Abstract
AIM
To investigate the correlation between rs2910164, miR-196a rs11614913, miR-221 rs113054794 and miR-224 rs188519172 polymorphisms with anti-TNF treatment response in patients with Crohn’s disease (CD).

METHODS
One hundred seven patients with CD based on standard...
MicroRNAs (miRNAs) are small, single stranded, non-coding RNA molecules comprising of 19-25 nucleotides exerting post-transcriptional gene expression regulation in response to cellular or environmental changes[7]. MiRNAs have begun to attract scientists’ attention as biomarkers of prognosis or response to treatment in various diseases in part due to some unique advantages they possess: They are practically noninvasive, stable in serum, and can be promptly and repeatedly detected from archived sera[9].

A series of studies have focused on miRNA expression and its impact on response to anti-TNF agents in autoimmune diseases - sharing many commons with IBD-such as rheumatoid arthritis[9,10] or psoriasis[11,12]. MiRNA expression has been investigated in differentiation of CD from UC[13,14] or in distinction of CD phenotypes[15,16] while only one study in Asian population has been done so far searching an association between miRNA expression and response to infliximab in patients with CD[17]. As far as miRNA single nucleotide polymorphisms (SNPs) in mature rs188519172 polymorphisms with anti-TNF treatment response in a Greek population with Crohn’s disease. World J Gastrointest Pharmacol Ther 2017; 8(4): 193-200 Available from: URL: http://www.wjgnet.com/2150-5349/full/v8/i4/193.htm DOI: http://dx.doi.org/10.4292/wjgpt.v8.i4.193

INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic relapsing disease of unknown etiology. It is hypothesized that it arises from a combination of genetic susceptibility and environmental factors that trigger an inappropriate mucosal inflammatory response[4]. Anti-TNF agents have revolutionized IBD therapy since their induction in the market almost 20 years ago and are nowadays considered the cornerstone of IBD treatment strategy[2]. Although their undisputable effectiveness, almost one third of patients will never respond in the first 3-6 mo of therapy (primary non response)[3]. Taking into consideration their known side-effects and the cost-effectiveness of such expensive medications it becomes clear that research and identification of novel reliable biomarkers of response is of paramount importance. Moreover, with several new therapeutic drugs lying ahead of us [anti-interleukin (IL)-12/23 monoclonal antibodies, janus kinase inhibitors, agents targeting leukocyte trafficking] options will expand rendering prediction of response to a specific drug crucial for the patient. Until now, existing clinical or serologic markers have failed to accurately predict a patient’s response to anti-TNF treatment[5]. Genetic or epigenetic markers are under vigorous study in an attempt to improve our understanding of the disease and enhance the prospect of personalized medicine according to each patient’s likelihood to respond to different drug classes, especially anti-TNF agents[5,6].

MicroRNAs (miRNAs) are small, single stranded, non-coding RNA molecules comprising of 19-25 nucleotides exerting post-transcriptional gene expression regulation in response to cellular or environmental changes[7]. MiRNAs have begun to attract scientists’ attention as biomarkers of prognosis or response to treatment in various diseases in part due to some unique advantages they possess: They are practically noninvasive, stable in serum, and can be promptly and repeatedly detected from archived sera[9].

A series of studies have focused on miRNA expression and its impact on response to anti-TNF agents in autoimmune diseases - sharing many commons with IBD-such as rheumatoid arthritis[9,10] or psoriasis[11,12]. MiRNA expression has been investigated in differentiation of CD from UC[13,14] or in distinction of CD phenotypes[15,16] while only one study in Asian population has been done so far searching an association between miRNA expression and response to infliximab in patients with CD[17]. As far as miRNA single nucleotide polymorphisms (SNPs) in mature
or pre-miRNA are concerned no assiduous research has been done trying to unravel a possible association between autoimmune diseases and anti-TNF response. That is in contrast with cancer, where a wealth of studies exists upon miRNA variants and response to treatment\cite{18-22}, with promising results that haven’t though found their role yet in clinical every day practice. Regarding autoimmune diseases and SNPs predicting treatment efficacy, only one recently published study seeks to correlate miR-146a expression and rs2910164 polymorphism to rheumatoid arthritis development and clinical outcome after anti-TNF therapy\cite{23}.

It is well known that miR-146a is implicated in regulation of immune responses through NF-κB pathway and has been extensively studied in autoimmune diseases pathogenesis\cite{23-27} including IBD\cite{28,29}. Concerning miR-196, apart from having been extensively studied in cancer\cite{18-20,195}, it has also been reported to negatively regulate IGRM, a gene associated with autophagy, thus facilitating epithelial inflammation in CD\cite{30} while its variant rs11614913 has been recently related as possibly contributing to IBD-related colorectal cancer development\cite{31}. Last, miR-221 and miR-224 have been detected to be up-regulated after anti-TNF treatment in patients with CD in Fujjoka et al\cite{32} work while both have been shown to interfere in IBD related pathways; miR-221 as a down regulator of ICAM1 gene the protein of which has been widely studied in IBD pathogenesis\cite{32-34} and miR-224 inducing cell proliferation in ovarian murine cells through SMAD/TGF-β pathway\cite{35}. Assuming that SNPs in the aforementioned miRNAs would exert an alteration in their functional capacity, we chose to examine whether rs2910164 of mir-146a, rs11614913 of mir-196a, rs113054794 of miR-221, and rs188519172 of miR-224 can predict response to anti-TNF treatment in a cohort of Greek patients with CD.

**MATERIALS AND METHODS**

**Patients**

One hundred and seven patients diagnosed with CD attending the IBD Clinic at Aretaieio Hospital, Athens, Greece were enrolled in the study. The diagnosis of CD was based on standard clinical, endoscopic, radiological, and pathological criteria\cite{36}. Patients, who were due to receive anti-TNF therapy-infliximab (IFX) or adalimumab (ADA) - and were naïve to these or any other anti-TNF agent, were eligible for the study. Patients could receive in parallel other disease related drugs as long as there was no dose change 8 wk before enrollment. Patients with the following characteristics were excluded from the study: < 18 or > 80 years old, IBD-unclassified, and malignancy.

IFX was administered intravenously at a dose of 5 mg/kg at weeks 0, 2, 6 and every 8 wk thereafter. ADA was administered subcutaneously at a dose of 160 mg at week 0, 80 mg at week 2 and 40 mg every 2 wk thereafter. Clinical and serological response was assessed with Harvey-Bradshaw Index (HBI) and CRP, respectively at various time points: At baseline (before 1st infusion or injection), the day before each subsequent drug administration and at week 12 of treatment. Ileocolonoscopy was performed at baseline and after 12-20 wk of therapy to assess mucosal healing. Changes of endoscopic image compared to baseline were classified in four categories and patients were classified as responders or not to anti-TNF therapy as previously described\cite{37}.

**Genotyping**

Genomic DNA from whole peripheral blood containing EDTA was extracted using validated techniques (NucleoSpin Blood kit; Macherey-Nagel, Germany). PCR–RFLP was used to determine the rs2910164 and rs11614913 was performed using T-ARMS-PCR assay as described previously genotypes as previously described\cite{38,39}. Regarding the rs113054794 we used PCR-RFLP method. Forward primer: 5’CAGAAACATTAGGTTAGCA3’ and reverse: 5’GGTAGTAGGTAAGTCCAGA3’. Annealing was done at 62 °C. PCR products were digested with MvaI. For rs188519172 polymorphism we used allele-specific PCR. Two different PCR reactions are performed with one or the other allele specific primer. The primers used were a common forward 5’CCTCAAGAATCCTCCTCACT3’ and a reverse for the G-allele: 5’GTGGTCTCGTTAGTAGATGAC3’ and for the A-allele: 5’GTGGTCTCGTTAGTAGATGAT3’.

**Statistical analysis**

Genotype frequencies were compared with the $\chi^2$ test with Yate’s correction using S-Plus (v.6.2 Insightful, Seattle, WA, United States). Odds ratios (OR) and 95%CI were obtained with GraphPad (v.300, GraphPad Software, San Diego, CA, United States). The $P$ values are all two-sided. $P$ values of < 0.05 were considered to be significant.

**RESULTS**

Patients’ demographic and clinical characteristics are summarized in Table 1. From the 107 patients included in the study, 104 (97.19%) received infliximab while the rest received adalimumab. Seventy two (67.29%) were classified as complete responders while 22 (21.57%) were classified as partial responders and 13 (12.14%) as non-responders to anti-TNF therapy as far as patients’ clinical response. Thirteen patients (14.74%) did not respond and were classified as non-responders to anti-TNF therapy. Ileocolonoscopy was performed at baseline and after 12-20 wk of therapy to assess mucosal healing. Changes of endoscopic image compared to baseline were classified in four categories and patients were classified as complete, partial, and non-responders ($P = \chi^2$ test with Yate’s correction using S-Plus (v.6.2 Insightful, Seattle, WA, United States). Odds ratios (OR) and 95%CI were obtained with GraphPad (v.300, GraphPad Software, San Diego, CA, United States). The $P$ values are all two-sided. $P$ values of < 0.05 were considered to be significant.

The prevalence of rs2910164, rs11614913, and rs188519172 in patients with CD who responded fully, partially and those who didn’t respond to anti-TNF treatment are depicted in Table 2. Regarding the first polymorphism studied, rs2910164 C allele was not found to be significantly different between complete, partial, and non-responders ($P =$
Table 1  Patient demographic and clinical characteristic according to response to anti-TNF treatment

| Characteristics                     | Responders | Partial responders | Primarily non-responders |
|-------------------------------------|------------|-------------------|--------------------------|
| n (%)                               | 72 (67.29) | 22 (21.57)        | 13 (14.74)               |
| Age (yr, mean ± SD)                 | 34.10±11.63| 32.21±13.31       | 39.09±15.60              |
| Sex (%)                             | 30 (41.67) | 14 (63.64)        | 10 (76.92)               |
| Male                                | 42 (62.69) | 8 (36.36)         | 3 (23.08)                |
| C-reactive protein (mg/dL, mean ± SD) | 3.10±3.91 | 5.71±3.91         | 3.96±2.81                |
| After treatment                     | 0.88±1.84  | 2.21±2.69         | 3.96±2.81                |
| Duration of disease (yr)            | 6.38±5.91  | 5.71±3.77         | 4.00±3.13                |
| Infliximab dose (mg/kg)             | 5          | 5                 | 5                        |

DISCUSSION

Recent studies highlight the emerging role of circulating microRNAs as potential biomarkers in the pathogenesis or response to treatment of cancer and autoimmune diseases[9-22]. In the era when personalized medicine becomes the ultimate goal, vigorous research is carried out towards identification of biomarkers able to predict the exact outcome a therapy may have to a specific patient, according to his unique genetic fingerprints. In IBD, until today, no marker has achieved to fully foresee how patients will respond to anti-TNF treatment, the most popular therapy, which though will be ineffective in one out of three patients during the first months of drug administration[3].

Recently, a study from Japan investigated serum miRNA expression in CD patients receiving induction therapy with infliximab. They concluded that, among others, miR-221 and miR-224 increased during induction therapy with infliximab in patients considered as responders[17]. Castro-Villegas et al[8] studied serum miRNA levels as possible biomarkers of response to 6-month anti-TNFα therapy in patients with rheumatoid arthritis and concluded that, among others, miR-146a increased after anti-TNFα therapy in patients who responded. In addition, Bogunia-Kubik et al[9] have recently assessed miR-146 expression along with its rs2910164 polymorphism and their possible connection to rheumatoid arthritis pathogenesis and therapeutic outcome after 3 mo of anti-TNFα administration. Their results showed initially reduced miR-146 levels in patients compared to controls and restoration of these levels in patients receiving a 3 mo course of anti-TNFα. Moreover they concluded that although rs2910164 variant could be associated with miR-146 levels after treatment, overall this genetic variant didn’t influence neither predisposition to the disease nor efficacy of anti-TNFα therapy, in accordance to our results.

This is the first to our knowledge study to examine the association of polymorphisms in either pre- or mature miRNAs with response to induction therapy with anti-TNF agents in patients with CD.

Our results showed that of the SNPs genotyped, rs2910164 of mir-146, rs11614913 of mir-196a, and rs188519172 of mir-224 had no statistically significant association to anti-TNF treatment response in Greek patients with CD. Moreover, rs113054794 SNP of miR-221 was not detected in our population.

No significant difference was found for rs188519172 as well, between complete, partial, and non- responders. G allele was not statistically different between these groups (P = 0.44, OR = 1.56, 95%CI: 0.56-4.36; and P = 0.75, OR = 0.73, 95%CI: 0.19-2.78 respectively) with GG genotype not being statistically different either (P = 0.61, OR = 1.78, 95%CI: 0.28-11.33; and P = 0.58, OR = 2.86, 95%CI: 0.35-15.05). The rs113054794 SNP of miR-221 was not detected at all in our population.

Concerning rs11614913, again neither T allele nor TT genotype was found to be statistically associated with response to anti-TNF. Specifically, T allele was not found to be different between complete, partial, and non- responders (P = 0.11, OR = 2.7; 95%CI: 0.86-8.39 and P = 1, OR = 1.03; 95%CI: 0.27-3.91 respectively); similarly for TT genotype (P = 0.18, OR = 3.78; 95%CI: 0.8-17.73 and P = 0.34, OR = 2.83; 95%CI: 0.54-4.69 respectively).

0.55, OR = 1.67; 95%CI: 0.14-19.32 and P = 0.39, OR = 2.92; 95%CI: 0.25-34.76 respectively) while CC genotype was not found in any of the patients.

Concerning rs188519172, again neither T allele nor TT genotype was found to be statistically associated with response to anti-TNF. Specifically, T allele was not found to be different between complete, partial, and non- responders (P = 0.11, OR = 2.7; 95%CI: 0.86-8.39 and P = 1, OR = 1.03; 95%CI: 0.27-3.91 respectively); similarly for TT genotype (P = 0.18, OR = 3.78; 95%CI: 0.8-17.73 and P = 0.34, OR = 2.83; 95%CI: 0.54-4.69 respectively).

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clinical, serological and endoscopic markers. Endoscopy at the end of the study was performed to measure primary response to therapy with the most objective marker of treatment efficacy, which is mucosal healing[3].

MiR-146a has been demonstrated to be an integral part of the immunological responses observed in many autoimmune diseases through NF-κB pathway. Specifically, it participates in a negative feedback system induced by microbial constituents like LPS or other pro-inflammatory elements resulting in inhibition of protein production by specific genes. These genes were shown to be interleukin-1 receptor-associated kinase (IRAK) 1 and tumor necrosis factors receptor associated factor (TRAF) 6[34]. Moreover, miR-146a was shown to be overexpressed - upon nitric oxide (NO) trigger - in the nucleotide-binding oligomerization domain (NOD2) signaling pathway thus facilitating further activation of various inflammatory genes like IL-12, TNF-α, Il-6[28]. Both abovementioned mechanisms have been implicated in IBD pathogenesis[35,36] with NF-κB pathway actually being one of the targets of anti-inflammatory effects exerted by steroids and anti-TNF agents[41,43].

MiR-196a has been demonstrated to be related to IBD pathogenesis[29] and IBD phenotype[44] with the target molecular pattern through which it exerts its effect probably being related to autophagy. Specifically it has been reported to negatively regulate Immunity Related GTPase M (IGRM)[30], a gene that has been associated to IBD susceptibility[45]. In addition, its rs11614913 SNP has only recently been implicated in IBD related colorectal cancer progression[33] while its association to other forms of cancer, mainly colorectal, has already been established[46].

MiR-221 has been shown to mediate down regulation of ICAM-1 translation in human cholangiocytes with ICAM-1 playing a major role in regulation of a balanced inflammatory response in biliary cells. MiR-221 related ICAM-1 expression has also been implicated in T cells adhesion during local inflammation[33]. Furthermore, ICAM expression in human umbilical vein endothelial cells was demonstrated to be regulated by miR-221 in response to HIV again influencing monocytes adherence[37]. Zhao et al.[48] have connected miR-221 to TLR4 mediated production of pro-inflammatory cytokines in lung cells with simultaneous increased TNFα and IL-6 expression through NF-κB signaling, a key pathway in IBD pathogenesis.

Apart from the aforementioned cell lines, miR-221 has also been studied in colonic epithelial cells with similar results. Fang et al.[49] have shown that down regulation of miR-221 leads to amplification of experimental colitis and increase of TNFα in histological specimens. All the above highlight a plausible role of miR-221 in inflammatory response either through ICAM regulation, a molecule suggested to interfere with IBD inflammation facilitation[34,50,51], either through still unraveled mechanisms.

Last, miR-224 expression has been shown to be up-regulated in hepatocellular cancer patients with its possible target being apoptosis inhibitor-5 (API-5), thus mediating its role by inducing apoptosis[52]. Over expression of miR-224 has also been displayed in T cells of systemic lupus erythematosus patients again through suppression of API-5 leading to T cell apoptosis[53]. Interestingly, one report has presumed AIP5 involvement in IBD inflammation and progression to neoplasia, as quick epithelial cell turnover, cell proliferation and finally apoptosis are present in both these situations[54]. Moreover, Olaru et al.[55] have proved involvement of miR-224 in down-regulation of p21, a tumor suppressor gene through which miR-224 coordinates neoplasia initiation and progression through dysplasia to IBD related colorectal cancer. MiR-224 is implicated in inflammatory pathways, also linked to IBD pathogenesis. Scisciani et al.[56] reported p65/NF-κB to be a target pathway of miR-224 in liver cells while TNFα inflammatory pathway is activated with up-regulation of miR-224. Two other reports have demonstrated that SMAD4 is the target of miR-224 with SMAD4 being a pivotal component of TGF-β pathway leading to cell proliferation[35,57]. TGF-β pathway dysregulation has long been shown to be a contributor to IBD pathogenesis[58].

Table 2 Genotype and allele frequencies of rs2910164, rs11614913, and rs188519172 polymorphisms in Crohn’s disease patients according to response to anti-TNF treatment

| Genotype      | Complete responders (n = 72) | Partial responders (n = 22) | P value; OR (95%CI) | Non-responders (n = 13) | P value; OR (95%CI) |
|---------------|-----------------------------|-----------------------------|---------------------|-------------------------|---------------------|
| miR-146a, rs2910164 |                             |                             |                     |                         |                     |
| GG            | 70 (97.22)                  | 21 (95.45)                  | 1.0 (reference)     | 12 (92.31)              | 1.0 (reference)     |
| GC            | 2 (2.78)                    | 1 (4.54)                    | 0.55; 1.67 (0.14-19.32) | 1 (7.69)               | 0.39; 2.92 (0.25-34.76) |
| CC            | 0                           | 0                           | -                   | 0                       | -                   |
| miR-196a, rs11614913 |                             |                             |                     |                         |                     |
| CC            | 33 (45.83)                  | 5 (22.73)                   | 1.0 (reference)     | 5 (38.46)               | 1.0 (reference)     |
| CT            | 32 (44.45)                  | 13 (59.09)                  | 0.11; 2.27 (0.86-8.39) | 5 (38.46)              | 1.0; 1.03 (0.27-3.91) |
| TT            | 7 (9.73)                    | 4 (18.18)                   | 0.18; 3.78 (0.8-17.73) | 3 (23.08)              | 0.34; 2.83 (0.54-14.69) |
| miR-224, rs188519172 |                             |                             |                     |                         |                     |
| AA            | 38 (52.78)                  | 9 (40.9)                    | 1.0 (reference)     | 7 (53.84)               | 1.0 (reference)     |
| AG            | 29 (40.28)                  | 11 (50)                     | 0.44; 1.60 (0.59-4.37) | 4 (30.76)              | 0.75; 0.75 (0.20-2.80) |
| GG            | 5 (6.94)                    | 2 (9.09)                    | 0.62; 1.69 (0.28-10.16) | 2 (15.38)             | 0.59; 2.17 (0.35-13.51) |
Hypothesizing that miRNAs regulating IBD susceptibility genes would be a logical initial thought, we chose to study SNPs of such miRNAs, such as miR-196a, and their association to anti-TNF response in patients with CD. Among many, we presumed that SNPs of miR-146a, miR-221, and miR-224 would additionally be ideal based on previous work showing alteration of their expression after anti-TNF treatment in patients with CD and rheumatoid arthritis\(^\text{[9,17,23]}\). However, even though anti-TNF treatment interferes in pathophysiological pathways regulated by those miRNAs, no correlation was found. This is in agreement to what Lee et al\(^\text{[69]}\) have concluded in a very recent genome-wide association study in CD. Their data support the idea of different genetic loci contributing in susceptibility compared to prognosis - and thus potential therapeutic interventions - in adult patients with CD. Interestingly, this had been already shown in pediatric patients with CD being treated with anti-TNF agents\(^\text{[68]}\). Furthermore, absence of studied SNPs’ association to treatment response may denote that other SNPs or other transcriptional (ex, methylation of gene promoters) and post-transcriptional mechanisms (concerning miRNA stability or processing) interfere with alterations in miRNA expression. In addition, in the only study conducted in IB patients assessing miRNA expression alterations and anti-TNF response, the population studied was of a different ethnic group - Japanese-compared with ours. Ethnic and geographic differences representing distinct genetic and environmental background may influence frequency or even variety of polymorphisms detected.

We chose to include in our study patients receiving both infliximab or adalimumab. It has been established that their clinical and endoscopic efficacy is similar in Crohn’s disease\(^\text{[66,67]}\). Nevertheless, their different structure could have interfered with our results. Notwithstanding, with only 3 patients receiving adalimumab, we believe that our findings have only minimally been influenced.

Lastly, another factor contributing in our inability to show a positive association between studied SNPs and anti-TNF response may be that due to the small effect these variants may pose, we would require a larger sample to identify a statistically significant result. IBD genetic background has not been fully elucidated but we know that a variety of risk factors contribute with a small or modest effect and not one highly penetrant, suggesting a difficulty of uncovering this effect.

Nevertheless, we believe that miRNAs constitute a small but rather attractive pawn in our effort to delineate epigenetic regulation of gene expression and its contribution to IBD susceptibility, prognosis and therapeutic possibilities. Genetic markers may need to be used as biomarkers of therapy response in combination to other clinical or serological ones to attain the maximum benefit and accurately distinguish the ideal patient for each therapeutic treatment.

In conclusion, our results demonstrate for the first time that mir-146 rs2910164, mir-196a rs11614913, mir-221 rs113054794 and mir-224 rs188519172 are not correlated with anti-TNF treatment response in Caucasian patients with CD. Hence, they cannot be used as biomarkers to predict anti-TNF drug response in candidate patients with CD. Further independent studies are required to validate our findings in a larger scale or possibly to a different ethnic population.

**Peer-review**

The result of this study demonstrates for the first time that mir-146 rs2910164, mir-196a rs11614913, mir-221 rs113054794 and mir-224 rs188519172 are not correlated with anti-TNF treatment response in Caucasian patients with CD. Hence, these markers can be used as biomarkers to predict anti-TNF drug response in candidate patients with CD.

**REFERENCES**

1. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. *Ann Rev Immunol* 2010; 28: 573-621 [PMID: 20192811 DOI: 10.1146/annurev-immunol-030409-101225]
2. Rutgeerts P, Vermeire S, Van Assche G. Biological therapies for inflammatory bowel diseases. *Gastroenterology* 2009; 136: 1182-1197 [PMID: 19249397 DOI: 10.1053/j.gastro.2009.02.001]
3. Ding NS, Hart A, De Cruz P. Systematic review: predicting and optimising response to anti-TNF therapy in Crohn’s disease - algorithm for practical management. *Aliment Pharmacol Ther* 2016; 43: 30-51 [PMID: 26515897 DOI: 10.1111/apt.13445]
4. Billiet T, Ferrante M, Van Assche G. The use of prognostic factors in inflammatory bowel diseases. *Curr Gastroenterol Rep* 2014; 16: 416 [PMID: 25262067 DOI: 10.1007/s11894-014-0416-y]
5. McGovern DP, Kugathasan S, Cho JH. Genetics of Inflammatory Bowel Diseases. *Gastroenterology* 2015; 149: 1163-1176.e2 [PMID: 26255561 DOI: 10.1053/j.gastro.2015.08.001]
6. Prieto-Pérez R, Almoguera B, Caballeiro T, Hakonarson H, Abad-Santos F. Association between Genetic Polymorphisms and Response to Anti-TNFs in Patients with Inflammatory Bowel Disease. *Int J Mol Sci* 2016; **17**: 225 [PMID: 26863132 DOI: 10.3390/ijms17020225]

7. Winter J, Sarker S, Keller S. Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 2009; **11**: 228-234 [PMID: 19255566 DOI: 10.1038/ncb0309-228]

8. Ginge C, Claus S, Boddum K, Jabbri R, Jisragard B, Tompsits P, Hildebrand B, Kääb S, Wakiš R, Jespersen T, Tfelt-Hansen J. Stability of Circulating Blood-Based Micro-RNAs - Pre-Analytical Methodological Considerations. *PLoS One* 2017; **12**: e0167690 [PMID: 28571938 DOI: 10.1371/journal.pone.0167690]

9. Castro-Villegas C, Pérez-Sánchez C, Escudero A, Filipescu I, Verdu M, Ruiz-Limón P, Aguirre MA, Jiménez-Gómez Y, Font P, Rodríguez-Ariza A, Peinado JR, Collantes-Estévez E, González-Conejero R, Martínez C, Barbero-N López-Pedrera C. Circulating miRNAs as potential biomarkers of therapy effectiveness in rheumatoid arthritis patients treated with anti-TNFα. *Arthritis Res Ther* 2015; **17**: 49 [PMID: 25860297 DOI: 10.1186/s13075-015-0555-z]

10. Cuppen BV, Rosatto M, Frisch-Stork RD, Concepcion AN, Schenk Y, Biglavs JW, Radtaste TR, Laléfer FP, all SRU investigators. Can baseline serum microRNAs predict response to TNF-α inhibitors in rheumatoid arthritis? *Arthritis Res Ther* 2016; **18**: 189 [PMID: 27558398 DOI: 10.1186/s13075-016-0985-z]

11. Raabý L, Langkilde A, Kjellérup RB, Vinter H, Khalib SB, Hjuler KF, Johansen C, Iversen L. Changes in mRNA expression precede changes in microRNA expression in lesional psoriatic skin during treatment with adalimumab. *Br J Dermatol* 2015; **173**: 436-447 [PMID: 25662483 DOI: 10.1111/bjd.13721]

12. Pivarevi A, Meigen F, Xu N, Stähle M, Sonkoly E. Changes in the level of serum microRNAs in patients with psoriasis after antitumour necrosis factor-α therapy. *Br J Dermatol* 2016; **176**: 563-570 [PMID: 23600954 DOI: 10.1111/bjd.12381]

13. Scharfer JS, Attumi T, Opekun AR, Abraham B, Hou J, Shelby H, Graham DY, Streckfus C, Klein JR. MicroRNA signatures differentiate Disease Behavior Phenotypes of Crohn’s disease and may herald a switch to Anti-TNFs in Patients with Inflammatory Bowel Disease. *J Biol Chem* 2013; **288**: 33037-33048 [PMID: 24092752 DOI: 10.1074/jbc.M113.492496]

14. Gazouli M, Papacostantinou I, Stamatis K, Vaiopoulou A, Zeglinas C, Vassiliou I, Giokas G, Tzathas C. Association study of genetic variants in miRNAs in patients with inflammatory bowel disease: preliminary results. *Dig Dis Sci* 2013; **58**: 2324-2338 [PMID: 23543085 DOI: 10.1007/s10620-013-3240-2]

15. Brest P, Lapaquette P, Soudi M, Lebrigand K, Cesaro A, Vuoret-Cravatsi V, Mann B, Barby P, Mosnier JF, Hébuterne X, Harel-Bellan A, Mograbi B, Darfauf-Michaud A, Hofman P. A synonymous variant in IRGM alters a binding site for miR-196 and causes deregulation of IRGM-dependent xenophagy in Crohn’s disease. *Arch Immunol Ther Exp (Warsz)* 2016; **64**: 131-136 [PMID: 28083614 DOI: 10.1007/s00005-014-0443-5]

16. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci U S A* 2006; **103**: 12481-12486 [PMID: 16885212 DOI: 10.1073/pnas.0605298103]

17. Pauley KM, Satoh M, Chan AL, Bubb MR, Reeves WH, ChanEK. Unregulated miR-146a expression in peripheral blood mononuclear cells from rheumatoid arthritis patients. *Arthritis Res Ther* 2008; **10**: R101 [PMID: 18759964 DOI: 10.1186/ar2493]

18. Abou-Zeid A, Saad M, Solomon E. MicroRNA 146a expression in rheumatoid arthritis: association with tumor necrosis factor-alpha and disease activity. *Genet Test Mol Markers* 2011; **15**: 807-812 [PMID: 21810022 DOI: 10.1016/j.gtmm.2011.02.006]

19. Park R, Lee WJ, Ji JD. Association between the three functional miR-146a single-nucleotide polymorphisms, rs2910146, rs7095329, and rs2431697, and autoimmune disease susceptibility: A meta-analysis. *Autoimmunity* 2016; **49**: 451-458 [PMID: 27088222 DOI: 10.1016/j.autoimm.2016.07.011]

20. Zhu M, Li D, Jin M, Li M. Association between microRNA polymorphisms and the risk of inflammatory bowel disease. *Mol Med Rep* 2016; **13**: 5297-5308 [PMID: 27109937 DOI: 10.3822/mm.2016.5157]

21. Bai J, Li Y, Shao T, Zhao Z, Wang Y, Wu A, Chen H, Li S, Jiang C, Xu J, Li X. Integrating analysis reveals microRNA-mediated pathway crosstalk among Crohn’s disease, ulcerative colitis and colorectal cancer. *Mol Biosyst* 2014; **10**: 2317-2328 [PMID: 24949825 DOI: 10.1039/c4mb00169a]

22. Hu G, Gong AY, Liu J, Zhou R, Deng C, Chen XM. miR-221 suppresses IRGM-1 translation and regulates interferon-gamma-induced IRGM-1 expression in human cholangiocytes. *Ant J Physiol Gastrointest Liver Physiol* 2010; **298**: G542-G550 [PMID: 20104603 DOI: 10.1152/ajpgi.00490.2009]

23. Song BW, Lv YH, Zhang ZS, Li YN, Xiao LP, Yu XP, Wang YY, Ji HL, Ma L. Soluble intercellular adhesion molecule-1, D-lactate and soluble intercellular adhesion molecule-1 expression in human cholangiocytes. *World J Gastroenterol* 2009; **15**: 3916-3919 [PMID: 19701972 DOI: 10.3748/wjg.15.3916]
granulosa cell proliferation and granulosa cell function by targeting Smad4. Mol Endocrinol 2010; 24: 540-551 [PMID: 20118412 DOI: 10.1202/mce.2009-0432]

Podolsky DK. Inflammatory bowel disease. N Engl J Med 2002; 347: 417-429 [PMID: 12167685 DOI: 10.1056/NEJMoa020831]

Papamichael K, Gouzoli M, Karakoidas C, Panayotou I, Roman-Giannikou E, Mantzaris GJ. Association of TNF and FcRγIIA gene polymorphisms with differential response to infliximab in a Greek cohort of Crohn’s disease patients. Ann Gastroenterol 2011; 24: 35-40 [PMID: 24714240]

Maharaj NR, Ramkaran P, Pillay S, Chuturagoon AA. MicroRNA-146a rs2910164 is associated with severe preeclampsia in Black South African women on HAART. BMC Genet 2017; 18: 5 [PMID: 28103790 DOI: 10.1186/s12863-016-0469-z]

Song ZS, Wu Y, Zhao HG, Liu CX, Cai HY, Gao BZ, Xie YA, Shi HR. Association between the rs16114913 variant of miRNA-196a-2 and the risk of epithelial ovarian cancer. Oncol Lett 2016; 11: 194-200 [PMID: 26870188 DOI: 10.3892/ol.2015.3577]

Nguyen-Dien GT, Smith RA, Haupt LM, Griffiths LR, Nguyen HT. Genetic polymorphisms in miRNAs targeting the estrogen receptor and their effect on breast cancer risk. Mol Diagn Ther 2016; 20: 59-66 [PMID: 25664064 DOI: 10.1007/s12028-014-9315-4]

Schreiber S, Nikolaus S, Hampe J. Activation of nuclear factor kappa B inflammatory bowel disease. Gut 1998; 42: 477-484 [PMID: 9616307 DOI: 10.1136/gut.42.4.477]

Hugot JP, Charmaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Alenier S, Tysk C, O’Morain CA, Cassell M, Binder V, Finkiel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Almer S, Tysk C, O’Morain CA, Gassull M, Binder V, Finkel Y, Franssen KD, Rotter JI. Genome wide association (GW A) predictors of anti-TNFα treatment failure in inflammatory bowel disease. Gut 2007; 56: 155-164 [PMID: 17568520 DOI: 10.1136/gut.2006.098639]

Cicciazi C, Polistì C, Biancone L, Latini A, Novelli G, Calabrese E, Borgiani P. Polymorphisms in MIR122, MIR196A2, and MIR124A Genes are Associated with Clinical Phenotypes in Inflammatory Bowel Diseases. J Mol Diagn Ther 2017; 21: 107-114 [PMID: 27718165 DOI: 10.1007/s40291-016-0240-1]

Brest P, Corcèlle EA, Cesaro A, Charrigu A, Behald K, Klionsky DJ, Bouvet-Craviari V, Heuterman H, Hofman P, Mograbi B. Autophagy and Crohn’s disease: at the crossroads of infection, inflammation, immunity, and cancer. Curr Mol Med 2010; 10: 486-502 [PMID: 20540703 DOI: 10.2174/156652410791608252]

Schimanski CC, Freericks K, Rahman F, Berger M, Lang H, Galea PR, Moehler M, Gockel I. High mir-196a levels promote the oncogenic phenotype of colorectal cancer cells. World J Gastroenterol 2009; 15: 2089-2096 [PMID: 19418581 DOI: 10.3748/wjg.v15.2089]

Duan M, Yao H, Hu G, Chen X, Lund AK, Buch S. HIV Tat induces expression of ICAM-1 in HIV-1 infected CD4+ T cells and ICAM-1 mediates leukocyte-endothelial cell adhesion in rat experimental colitis. Gastroenterology 1999; 116: 874-883 [PMID: 10092309 DOI: 10.1016/s0016-5085(99)70070-3]

Taniguchi T, Tsukada H, Nakamura H, Kodama M, Fukuda K, Saito T, Miyasaka M, Seino Y. Effects of the anti-ICAM-1 monoclonal antibody on dextran sodium sulphate-induced colitis in rats. J Gastroenterol Hepatol 1998; 13: 945-949 [PMID: 9794195 DOI: 10.1111/j.1440-1746.1998.tb00766.x]

Wang Y, Lee AT, Ma JZ, Wang J, Ren J, Yang Y, Tantoso E, Li KB, Ooi LL, Tan P, Lee CG. Profiling microRNA expression in hepatocellular carcinoma reveals microRNA-224 up-regulation and apoptosis inhibitor-3 as a microRNA-224-specific target. J Biol Chem 2008; 283: 13205-13215 [PMID: 18319255 DOI: 10.1074/jbc.M707629200]

Lu MC, Lai NS, Chen HC, Yu HC, Huang KY, Tung CH, Huang HB, Yu CL. Decreased microRNA(miR)-145 and increased miR-224 expression in T cells from patients with systemic lupus erythematosus involved in lupus immunopathogenesis. Clin Exp Immunol 2013; 171: 91-99 [PMID: 23199328 DOI: 10.1111/cei.12492.2013.04676.x]

Pekow JR, Dougherty U, Mustafi R, Zhu H, Kocherginsky M, Rubin DT, Hanauer SB, Hart J, Chang EB, Fishman A, Joseph LJ, Bissoum E. miR-143 and miR-145 are downregulated in ulcerative colitis: putative regulators of inflammation and protooncogenes. Inflamm Bowel Dis 2012; 18: 94-100 [PMID: 21557394 DOI: 10.1002/ibd.21742]

Olaru AV, Yamanaka S, Vazquez C, Mori Y, Cheng Y, Abraham JM, Bayless TM, Harpur N, Selaru FM, Meltzer SJ. MicroRNA-224 negatively regulates p21 expression during late neoplastic progression in inflammatory bowel disease. Inflamm Bowel Dis 2013; 19: 471-480 [PMID: 23399735 DOI: 10.1097/MIB.0b013e31827e76b]

Seiceliani C, Vossio S, Guerrieri F, Schinzari V, De Iaco R, D’Onorio de Meo P, Cerovello M, Montalto G, Pollicino T, Raimondo G, Levrevo M, Pediconi N. Transcriptional regulation of miR-224 upregulated in human HCCs by NFκB inflammatory pathways. J Hepatol 2012; 56: 855-861 [PMID: 22178270 DOI: 10.1016/j.jhep.2011.11.007]

Wang Y, Ren J, Gao Y, Ma JZ, Toh HC, Chow P, Chung AY, Ooi LL, Lee CG. MicroRNA-224 targets SMAD family member 4 to promote cell proliferation and negatively influence patient survival. PLoS One 2013; 8: e68744 [PMID: 23922662 DOI: 10.1371/journal.pone.0068744]

Hahn KB, Im YH, Parks TW, Park SH, Markowitz S, Jung HY, Green J, Kim SJ. Loss of transforming growth factor beta signalling in the intestine contributes to tissue injury in inflammatory bowel disease. Gut 2001; 49: 190-198 [PMID: 11454793]

Lee JC, Biasci D, Roberts R, Gecary RB, Mansfield JC, Ahmad T, Prescott NJ, Satsangi J, Wilson DC, Jordan J, Anderson CA, UK IBD Genetics Consortium, Traherne JA, Lyons PA, Parkes M, Smith KG. Genome-wide association study identifies distinct genetic contributions to prognosis and susceptibility in Crohn’s disease. Nat Genet 2017; 49: 262-268 [PMID: 28067912 DOI: 10.1038/ng.3755]

Dubinsky MC, Mei L, Friedman M, Dhere T, Haritunians T, Hakonarson H, Kim C, Glesner J, Targan SR, McDevitt DP, Taylor KD, Rotter J. Genome-wide association (GWA) predictors of anti-TNFα therapeutic responsiveness in pediatric inflammatory bowel disease. Inflamm Bowel Dis 2010; 16: 1357-1366 [PMID: 20014019 DOI: 10.1002/ibd.21174]

Stidham RW, Lee TC, Higgins PD, Deshpande AR, Sussman DA, Singal AG, Elmunzer BJ, Saini SD, Vijan S, Waljee AK. Systematic review with network meta-analysis: the efficacy of anti-TNF agents for the treatment of Crohn’s disease. Aliment Pharmacol Ther 2014; 39: 1349-1362 [PMID: 24749763 DOI: 10.1111/apt.12749]

Chopra A, Hazlewood GS, Kaplan GG, Peyrin-Biroulet L, Ananthakrishnan AN. Systematic review with meta-analysis: comparative efficacy of biologics for induction and maintenance of mucosal healing in Crohn’s disease and ulcerative colitis controlled trials. Aliment Pharmacol Ther 2017; 45: 1291-1302 [PMID: 28326566 DOI: 10.1111/apt.14030]
