

Low molecular weight PTP–IL-4RA interaction in atopy predisposition

We recently described a protective effect of the low molecular weight protein tyrosine phosphatase (LMPTP) BC genotype, associated with the highest total enzymatic activity, against high serum IgE levels both in the English and the Italian populations. Here we test the hypothesis of a role of LMPTP in the negative modulation of IL-4 signal transduction checking for genetic interaction between interleukin-4 receptor alpha chain (IL-4RA) genetic polymorphisms and LMPTP polymorphism in the predisposition to high total IgE levels in the English population. We find a significant interaction between LMPTP polymorphism and the intracellular Gln/Arg polymorphism in position 551 of IL-4RA. Our data support the hypothesis of a direct or indirect biochemical interaction between LMPTP and IL-4RA resulting in different modulation of IL-4 signal transduction among joint genotypes.

**N. Bottini^{1,2}, X.-Q. Mao¹,
P. Borgiani^{1,3}, P. Saccucci^{1,3},
L. Stefanini^{1,3}, E. Greco^{1,4},
L. Fontana², T. Shirakawa^{1,5},
J. M. Hopkin¹**

¹Experimental Medicine Unit, University of Wales Swansea, Swansea, UK; ²Department of Internal Medicine, University of Rome 'Tor Vergata', Rome, Italy; ³Department of Biopathology and Image Diagnostic, University of Rome 'Tor Vergata', Rome, Italy; ⁴Post-graduate School of Allergy and Clinical Immunology, University of Rome 'Tor Vergata', Rome, Italy; ⁵Department of Health Promotion and Human Behavior, Kyoto University Graduate School of Public Health, Kyoto, Japan

Key words: ACP1, LMPTP, IL4RA, polymorphism, atopy, genetic interaction, IgE level.

Nunzio Bottini
Department of Internal Medicine
University of Rome 'Tor Vergata'
135, via di Tor Vergata
I-00133 Rome
Italy

Introduction

The low molecular weight protein tyrosine phosphatase (LMPTP or cLMWPTPase), also known as acid phosphatase locus 1 (ACP1), is a cytosolic enzyme involved in the modulation of signal transduction by insulin and PDGF receptors (1) and by the T cell receptor (2). LMPTP is present ubiquitously in human tissues in two isoforms called A and B (also called ACP1 F and S) (3), and shows a genetic polymorphism with strong variations in total enzymatic activity and in the ratio between the activity of the two isoforms among the genotypes (4).

We recently described a protective effect of the BC genotype associated with the highest total enzymatic activity against high total IgE levels in two independent samples from the English and the Italian populations (Bottini N et al. submitted). Our finding suggests a possible modulatory role of LMPTP on some transduction pathway relevant for IgE hyperproduction. We

consider the IL-4 signal transduction one of the candidates for modulation by LMPTP and in this paper we test for genetic interaction between LMPTP polymorphism and some IL-4RA polymorphisms capable to influence IL-4 signal transduction.

Subjects and methods

300 subjects have been considered from the English population (Oxford District). 150 subjects were affected by atopic asthma and 150 were local population controls. Total IgE levels, specific IgE levels for aeroallergens, and the results of skin prick tests were available both for patients and controls from the English population. LMPTP polymorphism was determined with a RFLP-PCR method (Sensabaugh G, unpublished data), as follows: a T to A transversion at nucleotide 24 of exon 6 creates in the *A allele a restriction site for *TaqI*, while a C to T transition at

Table 1. Interaction among IL-4R, LMPTP and IgE level

IgE (U/ml)	LMPTP genotype	Enzymatic activity	IL-4R-551			IL-4R-478			IL-4R-50		
			1-1 Gln-Gln	1-2 Gln-Arg	2-2 Arg-Arg	1-1 Ser-Ser	1-2 Ser-Pro	2-2 Pro-Pro	1-1 Ile-Ile	1-2 Ile-Val	2-2 Val-Val
100	AA	Very low	14	5	0	14	5	0	5	12	2
	Others	Intermediate	71	44	8	78	39	6	40	54	29
	BC	Very high	13	4	2	13	5	1	6	8	5
> 100	AA	Very low	10	4	3	12	3	2	7	7	3
	Others	Intermediate	73	42	1	82	32	2	38	44	34
	BC	Very high	3	2	0	3	2	0	3	1	1

Three way contingency table analysis by a log-linear model (x =IL-4R-551, y =LMPTP, z =IgE)

Interaction $x-y-z$	G (d.f)	10.785 (4)	5.703 (4)	1.985 (4)
IL-4R 1-1 vs. 1-2 vs. 2-2	P	<0.02	N.S.	N.S.
Interaction $x-y-z$	G (d.f)	10.081 (2)	4.962 (2)	0.760 (2)
IL-4R 1-1 vs. 2-2	P	<0.01	<0.10	N.S.

nucleotide 15 of the exon 3 removes in the *C allele a restriction site for *HhaI*. Both the *TaqI* and *HhaI* restriction polymorphism were determined by RFLP-PCR after amplification, respectively, of a 300-bp fragment of the exon 6 and of a 341-bp fragment of the exon 3 and digesting the PCR product with an excess of the relative restriction enzyme. The determination of the Ile50Val, Ser478Pro and Gln551Arg polymorphism of the IL-4 receptor alpha chain has been performed by RFLP-PCR.

Total IgE levels were measured by immunoassay (Pharmacia, Sweden).

Chi square tests were performed using the SPSS computing package (5) on a PC computer. The log-linear analysis has been performed according to Sokal and Rohlf (6).

Results

No significant deviation from Hardy-Weinberg expectation was detected in cases and controls from the English population for each of the polymorphisms investigated.

We already reported the association between BC genotype and low serum IgE levels in the English sample. No significant association is present between each of the IL-4RA polymorphisms and serum IgE levels or asthma symptoms in our sample (unpublished data).

Table 1 shows the three way contingency table with the combined distribution of IL-4RA polymorphisms and LMPTP polymorphism in subjects with total IgE levels lower or equal than 100 U/ml and higher than 100 U/ml. LMPTP genotypes have been grouped according to the associated enzymatic activity (4). A significant interaction is present between LMPTP and IL-4RA-551 polymorphism concerning the predisposition to high total IgE levels. Most of interaction is due to differences between IL-4RA-551 Gln/Gln and

Arg/Arg genotypes. Interestingly the strength of the interaction between LMPTP polymorphism and the intracellular IL-4RA Ser478Pro polymorphism is lower than that between LMPTP and IL-4RA Gln551Arg while there is practically no interaction between LMPTP and the extracellular IL-4RA Ile50Val polymorphism.

There is a significant association between the joint LMPTP/IL-4RA-551 genotype and the IgE level ($\chi^2=16.542$, d.f.=8, $P=0.035$) (unpublished data). The maximum protection against high IgE level is observed in the high activity BC LMPTP genotype when joined with IL-4RA-551 Gln/Gln and in genotypes with LMPTP medium activity when joined with IL-4RA-551 Arg/Arg genotype (Table 2).

Discussion

Among the immune signal transduction pathways affecting IgE production the IL-4 receptor signal transduction is one of the more important and more extensively studied (7). The role of IL-4 signal transduction genetic variability in the predisposition to atopy is object of intense investigation (8). Some transduction pathways activated by the IL-4 receptor stimulation are similar to pathways activated during insulin signal transduction, which is *in vitro* modulated by LMPTP B isoform (9). A recent paper by Bucciantini et al. suggests that phosphorylated Tyr residues preceded by a couple of not bulky hydrophobic residues

Table 2. Association between joint LMPTP-IL4R - 551 genotype and IgE level

	IL-4R Gln/Gln-high LMPTP activity + IL-4R Arg/Arg-medium LMPTP activity	Other joint genotypes
IgE = 100 U/ml	21	140
IgE > 100 U/ml	4	134

Chi-square test of independence, $P=0.001$. Odds ratio (risk of high IgE for other joint types), 5.025. 95% confidence interval, 1.58-14.69

could represent the preferential target for LMPTP-B dephosphorylating action (10); the IL-4R alpha chain is phosphorylated on 5 Tyr residues after stimulation with IL-4; the three residues in the STAT domain are all preceded by a glycine residue. The Tyr 713 residue is preceded by a Ile-Val couple of aminoacids and lies in a sequence similar to the one which is *in vitro* dephosphorylated by LMPTP-B on the PDGF receptor. The Tyr 713 residue is also considered to be important for the recruitment of other PTPases like SHP-1 to the IL-4RA (7).

We performed the genetic interaction study in order to get some more information before starting a biochemical study of the direct or indirect interaction between LMPTP and IL-4RA. Our data suggest that one or both LMPTP isoforms could interact preferentially at the intracellular level with variants of the IL-4 receptor alpha chain modulating its signal transduction. Interestingly the Gln/Arg polymorphism at position 551 of the IL-4RA has been proposed to affect the biochemical interaction of another PTPase (SHP-1) with the IL-4RA (11).

Due to the low frequency of the IL-4RA Arg551

variant the overall role of the interaction LMPTP $\times$ IL-4RA in explaining the variance of IgE levels in human populations is probably low. Anyway the preferential interaction between variants of biochemically molecule is very likely a widely diffused mechanism. The genetic analysis indicates epistasis but does not give information about the direct or indirect nature of such interaction. Therefore, genetic studies can only give some concrete *a priori* hypotheses and some directions for the study of biochemical interactions between molecules in signal transduction pathways. We propose to use the genetic interaction investigation as *a priori* test before approaching the study of biochemical direct or indirect interactions between polymorphic molecules involved in signal transduction pathways.

Acknowledgments

This study was conducted while N.B. was at the University of Wales Swansea, Experimental Medicine Unit as UCB Fellow. We wish to thank Professor G. Sensabaugh (University of California Berkeley) for sharing unpublished results and for precious technical advice on LMPTP genotyping.

References

1. RAMPONI G, STEFANI M. Structure and function of the low Mr phosphotyrosine protein phosphatases. *Biochim Biophys Acta* 1997;**1341**:137–156.
2. TAILOR P, GILMAN J, WILLIAMS S, et al. Regulation of the low molecular weight phosphotyrosine phosphatase by phosphorylation at tyrosines 131 and 132. *J Biol Chem* 1997;**272**:5371–5374.
3. CIRRI P, FIASCHI T, CHIARUGI P, et al. The molecular basis of the differing kinetic behavior of the two low molecular mass phosphotyrosine protein phosphatase isoforms. *J Biol Chem* 1996;**271**:2604–2607.
4. DISSING J. Human 'red cell' acid phosphatase (ACP1) genetic, catalytic and molecular properties. PhD Thesis, 1993, Copenhagen University, Denmark.
5. SPSS/PC+, Version 5.0. Chicago, IL: SPSS Inc., 1992.
6. SOKAL RR, ROHLF FJ. *Biometry*. New York: W.H. Freeman, 1981: 253–261.
7. NELMS K, KEEGAN AD, ZAMORANO J, et al. The IL-4 receptor: signaling mechanisms and biologic functions. *Annu Rev Immunol* 1999;**17**:701–738.
8. SHIRAKAWA T, DEICHMANN KA, IZUHARA K, et al. Atopy and asthma: genetic variants of IL-4 and IL-13 signalling. *Immunol Today* 2000;**21**:60–64.
9. CHIARUGI P, CIRRI P, MARRA F, et al. LMW-PTP is a negative regulator of insulin-mediated mitotic and metabolic signalling. *Biochem Biophys Res Commun* 1997;**238**:676–682.
10. BUCCIANINI M, STEFANI M, TADDEI N, et al. Sequence-specific recognition of peptide substrates by the low Mr phosphotyrosine protein phosphatase isoforms. *FEBS Lett* 1998;**422**:213–217.
11. HERSHEY GK, FRIEDRICH MF, ESSWEIN LA, et al. The association of atopy with a gain-of-function mutation in the alpha subunit of the interleukin-4 receptor. *N Engl J Med* 1997;**337**:1720–1725.