Title:

Genes implicated in thiopurine-induced toxicity: Comparing TPMT enzyme activity with clinical phenotype and exome data in a paediatric IBD cohort

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Supplementary tables

Table S1: Genes implicated in thiopurine metabolism and toxicity

	HUGO Gene	Symbol	Chromosome	Function	Percentage coverage Agilent v5	Percentage coverage Agilent v4	References
1	Aldehyde oxidase 1	*AOX1*	2	Catabolism of 6-MP to 6-thiouracil (6-TU)	100	88.87	[1-3]
2	ATP-binding cassette, sub-family C (CFTR/MRP), member 4	*ABCC4*	13	Efflux pump transporting a wide variety of endogenous and xenobiotic organic anionic compounds including 6-MP and 6-TGN out of the cell	95.61	72.00	[4]
3	Follistatin-Like 5	*FSTL5*	4	Involved in calcium ion binding and interacting with metalloproteases at the extracellular matrix level	91.40	57.40	[5]
4	Guanine monphosphate synthase	*GMPS*	3	Converts 6-TIMP to 6-thioguanines (6-TGN)	100	96.78	[6]
5	Glutathione-s-transferase	*GSTM1*	1	Detoxification enzymes catalysing the conjugation of electrophilic substrates to glutathione	100	42	[7-10]
6	Hypoxanthine phosphoribosyl transferase 1	*HPRT1*	X	Conversion of 6-MP to 6-TIMP	100	76.81	[6, 11-15]
7	Inosine-5-Monophosphate Dehydrogenase 1	*IMPDH1*	7	Converts 6-TIMP to 6-thioguanines (6-TGN)	99.39	77.41	[6, 16]
8	Inosine-5-Monophosphate Dehydrogenase 2	*IMPDH2*	X	Converts 6-TIMP to 6-thioguanines (6-TGN)	100	100	[6, 16]
9	Inosine triphosphatase (nucleoside triphosphate pyrophosphatase)	*ITPA*	20	De-phosphorylation of 6-thioinosine triphosphate (6-TITP) to 6-thioinosine monophosphate (6-TIMP) in the thiopurine metabolic pathway	89.65	76.99	[4, 6, 7, 11, 17-31]
10	Interleukin 6 signal transducer	*IL6ST*	5	Signal transducer protein shared by several cytokines including IL-6	96.55	32.14	[5]
11	Methylenetetrahydrofolate reductase	*MTHFR*	1	Intracellular folate metabolism and purine-pyrimidine synthesis	95.3	65.70	[32]
12	Molybdenum cofactor sulfurase	*MOCOS*	18	Sulfuration of molybdenum co-factor in the enzymes XDH and AOX1; essential for their enzymatic activities	100	100	[2, 3]
13	Nudix (nucleoside diphosphate linked moiety X)-type motif 15	*NUDT15*	13	Involved in DNA repair	95.99	43.12	[33]

14	Protein kinase C and casein kinase substrate in neurons 2	*PACSIN2*	22	Family of proteins, involved in various biological processes including endocytosis, cell-cycle control and autophagy	89.01	49.78	[34]
15	Xanthine dehydrogenase (Synonyms- XO)	*XDH*	2	Catabolism of 6-MP to 6-thiouracil (6-TU)	100	75.94	[1-3, 12, 35, 36]

Fifteen genes implicated in thiopurine metabolism and toxicity identified through a systematic search shown in the table (tabulated in an alphabetical order). The *HLA-DQA1-HLA-DRB1* locus although implicated in thiopurine toxicity, was not included in our analysis due to inherent difficulties in analysis of this highly polymorphic region through whole-exome sequencing data.

Table S2: Specificity and sensitivity for predicting normality of the biochemical test

		TPMT value < 67	TPMT value > 67	Sensitivity	Specificity
Deleterious *TPMT* variants	+	10	0	19.23%	100%
Non-deleterious *TPMT* variants	-	42	48		
Deleterious *MOCOS* variants	+	28	30	60.86%	37.50%
Non-deleterious *MOCOS* variants	-	24	18		

In this table, the sensitivity and specificity values for predicting the normality of the TPMT enzyme activity (with 67 mU/L as the cut-off between low or intermediate and normal) through application of the TPMT variants alone or in combination with the MOCOS variants. Inclusion of the MOCOS variants to the TPMT variation improves the sensitivity for the biochemical activity level of TPMT, but a corresponding loss of specificity is observed when compared with the TPMT variation alone.

Table S3: Specificity and sensitivity for Responders and non-responders

		Responders	Non-Responders	Sensitivity	Specificity
Deleterious *IL6ST* variants	+	14	2	27.45%	84.62%
Non-deleterious *IL6ST* variants	-	37	11		
Deleterious *ABCC4* variants	+	4	6	7.84%	53.85%
Non-deleterious *ABCC4* variants	-	47	7		

Table S3 demonstrates the sensitivity and specificity of application of IL6ST and ABCC4 variations with clinical response to thiopurines as the gold standard.

Table S4: Clinical Information on the 100 patients recruited to the study

Patients	Gender	Diagnosis	TPMT mU/L	Any blood transfusions before the test (<3 months)	On 5-ASA drugs at or before the time of test	On Azathioprine/6-Mercaptopurine	Dose (mg/kg)	Abnormal counts requiring withdrawal of the drug	Abnormal LFTS after starting the drug	Pancreatitis	Other complications	Drug withdrawn	Possible Responder	Possible Nonresponder or Poor responder	Therapy intolerant
1	M	CD	34	X	X	Azathioprine	2.75	X	X	X	X	X	X	✓	X
2	F	CD	31	X	X	Azathioprine	2	X	X	X	X	X	✓	X	X
3	M	CD	32	X	X	Azathioprine	2.3	X	X	X	X	X	✓	X	X
4	M	CD	55	X	X	Azathioprine	2.2	X	X	X	X	X	✓	X	X
5	M	CD	32	X	X	Azathioprine	2	X	X	X	X	X	X	✓	X
6	M	CD	21	X	X	Azathioprine	1.5	X	X	X	Leucoencephalopathy	X	X	X	✓
7	M	CD	38	X	X	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
8	M	UC	33	X	✓	Azathioprine	2.7	X	X	X	X	X	✓	X	X
9	M	CD	25	X	X	Azathioprine	2	X	X	X	X	X	✓	X	X
10	F	CD	18	X	X	Azathioprine	2	✓	X	X	Neutropenia	✓	X	X	✓
11	F	CD	49	X	✓	Azathioprine	2.5	X	X	X	X	X	X	✓	X
12	M	CD	29	X	✓	Azathioprine	2.5	X	X	X	X	✓	X	✓	X
13	M	CD	29	X	✓	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
14	F	CD	25	X	X	Azathioprine	2	X	X	X	X	X	X	✓	X
15	M	UC	101	X	✓	Azathioprine	2.5	X	X	X	X	X	✓	X	X
16	M	UC	77	X	✓	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
17	F	CD	85	X	X	Azathioprine	2	X	X	X	X	X	✓	X	X
18	M	CD	32	X	X	Azathioprine	2.5	X	X	X	X	X	X	✓	X
19	M	CD	32	X	X	Azathioprine	2	X	X	X	X	X	X	✓	X
20	M	CD	29	X	X	Azathioprine	2	X	X	X	X	X	✓	X	X
21	F	CD	32	X	✓	Azathioprine	2.25	X	X	X	X	X	✓	X	X
22	M	UC	26	X	✓	Azathioprine	2	X	X	X	Elevated amylase	✓	X	X	✓
23	F	CD	30	X	✓	Azathioprine	2	X	X	X	Severe nausea	✓	X	X	✓
24	M	CD	138	X	✓	Azathioprine	2	X	X	X	X	✓	✓	X	X
25	M	UC	37	✓	X	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
26	M	CD	127	X	X	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
27	M	UC	29	X	X	Azathioprine	2	X	X	X	X	X	✓	X	X
28	M	CD	93	X	✓	Azathioprine	1	X	X	X	Severe nausea	✓	X	X	✓
29	M	UC	17	X	X	Azathioprine	2.5	X	X	X	X	X	✓	X	X
30	M	CD	37	X	✓	Azathioprine	1.5	X	X	X	X	X	✓	X	X
31	M	CD	34	X	X	Azathioprine	1.5	X	X	X	X	X	✓	X	X
32	F	UC	34	X	✓	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
33	M	CD	18	X	✓	Azathioprine	2	X	X	X	X	X	✓	X	X
34	F	UC	29	X	✓	6-Mercaptopurine	1	X	✓	X	X	X	X	✓	X
35	F	IBDU	105	X	X	6-Mercaptopurine	1	X	X	X	X	X	✓	X	X
36	F	UC	92	X	✓	6-Mercaptopurine	2	X	X	X	X	X	✓	X	X
37	F	CD	41	X	X	Azathioprine	2	X	X	X	X	X	✓	X	X
38	F	UC	30	X	X	6-Mercaptopurine	2	X	X	X	X	X	✓	X	X
39	M	CD	16	X	X	Azathioprine	1.5	✓	X	X	Pancytopenia	✓	X	X	✓
40	M	CD	52	X	✓	6-MP	1	X	X	X	Severe nausea	✓	X	X	✓
41	M	UC	114	X	✓	Azathioprine	1	X	✓	X	Abnormal LFTs	✓	X	X	X
42	M	CD	93	X	✓	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
43	F	UC	50	X	✓	Azathioprine	1.5	X	X	X	X	X	✓	X	X
44	F	CD	36	X	X	6-Mercaptopurine	1	X	X	X	X	X	✓	X	X
45	M	UC	25	X	X	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
46	F	UC	31	X	X	6-Mercaptopurine	1.5	X	X	X	X	X	X	✓	X
47	F	CD	96	X	X	Azathioprine	1.5	X	X	X	X	X	✓	X	X
48	F	UC	105	X	✓	Azathioprine	2	X	X	X	X	X	✓	X	X
49	F	CD	27	X	X	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable

50	F	IBDU	91	**X**	✓	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
51	F	IBDU	82	**X**	✓	6-Mercaptopurine	1.5	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
52	M	CD	14	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
53	M	CD	85	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
54	F	UC	152	**X**	✓	Azathioprine	2.5	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
55	F	CD	86	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
56	F	CD	101	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
57	M	UC	26	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
58	F	CD	77	**X**	**X**	Azathioprine	2.5	**X**	**X**	**X**	**X**	**X**	**X**	✓	**X**
59	F	CD	119	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
60	F	UC	38	**X**	✓	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
61	F	IBDU	92	**X**	**X**	Azathioprine	2.5	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
62	F	IBDU	95	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
63	M	CD	73	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
64	F	CD	66	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
65	M	CD	30	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
66	M	CD	38	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
67	F	CD	66	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
68	F	CD	124	**X**	✓	Azathioprine	2.5	**X**	✓	**X**	Autoimmune sclerosing cholangitis	✓	✓	**X**	**X**
69	F	CD	80	**X**	**X**	6-Mercaptopurine	2.5	**X**	**X**	**X**	Severe nausea	**X**	**X**	**X**	✓
70	M	CD	96	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	✓	**X**	✓	**X**
71	M	CD	90	**X**	**X**	6-Mercaptopurine	1	**X**	✓	**X**	**X**	**X**	✓	**X**	**X**
72	F	IBDU	35	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
73	M	CD	128	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	Severe nausea	✓	**X**	**X**	✓
74	F	CD	55	**X**	**X**	Azathioprine	1	**X**	**X**	**X**	Severe nausea	✓	**X**	**X**	✓
75	F	CD	97	**X**	**X**	Azathioprine	2	**X**	✓	**X**	**X**	**X**	✓	**X**	**X**
76	M	CD	70	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
77	M	IBDU	99	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
78	F	CD	82	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
79	F	CD	87	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
80	M	UC	53	**X**	**X**	Azathioprine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
81	M	CD	110	**X**	✓	Azathioprine	2.5	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
82	F	IBDU	100	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
83	F	CD	99	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
84	M	CD	28	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
85	M	CD	85	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
86	M	CD	101	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
87	M	CD	100	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
88	M	CD	83	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	Severe nausea	✓	**X**	**X**	✓
89	M	UC	96	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	□	**X**	✓	**X**	**X**
90	M	CD	145	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
91	M	CD	40	**X**	**X**	Azathioprine	1.5	**X**	**X**	**X**	**X**	**X**	**X**	✓	**X**
92	M	CD	47	**X**	**X**	Azathioprine	1.5	**X**	✓	**X**	Abnormal LFTs	✓	**X**	**X**	✓
93	M	CD	35	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
94	F	CD	102	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	**X**	**X**	**X**	✓	**X**
95	M	CD	113	**X**	**X**	6-Mercaptopurine	1.5	**X**	**X**	✓	Pancreatitis	✓	**X**	**X**	✓
96	F	UC	89	**X**	**X**	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
97	F	IBDU	56	**X**	✓	6-Mercaptopurine	1	**X**	**X**	**X**	**X**	**X**	✓	**X**	**X**
98	M	CD	86	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
99	F	CD	80	**X**	**X**	None	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable	Not Applicable
100	M	UC	99	**X**	**X**	Azathioprine	2	**X**	**X**	**X**	□	**X**	□	**X**	**X**

Table S4 shows the clinical details on all 100 patients within the cohort

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