THE ROLE OF THIOPURINE METHYLTRANSFERASE PROMOTER METHYLATION IN AZATHIOPRINE BIOTRANSFORMATION IN EARLY-ONSET PEDIATRIC PATIENTS WITH INFLAMMATORY BOWEL DISEASE

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Introduction: Inflammatory bowel disease (IBD) is a chronic inflammation of the intestinal tract, that comprises 2disorders, Crohn's disease (CD) and ulcerative colitis (UC) that may develop at relatively young ages. In early-onset (EO) IBD patients (age < 6years old) generally the pathology is more aggressive, with a high probability of complications. At present, a curative therapy for IBD does not exist, but many drugs are employed to induce and maintain disease remission, such as mercaptopurine (MP) and its pro-drug azathioprine (AZA) alone or in combination with other drugs. The response to AZA and MP is highly variable and can be influenced by various factors. In particular the enzyme thiopurine methyltransferase (TPMT) is well known for its role in the inactivation of MP. Alterations in the TPMT gene can lead to decreased inactivation of MP, increased concentration of active metabolites, determining enhanced bone marrow toxicity. Recently, age has been demonstrated to affect the efficacy of thiopurines in pediatric IBD patients, with effects particularly strong in EO patients, who need increased AZA doses, putatively because of increased TPMT activity. Our hypothesis is that the increase in TPMT activity in EO-IBD patients is caused by a low TPMT promoter methylation. For this purpose, TPMT promoter methylation and TPMT gene expression, and their associations with patients' age and therapy duration has been evaluated.

Materials and methods: Patients with pediatric IBD, under AZA therapy for at least 3months were enrolled. TPMT methylation was analyzed using Infinium MethylationEPIC BeadChip Array in 10 EO and 10 nonearly-onset (NEO) IBD patients (aged >12and <18years). Five candidate CpG sites potentially involved in TPMT gene expression, located in TPMT's promoter and neighboring region, were selected in the current analysis. AZA metabolites and TPMT activity were assessed in lysates of patients' erythrocytes by HPLC-UV. TPMT gene expression analysis was performed using TaqMan Gene Expression Assays in a different subset of IBD patients composed by 7EO and 15NEO. DNA and RNA samples were obtained from patients' peripheral blood mononuclear cells (PBMCs). Statistical analysis was performed using linear models.

Results: This study recruited 10 EO (average age: 4.71years, average AZA duration: 512days , 7females, 5UC, 2CD and 3undetermined colitis) and 10 NEO (average age: 15.6years, average AZA duration: 1311days , 3females, 6UC, 4CD) pediatric IBD patients for the DNA methylation analysis. AZA duration was different between EO and NEO patients (p-value: 0.0007), while AZA metabolites levels, AZA doses and TPMT activity showed no difference. AZA therapy duration was associated with decreased methylation level in 2CpG sites (cg04231636, cg00772000) (p-value: 0.040 and 0.046respectively). CpG site cg04231636in the neighboring region of TPMT resulted also less methylated in NEO (average methylation 0.30 ± 0.010) compared to EO patients (0.35± 0.016) (p-value: 0.035). Preliminary results of TPMT gene expression and its association with therapy duration or patients' age, showed no difference between 7EO (average age: 4.1years, average AZA duration: 387days , 5females, 4UC, 1CD, 1undetermined colitis and data not available for 1patient) and 15NEO (average age: 15.1years, average AZA duration: 1072days , 7females, 7UC, 6CD and data not available for 2patients). All the patients analyzed were carrying wild-type TPMT gene.

Discussion and conclusion: AZA therapy decreased the methylation level in TPMT gene neighboring region. NEO patients presented a lower methylation level of the cg04231636compared to EO even though it is still not clear if this reduction is independent from the effect of AZA duration. Further studies will increase the number of patients for all the analysis in the way of further exploring AZA biotransformation in EO IBD patients.