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RESEARCH LETTER

WILEY

Multi-omic analysis of *Tyrophagus putrescentiae* reveals insights into the allergen complexity of storage mites

To the Editor,

Tyrophagus putrescentiae, commonly referred to as the mould mite or cheese mite, is especially well-known as a storage mite that causes human allergic diseases.[1] However, it has fewer reported allergen groups compared to the house dust mites of the *Dermatophagoides* genus in the WHO/IUIS allergen nomenclature database.[2,3]

In this omics era, multiple genome-based approaches have been boosting our understanding of the medically important mites.[4–6] Using the mite allergens reported in the WHO/IUIS database as reference genes, 37 allergen groups (up to group 42) were predicted in the genome of *T. putrescentiae*[6] and composed an allergen profile encompassing up to 85 predicted genes (Table S1). Unlike the allergen gene expression of *D. farinae* and *D. pteronyssinus*,[5] the group 1 allergens (cysteine proteases) of *T. putrescentiae* were expressed at low levels, while the homologue of group 13 allergen, pTyr p 13.0201, exhibited the highest expression level (Figure 1A). To evaluate the IgE-binding reactivity of novel allergens, recombinant proteins were cloned, expressed and assessed by ELISA with *T. putrescentiae*-sensitized patient sera (Table S2). Five proteins, rTyr p 6.0101, 9.0101, 18.0101, 20.0101 and 26.0101, were suggested to be novel allergens by ELISA experiments with 11.1%, 22.2%, 11.1%, 44.4% and 50.0% positive rates, but with low IgE levels. Additional information about study methods and findings is available in the following repository https://zenodo.org/record/8429480.

Group 2 allergens belong to the Niemann-Pick protein type C2 (NPC2) family and are considered the major allergens of storage mites including *T. putrescentiae*.[7,8] We identified up to six homologues of Tyr p 2, including the two homologous genes (Tyr p 2.0101 and 2.0201) which shared identical sequences, and as high as 99.3% identity with the reported Tyr p 2.

To investigate the NPC2 family, encompassing group 2, 22 and 25 allergens of mites, we gathered the genes of six astigmatic mites. The resultant phylogenetic tree revealed eight distinct clusters, named N1-3 and C1-5 (Figure 1B). All reported allergen genes were in Cluster N1-3, while none of the genes in Cluster C1-5 were reported to be allergens. Cluster N1 contained Blo t 2, Tyr p 2, Lep d 2 and Gly d 2 and Der f 35, as well as Pso o 2 (UniProt ID: Q965E2) of *Psoroptes ovis*. Cluster N2 covered group 2 allergens of house dust mites including Der f 2, Der p 2 and Eur m 2, while Cluster N3 contained Der f 22. The gene synteny alignment (Figure 1C) suggested that the NPC2 gene of *S. scabiei* in N1 decayed, while that of *B. tropicalis* was tandemly duplicated. Cluster N2 is proposed to be unique in psoroptid mites, but decayed in *P. ovis*, while a 38-aa insertion was identified in the N-terminus of SS_011027.01 in *S. scabiei*.

Proteomic identification was performed using the pooled sera of allergy patients (Figure 1D). In total, 31 protein spots of *T. putrescentiae* bound by IgE underwent peptide sequencing by MALDI-TOF mass spectrometry (Table 1). A range of our identified allergens could be found in the spots, including members of Tyr p 1, 2, 3, 8, 10, 13, 20, 21, 25, 28 and 39. Among them, the best-matched homologues in the spots included Tyr p 2.0101, Tyr p 10.0101, pTyr p 20.0101, pTyr p 21.0101, pTyr p 25.0101, pTyr p 28.0101 and Tyr p 34.0101. PTyr p 21.0101 was matched by four spots, while Tyr p 10.0101, pTyr p 20.0101 and pTyr p 25.0101 were each found in two spots. Some genes were suggested as allergen homologues but shared relatively low identity with the reported allergens, so they were labelled ungroup allergens (Table 1). However, in Tyr p 2, Tyr p 2.0101/Tyr p 2.0201, pTyr p 2.0601 and an ungrouped Tyr p 2 (gene locus: TP_020235.01) were matched. For Tyr p 28, pTyr p 28.0101, pTyr p 28.0201 and an ungrouped Tyr p 28 (gene locus: TP_006940.01) were identified.

The ungrouped Tyr p 1 (gene locus: TP_008599.01) was identified and shared only 28.2% identity with the reported Tyr p 1 (Table 1). This cysteine protease homologue (gene locus: TP_008599.01) was estimated to be expressed over two hundred times more than the best-matched Tyr p 1.0101 and lower than 50% of that of pTyr p 3.0401 (Figure 1A). Similarly in Tyr p 13, the ungrouped homologue (gene locus: TP_011560.01) was expressed at over 50% higher levels than the highly expressed pTyr p 13.0201. Therefore, we proposed that the gene expression level was a crucial factor in proteomic identification. In addition, an ungrouped homologue of Tyr p 8 (gene locus: TP_014174.01) was identified, but not the *in-silico*-predicted pTyr p 8.0101 (GenBank accession: AGG10560). Other proteins such as the FK506-binding protein (gene locus: TP_004518.01) were identified in one spot (Table 1). The FK506-binding protein was assessed to be 22.2% and positive in four patient sera. The pooled sera were found to test positive for *D. pteronyssinus*, while their status for *T. putrescentiae* remained unknown. This raises concerns about potential cross-reactivity. However, it is important to note that a significant limitation of

this study is the absence of immunological investigations of these allergens.

In comparison to previous omics studies focused on *T. putrescentiae* allergens,[9,10] our research integrates multi-omic data and highlights the presence of multiple allergen homologues in *T. putrescentiae* genome. Through our multi-omic approaches, a comprehensive allergen profile of *T. putrescentiae* was revealed and the integrative analysis provides a systematic understanding of the allergen complexity of storage mites.

Summary Box

- A multi-omic analysis reveals the highly complex allergen profile of *Tyrophagus putrescentiae*.
- This comprehensive analysis of *Tyrophagus putrescentiae* can improve the component-resolved diagnosis of mite allergy.

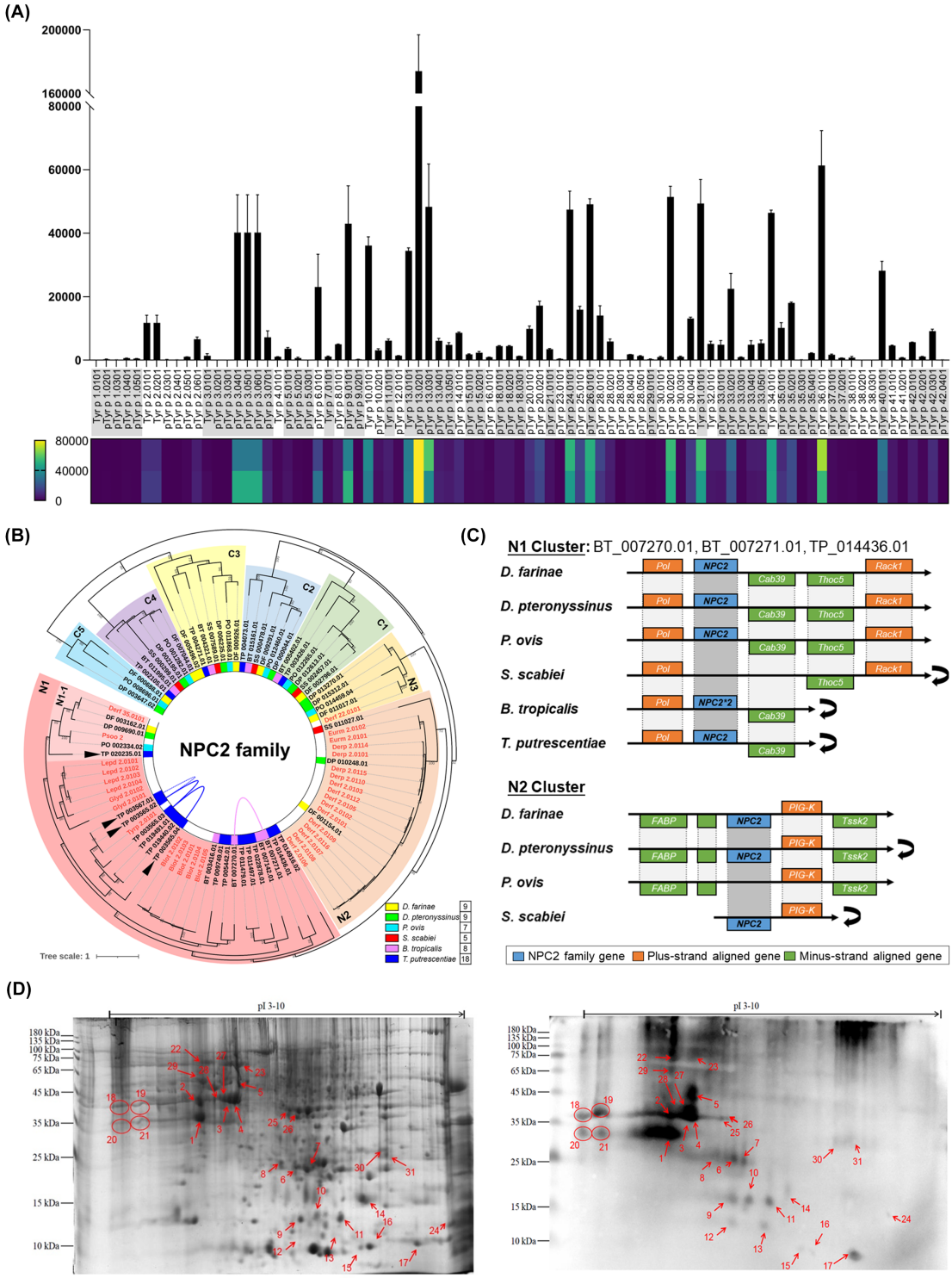

FIGURE 1 Multi-omic analysis of *T. putrescentiae* allergens. (A) The expression levels of all identified allergens in *T. putrescentiae* were quantified as transcripts per million (TPM) using two transcriptome data of adult mites (TP1 and TP3 of SRR13837414). In the bar chart, the *y*-axis represents the TPM (Transcripts Per Million) value, while the error bars correspond to the standard deviation. In the lower heatmap, which represents the TPM (Transcripts Per Million) values of TP1 and TP3, the maximum value has been set at 80,000. The transcriptomic reads were unable to differentiate identical genes, such as Tyr p 2.0101 and Tyr p 2.0201, which shared identical protein and coding sequences. Consequently, Tyr p 2.0101 and Tyr p 2.0201 exhibited the same expression level. (B) Phylogenetic analysis of NPC2 family of six astigmatic mites, including two house dust mites, *D. farinae* and *D. pteronyssinus*, two parasitic mites, *P. ovis* and *S. scabiei*, and two canonical storage mites, *B. tropicalis* and *T. putrescentiae*. The NPC2 genes were divided into 8 clusters, that is N1-3 and C1-5. The NPC2 allergens (highlighted in red) include group 2, 22 and 35 of astigmatic mites collected from the WHO/IUIS nomenclature database and Pso o 2 of *P. ovis* from UniProt database (ID: Q965E2). Tandemly arrayed genes and proximally arrayed genes (separated by no more than 10 genes) were connected by curved solid lines and dotted lines, respectively. Four black triangles marked the four NPC2 genes identified in MS (Table 1). (C) Gene synteny alignment of N1 and N2 Cluster. The black turnover arrow means after reverse complement. (D) *T. putrescentiae* proteins in two-dimensional (2D) gel electrophoresis. The left panel is a SDS-PAGE image. The Coomassie blue stained 2D gel showed the success of the separation of more than 60 *T. putrescentiae* proteins. The right panel is a western blotting image. The results of immunoblotting from the 2D gel showed that there were 31 protein spots in the total protein bound by the specific IgE in the patient sera (Table S3), numbered 1–31. All the protein spots could be found correspondingly in the 2D gel.

TABLE 1 Proteomic results of IgE-bound *T. putrescentiae* proteins. Within the 31 spots that exhibited IgE-binding activity (Figure 1D), we have successfully identified all the corresponding peptides through mass spectrometry analysis. The list of these identified peptides is provided below.

ID on 2D gel	Accession	Allergen	Protein	Score	MW (kDa)	pI	No. of peptides	Coverage (%)
1	TP_005966.01	Tyr p 10.0101	Tropomyosin	334.36	33	4.78	6	21.8
2	TP_005966.01	Tyr p 10.0101	Tropomyosin	265.73	33	4.78	4	14.8
6	TP_010799.01	pTyr p 25.0101	Triosephosphate isomerase	674.93	26.8	6.13	6	41.3
8	TP_010799.01	pTyr p 25.0101	Triosephosphate isomerase	610.06	26.8	6.13	6	41.3
	TP_007838.01	pTyr p 3.0701	Trypsin	57.33	29.9	7.1	2	7.7
9	TP_004226.01	–	Sod1 Superoxide dismutase [Cu-Zn]	576.51	15.6	5.92	5	51
10	TP_003565.04	pTyr p 2.0601	NPC2 family	51.72	15.1	6.99	1	7.7
11	TP_003565.04	pTyr p 2.0601	NPC2 family	354.79	15.1	6.99	4	41.5
	TP_004226.01	–	Sod1 Superoxide dismutase [Cu-Zn]	115.71	15.6	5.92	3	23.2
	TP_014856.01	–	Unknown	42.84	24.9	6.93	1	6.2
13	TP_020235.01	Ungrouped Tyr p 2[a] (44.0%, CAA73221)	NPC2 family	60.89	15.6	7.1	2	17.1
15	TP_004518.01	–	FK506-binding protein	296.13	11.5	6.72	4	40.7
	TP_014185.01	pTyr p 21.0101	Unknown	180.4	15.3	7.83	3	30.4
16	TP_014185.01	pTyr p 21.0101	Unknown	538.38	15.3	7.83	6	46.4
17	TP_011560.01	Ungrouped Tyr p 13[a] (37.4%, AAU11502)	Fatty acid-binding protein	392.65	17.5	8.61	5	42.9
	TP_003565.02/ TP_003567.01	Tyr p 2.0101/Tyr p 2.0201	NPC2 family	184.57	14.8	8.51	2	27.7
18	TP_014185.01	pTyr p 21.0101	Unknown	161.6	15.3	7.83	3	30.2
19	TP_014185.01	pTyr p 21.0101	Unknown	46.76	15.3	7.83	1	10.1
20	TP_004953.01	–	Unknown	132.75	34.2	9.96	3	17.9
	TP_005006.01	–	Unknown	61.66	34.2	6.9	2	11.5
23	TP_011350.01	pTyr p 28.0201	Heat shock protein 70	267.96	71.5	5.29	4	8.1
	TP_011342.01	pTyr p 28.0101	Heat shock protein 70	141.09	122	5.33	3	3.2
	TP_006940.01	Ungrouped Tyr p 28[a] (51.3%, AOD75395)	Heat shock protein 70	65.45	74.4	5.64	2	3.8

(Continues)

TABLE 1 (Continued)

ID on 2D gel	Accession	Allergen	Protein	Score	MW (kDa)	pI	No. of peptides	Coverage (%)
24	TP_001283.01	–	Nucleoside diphosphate kinase A1	280.29	23.3	8.32	5	24.2
	TP_006307.01	–	HEXBP DNA-binding protein HEXBP	173.74	14.1	8.26	3	40.8
	TP_020843.01	–	CYPA Peptidyl-prolyl cis-trans isomerase	81.44	28.8	10.08	1	5.3
	TP_005620.02	Tyr p 34.0101	Troponin C	52.78	17.7	4.04	1	9.8
25	TP_001138.03	pTyr p 20.0101	Arginine kinase	421.42	40.1	6.23	5	21.7
	TP_009769.01	–	FBPA Fructose-bisphosphate aldolase	84.84	38.7	6.55	3	14.3
	TP_008599.01	Ungrouped Tyr p 1[a] (28.2%, ABM53753)	Cysteine protease	69.95	39.5	5.9	2	10
26	TP_001138.03	pTyr p 20.0101	Arginine kinase	427.58	40.1	6.23	5	21.7
	TP_009769.01	–	FBPA Fructose-bisphosphate aldolase	71.34	38.7	6.55	2	6.6
29	TP_007523.01	–	pdi-2 Protein disulfide-isomerase 2	236.42	54.9	4.95	4	10.5
	TP_006736.01	–	ATPsynbeta ATP synthase subunit beta, mitochondrial	99.41	56.1	5.13	3	8.4
31	TP_013474.01	–	Gpx5 Epididymal secretory glutathione peroxidase	139.51	25.8	7.12	2	9.7
	TP_014174.01	Ungrouped Tyr p 8[a] (50.7%, AGG10560)	Glutathione S-transferase	103.13	24.9	8.95	2	12.7
	TP_019125.01	–	Cuticle protein 16.8	87.72	25.6	8.92	1	11.6

[a]These genes were suggested as allergen homologues but shared relatively low identity with the reported allergens so that they were not listed in Figure 1A. The identity percentage and the GenBank accession of the reference allergen were noted in the bracket.

KEYWORDS

allergens, human allergic diseases, storage mites

AUTHOR CONTRIBUTIONS

ATYW, QX, and XX designed the experiments, analyzed the data, and wrote the manuscript. KFKA, SWJ, BSHW, MW, QC, CSHF, SMN performed the experiments and analyzed the data. FTC, BS, TFL and KYJ provided the resources. XL and SKWT supervised the study and revised the manuscript.

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CONFLICT OF INTEREST STATEMENT

We have no competing interest to disclose.

DATA AVAILABILITY STATEMENT

The genome and sequencing data of *Tyrophagus putrescentiae* are deposited in NCBI database under BioProject accession PRJNA706095. The in-silico-identified allergen sequences were uploaded to NCBI GenBank database under accessions OP558975–OP559059.

IRB STATEMENT

This study was approved by the institutional review board (IRB no. 4-2013-0397) of Institute of Allergy, College of Medicine, Yonsei University for using the patient sera in ELISA experiments and the hospital ethics committee of The First Affiliated Hospital of Guangzhou Medical University (reference no. 2017018) for using the pooled patient sera in immunoblotting (western blotting) following the 2D gel electrophoresis.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.