Chapter 7

Role for *ITPA* variants in the clinical course of pulmonary Langerhans'cell histiocytosis?

ITPA polymorphisms in PLCH

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Interstitial lung diseases (ILD) comprise a number of clinical conditions including sarcoidosis, pulmonary fibrosis and pulmonary Langerhans' cell histiocytosis (PLCH, Histiocytosis X). The diagnostic work-up for the classification of ILD is often complicated and tedious. PLCH is characterized by the proliferation and infiltration of Langerhans' cells into pulmonary tissues, in disseminated LCH additional tissues are affected. PLCH is strongly associated with smoking and the clinical outcome depends on cessation of smoking[1].

Treatment of ILD often requires immunosuppressant drugs: corticosteroids, immunosuppressant drugs, including thiopurines and biologicals. It is mandatory to exclude genetic polymorphisms in genes involved in metabolism of thiopurine metabolism, because of the pharmacogenetic consequences of the use of thiopurines. The prospective measuring of erythrocyte enzyme activity of thiopurine-S-methyltransferase (TPMT) and inosine triphosphatase (ITPase) or molecular analyses of the genes encoding for these enzymes is recommended in clinical practice[2].

Aberrant enzyme activity of either TPMT or ITPase gives rise to a broad spectrum of adverse drug reactions (ADR) associated with thiopurines, ranging from therapeutic failure to life threatening leucopenia[3,4].

In order to avoid these ADR, we have incorporated pre-treatment screening of TPMT and ITPase of patients with ILD, who are candidates for treatment with thiopurines, in our hospital.

An unexpected high number of decreased ITPase activities in patients with PLCH was found, all of which had an unfavourable outcome. We hypothesized about the role of the *ITPA* gene in relation to immunity, which might play a key role in this phenomenon.

We examined a total of 105 patients with ILD, referred to the ild care team of the Maastricht University Medical Centre, a tertiary referral centre in The Netherlands for ILD (Table 7.1).

Table 7.1 Distribution of *ITPA* polymorphisms in interstitial lung disease (ILD).

	N	Lowered ITPase activity	*ITPA* polymorphism	% *ITPA* polymorphisms
Pulmonary sarcoidosis (chest X-ray stage IV)	41	8	8	19.5
Idiopathic pulmonary fibrosis (IPF)	50	3	3	6.0
PLCH	6	3	3	50
Other causes of ILD	8	4*	4	50
Reference population #	100	11	11	11

*The four cases with an *ITPA* polymorphism were suffering from drug-induced pneumonitis (n=2) due to azathioprine, chronic extrinsic allergic alveolitis (EAA; n=1), and Lymphangioleiomyomatosis **(**LAM; n=1), respectively. # Definition of reference population see text

According to the local protocol for the workup of patients with ILD where thiopurine medication is considered, TPMT and ITPase activities in erythrocytes were measured prior to the start of the treatment. When a lowered activity of TPMT or ITPase was detected molecular analysis of the *TPMT* and *ITPA* gene were performed. Reference values for ITPase and *ITPA* polymorphism distribution were established in a group of 100 anonymous patients from a general hospital population.
TPMT and ITPase activities were measured in erythrocyte lysates as described earlier[5]. Molecular analyses of *TPMT* and *ITPA* genes were performed using previously published protocols[6].

The results of the TMPT and ITPase phenotyping and genotyping in the ILD population are displayed in Table 7.1. Erythrocyte TPMT activity was normal in the PLCH patients, confirmed by molecular analysis, revealing the wild type *1/*1 genotypes in all patients. ITPase activity was decreased in 50% (3/6) PLCH patients. The patients were genotyped and in patients with a decreased ITPase activity this was confirmed by the finding of polymorphic *ITPA* genotypes, either heterozygous or homozygous (Table 7.2). The distribution of ITPase activity and *ITPA* polymorphisms in the reference population were according earlier published results[6].

Table 7.2 ITPase activity, *ITPA* genotype and clinical outcome in PLCH patients.

Sex	Age at diagnosis (years)	Follow up (months)	Smoking status	ITPase activity (mmol/mmol Hb/hr)*	*ITPA* genotype	Clinical outcome
F	17	70	Stopped	3.15	Wt	Favourable**; clinically improved
M	38	19	Stopped	4.60	Wt	Favourable**; clinically improved
M	52	21	Smoking	2.89	Wt	Unchanged
F	32	27	Stopped	0.03	c.94AA	Unfavourable#; awaiting lung transplantation
M	36	49	Stopped	0.79	c.94CA	Unfavourable#
M	54	37	Stopped	0.28	c.94CA/g.IVS2+21AC	Unfavourable#

*reference values : 4.0 – 10.0 mmol/mmol Hb/hr (n=100) ; ** Favourable: substantial improvement of the HRCT and >10% improvement of the DLCO; # Unfavourable: worse evaluation of the HRCT and more than 10% decrease of the DLCO and desaturation during a 6 minute walking test.

Although the number of patients with PLCH in our cohort is limited, we detected an unusual high and intriguing prevalence of low erythrocyte ITPase activities and a concomitant higher number of *ITPA* polymorphisms, i.e. 50%, compared to the reference population (11%).

It is unclear whether there is a connection between the absolute ITPase activity and the occurrence of the disease. Earlier reports referring to patients with a complete ITPase deficiency reported no clinical abnormalities, classifying it as a benign condition[7]. Interestingly, the PLCH cases carrying an *ITPA* polymorphism all demonstrated an unfavourable outcome despite that they all stopped smoking. To date, smoking is associated with the clinical course of PLCH. In general, the clinical features improve after the patient stops smoking (Figure 7.1). However, our three patients carrying an *ITPA* polymorphism all deteriorated and no clinical improvement occurred compared to the PLCH patients with the normal genotype. This finding is also clinically relevant regarding the therapeutic options, as patients who do not improve after they quit smoking are sometimes candidates for azathioprine. Therefore, we strongly recommend to assess erythrocyte ITPase activity, in addition to TPMT activity, prior to initiating treatment with azathioprine in patients with PLCH.

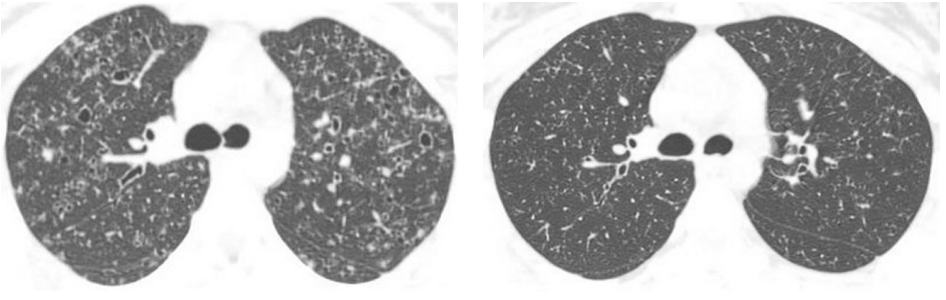

Figure 7.1 HRCT scan of the thorax of a patient (case #1) with Langerhanscel histiocytosis at presentation showing cyst and micro noduli (left panel) and normalized scan 4 months after stopping with smoking (richt panel)

To the best of our knowledge ITP does not accumulate in patients with a partial ITPase deficiency. Therefore, clinical pathology cannot merely be explained by accumulation of ITP in carriers of *ITPA* polymorphisms, but is more likely to be associated with housekeeping or moonlighting functions of the gene or its protein(s).

The role of ITPase in mammalian metabolism is still poorly understood[8]. Its primary role is the pyrophosphohydrolysis of ITP (and dITP) to maintain the balance between ITP and IMP, IMP being the key metabolite in the purine interconversion pathway. Tissue distribution of ITPase varies widely, the highest activity is present in tissues with an exocrine function[9].

The other, less highlighted, function of *ITPA* is its role as a house keeping gene. ITPase is involved in the maintenance of the degradation of non-canonical purine nucleotide triphosphates, hereby preventing the incorporation of these metabolites into RNA and

DNA. Under abnormal physiological circumstances, such as metabolic stress or an inflammatory response, proper functioning of housekeeping genes is pivotal for cellular maintenance. In patients carrying one or more polymorphic *ITPA* alleles, it can be speculated that the house keeping function may be compromised and inflammatory responses cannot be countered properly, resulting in enhanced disease activity. In PLCH there is excessive eosinophilic granuloma formation, thereby corrupting the immune response. Although little is known about ITPase activity in progenitor cells of eosinophils and macrophages, it can be speculated that a wild type *ITPA* genotype is crucial to maintain adequate housekeeping in these cells. Comparing ITPase deficiency with two other inherited disorders in purine metabolism, adenosine deaminase (ADA) and purine nucleoside phosphorylase (PNP) deficiency, makes this line of thought even more compelling. Both conditions cause severe immune disease, resulting in SCID and T-cell immunodeficiency respectively. The concept of a potential role of *ITPA* in immunity is further strengthened by the fact that two proteins, encoded by *ITPA*, were recognized by cytotoxic T-cells. Whether or not these immunogenic proteins are the products of alternative splicing remains unclear. However, as was shown by Arenas et al., alternative mRNA splice variants are present in significantly higher amounts in patients carrying the *ITPA* 94C>A polymorphism than in wild type individuals[10]. A direct link between ITPase and smoking seems highly unlikely. One may argue, however, that toxic compounds originating from (tobacco) smoke cause oxidative stress in exposed tissues, generating non-canonical nucleotides. ITPase is required to scavenge these toxic compounds. Expression of ITPase in lung tissue is low, which may imply that under metabolic stress the presence of *ITPA* polymorphisms results in insufficient ITPase activity thus compromising the cellular capability to neutralize toxic, non-canonical, nucleotides.

In conclusion, we hypothesize that *ITPA* polymorphisms are associated with an unfavourable clinical outcome in PLCH. Future investigations are required to confirm the high prevalence of *ITPA* polymorphisms in PLCH patients. Moreover, the prognostic value should be evaluated in a larger cohort, focussing especially on the association with smoking. Furthermore, the potential role of ITPase in inflammatory modulating cells needs to be elucidated. These investigations may gain more insight in the real physiological role of ITPase and the clinical effect of polymorphisms in the *ITPA* gene.

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