 (%)	With N=8 (%)	P (1 df)	OR (95% CI)
CC	176 (82.6)	11 (61.1)	**0.026**	3.03 (1.1–8.3)	152 (84.9)	5 (62.5)	0.09	3.38 (0.76–14.97)
CT	37 (17.4)	7 (38.9)			24 (13.4)	3 (37.5)		
TT					3 (1.7)			
C	389 (91.3)	29 (80.6)	**0.043**	2.54 (1.04–6.19)	328 (91.6)	13 (81.3)	0.16	2.52 (0.68–9.35)
T	37 (8.7)	7 (19.4)			30 (8.4)	3 (18.7)		
Rs13190932	Without N=213 (%)	With N=18 (%)	P (1 df)	OR (95% CI)	Without N=179 (%)	With N=8 (%)	P (1 df)	OR (95% CI)
GG	191 (89.7)	14 (77.8)	0.13	2.48 (0.75–8.2)	160 (89.4)	5 (62.5)	**0.02**	5.05 (1.12–22.83)
GA	22 (10.3)	4 (22.2)			18 (10.1)	3 (37.5)		
AA					1 (0.5)			
G	404 (94.8)	32 (88.9)	0.25	2.3 (0.75–7.07)	338 (94.4)	13 (81.3)	0.07	3.9 (1.03–14.8)
A	22 (5.2)	4 (11.1)			20 (5.6)	3 (18.7)		
Rs13196377	Without N=212 (%)	With N=18 (%)	P (1 df)	OR (95% CI)	Without N=179 (%)	With N=8 (%)	P (1 df)	OR (95% CI)
GG	183 (86.3)	16 (88.9)	0.76	0.79 (0.17–3.6)	156 (87.2)	5 (62.5)	**0.049**	4.1 (0.91–18.18)
GA	28 (13.2)	2 (11.1)			22 (12.3)	3 (37.5)		
AA	1 (0.5)				1 (0.5)			
G	394 (92.9)	34 (94.4)	1	0.77 (0.18–3.37)	334 (93.3)	13 (81.3)	0.1	3.21 (0.66–12.05)
A	30 (7.1)	2 (5.6)			24 (6.7)	3 (18.7)		

Heterozygotes and variant homozygotes were considered together (1 df) in the comparisons between genotypes by Chi square test.
When required, P value was evaluated by Fisher exact test. Significant P-values are reported in bold.

correlation was observed between *TRAF3IP2* variants and presence of cutaneous manifestations related to both CD and UC (Table 3). As shown in Table 3, a significant association was observed in CD, when considering the rs33980500 variant allele (OR 2.54, [95% CI 1.04–6.19]; P = 0.043). Differently, the association for the rs13190932 and rs13196377 variant alleles did not reach statistical significance in our CD population (OR 2.48; P = 0.13 and OR 0.79; P = 0.76, respectively). In UC, a significant association was observed for 2 SNPs: particularly for the rs13190932 (OR 5.05 [95% CI 1.12–22.83]; P = 0.02), although a significant association was also observed for the rs13196377 (OR 4.1 [95% CI 0.91–18.18]; P = 0.049). The association for the rs33980500 variant was not significant in our UC population (OR 3.38; P = 0.09) (Table 3).

When including also psoriasis among the cutaneous manifestations of IBD, the associations, as expected, were strengthened, confirming the high association already described between this gene variants and psoriasis. In particular, the association with rs13190932 was observed both in CD and in UC (CD: OR 2.53; P = 0.049; UC: OR 3.83; P = 0.016, respectively). A significant association was also observed in CD for the rs33980500 (OR 3; P = 0.006) and in UC for the rs13196377 variant (OR 3.08; P = 0.044).

Differently from the cutaneous manifestations, no significant correlations were observed between *TRAF3IP2* variants and arthralgias in IBD, although this extraintestinal manifestation was observed in a high proportion of patients (CD: 46.8%; UC: 24% of patients, respectively) (Table 1).

3.4. Haplotypes analysis

Considering the genotypes frequencies in the 3 SNPs, we have inferred[27] the haplotypes distribution in CD, UC and healthy controls. The haplotypes analysis was conducted in both in CD and UC patients, comparing the haplotypes distribution versus controls. A significant difference in the haplotypes distribution was observed between CD patients and controls (P = 0.0087) (Table 4). Individuals carrying the wild-type haplotype (CGG) showed a significantly decreased risk of CD (OR = 0.58 with P = 0.013). Individuals carrying rare haplotypes (with one or two variant alleles: CGA, TAG, TGA and CAA) showed a five-fold increased risk to develop CD (OR = 5.51, 95% CI 1.60–19; P = 0.0025). This finding suggests that specific allele combinations increased the risk of CD. Differences between haplotypes distribution in UC patients and controls did not achieve statistical significance.

3.5. CD sub-phenotype correlations

Since no correlation was observed between genotypes and patients with IBD showing different clinical characteristics (i.e. IBD localization, behavior, extent), we performed the same analysis when considering subgroups of CD patients showing specific clinical characteristics *vs* controls. Significant correlations were observed between genotypes and the following CD subtypes: ileal disease, fibrostricturing behavior or perianal disease (Table 5). In particular, a significantly increased risk of ileal localization of CD was observed for the rs13196377 SNP, with an OR equal to 1.92 (95%CI 1.05–3.5; P = 0.03), while the same association was not observed for the rs33980500 SNP (OR = 1.63 [95% CI 0.96–2.77]; P = 0.067). In CD, the rs33980500 SNP also showed to increase the risk of both fibrostricturing disease (OR = 1.91 [95% CI 1.09–3.33]; P = 0.021) and perianal disease (OR = 2.03 [95% CI 1.08–3.82]; P = 0.026). No other significant correlations were observed between the SNPs rs13196377 and rs13190932 and CD subgroups (Table 5).

3.6. NOD2/CARD15 interaction

Since *CARD15* gene is considered the major genetic determinant for CD risk, possible interactions between *TRAF3IP2* SNPs and the 3 *CARD15*/*NOD2* SNPs in determining CD risk were also searched by log-linear model. No interactions between the 2 genes were observed, when considering either each *CARD15*/*NOD2* SNP separately or a compound *CARD15*/*NOD2* genotype.

4. Discussion

Chronic inflammatory diseases have been recently shown to share numerous susceptibility loci.[1,28] These growing observations support that common pathways may be involved in different chronic inflammatory diseases. Among these, genes products in the IL-23 pathway have been reported to be associated with both psoriasis and IBD (*IL23R*, *IL12B*, *TYK2*).[1,17,28,29] Recently, two GWAs identified the *TRAF3IP2* gene as a new susceptibility gene for PsV and the association was confirmed for PsA.[21,22] Several epidemiological, clinical and "in vitro" evidences including the role of IL-23 in modulating the mucosal immune response[1,17] suggest an association between CD and psoriasis. Common pathogenetic mechanisms may also be involved in the development of intestinal and cutaneous manifestations of IBD (i.e. pyoderma

Table 4 Comparisons between the haplotypes distribution in CD and controls.

Haplotypes	CD	Controls	Comparisons	P	OR	(95% CI)
CGG	456 (88.4%)	483 (92.9%)	CGG vs others	**0.013**	0.58	0.38–0.89
TGG	17 (3.3%)	10 (1.9%)	TGG vs others	0.17	1.74	0.79–3.83
TAA	27 (5.2%)	24 (4.6%)	TAA vs others	0.65	1.14	0.65–2
Rare haplotypes [a]	16 (3.1%)	3 (0.6%)	Rare haplotypes vs others	**0.0025**	5.51	1.60–19
Total	516	520	All haplotypes (3 df)	0.0087		

The haplotypes correspond to the following order of SNPs: rs33980500, rs13190932, rs13196377.
[a] Rare haplotypes include all haplotypes with a frequency > 1.5% (CGA, TAG, TGA and CAA). Significant P-values are reported in bold.

Table 5 CD sub-phenotypes associated with *TRAF3IP2* variants. Significant P-values are reported in bold.

	Controls	Ileum CD			Fibrostricturing CD			Perianal CD		
Rs33980500	N = 278 (%)	N = 146 (%)	P (1 df)	OR (95% CI)	N = 112 (%)	P (1 df)	OR (95% CI)	N = 74 (%)	P (1 df)	OR (95% CI)
CC	240 (86.3)	116 (79.5)	0.067	1.63 (0.96–2.77)	86 (76.8)	**0.021**	1.91 (1.09–3.33)	56 (75.7)	**0.026**	2.03 (1.08–3.82)
CT	38 (13.7)	30 (20.5)			26 (23.2)			18 (24.3)		
C	518 (93.2)	262 (89.7)	0.08	1.56 (0.95–2.58)	198 (88.4)	**0.028**	1.79 (1.06–3.03)	130 (87.8)	**0.033**	1.89 (1.04–3.41)
T	38 (6.8)	30 (10.3)			26 (11.6)			18 (12.2)		
Rs13190932	N = 271 (%)	N = 146 (%)	P (1 df)	OR (95% CI)	N = 112 (%)	P (1 df)	OR (95% CI)	N = 74 (%)	P (1 df)	OR (95% CI)
GG	243 (89.7)	127 (87)	0.41	1.3 (0.7–2.42)	96 (85.7)	0.27	1.45 (0.75–2.79)	66 (89.2)	0.92	1.05 (0.46–2.42)
GA	28 (10.3)	19 (13)			16 (14.3)			8 (10.8)		
G	514 (94.8)	273(93.5)	0.42	1.28 (0.7–2.33)	208 (92.9)	0.29	1.41 (0.75–2.66)	140 (94.6)	0.92	1.05 (0.47–2.35)
A	28 (5.2)	19 (6.5)			16 (7.1)			8 (5.4)		
Rs13196377	N = 269 (%)	N = 146 (%)	P (1 df)	OR (95% CI)	N = 112 (%)	P (1 df)	OR (95% CI)	N = 73 (%)	P (1 df)	OR (95% CI)
GG	244 (90.7)	122 (83.5)	**0.03**	1.92 (1.05–3.5)	96 (85.7)	0.15	1.63 (0.83–3.2)	63 (86.3)	0.27	1.55 (0.71–3.39)
GA	25 (9.3)	23 (15.8)			16 (14.3)			10 (13.7)		
AA	0	1 (0.7)								
G	513 (95.4)	267 (91.4)	**0.02**	1.92 (1.08–3.41)	208 (92.9)	0.16	1.58 (0.83–3.02)	136 (93.2)	0.28	1.51 (0.71–3.22)
A	25 (4.6)	25 (8.6)			16 (7.1)			10 (6.8)		

gangrenosum and erythema nodosum), as also suggested by the marked responsiveness to anti-TNFs for treating both luminal and cutaneous manifestations of IBD. Blocking TNF-α has also been proven to be highly effective for treating psoriasis. On the basis of these observations, we aimed to assess, in a case/control association study and a phenotype/genotype correlation analysis, whether *TRAF3IP2* gene is involved in the susceptibility to develop IBD and/or IBD cutaneous extraintestinal manifestations. According to GWAs findings,[20–22] the *TRAF3IP2* SNPs showed no significant association with IBD, when considering patients with CD or UC. When considering all IBD patients and combining together the allelic frequencies, only the rs13196377 resulted associated (OR = 1.62; P = 0.04). However, the major finding of the present study was the significant correlation between cutaneous extraintestinal manifestations of IBD (pyoderma gangrenosum and erythema nodosum) and *TRAF3IP2* SNPs: two out of the three *TRAF3IP2* SNPs were significantly associated with cutaneous manifestations in UC, while in CD a significant association was observed only for the rs33980500 SNP. In particular, a strong association was observed between the rs13190932 SNP and UC patients with cutaneous manifestations, as patients carrying variant alleles showed an increased risk to develop pyoderma gangrenosum or erythema nodusum of about five folds. CD patients carrying variant alleles of rs33980500, increased the risk to develop pyoderma gangrenosum or erythema nodusum of about three folds. These findings need to be confirmed by studies including a higher number of patients with cutaneous extraintestinal manifestations of IBD.

Moreover, our results also confirm the already described association between *TRAF3IP2* gene and psoriasis.

Although no significant association was observed between *TRAF3IP2* and all patients with CD, the stratification of CD patients by characteristics of the disease has shown a significant correlation between *TRAF3IP2* and ileal involvement, fibrostricturing behaviour and perianal disease. As no previous studies investigated the association between *TRAF3IP2* and subgroups of patients with IBD, including the presence of extraintestinal cutaneous manifestations or other characteristics of the diseases, no comparisons can be made with the present findings. Differently, clinical characteristics of tested IBD patients were comparable to the general IBD population, including the frequency of cutaneous extraintestinal manifestations in UC and CD,[12,30] thus suggesting that present findings may be confirmed by independent studies including different IBD populations.

The functional role of *TRAF3IP2* gene in IBD may be hypothesized since TRAF3IP2 is recruited to the IL-17 receptor upon IL-17 stimulation.[31] Deletions and point mutations of the ubiquitin-box in *TRAF3IP2* gene in mice abolished the ubiquitination of TRAF6 and impaired the ability to restore IL-17 dependent signaling.[31] Functional studies on the rs33980500 variant allele show a nearly complete loss of ability to interact with TRAF6.[32] Accordingly, TRAF3IP2 allows the incorporation of TRAF6 into the signaling complex, followed by downstream activation of NF-κB, representing a crucial step to switch on antibacterial mechanisms.

The known role of CARD15-NOD2 both in the susceptibility to CD and in the signaling pathway of NF-kB[1] prompted us to search for possible interactions between *CARD15* and *TRAF3IP2* genes. In our analysis, no interaction between the two genes was observed in terms of CD susceptibility. As for the observed association between *TRAF3IP2* and cutaneous manifestations in IBD, by our knowledge no studies investigated the possible interaction between *CARD15* and *TRAF3IP2* genes in determining the risk of CD, thus not allowing comparisons with previous findings. The observed association between cutaneous manifestations of IBD and *TRAF3IP2* may be related to the role of TRAF3IP2 in the IL-17 pathways.[32] In an atopic dermatitis-like skin disease in mice, a malfunction of the TRAF3IP2 protein has been shown to determine hyper-IgE-emia through the CD40- and B cell-activating factor-mediated pathway in B cells, thus causing skin inflammation through the IL-17-mediated pathway.[33] This observation may support the hypothesis that this locus may contribute to the development of both psoriasis and cutaneous extraintestinal manifestations of IBD, as suggested by the present findings.

In summary, by our knowledge this is the first report indicating the contribution of the *TRAF3IP2* gene in determining susceptibility to develop cutaneous extraintestinal manifestations in IBD. In particular, our findings suggest that *TRAF3IP2* variants contribute to cutaneous extarintestinal manifestations of both CD and UC. The present findings suggest that the analysis of the *TRAF3IP2* variants may be useful for identifying IBD patients at risk to develop cutaneous manifestations of the disease. Additional independent studies including a higher number of IBD patients from different study populations are however required in order to confirm and clarify the possible contribution of this locus to susceptibility to develop cutaneous manifestations related to IBD. Moreover, further functional studies are required to detect the effects of genetic variations in *TRAF3IP2* gene, to clarify its role in TRAF6 and NF-κB signaling pathway and to evaluate models for the pathogenesis in inflammatory diseases.

Conflict of interest

None declared.

Acknowledgments

This study was supported by funds from the Italian Ministry of University and Research—MIUR (PRIN 2008X8NRH4).

We thank Professor Giovanni Monteleone for the helpful discussions.

Authorship details

CC designed the research, performed the genetic analysis and wrote the manuscript.

LB supervised the clinical diagnosis/follow up and wrote the manuscript.

DDF performed the genetic analysis.

MR collected the patients' blood sample and the clinical features.

GC provided the clinical follow up and collected the patient blood sample.

EG provided control samples and revised the manuscript.

SO provided the clinical follow up and completed the database.

TL provided control samples and contributed to genotyping.

FP revised the manuscript.

GN revised the manuscript.
PB supervised the research and wrote the manuscript.
All authors read and approved the final manuscript.

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