

# A role for *ITPA* variants in the clinical course of pulmonary Langerhans' cell histiocytosis?

To the Editors:

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

Treatment of ILD often requires corticosteroids and 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 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 leukopenia [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 unexpectedly high number of decreased ITPase activities in patients with PLCH was found, all of which had an unfavourable outcome. We hypothesised 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 Maastricht, The Netherlands for ILD (table 1).

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* genes 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 previously [5]. Molecular analyses of *TPMT* and *ITPA* genes were performed using previously published protocols [6].

The results of the TPMT and ITPase phenotyping and genotyping in the ILD population are displayed in table 1.

ITPase activity was decreased in 50% (three out of six) 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 2). The distribution of ITPase activity and *ITPA* polymorphisms in the reference population were consistent with earlier published results [6]. Erythrocyte TPMT activity was normal in the PLCH patients, as confirmed by molecular analysis, revealing the wild type \*1/\*1 genotypes in all patients.

Although the number of patients with PLCH in our cohort is limited, we detected an unusually 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 at 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 the fact 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. 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 the assessment of erythrocyte ITPase activity, in addition to TPMT activity, prior to initiating treatment with azathioprine in patients with PLCH.

To the best of our knowledge inosine triphosphate (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 deoxy-ITP) to maintain the balance between ITP and inosine monophosphate (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 housekeeping gene. ITPase is involved in the maintenance of the degradation of noncanonical 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

**TABLE 1** Distribution of *ITPA* polymorphisms in interstitial lung diseases (ILD)

	All patients	Lowered ITPase activity	<i>ITPA</i> polymorphisms
<b>Pulmonary sarcoidosis (chest radiograph stage IV)</b>	41	8	8 (19.5)
<b>IPF</b>	50	3	3 (6.0)
<b>PLCH</b>	6	3	3 (50)
<b>Other causes of ILD</b>	8	4 <sup>†</sup>	4 (50)
<b>Reference population<sup>#</sup></b>	100	11	11 (11)

Data are presented as n or n (%). ITPase: inosine triphosphatase; IPF: idiopathic pulmonary fibrosis; PLCH: pulmonary Langerhans' cell histiocytosis. <sup>#</sup>: definition of reference population presented in the main text. <sup>†</sup>: the four cases with an *ITPA* polymorphism were suffering from drug-induced pneumonitis (n=2) due to azathioprine, chronic extrinsic allergic alveolitis (n=1), and lymphangioleiomyomatosis (n=1).

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 housekeeping 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 and purine nucleoside phosphorylase deficiency, makes this line of thought even more compelling. Both conditions cause severe immune disease, resulting in severe combined immune deficiency 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.* [10], alternative mRNA splice variants are present in significantly higher amounts in patients carrying the *ITPA* 94C>A polymorphism than in wild type individuals. 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 noncanonical 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 neutralise toxic, noncanonical, nucleotides.

In conclusion, we hypothesise 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 give more insight in the real physiological role of ITPase and the clinical effect of polymorphisms in the *ITPA* gene.

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**TABLE 2** Inosine triphosphatase (ITPase) activity, *ITPA* genotype and clinical outcome in pulmonary Langerhans' cell histiocytosis (PLCH) patients

Sex	Age at diagnosis yrs	Follow-up months	Smoking status	ITPase activity <sup>#</sup> $\mu\text{mol}\cdot\text{mmol}^{-1}\text{Hb}\cdot\text{h}^{-1}$	<i>ITPA</i> genotype	Clinical outcome
F	17	70	Stopped	3.15	Wt	Favourable <sup>†</sup> ; clinically improved
M	38	19	Stopped	4.60	Wt	Favourable <sup>†</sup> ; clinically improved
M	52	21	Smoking	2.89	Wt	Unchanged
F	32	27	Stopped	0.03	c.94AA	Unfavourable <sup>+</sup> ; awaiting lung transplantation
M	36	49	Stopped	0.79	c.94CA	Unfavourable <sup>+</sup>
M	54	37	Stopped	0.28	c.94CA/ g.IVS2+21AC	Unfavourable <sup>+</sup>

<sup>#</sup>: reference values: 4.0–10.0  $\mu\text{mol}\cdot\text{mmol}^{-1}\text{Hb}\cdot\text{h}^{-1}$  (n=100); <sup>†</sup>: favourable is defined as substantial improvement of the high resolution computed tomography (HRCT) and >10% improvement of the diffusing capacity of the lung for carbon monoxide ( $D_{LCO}$ ); <sup>+</sup>: unfavourable is defined as a worse evaluation of the HRCT, a >10% decrease of the  $D_{LCO}$  and desaturation during a 6-min walking test.

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# Quadriceps myostatin expression in COPD

To the Editors:

Quadriceps muscle dysfunction is well recognised in chronic obstructive pulmonary disease (COPD), and is associated with impaired exercise capacity and increased mortality [1]. Functionally, the quadriceps is characterised by reduced strength, increased fatigability and decreased endurance, associated with muscle fibre atrophy and a switch towards more glycolytic muscle fibres (types 1–2). The underlying molecular mechanisms of quadriceps dysfunction in COPD are not well clarified.

Myostatin is a member of the transforming growth factor- $\beta$  family, and is a potent negative regulator of muscle mass, as demonstrated in naturally occurring animal and human genetic mutations, and genetic murine models [2]. Myostatin may therefore be a candidate regulator of muscle mass in disorders characterised by muscle atrophy, including COPD.

Few data exist regarding myostatin in COPD, but a recent cross-sectional study showed a three-fold increase in *vastus lateralis* myostatin mRNA transcripts in COPD patients with significant quadriceps weakness compared to healthy controls [3]. However, the relationship between quadriceps myostatin expression and functional characteristics of the muscle is not known. We hypothesised that myostatin expression would negatively correlate with quadriceps strength and exercise capacity in COPD.

18 patients with COPD were enrolled from clinics at the Royal Brompton Hospital (London, UK). Exclusion criteria included exacerbation in the previous 4 weeks, coexisting heart, renal or liver failure, or a systemic inflammatory or metabolic disorder. 16 healthy, age-matched controls were recruited by advertisement. All participants gave written informed consent and the protocol was approved by the local Research Ethics Committee.

The following were measured in study participants: lung function tests; fat-free mass index, calculated from bioelectrical impedance and normalised to height squared; quadriceps muscle

**TABLE 1** Clinical characteristics of subjects

	COPD	Controls	p-value
Age yrs	65 $\pm$ 7	65 $\pm$ 7	NS
Males:females n	12:6	9:7	NS
BMI kg·m <sup>-2</sup>	24.5 $\pm$ 4.7	25.7 $\pm$ 4.8	NS
Fat free mass index	15.8 $\pm$ 2.3	17.4 $\pm$ 2.5	NS
FEV <sub>1</sub> L	0.93 $\pm$ 0.40	3.03 $\pm$ 0.63	<0.0001
FEV <sub>1</sub> % pred	35 $\pm$ 15	112 $\pm$ 13	<0.0001
DL <sub>CO</sub> % pred	37.9 $\pm$ 14.3	91.3 $\pm$ 16.1	<0.0001
TLC % pred	120 $\pm$ 19	108 $\pm$ 11	0.03
RV % pred	193 $\pm$ 51	103 $\pm$ 16	<0.0001
Quadriceps MVC kg	29.8 $\pm$ 8.6	37.7 $\pm$ 9.8	0.02
MVC/BMI	1.2 $\pm$ 0.3	1.5 $\pm$ 0.5	0.04
Quadriceps endurance <sup>#</sup> s	91 $\pm$ 47	127 $\pm$ 72	NS
6MWD m	362 $\pm$ 145	630 $\pm$ 86	<0.0001
SGRQ total score	57 $\pm$ 11	3 $\pm$ 4	<0.0001
Locomotion time min	48 $\pm$ 45	106 $\pm$ 53	0.004

Data are presented as mean  $\pm$  SD, unless otherwise stated. COPD: chronic obstructive pulmonary disease; BMI: body mass index; FEV<sub>1</sub>: forced expiratory volume in 1 s; % pred: % predicted; DL<sub>CO</sub>: diffusing capacity of the lung for carbon monoxide; TLC: total lung capacity; RV: residual volume; MVC: maximal voluntary contraction; 6MWD: 6-min walk distance; SGRQ: St George's Respiratory Questionnaire, NS: nonsignificant *i.e.*  $p > 0.05$ . #: defined as the time for force to fall to 80% of baseline.

This article has supplementary material accessible from [www.erj.ersjournals.com](http://www.erj.ersjournals.com)