



Characterization of the honeybee venom proteins C1q-like protein and PVF1 and their allergenic potential

Dennis Russkamp^{a,1}, Matthias Van Vaerenbergh^{b,1}, Stefanie Etzold^a, Bernadette Eberlein^c, Ulf Darsow^c, Maximilian Schiener^a, Lina De Smet^b, Magdalena Absmaier^c, Tilo Biedermann^c, Edzard Spillner^d, Markus Ollert^{e,f}, Thilo Jakob^{g,h}, Carsten B. Schmidt-Weber^a, Dirk C. de Graaf^b, Simon Blank^{a,*}

^a Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Ingolstädter Landstraße 1, 85764 Munich, Germany

^b Laboratory of Molecular Entomology and Bee Pathology, Ghent University, Krijgslaan 281, 9000 Gent, Belgium

^c Department of Dermatology and Allergy Biederstein, Technical University of Munich, Am Biederstein 29, 80802 Munich, Germany

^d Immunological Engineering, Department of Engineering, Aarhus University, Gustav Wieds Vej 10, 9000 Aarhus C, Denmark

^e Department of Infection and Immunity, Luxembourg Institute of Health (LIH), 29, Rue Henri Koch, 4354 Esch-sur-Alzette, Luxembourg

^f Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis, University of Southern Denmark, Sdr. Boulevard 29, 5000 Odense C, Denmark

^g Department of Dermatology and Allergology, University Medical Center Gießen-Marburg, Justus Liebig University Gießen, Gaffkystraße 14, 35395 Gießen, Germany

^h Allergy Research Group, Department of Dermatology, University Freiburg Medical Center, Hauptstrasse 7, 79104 Freiburg, Germany

ARTICLE INFO

Keywords:

C1q-like protein

Honeybee venom

Hymenoptera venom allergy

PDGF/VEGF-like factors 1

PVF1

Venom allergen

ABSTRACT

Honeybee (*Apis mellifera*) venom (HBV) represents an ideal model to study the role of particular venom components in allergic reactions in sensitized individuals as well as in the eusociality of Hymenoptera species. The aim of this study was to further characterize the HBV components C1q-like protein (C1q) and PDGF/VEGF-like factor 1 (PVF1). C1q and PVF1 were produced as recombinant proteins in insect cells. Their allergenic properties were examined by determining the level of specific IgE antibodies in the sera of HBV-allergic patients (n = 26) as well as by their capacity to activate patients' basophils (n = 11). Moreover, the transcript heterogeneity of PVF1 was analyzed. It could be demonstrated that at least three PVF1 variants are present in the venom gland, which all result from alternative splicing of one transcript. Additionally, recombinant C1q and PVF1 from *Spodoptera frugiperda* insect cells exhibited specific IgE reactivity with approximately 38.5% of sera of HBV-allergic patients. Interestingly, both proteins were unable to activate basophils of the patients, questioning their role in the context of clinically relevant sensitization. Recombinant C1q and PVF1 can build the basis for a deeper understanding of the molecular mechanisms of Hymenoptera venoms. Moreover, the conflicting results between IgE sensitization and lack of basophil activation, might in the future contribute to the identification of factors that determine the allergenic potential of proteins.

1. Introduction

The venom of the European honeybee *Apis mellifera* is a complex mixture of various substances such as biogenic amines, peptides and proteins. The honeybee venom gland has evolved from an ancestral female accessory reproductive gland. The venom represents an important defense mechanism of the queen and the worker bees (de Graaf et al., 2010). Moreover, it has been hypothesized that the venom has an additional function for the social immunity of the highly eusocial honeybee (Baracchi et al., 2011). While honeybee venom (HBV) is

lethal for other insects, the normal sting reaction in higher animals consists of pain and inflammation (swelling, redness and itching) (de Graaf et al., 2009). Only massive attacks, for instance by the aggressive Africanized honeybees, can be fatal for humans due to the toxicity of HBV (Tunget and Clark, 1993).

However, already one honeybee sting can lead to very severe and even fatal reactions in allergic patients. In these venom-sensitized individuals, certain HBV components that act as allergens lead to the cross-linking of preformed receptor-bound IgE antibodies on the surface of mast cells and basophils. The immune response following the cross-

* Corresponding author. Center of Allergy and Environment (ZAUM), Technical University of Munich and Helmholtz Center Munich, Ingolstädter Landstraße 1, D-85764 Munich, Germany.

E-mail address: simon.blank@tum.de (S. Blank).

¹ The authors contributed equally to this work.

Abbreviations

BAT	basophil activation test
C1q	C1q-like protein
CCD	cross-reactive carbohydrate determinant
EST	expressed sequence tag
GNA	<i>Galanthus nivalis</i> agglutinin
HBV	honeybee venom

HRP	horseradish peroxidase
PDGF/VEGF	platelet-derived growth factor/vascular endothelial growth factor
PVF1	PDGF/VEGF-like factor 1
SD	standard deviation
sIgE	specific IgE
tIgE	total IgE
VIT	venom immunotherapy

linking of IgE, is characterized by the degranulation of activated effector cells and can result in systemic reactions and even fatal anaphylaxis (Ozdemir et al., 2011). Systemic allergic reactions to Hymenoptera stings (also including vespid and ant species) have been reported in up to 7.5% of adults and up to 3.4% of children (Bilo and Bonifazi, 2008; Jennings et al., 2010). However, venom immunotherapy (VIT) represents a very effective curative treatment for venom allergy and is able to reduce the risk of subsequent systemic sting reactions (Schiener et al., 2017b). VIT is reported to be effective in 77%–84% of patients treated with HBV and in 91%–96% of patients receiving vespid venom (Sturm et al., 2018). A successful VIT depends on the choice of the correct venom. Advanced diagnostic approaches are mandatory for the correct identification of the culprit venom (Antolin-Amerigo et al., 2017; Blank et al., 2018; Jakob et al., 2017a). Hence, both, the design of proper therapeutic venom extracts containing all relevant allergens and the development of superior diagnostic tests rely on an extensive knowledge of the venom composition and of the relevance of particular components as allergens (Blank et al., 2017, 2018; Frick et al., 2015, 2016; Jakob et al., 2017a).

The certainly best characterized Hymenoptera venom is that of the honeybee. This is most likely due to the outstanding importance of honeybees as pollinators and, hence, as elicitors of venom allergy all over the world (Ollert and Blank, 2015; Spillner et al., 2014). Moreover, detailed proteomic data of the pure venom (Danneels et al., 2015; Peiren et al., 2005; Van Vaerenbergh et al., 2014) as well as genomic information of the honeybee are available (Honeybee Genome Sequencing, 2006). So far, 113 proteins were identified as venom components. Several of these components have a function as toxin or play a role in social immunity. Others belong to the so called venom trace molecules which have functions in the extracellular space or are unintentionally released into the venom from disrupted cells (Van Vaerenbergh et al., 2014). Moreover, it could be shown that the venom of queens, winter bees and summer bees differs in composition according to the respective needs and functions (Danneels et al., 2015; Van Vaerenbergh et al., 2013).

To date, 12 proteins of HBV were characterized as allergens and included in the official allergen nomenclature list (Blank et al., 2018; Radauer et al., 2014). Out of these allergens, only very few, such as the major allergen phospholipase A2 (Api m 1) or the minor peptidic allergen melittin (Api m 4), are contained in the venom in substantial amounts. All other allergens make up only low to moderate amounts of the venom (Spillner et al., 2014). Nevertheless, in the last years it has become increasingly evident that also components that are contained in the venom in only minimal amounts, such as icarapin (Api m 10), a protein of unknown function, can have a high relevance for allergic sensitization as well as for the therapeutic outcome of VIT (Blank et al., 2011b; Frick et al., 2015, 2016). However, since such proteins of low abundance are difficult to isolate in larger quantities and in pure form from the crude venom, their recombinant production is a prerequisite for both, their detailed characterization, as well as the evaluation of their relevance as allergen (Blank et al., 2010, 2011b, 2012, 2013; Grunwald et al., 2006; Michel et al., 2012).

Several proteins that have been known to be present in honeybee venom for several years have never been characterized in detail. One of these proteins which is present in HBV as venom trace molecule in low

amounts is C1q-like protein (C1q) (de Graaf et al., 2010). C1q, which is also expressed in various other tissues of honeybee workers and drones, is characterized by an ancient C1q-like domain. In higher organisms, by antibody-antigen-complexes activated C1q initiates the classical complement pathway and, hence, represents a major link between adaptive and innate immunity. Another yet uncharacterized venom and venom gland component is PDGF/VEGF-like factor 1 (PVF1), a protein containing a platelet-derived and vascular endothelial growth factor domain (Peiren et al., 2005, 2008; Van Vaerenbergh et al., 2014). The PVF1 spot density from 2D-gel separated venom indicates that it is a venom compound of moderate abundance (Peiren et al., 2005).

As HBV represents an ideal model for both the role of Hymenoptera venoms in insect eusociality as well as for allergic sensitization to Hymenoptera venoms, the component-resolved understanding of the honeybee venom is a prerequisite for the understanding of the underlying molecular mechanisms. Therefore, the aim of this study was the recombinant production as well as detailed immunological characterization of both proteins. The study revealed that at least three PVF1 alternative splice variants are produced by the HBV gland. Interestingly, although a minor group of HBV-allergic patients exhibited pronounced specific IgE (sIgE) reactivity with the two proteins, both were not able to activate basophils of the patients. Hence, in the future, the conflicting results on sIgE sensitization and effector cell activation might help to understand which parameters determine the allergic potential of proteins. Moreover, the recombinant availability of C1q and PVF1 can help to gain further insights into the molecular mechanisms of Hymenoptera venoms.

2. Methods

2.1. Screening of PVF1 transcript heterogeneity

RNA isolation from honeybee venom gland tissue, cDNA synthesis and reverse transcription PCR were performed as described before (Van Vaerenbergh et al., 2014). DNA elongation in PCR was adapted to 1 min at 72 °C. The coding sequence of mature PVF1 (GenBank: XM_392204.4) was amplified by PCR (Q5, NEB, Frankfurt am Main, Germany) using primers introducing sequences for ligation-independent cloning (forward primer: **GAC GAC GAC AAG ATG CAA CTC GAG GAT ACC AGA TAC**; reverse primer: **GAG GAG AAG CCC GGT TAT TCT GGA TCT GGT TTA GGT**; sequences for cloning in bold). Subcloning for sequencing was done in the pIEx-7 Ek/LIC vector according to the instructions of the Ek/LIC cloning kit (Novagen, Madison, WI, USA). The obtained different transcript variants were analyzed by multiple sequence alignment using the Clustal W software (Larkin et al., 2007).

2.2. Proteomic evidence

PVF1 was successfully identified in the honeybee venom proteome by a profound preceding mass spectrometric analysis (Van Vaerenbergh et al., 2014). A search of the generated MS/MS data against the honeybee RefSeq database extended with all translated PVF1 variant sequences was performed to identify isoform-specific tryptic peptides. Identical search parameters were used as those previously described (Van Vaerenbergh et al., 2014).

phosphatase conjugates of anti-mouse IgG (0.4 µg/mL), ExtrAvidin (1:20000) or anti-rabbit IgG (0.05 µg/mL) (Sigma, Taufkirchen, Germany). The bound secondary antibodies were visualized with nitroterazolium blue chloride and 5-bromo-4-chloro-3-indolyl phosphate (AppliChem, Darmstadt, Germany) as substrate.

3. Results

3.1. Heterogeneity of PVF1

Mature Pvf1 transcripts were amplified from HBV gland tissue by reverse transcription PCR. All cloned nucleotide sequences and their GenBank numbers are shown in Fig. S2A. Sequence analyses of the obtained Pvf1 amplicons revealed the existence of at least three variants (Fig. 1, Fig. S2B). The nucleotide sequence of variant 1 matched the GenBank record XM_392204.4, representing the predicted Pvf1 mRNA sequence. Only one nucleotide substitution was found between the cloned fragment and the predicted NCBI sequence (Fig. S2C). However, the sequences are identical at the protein level (Fig. S2D). Compared to variant 1, variant 2 lacks a sequence of four nucleotides, while variant 3 contains an additional internal sequence of 82 nucleotides. Variant 1 and 2 show five nucleotide substitutions (Fig. S2B), three of those resulting in an amino acid substitution (Fig. 1). Alignments of all three variants with the genome show that they are all generated by alternative splicing of the same gene (Fig. S3). The Pvf1 gene is positioned on chromosome LG2 and consists of six exons (GeneID: 408666). Three alternative 5'donor sites are present within exon 5. While variant 1 and variant 2 use an alternative canonical GT splice donor, variant 3 uses a non-canonical GC splice donor. In addition, BeeBase (Elsik et al., 2016; Honeybee Genome Sequencing, 2006) blasts confirmed the existence of these Pvf1 transcripts and their expression in honeybee tissues. A complete variant 1 (GenBank: gi|308418987) and variant 3 (GenBank: gi|308392023) expressed sequence tag (EST) was found in a brain/ovary and abdomen database, respectively. In contrast, only a partial variant 2 (GenBank: gi|10276753) EST is available in a honeybee antennae database.

The variant 1 transcript codes for a protein of 292 amino acids. In contrast, due to the alternative splice events, variant 2 and variant 3 use alternative stop codons, which generate C-terminally truncated proteins with lengths of 272 and 249 amino acids, respectively (Fig. 2, Fig. S2E). In order to obtain proteomic evidence for the presence of particular PVF1 variants within HBV, honeybee venom MS/MS data from preceding proteomic analyses (Van Vaerenbergh et al., 2014) were analyzed for the presence of variant-specific tryptic peptides of the C-terminal sequence which differs in all three variants. This analysis identified the peptide ETQECSTGFYFDQNSCR which is found in the C-

terminal region of both variant 1 and variant 3, but not of variant 2 (Fig. 1). Unfortunately, additional distinguishing peptides could not be found.

3.2. Recombinant production of C1q and PVF1

In order to further characterize C1q (Fig. 3) and PVF1 and to address the putative allergic potential of the two proteins, both were recombinantly produced by the baculoviral infection of *Spodoptera frugiperda* (Sf9) insect cells. As PVF1 variant 1 is the longest variant and as it was proven that it is contained in HBV, it seems to be the most suitable variant for assessing the allergenic potential of PVF1. Hence, this variant was selected for recombinant production. Ni²⁺-affinity chromatography yielded soluble recombinant C1q and PVF1 from culture supernatants as shown by Coomassie staining and reactivity with an antibody directed against the V5-epitope tag added for recombinant expression (Fig. 4). Both proteins contain one predicted N-glycosylation site (Gupta and Brunak, 2002) and reactivity with *Galanthus nivalis* agglutinin (GNA) (reactive with terminal 1,2-, 1,3- and 1,6-linked mannose residues) proves the presence of N-linked glycans (Fig. 4). However, staining with rabbit polyclonal horseradish peroxidase (HRP) antiserum, specific for α-1,3-core fucosylation, the structure responsible for clinically irrelevant cross-reactive carbohydrate determinant (CCD)-based cross-reactivity, demonstrates the lack of CCDs, as shown previously for other allergens produced in Sf9 insect cells (Blank et al., 2010, 2011a, 2011b; Schiener et al., 2018; Seismann et al., 2010). Hence, proteins produced in Sf9 cells are ideal tools to assess their allergenic potential on protein level independent of interfering CCD-reactivity (Ollert and Blank, 2015).

3.3. Allergenic potential of C1q and PVF1

To evaluate the allergenic potential of C1q and PVF1, sIgE reactivity of 26 HBV-allergic patients with the two proteins was addressed by ELISA (Fig. 5A). HBV allergy was confirmed by a combination of clinical history, skin test and sIgE level to HBV (Table 1).

Thereby, 10/26 patients (38.5%) exhibited pronounced sIgE-reactivity with C1q above the cut-off of the ELISA. Likewise, 10/26 (38.5%) patients exhibited pronounced sIgE reactivity with PVF1. HBV allergy of all patients again was confirmed by the strong sIgE-reactivity of 25/26 (96.2%) of the patients with the HBV major allergen Api m 1 (phospholipase A2). Interestingly, eight patients were reactive with both C1q and PVF1 (Fig. 5B, Table 1). Intriguingly, the patients who were reactive with C1q and/or PVF1 showed a tendency to higher sIgE levels to whole HBV (i1) in ImmunoCAP measurements (Table 1). Two of the patients had CAP class 6 (> 100 kU_A/L), two class 5 (50.1–100

```

PVF1 variant 1  QLEDTRYPDQRIVFPDRGRE*ETANPALEGGPSSGGGIGELAK*SIQLAKKISSINSRDDFLKL
PVF1 variant 2  QLEDTRYPD*RIVFPDRGRE*ETANPALEGGPSSGGGIGELAK*SIQLAKKISSINSRDDFLKL
PVF1 variant 3  QLEDTRYPDQRIVFPDRGRE*ETANPALEGGPSSGGGIGELAK*SIQLAKKISSINSRDDFLKL
*****:*****

PVF1 variant 1  VKDVPKDISFFSSSSRMGETERSNAERPNOALCMPELQTVLLENEP*SVIYYPTCTRIK*R
PVF1 variant 2  VKDVPKDISFFSSSSRMGETERSNAERPNOALCMPELQTVLLENEP*SVIYYPTCTRIK*R
PVF1 variant 3  VKDVPKDISFFSSSSRMGETERSNAERPNOALCMPELQTVLLENEP*SVIYYPTCTRIK*R
*****:*****

PVF1 variant 1  CGGCGCTHSLLS*COPTATEIRNFEILV*TILESSGK*LKYQGKRIVPIEHTQCT*CDCKIKET
PVF1 variant 2  CGGCGCTHSLLS*COPTATEIRNFEILV*TILESSGK*LKYQGKRIVPIEHTQCT*CDCKIKET
PVF1 variant 3  CGGCGCTHSLLS*COPTATEIRNFEILV*TILESSGK*LKYQGKRIVPIEHTQCT*CDCKIKET
*****:*****

PVF1 variant 1  DCNKKQ*SYVPEECTACANNVDEQ*KK*CNESNIK*MWHPDLCSFCFRETQECSTGFYFDQNSC
PVF1 variant 2  DCNKKQ*SYVPEECTACANNVDEQ*RKCNESNIK*MWHPDLCSFCFRETQECSTGFYFDQNSY
PVF1 variant 3  DCNKKQ*SYVPEECTACANNVDEQ*KK*CNESNIK*MWHPDLCSFCFRETQECSTGFYFDQNSC
*****:*****

PVF1 variant 1  RCLQVPLSRTWFTSTKGS*DYRFGQTQRPDNPVPIIALDSDDPRKPKPDPE
PVF1 variant 2  ACKYRYLEHGLH*FPQKVLII*DSKHKDQIMYHR-----
PVF1 variant 3  RCERNNKDL-----

```

Fig. 1. Protein sequence alignment of the PVF1 variants. Asterisks, colons, and periods indicate fully conserved, strongly similar, and weakly similar residues, respectively. Peptides identified by mass spectrometry (Van Vaerenbergh et al., 2014) are shown in yellow. Amino acid substitutions are indicated in red. The eight conserved cysteine residues of the central platelet-derived and vascular endothelial growth factor domain are shown boxed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

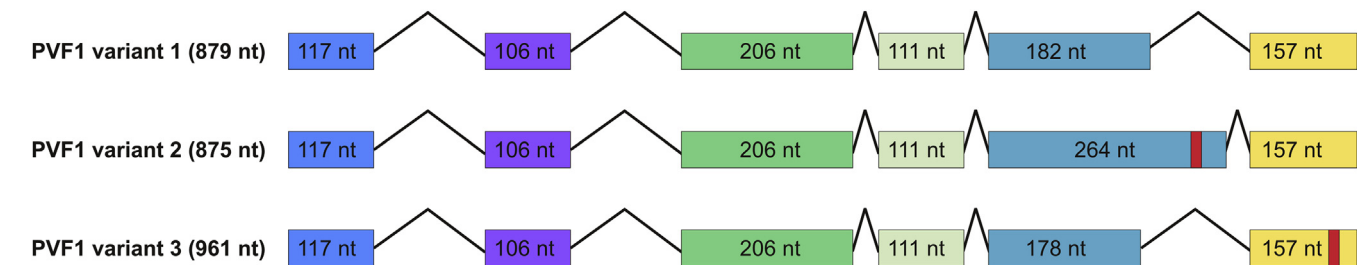


Fig. 2. Schematic representation of the exon-intron-structure of the three *Pvf1* amplicons. Exons are shown as colored boxes, while introns are presented as lines. Red boxes present alternative stop codons that generate C-terminally truncated variants. nt = nucleotides. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

C1q AIPDPPNSKISTVNPSKLSTAVDCTGIIAFSATHASVDHAKAVFAETLVDKGVGYVPQTG
I FITNCPGLYQFSFAGYGSTDLRLTLKKKQNNSDSWRPVVG TGAGGGANLILLEVDVGDQ
LAVFVDSGKISDGVTFSGYRIAKI

protein family (Tom Tang et al., 2005) are shown in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 3. Protein sequence of C1q. Shown is the mature sequence of C1q. Conserved residues that clearly define the protein as member of the C1q domain-containing

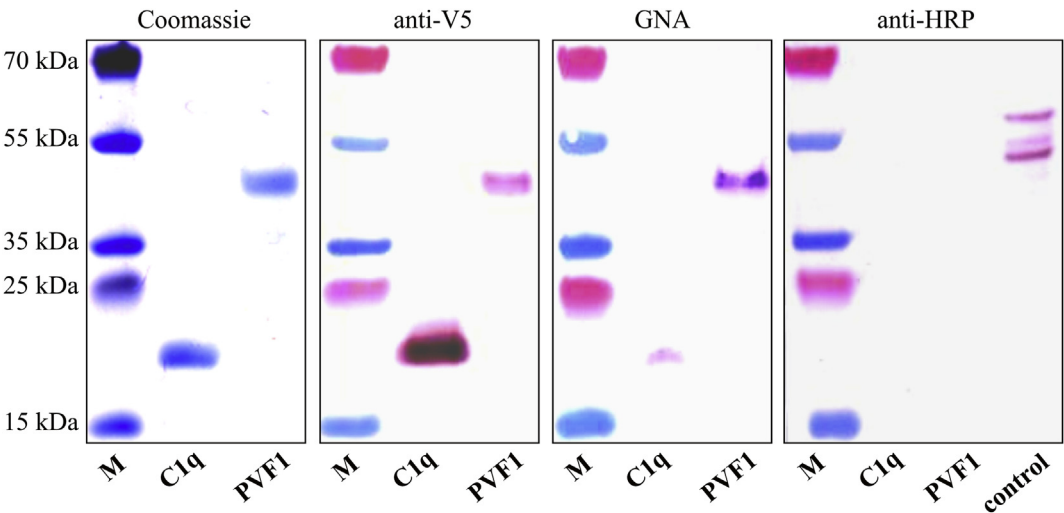


Fig. 4. Recombinant expression and characterization of C1q and PVF1. SDS-PAGE and Western blot analyses of C1q and PVF1, recombinantly produced in Sf9 insect cells, visualized either by Coomassie blue staining or anti-V5 epitope antibody, GNA (*Galanthus nivalis* agglutinin) or anti-HRP (horseradish peroxidase) antiserum. For anti-HRP staining a CCD-bearing protein served as positive control (Seismann et al., 2010). M, molecular weight marker.

kU_A/L), three class 4 (17.6–50 kU_A/L), two class 3 (3.6–17.5 kU_A/L), two class 2 (0.71–3.5 kU_A/L) and one class 1 (0.35–0.7 kU_A/L). In contrast, in the group of patients without reactivity to C1q and PVF1 one had class 6, three class 4, five class 3, four class 2 and one class 1. Control patients without clinical history of HBV allergy exhibited no reactivity with C1q or PVF1 (Fig. S4).

However, since sIgE sensitization does not imply a clinically relevant allergy to the allergen *per se*, the capability of C1q and PVF1 to cross-link receptor-bound IgE antibodies and, thus, to activate effector cells, was analyzed. For this purpose, basophil activation tests (BATs) were performed (Fig. 6) as previously described (Eberlein et al., 2012; Schiener et al., 2017a, 2018). Two patients (5 and 11) with sIgE to C1q only and four patients (9, 18, 20 and 24) with sIgE to C1q and PVF1 were analyzed in BAT. Interestingly, none of the patients showed any basophil activation by C1q and PVF1, hinting to the fact that both proteins are not able to cross-link receptor-bound IgE antibodies. In contrast, Api m 1 induced a dose-dependent basophil activation in all patients. This could even be observed in patient 24 who showed a very low level of Api m 1-specific sIgE that was only detectable by ImmunoCAP measurement but not by ELISA (Table 1). In contrast, sIgE to C1q and PDV1 were well detectable in ELISA, however, both proteins were unable to activate basophils. Basophil activation by C1q and PVF1

was also negative in five other patients (6, 10, 16, 17, 19) and two non-allergic controls (data not shown).

4. Discussion

Hymenoptera venoms contain an enormous diversity of different components playing roles in toxicity as well as eusociality (Baracchi et al., 2011; de Graaf et al., 2010). The European honeybee, *Apis mellifera*, is the most important pollinator and domesticated by humans for the production of honey all over the world. Therefore, a honeybee sting has been an unpleasant experience for many people and many researchers have become intrigued by the HBV composition and toxic effects of individual venom components. Moreover, HBV is one of the most common elicitors of IgE-mediated venom allergy and can lead to severe systemic and even fatal reactions in sensitized individuals (Blank et al., 2018; Sturm et al., 2018).

The two highest abundant HBV components, melittin (Api m 4) and phospholipase A2 (PLA2, Api m 1) already make up more than 60% of the venom dry weight (Hider, 1988). Besides these two components, HBV contains multiple proteins of lower abundance (Peiren et al., 2008; Van Vaerenbergh et al., 2014), out of which only few have been characterized in detail. To date, 113 proteins have been identified as

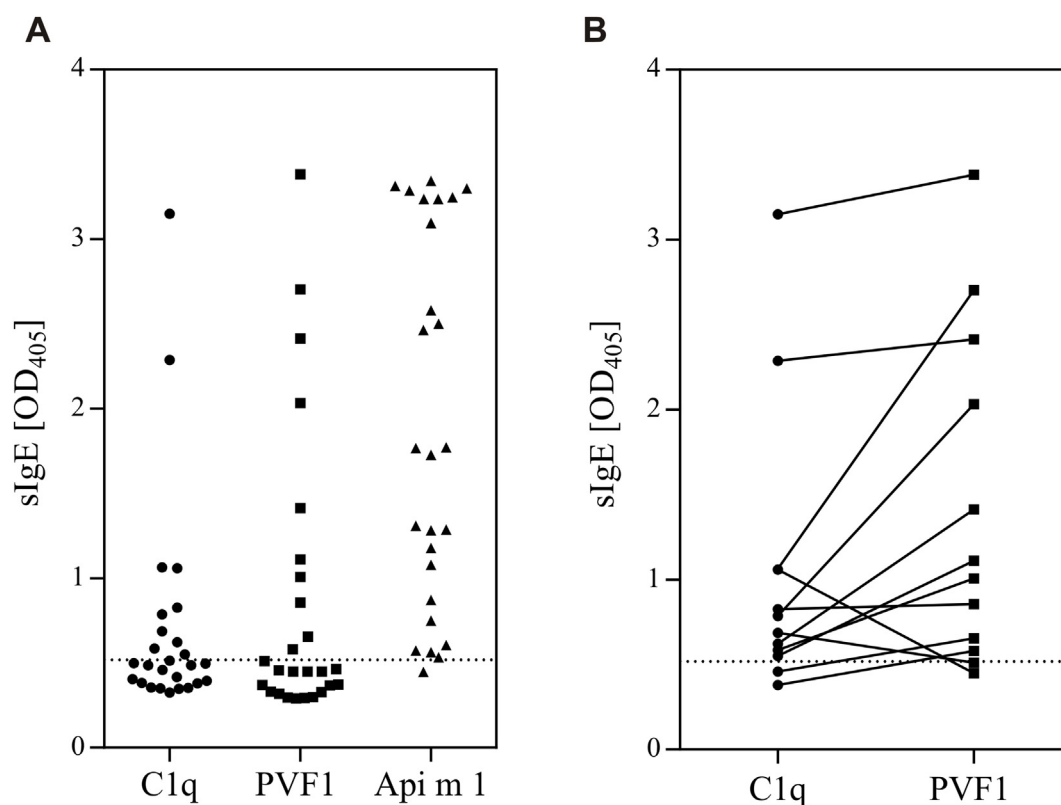


Fig. 5. sIgE reactivity of HBV-allergic patients with recombinant C1q and PVF1 in ELISA. (A) sIgE immunoreactivity of HBV-allergic patients ($n = 26$) with recombinant C1q and PVF1 as well as native Api m 1 (phospholipase A2). (B) Comparative sIgE immunoreactivity of C1q- and/or PVF1-positive HBV-allergic patients ($n = 12$) with C1q and PVF1. The lower end cut-off of the ELISA is represented by dotted lines.

Table 1

Clinical and serological data of HBV-allergic patients analyzed in ELISA and basophil activation test.

Patient ID	Sting reaction grade ^a	Skin test ^b (i.c.) HBV	tIgE [kU/L]	sIgE HBV (i1) [class]	sIgE Api m 1 (i208) [class]	sIgE C1q [OD ₄₀₅]	sIgE PVF1 [OD ₄₀₅]	sIgE Api m 1 [OD ₄₀₅]	Negative control
1	4	0.0001	226	4	3	3.15	3.38	2.50	0.33
2	2	0.001	222	4	4	0.51	0.33	3.34	0.22
3	2	0.001	16.1	3	n.d.	0.40	0.36	1.28	0.23
4	3	0.01	150	2	n.d.	0.45	0.65	1.17	0.25
5	3	0.0001	306	5	2	1.05	0.44	3.28	0.27
6	2	0.001	164	6	5	0.38	0.58	3.23	0.23
7	2	0.1	160	2	n.d.	0.38	0.3	0.56	0.22
8	3	0.001	7.85	2	2	0.34	0.29	1.28	0.27
9	1	0.0001	345	5	2	1.06	2.70	3.29	0.22
10	2	0.01	1102	6	2	0.49	0.44	1.76	0.25
11	2	0.001	334	6	4	0.68	0.51	3.31	0.29
12	2	0.001	22.1	3	1	0.55	1.11	0.60	0.20
13	2	0.001	78.8	2	0/1	0.82	0.85	0.74	0.33
14	4	0.001	137	4	3	0.39	0.32	3.09	0.27
15	3	0.0001	10.3	2	1	0.35	0.29	0.53	0.32
16	3	0.1	517	3	2	0.48	0.36	1.30	0.25
17	2	0.0001	105	3	2	0.35	0.44	3.24	0.37
18	2	0.001	38.6	3	2	2.28	2.41	2.57	0.23
19	3	0.1	419	4	2	0.41	0.37	1.77	0.22
20	1	0.0001	42	4	0/1	0.62	1.41	1.72^c	0.23
21	2	0.0001	171	4	3	0.58	1.00	3.23	0.44
22	2	0.001	34	1	1	0.49	0.45	0.87	0.22
23	3	0.1	68.3	3	3	0.35	0.29	0.57	0.41
24	3	0.1	350	1	1	0.78	2.03	0.44	0.30
25	3	0.0001	143	2	n.d.	0.32	0.29	1.07	0.24
26	3	0.001	11.5	3	n.d.	0.48	0.46	2.46	0.30

The sIgE levels to HBV (i1) and Api m 1 (i208) as well as the total IgE (tIgE) levels were determined with the UniCAP250 system (Thermo Fisher Scientific). The sIgE levels to recombinant C1q and PVF1 as well as native Api m 1 were measured by ELISA. sIgE levels above the lower end cut-off of the ELISA are displayed in bold.

^a According to Ring and Messmer (1977).

^b For intradermal skin tests the lowest venom concentration [$\mu\text{g/mL}$] that gave a positive result is displayed.

^c The slightly discrepant results between the higher level of sIgE to native Api m 1 in ELISA and the lower level of sIgE to recombinant Api m 1 in the ImmunoCAP measurement can be explained by the presence of CCD-specific IgE (class 2), since CCDs are present on native Api m 1 but not on recombinant Api m 1.

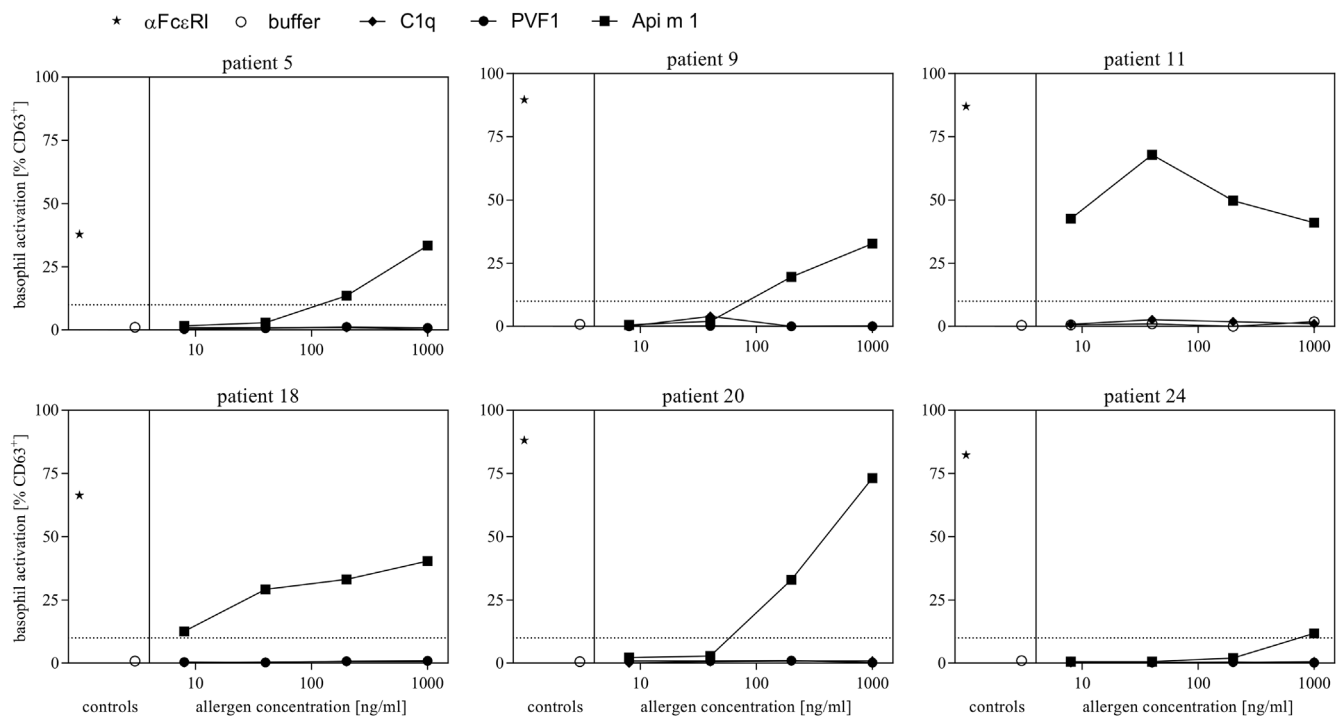


Fig. 6. Basophil activation tests of HBV-allergic patients. Basophils were exposed to different concentrations of recombinant C1q and PVF1 as well as native Api m 1 (phospholipase A2). Additionally, stimulation with anti-FcεRI antibody (positive control) and plain stimulation buffer (negative control) is shown. Activation is shown as percentage of CD63⁺ out of total basophilic cells. The cut-off of the assay (10%) is represented as dotted line.

components of HBV and, so far, the allergenic properties of only 12 of them have been addressed (Blank et al., 2018; Radauer et al., 2014). However, research conducted within the last years indicated that the knowledge of venom allergens on a molecular level is able to contribute to advanced diagnostic and therapeutic approaches for HBV allergy (Antolin-Amerigo et al., 2017; Blank et al., 2018; Jakob et al., 2017a). Today, it is widely accepted that the component-resolved analysis of the honeybee venom is able to contribute to a better understanding of the role of individual proteins in the function of the venom and of their contribution to the allergic potential of the venom. Hence, aim of this study was the immunological characterization of the novel HBV components C1q-like protein and PVF1.

PVF1 belongs to the PDGF/VEGF (platelet-derived growth factor/vascular endothelial growth factor) family. Our analyses demonstrated the presence of at least three different transcript variants in the venom gland tissue that are generated by alternative splicing. While in vertebrates two networks, PDGF/PDGFR (PDGF receptor) and VEGF/VEGFR (VEGF receptor), have evolved, invertebrates possess a single PVF/PVR (PDGF/VEGF-like factor/PVF receptor) network (Hoch and Soriano, 2003). In the fruit fly *Drosophila melanogaster*, three *Pvf* genes were found (*Pvf1*, *Pvf2* and *Pvf3*), which are ligands for a single PDGF/VEGF receptor (Cho et al., 2002). The *Pvf* genes have distinct expression patterns in the fruit fly embryo (Harris et al., 2007) and execute different functions during development (Hoch and Soriano, 2003). HBV PVF1 shows the highest similarity to the fruit fly PVF1. Also two splice variants of the fruit fly PVF1 have been described, but in comparison to the honeybee homologue these differ at their N-termini (Cho et al., 2002). Alternative splicing of *Pvf1* transcripts in both species does not affect the central PDGF/VEGF domain, which includes the typical eight conserved cysteines important for dimerization and functional activity (Tarsitano et al., 2006). Two additional predicted genes containing a PDGF/VEGF domain were found in the honeybee genome, while these compounds were detected in none of the honeybee venom proteome analyses. These predicted genes (GeneIDs:100577397 and 100579031) are positioned on different chromosomes and both show highest sequence similarity to fruit fly *Pvf3*. While fruit fly *Pvf2* and *Pvf3* were

suggested to be generated by gene duplication due to their close proximity in the genome (Cho et al., 2002), no additional *Pvf* gene was found within the honeybee genome in addition to the two *Pvf3*-resembling genes.

A function of PVF1 in HBV might be suggested based on similar venom components from other species. PVF1 may act similar to snake venom VEGF-like molecules, which are the most potent known vascular permeability factors and which facilitate venom spreading (Francischetti et al., 2004). Certainly, the here recombinantly produced HBV PVF1 can build the basis for future functional studies.

The second venom component evaluated in this study, C1q-like protein, contains a C1q domain referring to the C1q complex that initiates the activation of the complement system in higher organisms (de Graaf et al., 2010). Expression of a homologous C1q-like protein was also demonstrated for the venom gland of the jewel wasp *Nasonia vitripennis* (de Graaf et al., 2010). Both venom C1q-like proteins contain no additional conserved domains and were suggested to be members of the venom trace molecules (de Graaf et al., 2010; Van Vaerenbergh et al., 2014). As the human globular C1q domain directly interacts with lipopolysaccharides from Gram-negative bacteria (Roumenina et al., 2008) it was suggested to further explore possible functions of the insect C1q-like proteins in the cellular immune response, particularly as opsonizing molecules (de Graaf et al., 2010). For such purposes the here recombinantly produced HBV C1q might serve as ideal agent.

A particular aim of this study was to evaluate the role of HBV C1q and PVF1 as allergens responsible for allergic sensitization of humans. So far, the HBV components phospholipase A2 (Api m 1), hyaluronidase (Api m 2), acid phosphatase (Api m 3), dipeptidyl peptidase IV (Api m 5) and icarapin (Api m 10) were demonstrated to be major allergens of HBV to which a majority of allergic patients exhibits sIgE antibodies (Arzt et al., 2017; Blank et al., 2010, 2011b; Frick et al., 2015, 2016; Grunwald et al., 2006; Kohler et al., 2014; Seismann et al., 2010). Other venom components such as serine protease inhibitor (Api m 6), major royal jelly proteins (Api m 11) and vitellogenin (Api m 12) are minor allergens with putative importance for a minor fraction of allergic patients (Blank et al., 2012, 2013; Michel et al., 2012).

In this study, approximately 38.5% of HBV-allergic patients exhibited sIgE reactivity with C1q and PVF1, respectively. The sIgE-reactivity clearly was directed against protein epitopes of C1q and PVF1 and independent of interfering sIgE antibodies directed against cross-reactive carbohydrate determinants (CCDs) as both proteins were produced as CCD-free molecules in Sf9 insect cells (Seismann et al., 2010). However, all reactive patients additionally showed sIgE sensitization to Api m 1 and/or HBV extract. In this context sensitization profiles of patients with a clear history of HBV allergy but negative sIgE test results to currently available HBV allergens may be of particular interest. Here novel allergens such as C1q and PVF1 might help to close the diagnostic gap that still exists in the molecular diagnostics of HBV allergy (Blank et al., 2018; Jakob et al., 2017a, 2017b; Kohler et al., 2014). Component resolved analysis of the sensitization profile in a large population of HBV allergic patients in a previous study revealed 5.6% that did not show IgE reactivity to the currently available allergens. Among these, 25% displayed IgE reactivity to C1q (Kohler et al., 2014) suggesting that sIgE to C1q and/or PVF1 might be of relevance for the molecular diagnostics of particular HBV-allergic patients.

Of note, the C1q- and/or PVF1-reactive patients showed a tendency to higher sIgE levels to whole HBV compared to the non-reactive patients. Moreover, eight out of 12 patients with positive sIgE to C1q and/or PVF1 exhibited sIgE reactivity to both proteins. These observations might lead to the hypothesis that higher sIgE levels to HBV might be associated with sensitization to a broader panel of venom proteins, as has been suggested previously (Kohler et al., 2014).

The lack of basophil activation in patients with sIgE to C1q and/or PVF1 seems to indicate a missing relevance of these two venom proteins for the allergic reaction. Detectable levels of sIgE to a certain protein do not automatically render it an allergen. By fact, only the capability of a protein to activate mast cells and basophils via cross-linking of receptor-bound IgE antibodies on their surface determines its allergenic properties. The lack of basophil activation by C1q and PVF1 might be explained by the number and distribution of IgE epitopes on the two proteins or the level of sIgE in the context of total IgE. Moreover, even though insect cells were shown to be a suitable expression system for biologically active allergens (Seismann et al., 2010; Soldatova et al., 1998), it never can be fully excluded that the recombinant allergens might show another folding compared to their native counterparts, resulting in different effector cell activation capacity. Although these hypotheses clearly need further careful investigation, the discrepancy between sIgE sensitization and lack of basophil activation certainly has the potential to contribute to the understanding of the factors that determine the allergenic potential of a protein.

5. Conclusion

C1q and PVF1 are components of honeybee venom of low to moderate abundance. Both proteins are able to induce sIgE antibodies in a minor fraction of venom-allergic individuals. Hence, they might be of importance for accurate diagnosis of the small number of patients who are not covered by the currently available allergen components. However, the missing capability of both venom proteins to activate basophils of sIgE-sensitized allergic patients renders their relevance in the context of clinically relevant sensitization questionable. Nevertheless, the discrepancy between sIgE-inducing potential and lacking basophil activation might in the future help to determine factors that define the allergenic potential of proteins.

Conflicts of interest

The authors declare no conflict of interest in relation to this publication.

Funding

The research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Transparency document

Transparency document related to this article can be found online at <http://dx.doi.org/10.1016/j.toxicol.2018.05.017>.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.toxicol.2018.05.017>.

References

- Antolin-Amerigo, D., Ruiz-Leon, B., Boni, E., Alfaya-Arias, T., Alvarez-Mon, M., Barroja-Escudero, J., Gonzalez-de-Olano, D., Moreno-Aguilar, C., Rodriguez-Rodriguez, M., Sanchez-Gonzalez, M.J., Sanchez-Morillas, L., Vega-Castro, A., 2017. Component-resolved diagnosis in hymenoptera allergy. *Allergologia et immunopathologia*.
- Arzt, L., Bokanovic, D., Schrautner, C., Schwarz, I., Laipold, K., Aberer, W., Sturm, G.J., 2017. Questionable diagnostic benefit of the commercially available panel of bee venom components. *Allergy* 72, 1419–1422.
- Baracchi, D., Francese, S., Turillazzi, S., 2011. Beyond the antipredatory defence: honey bee venom function as a component of social immunity. *Toxicol. Off. J. Int. Soc. Toxicol.* 58, 550–557.
- Bilo, B.M., Bonifazi, F., 2008. Epidemiology of insect-venom anaphylaxis. *Curr. Opin. Allergy Clin. Immunol.* 8, 330–337.
- Blank, S., Bantleon, F.I., McIntyre, M., Ollert, M., Spillner, E., 2012. The major royal jelly proteins 8 and 9 (Api m 11) are glycosylated components of *Apis mellifera* venom with allergenic potential beyond carbohydrate-based reactivity. *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* 42, 976–985.
- Blank, S., Bilo, M.B., Ollert, M., 2018. Component-resolved diagnostics to direct in venom immunotherapy: important steps towards precision medicine. *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* 48 (4), 354–364.
- Blank, S., Etzold, S., Darsow, U., Schiener, M., Eberlein, B., Russkamp, D., Wolf, S., Graessel, A., Biedermann, T., Ollert, M., Schmidt-Weber, C.B., 2017. Component-resolved evaluation of the content of major allergens in therapeutic extracts for specific immunotherapy of honeybee venom allergy. *Hum. Vaccines Immunother.* 13, 2482–2489.
- Blank, S., Michel, Y., Seismann, H., Plum, M., Greunke, K., Grunwald, T., Bredehorst, R., Ollert, M., Braren, I., Spillner, E., 2011a. Evaluation of different glycoforms of honeybee venom major allergen phospholipase A2 (Api m 1) produced in insect cells. *Protein Pept. Lett.* 18, 415–422.
- Blank, S., Seismann, H., Bockisch, B., Braren, I., Cifuentes, L., McIntyre, M., Ruhl, D., Ring, J., Bredehorst, R., Ollert, M.W., Grunwald, T., Spillner, E., 2010. Identification, recombinant expression, and characterization of the 100 kDa high molecular weight hymenoptera venom allergens Api m 5 and Ves v 3. *J. Immunol.* 184, 5403–5413.
- Blank, S., Seismann, H., McIntyre, M., Ollert, M., Wolf, S., Bantleon, F.I., Spillner, E., 2013. Vitellogenins are new high molecular weight components and allergens (Api m 12 and Ves v 6) of *Apis mellifera* and *Vespula vulgaris* venom. *PLoS One* 8, e62009.
- Blank, S., Seismann, H., Michel, Y., McIntyre, M., Cifuentes, L., Braren, I., Grunwald, T., Darsow, U., Ring, J., Bredehorst, R., Ollert, M., Spillner, E., 2011b. Api m 10, a genuine *A. mellifera* venom allergen, is clinically relevant but underrepresented in therapeutic extracts. *Allergy* 66, 1322–1329.
- Cho, N.K., Keyes, L., Johnson, E., Heller, J., Ryner, L., Karim, F., Krasnow, M.A., 2002. Developmental control of blood cell migration by the *Drosophila* VEGF pathway. *Cell* 108, 865–876.
- Danneels, E.L., Van Vaerenbergh, M., Debyser, G., Devreese, B., de Graaf, D.C., 2015. Honeybee venom proteome profile of queens and winter bees as determined by a mass spectrometric approach. *Toxins (Basel)* 7, 4468–4483.
- de Graaf, D.C., Aerts, M., Danneels, E., Devreese, B., 2009. Bee, wasp and ant venomics pave the way for a component-resolved diagnosis of sting allergy. *J. Proteomics* 72, 145–154.
- de Graaf, D.C., Brunain, M., Scharlaken, B., Peiren, N., Devreese, B., Ebo, D.G., Stevens, W.J., Desjardins, C.A., Werren, J.H., Jacobs, F.J., 2010. Two novel proteins expressed by the venom glands of *Apis mellifera* and *Nasonia vitripennis* share an ancient C1q-like domain. *Insect Mol. Biol.* 19 (Suppl. 1), 1–10.
- Eberlein, B., Krischan, L., Darsow, U., Ollert, M., Ring, J., 2012. Double positivity to bee and wasp venom: improved diagnostic procedure by recombinant allergen-based IgE testing and basophil activation test including data about cross-reactive carbohydrate determinants. *J. Allergy Clin. Immunol.* 130, 155–161.
- Elsik, C.G., Tayal, A., Diesh, C.M., Unni, D.R., Emery, M.L., Nguyen, H.N., Hagen, D.E., 2016. Hymenoptera genome database: integrating genome annotations in hymenopteraMine. *Nucleic Acids Res.* 44, D793–D800.
- Francischetti, I.M., My-Pham, V., Harrison, J., Garfield, M.K., Ribeiro, J.M., 2004. *Bitis gabonica* (Gaboon viper) snake venom gland: toward a catalog for the full-length transcripts (cDNA) and proteins. *Gene* 337, 55–69.

- Frick, M., Fischer, J., Helbling, A., Rueff, F., Wiczorek, D., Ollert, M., Pfutzner, W., Muller, S., Huss-Marp, J., Dorn, B., Biedermann, T., Lidholm, J., Ruecker, G., Bantleon, F., Miehe, M., Spillner, E., Jakob, T., 2016. Predominant Api m 10 sensitization as risk factor for treatment failure in honey bee venom immunotherapy. *J. Allergy Clin. Immunol.* 138, 1663–1671 e1669.
- Frick, M., Muller, S., Bantleon, F., Huss-Marp, J., Lidholm, J., Spillner, E., Jakob, T., 2015. rApi m 3 and rApi m 10 improve detection of honey bee sensitization in Hymenoptera venom-allergic patients with double sensitization to honey bee and yellow jacket venom. *Allergy* 70, 1665–1668.
- Grunwald, T., Bockisch, B., Spillner, E., Ring, J., Bredehorst, R., Ollert, M.W., 2006. Molecular cloning and expression in insect cells of honeybee venom allergen acid phosphatase (Api m 3). *J. Allergy Clin. Immunol.* 117, 848–854.
- Gupta, R., Brunak, S., 2002. Prediction of glycosylation across the human proteome and the correlation to protein function. In: *Pac Symp Biocomput.*, pp. 310–322.
- Harris, K.E., Schnittke, N., Beckendorf, S.K., 2007. Two ligands signal through the *Drosophila* PDGF/VEGF receptor to ensure proper salivary gland positioning. *Mech. Dev.* 124, 441–448.
- Hider, R.C., 1988. Honeybee venom: a rich source of pharmacologically active peptides. *Endeavour* 12, 60–65.
- Hoch, R.V., Soriano, P., 2003. Roles of PDGF in animal development. *Development* 130, 4769–4784.
- Honeybee Genome Sequencing, C., 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* 443, 931–949.
- Jakob, T., Muller, U., Helbling, A., Spillner, E., 2017a. Component resolved diagnostics for hymenoptera venom allergy. *Curr. Opin. Allergy Clin. Immunol.* 17, 363–372.
- Jakob, T., Rafei-Shamsabadi, D., Spillner, E., Muller, S., 2017b. Diagnostics in Hymenoptera venom allergy: current concepts and developments with special focus on molecular allergy diagnostics. *Allergo J. Int.* 26, 93–105.
- Jennings, A., Duggan, E., Perry, I.J., Hourihane, J.O., 2010. Epidemiology of allergic reactions to hymenoptera stings in Irish school children. *Pediatr. Allergy Immunol. Off. Publ. Eur. Soc. Pediatr. Allergy Immunol.* 21, 1166–1170.
- Kohler, J., Blank, S., Muller, S., Bantleon, F., Frick, M., Huss-Marp, J., Lidholm, J., Spillner, E., Jakob, T., 2014. Component resolution reveals additional major allergens in patients with honeybee venom allergy. *J. Allergy Clin. Immunol.* 133, 1383–1389 e1381–1386.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and clustal X version 2.0. *Bioinformatics* 23, 2947–2948.
- Michel, Y., McIntyre, M., Ginglinger, H., Ollert, M., Cifuentes, L., Blank, S., Spillner, E., 2012. The putative serine protease inhibitor Api m 6 from *Apis mellifera* venom: recombinant and structural evaluation. *J. Invest. Allergol. Clin. Immunol.* 22, 476–484.
- Ollert, M., Blank, S., 2015. Anaphylaxis to insect venom allergens: role of molecular diagnostics. *Curr. Allergy Asthma Rep.* 15, 527.
- Ozdemir, C., Kucuksezer, U.C., Akdis, M., Akdis, C.A., 2011. Mechanisms of immunotherapy to wasp and bee venom. *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* 41, 1226–1234.
- Peiren, N., de Graaf, D.C., Vanrobaeys, F., Danneels, E.L., Devreese, B., Van Beeumen, J., Jacobs, F.J., 2008. Proteomic analysis of the honey bee worker venom gland focusing on the mechanisms of protection against tissue damage. *Toxicon Off. J. Int. Soc. Toxinol.* 52, 72–83.
- Peiren, N., Vanrobaeys, F., de Graaf, D.C., Devreese, B., Van Beeumen, J., Jacobs, F.J., 2005. The protein composition of honeybee venom reconsidered by a proteomic approach. *Biochim. Biophys. Acta* 1752, 1–5.
- Radauer, C., Nandy, A., Ferreira, F., Goodman, R.E., Larsen, J.N., Lidholm, J., Pomes, A., Raulf-Heimsoth, M., Rozynek, P., Thomas, W.R., Breiteneder, H., 2014. Update of the WHO/IUIS Allergen Nomenclature Database based on analysis of allergen sequences. *Allergy* 69, 413–419.
- Ring, J., Messmer, K., 1977. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. *Lancet* 1, 466–469.
- Roumenina, L.T., Popov, K.T., Bureeva, S.V., Kojouharova, M., Gadjeva, M., Rabheru, S., Thakrar, R., Kaplun, A., Kishore, U., 2008. Interaction of the globular domain of human C1q with *Salmonella typhimurium* lipopolysaccharide. *Biochim. Biophys. Acta* 1784, 1271–1276.
- Schiener, M., Eberlein, B., Moreno-Aguilar, C., Pietsch, G., Serrano, P., McIntyre, M., Schwarze, L., Russkamp, D., Biedermann, T., Spillner, E., Darsow, U., Ollert, M., Schmidt-Weber, C.B., Blank, S., 2017a. Application of recombinant antigen 5 allergens from seven allergy-relevant Hymenoptera species in diagnostics. *Allergy* 72, 98–108.
- Schiener, M., Graessel, A., Ollert, M., Schmidt-Weber, C.B., Blank, S., 2017b. Allergen-specific immunotherapy of Hymenoptera venom allergy - also a matter of diagnosis. *Hum. Vaccines Immunother.* 13, 2467–2481.
- Schiener, M., Hilger, C., Eberlein, B., Pascal, M., Kuehn, A., Revets, D., Planchon, S., Pietsch, G., Serrano, P., Moreno-Aguilar, C., de la Roca, F., Biedermann, T., Darsow, U., Schmidt-Weber, C.B., Ollert, M., Blank, S., 2018. The high molecular weight dipeptidyl peptidase IV Pol d 3 is a major allergen of *Polistes dominula* venom. *Sci. Rep.* 8, 1318.
- Seismann, H., Blank, S., Braren, I., Greunke, K., Cifuentes, L., Grunwald, T., Bredehorst, R., Ollert, M., Spillner, E., 2010. Dissecting cross-reactivity in hymenoptera venom allergy by circumvention of alpha-1,3-core fucosylation. *Mol. Immunol.* 47, 799–808.
- Soldatova, L.N., Cramer, R., Gmachl, M., Kemeny, D.M., Schmidt, M., Weber, M., Mueller, U.R., 1998. Superior biologic activity of the recombinant bee venom allergen hyaluronidase expressed in baculovirus-infected insect cells as compared with *Escherichia coli*. *J. Allergy Clin. Immunol.* 101, 691–698.
- Spillner, E., Blank, S., Jakob, T., 2014. Hymenoptera allergens: from venom to “venome”. *Front. Immunol.* 5, 77.
- Sturm, G.J., Varga, E.M., Roberts, G., Mosbech, H., Bilo, M.B., Akdis, C.A., Antolin-Amerigo, D., Cichocka-Jarosz, E., Gawlik, R., Jakob, T., Kosnik, M., Lange, J., Mingomataj, E., Mitsias, D.I., Ollert, M., Oude Elberink, J.N.G., Pfaar, O., Pitsios, C., Pravettoni, V., Rueff, F., Sin, B.A., Agache, I., Angier, E., Arasi, S., Calderon, M.A., Fernandez-Rivas, M., Halken, S., Jutel, M., Lau, S., Pajno, G.B., van Ree, R., Ryan, D., Spranger, O., van Wijk, R.G., Dhimi, S., Zaman, H., Sheikh, A., Muraro, A., 2018 Apr. EAACI guidelines on allergen immunotherapy: hymenoptera venom allergy. *Allergy* 73 (4), 744–764. <http://dx.doi.org/10.1111/all.13262>. Epub 2017 Dec 5.
- Tarsitano, M., De Falco, S., Colonna, V., McGhee, J.D., Persico, M.G., 2006. The *C. elegans* pvf-1 gene encodes a PDGF/VEGF-like factor able to bind mammalian VEGF receptors and to induce angiogenesis. *Faseb. J.* 20, 227–233.
- Tom Tang, Y., Hu, T., Arterburn, M., Boyle, B., Bright, J.M., Palencia, S., Emtage, P.C., Funk, W.D., 2005. The complete complement of C1q-domain-containing proteins in *Homo sapiens*. *Genomics* 86, 100–111.
- Tunget, C.L., Clark, R.F., 1993. Invasion of the ‘killer’ bees. Separating fact from fiction. *Postgrad. Med.* 94, 92–94 97–98, 101–102.
- Van Vaerenbergh, M., Cardoen, D., Formesyn, E.M., Brunain, M., Van Driessche, G., Blank, S., Spillner, E., Verleyen, P., Wenseleers, T., Schoofs, L., Devreese, B., de Graaf, D.C., 2013. Extending the honey bee venome with the antimicrobial peptide apidaecin and a protein resembling wasp antigen 5. *Insect Mol. Biol.* 22, 199–210.
- Van Vaerenbergh, M., Debyser, G., Devreese, B., de Graaf, D.C., 2014. Exploring the hidden honeybee (*Apis mellifera*) venom proteome by integrating a combinatorial peptide ligand library approach with FTMS. *J. Proteom.* 99, 169–178.

Figure S4: Gating strategy used in the basophil activation test. Basophilic cells were identified as CCR3^{pos} and SSC^{low}. Further, the percentage of activated basophils was calculated by the identification of CD63^{pos} basophils in the population of total basophils.

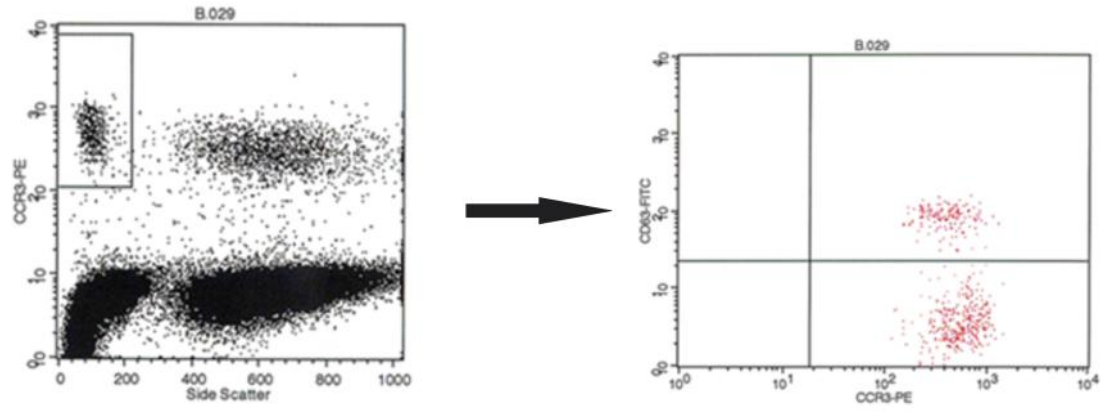


Figure S2: Identification of PVF1 variants.

A: Nucleotide sequences and GenBank accession numbers of sequenced mature PVF1 clones, generated by reverse transcription PCR on honeybee venom gland tissue.

>KF153240|PVF1_var1

```
CAACTCGAGGATACCAGATACCCGACCAAAGGATAGTGTTCCCCGATCGGGGGAGAGAAACGGCGAATCC
TGC GTTGGAGGGCGGGCCGAGTGGAGGAGGAATTGGCGAATTGGCGAAATCGATACAATTAGCCAAGAAAA
TCAGTTCCATTAATTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGCCGAAGGATATATCTTTCTTC
TCCTCGAGCAGTAGAATGGGAGAAACTGAACGTTCTGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCC
TGAATTGCAAAACAGTTCCTCTATTAGAAAATGAGCCATCTGTTATTTATTATCCAACCTTGACACGCGATCA
AAAGATGCGGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTCGAAATTC
GAGATTCTTGTGACAATTCTTGAATCCAGTGGTAAAGTTAAATATCAAGGCAAACGTATCGTACCGATAGA
GGAGCACACGCAATGCACGTGTGACTGCAAAATAAAGGAGACGGACTGTAACAAGAAACAGTCTTATGTAC
CAGAGGAATGTACATGTGCGTGCAACAACGTCGATGAACAGAAAAAGTGAATGAAAGCAACATAAAAAATG
TGGCACCCAGATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACTGGTTTTTATTTTCGACCA
AAATTCATGCAGATGCCTGCAAGTACCGCTATCTAGAACATGGTTTACATCCACAAAAGGTTCTGATTATA
GATTCGGACAAACACAAAGACCAGATAATGTACCACCGGTAATAATTGCCCTAGATTTCAGATGATCCTAGA
AGAAAACCTAAACCAGATCCAGAATAA
```

>KF153241|PVF1_var2

```
CAACTCGAGGATACCAGATACCCGACCGAAGGATAGTGTTCCCCGATCGGGGGAGAGAAACGGCGAATCC
TGC GTTGGAGGGCGGGCCGAGTGGAGGAGGAATTGGCGAATTGGCGAAATCGATACAATTAGCCAAGAAAA
TCAGTTCCATTAATTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGCCGAAGGATATATCTTTCTTC
TCCTCGAGCAGTAGAATGGGAGAAACTGAACGTTCTGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCC
TGAATTGCAAAACAGTTCCTCTATTAGAAAATGAGCCATCTGTTATTTATTATCCAACCTTGACACGCGATCA
AAAGATGCGGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTCGAAATTC
GAGATTCTTGTGACAATTCTTGAATCCAGTGGTAAATTTAAATATCAAGGCAAACGTATCGTACCGATAGA
GGAGCACACGCAATGCACGTGTGACTGCAAAATAAAGGAGACGGACTGTAACAAGAAACAGTCTTATGTAC
CAGAGGAATGTACATGTGCGTGCAACAACGTCGATGAACAGAGAAAGTGAATGAAAGCAACATAAAAAATG
TGGCACCCAGATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACTGGTTTTTATTTTCGACCA
AAATTCATATGCCTGCAAGTACCGCTATCTAGAACATGGTTTACATCCACAAAAGGTTCTGATTATAGATT
CGGACAAACACAAAGACCAGATAATGTACCACCGGTAATAATTGCCCTAGATTTCAGATGATCCTAGAAGAA
AACCTAAACCAGATCCAGAATAA
```

>KF153242|PVF1_var3

```
CAACTCGAGGATACCAGATACCCGACCAAAGGATAGTGTTCCCCGATCGGGGGAGAGAAACGGCGAATCC
TGC GTTGGAGGGCGGGCCGAGTGGAGGAGGAATTGGCGAATTGGCGAAATCGATACAATTAGCCAAGAAAA
TCAGTTCCATTAATTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGCCGAAGGATATATCTTTCTTC
TCCTCGAGCAGTAGAATGGGAGAAACTGAACGTTCTGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCC
TGAATTGCAAAACAGTTCCTCTATTAGAAAATGAGCCACCTGTTATTTATTATCCAACCTTGACACGCGATCA
AAAGATGCGGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTCGAAATTC
GAGATTCTTGTGACAATTCTTGAATCCAGTGGTAAATTTAAATATCAAGGCAAACGTATCGTACCGATAGA
GGAGCACACGCAATGCACGTGTGACTGCAAAATAAAGGAGACGGACTGTAACAAGAAACAGTCTTATGTAC
CAGAGGAATGTACATGTGCGTGCAACAACGTCGATGAACAGAAAAAGTGAATGAAAGCAACATAAAAAATG
TGGCACCCAGATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACTGGTTTTTATTTTCGACCA
AAATTCATGCAGGTGTGAACGGAACAACAAGGATTTGTAATACGGGAATAACATCTTTCCTTCTTTGCTGC
ATTTCTATTATCTTGTATATCTTATGCCTGCAAGTACCGCTATCTAGAACATGGTTTACATCCACAAAAGG
TTCTGATTATAGATTTCGGACAAACACAAAGACCAGATAATGTACCACCGGTAATAATTGCCCTAGATTTCAG
ATGACCCTAGAAGAAAACCTAAACCAGATCCAGAATAA
```

B: Sequence alignment of nucleotide sequences of the PVF1 variants. Nucleotide substitutions are indicated in red.

```

variant3      CAACTCGAGGATACCAGATACCCCGACCAAAGGATAGTGTTCCCGATCGGGGGAGAGAA 60
variant1      CAACTCGAGGATACCAGATACCCCGACCAAAGGATAGTGTTCCCGATCGGGGGAGAGAA 60
variant2      CAACTCGAGGATACCAGATACCCCGACCGAAGGATAGTGTTCCCGATCGGGGGAGAGAA 60
*****

variant3      ACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGTGGAGGAGGAATTGGCGAATTGGCGAAA 120
variant1      ACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGTGGAGGAGGAATTGGCGAATTGGCGAAA 120
variant2      ACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGTGGAGGAGGAATTGGCGAATTGGCGAAA 120
*****

variant3      TCGATACAATTAGCCAAGAAAATCAGTTCCATTAATTTCGAGAGACGATTTTCTTAAATTA 180
variant1      TCGATACAATTAGCCAAGAAAATCAGTTCCATTAATTTCGAGAGACGATTTTCTTAAATTA 180
variant2      TCGATACAATTAGCCAAGAAAATCAGTTCCATTAATTTCGAGAGACGATTTTCTTAAATTA 180
*****

variant3      GTCAAAGATGTGCCGAAGGATATATCTTTCTTCTCCTCGAGCAGTAGAATGGGAGAAACT 240
variant1      GTCAAAGATGTGCCGAAGGATATATCTTTCTTCTCCTCGAGCAGTAGAATGGGAGAAACT 240
variant2      GTCAAAGATGTGCCGAAGGATATATCTTTCTTCTCCTCGAGCAGTAGAATGGGAGAAACT 240
*****

variant3      GAACGTTTCGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCAAACAGTT 300
variant1      GAACGTTTCGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCAAACAGTT 300
variant2      GAACGTTTCGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCAAACAGTT 300
*****

variant3      CCTCTATTAGAAAATGAGCCACTGTTATTTATTATCCAATTGCACACGGATCAAAAAGA 360
variant1      CCTCTATTAGAAAATGAGCCATCTGTTATTTATTATCCAATTGCACACGGATCAAAAAGA 360
variant2      CCTCTATTAGAAAATGAGCCATCTGTTATTTATTATCCAATTGCACACGGATCAAAAAGA 360
*****

variant3      TGC GGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTCGA 420
variant1      TGC GGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTCGA 420
variant2      TGC GGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTCGA 420
*****

variant3      AATTTTCGAGATTCTTGTGACAATTCTTGAATCCAGTGGTAAATTAAAATATCAAGGCCAAA 480
variant1      AATTTTCGAGATTCTTGTGACAATTCTTGAATCCAGTGGTAAGTTAAAATATCAAGGCCAAA 480
variant2      AATTTTCGAGATTCTTGTGACAATTCTTGAATCCAGTGGTAAATTAAAATATCAAGGCCAAA 480
*****

variant3      CGTATCGTACCGATAGAGGAGCACACGCAATGCACGTGTGACTGCAAAAATAAAGGAGACG 540
variant1      CGTATCGTACCGATAGAGGAGCACACGCAATGCACGTGTGACTGCAAAAATAAAGGAGACG 540
variant2      CGTATCGTACCGATAGAGGAGCACACGCAATGCACGTGTGACTGCAAAAATAAAGGAGACG 540
*****

variant3      GACTGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGTGCAACAACGTC 600
variant1      GACTGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGTGCAACAACGTC 600
variant2      GACTGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGTGCAACAACGTC 600
*****

variant3      GATGAACAGAAAAAGTGTAATGAAAGCAACATAAAAAATGTGGCACCCAGATCTTTGTAGC 660
variant1      GATGAACAGAAAAAGTGTAATGAAAGCAACATAAAAAATGTGGCACCCAGATCTTTGTAGC 660
variant2      GATGAACAGAGAAAGTGTAATGAAAGCAACATAAAAAATGTGGCACCCAGATCTTTGTAGC 660
*****

variant3      TGT TTTTTCAGAGAAAACACAGGAATGTTCAACTGGT TTTTATTTTCGACCAAAATTCATGC 720
variant1      TGT TTTTTCAGAGAAAACACAGGAATGTTCAACTGGT TTTTATTTTCGACCAAAATTCATGC 720
variant2      TGT TTTTTCAGAGAAAACACAGGAATGTTCAACTGGT TTTTATTTTCGACCAAAATTCATGC -- 718
*****

variant3      AGGTGTGAACGGAACAACAAGGATTGTGAATACGGGAATAACATCTTTCCTTCTTTGCTG 780
variant1      AG----- 722
variant2      -----

```

variant3	CATTTCTATTATCTTGTATATCTTATGCCTGCAAGTACCGCTATCTAGAACATGGTTTAC	840
variant1	-----ATGCCTGCAAGTACCGCTATCTAGAACATGGTTTAC	758
variant2	-----ATGCCTGCAAGTACCGCTATCTAGAACATGGTTTAC	754

variant3	ATCCACAAAAGGTTCTGATTATAGATTCTGGACAAACACAAAGACCAGATAATGTACCACC	900
variant1	ATCCACAAAAGGTTCTGATTATAGATTCTGGACAAACACAAAGACCAGATAATGTACCACC	818
variant2	ATCCACAAAAGGTTCTGATTATAGATTCTGGACAAACACAAAGACCAGATAATGTACCACC	814

variant3	GGTAATAATTGCCCTAGATTCAGATGACCTAGAAGAAAACCTAAACCAGATCCAGAATA	960
variant1	GGTAATAATTGCCCTAGATTCAGATGATCCTAGAAGAAAACCTAAACCAGATCCAGAATA	878
variant2	GGTAATAATTGCCCTAGATTCAGATGATCCTAGAAGAAAACCTAAACCAGATCCAGAATA	874

variant3	A	961
variant1	A	879
variant2	A	875
	*	

C: The obtained nucleotide sequence of mature variant 1 matched with the GenBank record XM_392204.4, representing the predicted honeybee PVF1 mRNA sequence. Only one nucleotide substitution was found between the cloned fragment and the predicted NCBI sequence (indicated in red).

gi 328789530 ref XM_392204.4 variant1	GGCAGTCGATCGCGGCGCGAGCGCAGTCGAACCTGGTCAGGTGCGACTTT 50 -----
gi 328789530 ref XM_392204.4 variant1	CCTTCCGTTTTCTCGTGCATCGAGTGCGAAACACGCGAAACACGTCGGG 100 -----
gi 328789530 ref XM_392204.4 variant1	TGGATAAACACGATCGTGTGCAAACACGATCACC GTTTCCCGAATTTA 150 -----
gi 328789530 ref XM_392204.4 variant1	CCGCCGCAATATTGGATCGCCACGAGTTGCTGGCCGATCATCTGGGCCAC 200 -----
gi 328789530 ref XM_392204.4 variant1	GTGTTACCGTGTTTTTCAGAGAAGAGTGGCAACACGGTGAATATTTTATGA 250 -----
gi 328789530 ref XM_392204.4 variant1	CCGGAGGATAGGATAGGATAGAACTCGATTCCCTTTTCTTTTTTCGAGC 300 -----
gi 328789530 ref XM_392204.4 variant1	ACGGGTGGAGCACAGGTGAACAGCCAGACATGCCGTACTCTAAGTGTAGA 350 -----
gi 328789530 ref XM_392204.4 variant1	ACGTTTCTCCGTTTCTTCGCGATTTGTTGTTCTCGACTTGTGGACTGGT 400 -----
gi 328789530 ref XM_392204.4 variant1	GATGGCTCAACTCGAGGATACCAGATACCCCGACCAAAGGATAGTGTTC 450 -----CAACTCGAGGATACCAGATACCCCGACCAAAGGATAGTGTTC 43 *****
gi 328789530 ref XM_392204.4 variant1	CCGATCGGGGGAGAGAAACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGT 500 CCGATCGGGGGAGAGAAACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGT 93 *****
gi 328789530 ref XM_392204.4 variant1	GGAGGAGGAATTGGCGAATTGGCGAAATCGATACAATTAGCCAAGAAAAAT 550 GGAGGAGGAATTGGCGAATTGGCGAAATCGATACAATTAGCCAAGAAAAAT 143 *****
gi 328789530 ref XM_392204.4 variant1	CAGTTCCATTAATTTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGC 600 CAGTTCCATTAATTTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGC 193 *****
gi 328789530 ref XM_392204.4 variant1	CGAAGGATATATCTTTCTTCTCCTCGAGCAGTAGAATGGGAGAAACTGAA 650 CGAAGGATATATCTTTCTTCTCCTCGAGCAGTAGAATGGGAGAAACTGAA 243 *****
gi 328789530 ref XM_392204.4 variant1	CGTTCGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCA 700 CGTTCGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCA 293 *****
gi 328789530 ref XM_392204.4 variant1	AACAGTTCCTCTATTAGAAAAATGAGCCATCTGTTATTTATTATCCAACCT 750 AACAGTTCCTCTATTAGAAAAATGAGCCATCTGTTATTTATTATCCAACCT 343 *****

gi 328789530 ref XM_392204.4 variant1	GCACACGGATCAAAAGATGCGGAGGTTGTTGTACACACTCACTCCTCTCT 800 GCACACGGATCAAAAGATGCGGAGGTTGTTGTACACACTCACTCCTCTCT 393 *****
gi 328789530 ref XM_392204.4 variant1	TGTCAACCAACGGCTACGGAAATTCGAAATTTTCGAGATTCTTGTGACAAT 850 TGTCAACCAACGGCTACGGAAATTCGAAATTTTCGAGATTCTTGTGACAAT 443 *****
gi 328789530 ref XM_392204.4 variant1	TCTTGAATCCAGTGGTAAATTAAATATCAAGGCAAACGTATCGTACCGA 900 TCTTGAATCCAGTGGTAAATTAAATATCAAGGCAAACGTATCGTACCGA 493 *****
gi 328789530 ref XM_392204.4 variant1	TAGAGGAGCACACGCAATGCACGTGTGACTGCAAAATAAAGGAGACGGAC 950 TAGAGGAGCACACGCAATGCACGTGTGACTGCAAAATAAAGGAGACGGAC 543 *****
gi 328789530 ref XM_392204.4 variant1	TGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGTGCAA 1000 TGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGTGCAA 593 *****
gi 328789530 ref XM_392204.4 variant1	CAACGTCGATGAACAGAAAAAGTGAATGAAAGCAACATAAAAAATGTGGC 1050 CAACGTCGATGAACAGAAAAAGTGAATGAAAGCAACATAAAAAATGTGGC 643 *****
gi 328789530 ref XM_392204.4 variant1	ACCCAGATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACT 1100 ACCCAGATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACT 693 *****
gi 328789530 ref XM_392204.4 variant1	GGTTTTTATTTTCGACCAAAATTCATGCAGATGCCTGCAAGTACCGCTATC 1150 GGTTTTTATTTTCGACCAAAATTCATGCAGATGCCTGCAAGTACCGCTATC 743 *****
gi 328789530 ref XM_392204.4 variant1	TAGAACATGGTTTACATCCACAAAAGGTTCTGATTATAGATTTCGGACAAA 1200 TAGAACATGGTTTACATCCACAAAAGGTTCTGATTATAGATTTCGGACAAA 793 *****
gi 328789530 ref XM_392204.4 variant1	CACAAAGACCAGATAATGTACCACCGGTAATAATTGCCCTAGATTCAGAT 1250 CACAAAGACCAGATAATGTACCACCGGTAATAATTGCCCTAGATTCAGAT 843 *****
gi 328789530 ref XM_392204.4 variant1	GATCCTAGAAGAAAACCTAAACCAGATCCAGAATAATCAGATTGGTCATT 1300 GATCCTAGAAGAAAACCTAAACCAGATCCAGAATA----- 879 *****
gi 328789530 ref XM_392204.4 variant1	TATAGGCTAAATTTTAAACAATTTAATTTCTTATCTTTGTGCCATTTTG 1350 -----
gi 328789530 ref XM_392204.4 variant1	TTATACATAAAAGTATTGATATTATTTATGTATTAATATTAATATTAATA 1400 -----
gi 328789530 ref XM_392204.4 variant1	TTAGTTATTGCAAAAAATAAATGAATGTATTTTATATATCAAAAAATAT 1450 -----
gi 328789530 ref XM_392204.4 variant1	TGTAATATAAAGAAATACAATTATTTAATATAAATTATTTGTATATCATT 1500 -----
gi 328789530 ref XM_392204.4 variant1	TTTTAATCTTCATTTATAAAAAATATTTTATTAATTTTCTTTTCATTTAA 1550 -----
gi 328789530 ref XM_392204.4 variant1	AAAACATGTACAATTCTTAATCTTATTCTATGTTTTTTTAAATTATTTTG 1600 -----

gi|328789530|ref|XM_392204.4|
variant1

TACCTTTTGTTAATATAATGTATAAAAAAATATAAATACATTAAATATA 1650

gi|328789530|ref|XM_392204.4|
variant1

CAATG 1655

D: Amino acid sequence alignment of the obtained mature PVF1 variant 1 sequence with the GenBank honeybee PVF1 sequence (XP_392204.2).

```
variant1      -----QLEDTRYPDQRIVFPDRGRETANP 24
gi|328789531|ref|XP_392204.2|  MPYSKCRTFLRFFAICSFSTCGLVMAQLEDTRYPDQRIVFPDRGRETANP 50
                                *****

variant1      ALEGGPSGGGIGELAKSIQLAKKISSINSRDDFLKLVKDVPKDISFFSSS 74
gi|328789531|ref|XP_392204.2|  ALEGGPSGGGIGELAKSIQLAKKISSINSRDDFLKLVKDVPKDISFFSSS 100
                                *****

variant1      SRMGETERSNAERPQNQALCMPELQTVPLLENEPSVIYYPTCTRIKRCGGC 124
gi|328789531|ref|XP_392204.2|  SRMGETERSNAERPQNQALCMPELQTVPLLENEPSVIYYPTCTRIKRCGGC 150
                                *****

variant1      CTHSLLSCQPTATEIRNFEILVTILESSGKLKYQGKRIVPIEHTQCTCD 174
gi|328789531|ref|XP_392204.2|  CTHSLLSCQPTATEIRNFEILVTILESSGKLKYQGKRIVPIEHTQCTCD 200
                                *****

variant1      CKIKETDCNKKQSYVPEECTCACNNVDEQKKCNESNIKMWHPDLCSCFCR 224
gi|328789531|ref|XP_392204.2|  CKIKETDCNKKQSYVPEECTCACNNVDEQKKCNESNIKMWHPDLCSCFCR 250
                                *****

variant1      ETQECSTGFYFDQNSCRCLQVPLSRTWFTSTKGSDYRFGQTQRPDNPVPV 274
gi|328789531|ref|XP_392204.2|  ETQECSTGFYFDQNSCRCLQVPLSRTWFTSTKGSDYRFGQTQRPDNPVPV 300
                                *****

variant1      IIALDSDDPRRKPKPDPE 292
gi|328789531|ref|XP_392204.2|  IIALDSDDPRRKPKPDPE 318
                                *****
```

E: Translated amino acid sequences of the mature PVF1 variants.

>PVF1 variant1

QLEDTRYPDQRIVFPDRGRETANPALEGGPSGGGIGELAKSIQLAKKISSINSRDDFLKLVKDVPKDISFF
SSSSRMGETERSNAERPQNQALCMPELQTVPLENEPSVIYYPTCTRIKRCGGCCTHSLSCQPTATEIRNF
EILVTILESSGKLKYQGKRIVPIEEHTQCTCDCKIKETDCNKKQSYVPEECTCACNNVDEQKKCNESNIKM
WHPDLCSCFCRETQECSTGFYFDQNSCRCLQVPLSRTWFTSTKGSDYRFGQTQRPDNPVPPVIALDSDDPR
RKPKPDPE

>PVF1 variant2

QLEDTRYPDRRIVFPDRGRETANPALEGGPSGGGIGELAKSIQLAKKISSINSRDDFLKLVKDVPKDISFF
SSSSRMGETERSNAERPQNQALCMPELQTVPLENEPSVIYYPTCTRIKRCGGCCTHSLSCQPTATEIRNF
EILVTILESSGKLKYQGKRIVPIEEHTQCTCDCKIKETDCNKKQSYVPEECTCACNNVDEQQRKNESNIKM
WHPDLCSCFCRETQECSTGFYFDQNSYACKYRYLEHGLHPQKVLIIIDSKHKDQIMYHR

>PVF1 variant3

QLEDTRYPDQRIVFPDRGRETANPALEGGPSGGGIGELAKSIQLAKKISSINSRDDFLKLVKDVPKDISFFSSSS
RMGETERSNAERPQNQALCMPELQTVPLENEPPVIYYPTCTRIKRCGGCCTHSLSCQPTATEIRNFEILVTILE
SSGKLKYQGKRIVPIEEHTQCTCDCKIKETDCNKKQSYVPEECTCACNNVDEQKKCNESNIKMWHPDLCSCFCRE
TQECSTGFYFDQNSCRERNKDL

Figure S3: Alternative splicing of the *Pvf1* transcript. Alignment of the detected *Pvf1* transcript variants with the *Pvf1* gene (GeneID: 408666) on chromosome LG2. Intron 5'GT donor and 3'AG acceptor splice sites conform to the general canonical GT-AG splicing rule are presented in purple. The three alternative 5'donor sites present within exon 5 are shown in green. Alternative stop codons are indicated in red.

var3	-----	
var1	-----	
var2	-----	
genome	ATGCCGTACTCTAAGTGTAGAACGTTTCTCCGTTTCTTCGCGATTTGTTTCGTTCTCGACT	60
var3	-----CAACTCGAGGATACCAGATACCCCGACCGAAGGATAGTGTTTC	42
var1	-----CAACTCGAGGATACCAGATACCCCGACCAAAGGATAGTGTTTC	42
var2	-----CAACTCGAGGATACCAGATACCCCGACCAAAGGATAGTGTTTC	42
genome	TGTGGACTGGTGATGGCTCAACTCGAGGATACCAGATACCCCGACCAAAGGATAGTGTTTC	120

var3	CCCGATCGGGGGAGAGAAACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGTGGAGGAGGA	102
var1	CCCGATCGGGGGAGAGAAACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGTGGAGGAGGA	102
var2	CCCGATCGGGGGAGAGAAACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGTGGAGGAGGA	102
genome	CCCGATCGGGGGAGAGAAACGGCGAATCCTGCGTTGGAGGGCGGGCCGAGTGGAGGAGGA	180

var3	ATTGGCGAATTGGCG-----	117
var1	ATTGGCGAATTGGCG-----	117
var2	ATTGGCGAATTGGCG-----	117
genome	ATTGGCGAATTGGCGGTAAATCGATCGCATCATTTTCATCCGAACGCGCGTATTATTTCG	240

var3	-----	
var1	-----	
var2	-----	
genome	CGAACGGAGCGGGAACAGTCGTGGTACACAGTCGAGGAACAGCGTTGCAACACTCCCACG	300
var3	-----	
var1	-----	
var2	-----	
genome	TTTTCACTCTGATAAATAGCGCAATTATGTTATCCCGCGTAGCATTGTTAAGGAGAGCGT	360
var3	-----	
var1	-----	
var2	-----	
genome	ACGTGACGGACAAGGCAGCGGTAAAAATTTGAGGAAGGAAAAATTGATCCGATAGTCTCT	420
var3	-----	
var1	-----	
var2	-----	
genome	GAAAAAAGGAAGAAAAAGATATCTTTATAACTTTGGCATCGCGTGTTTGTATTTTTTAT	480
var3	-----	
var1	-----	
var2	-----	
genome	TTTTATTTTATCTTTATTTTTTTCTTTCCATTTTTTTTTTTTTTTTACAACATACCG	540
var3	-----	
var1	-----	
var2	-----	

genome	TTATTCAACGATTATTATTTTCGCTCGCTCGTTTCGTGTAAAATTTAAAATTCTGGCGATA	600
var3	-----	
var1	-----	
var2	-----	
genome	ACGATTTCACGAATATTTTGTGCGGAATAAAAAAGAAAAATAGCGGCGAATTACGCG	660
var3	-----	
var1	-----	
var2	-----	
genome	ATAAATTGTGCGGAAATCCTTCTCGATTTTGTGTGTCACCGTAATAGCGGTGGCCCCCTTT	720
var3	-----	
var1	-----	
var2	-----	
genome	TTTTTTTTCTATTATCATCGTCGGTGTTAACGTAAGCGATAAACGTGGAAACGTGGCGGC	780
var3	-----	
var1	-----	
var2	-----	
genome	GATTCCTTTTGTGACGACAGATGACGATTGTTATTACGAAATCCTGCGCGAGAACGGGA	840
var3	-----	
var1	-----	
var2	-----	
genome	GTATCGAGATCGTTTTTTCCCGCAAAAGATACGAAGGTCGTGTATATACCACTAATGTC	900
var3	-----	
var1	-----	
var2	-----	
genome	AAGGATGTATTGGAAGCGGCAGGAGAGATCGAGACAGAAAAGTGAAAACCTCGCCGTGCA	960
var3	-----	
var1	-----	
var2	-----	
genome	CGACACACTTAGAATTCTTGTGCAATACCTTGTGATTGTTATGTAGATAATTGATTAC	1020
var3	-----	
var1	-----	
var2	-----	
genome	CAACAACCGTCCGTGCGAATACCTACTCGATTAGTTCGCGAATTTTCTCTTTTTTTCGT	1080
var3	-----	
var1	-----	
var2	-----	
genome	AATTTTATCGAAACGATTTATCGATTTTATCAACTCGTGTATGAATACGAATTGAAC	1140
var3	-----	
var1	-----	
var2	-----	
genome	GATAGGTATTTATTTATTTATTTATACGTTCTACTCGACGAGAAGAAGAAAAGAGAAATT	1200
var3	-----	
var1	-----	
var2	-----	
genome	CAGGTTAGGAAAACGATTAGGAAATAATGCGGCGCGCGCGCGCAGCGTTATCGTTTGCA	1260

var3 -----
var1 -----
var2 -----
genome GCGGAGAACCATCCTTTTCGGCGCGATCGAGGATGGATAAACATTATTTTTTCGCGAAGAT 1320

var3 -----
var1 -----
var2 -----
genome AATATTTGTTTGGTCACGATCATTGTGTGCGCGCAATATTTTCATTGTCGCAATAGGGTTA 1380

var3 -----
var1 -----
var2 -----
genome TACGGTTGGAGCGCGAAGGCAGCGCAAGACGGACGACCTTACAATGCCGGCGATTAAATT 1440

var3 -----
var1 -----
var2 -----
genome CTTGGGAAGCGTCGAGAGGACGTTCTTGAAAGTACAATGTCTCGGGACATCGCGTTTCT 1500

var3 -----
var1 -----
var2 -----
genome CATTTATGCAAATTGTGCAACGTGCGTAATACGTTTATTTAAATGCTCGCCGTACCGTTA 1560

var3 -----
var1 -----
var2 -----
genome TCGATCCGAGTCAGCGCTCGTTCTCGCAAGTATTTGACTGGCAAAATGATGAAGAACT 1620

var3 -----
var1 -----
var2 -----
genome CATGGTCGATACGGAACGGATAAATATCGCTCGAGATTATCATCGATGAAATCTATCGCG 1680

var3 -----
var1 -----
var2 -----
genome TCTGGCCACGTCACCGCCGCTCGGAACAACCGTTTCGAAAGGGAAAACGCCTCGATCGAGA 1740

var3 -----
var1 -----
var2 -----
genome ACAGCTCGCGTTAACACCGAGGATGCTTCTCCTAATCGATTGCGGCTTTGCTGATTTTTT 1800

var3 -----
var1 -----
var2 -----
genome TTCAACGTGCACGCGATACCGGCTTGTTTAAACCTGTTCCAGATTGGAATGGTGGACGC 1860

var3 -----
var1 -----
var2 -----
genome GCGCGAGGTGAAGAACGCATCGAGTTATACACCTTCGTGCTCAACGTTTCGGCTTTAA 1920

var3 -----

var1	-----	
var2	-----	
genome	AATATTTTTTAAACGATATTTTAATACACTCGTGAAGATATCGATTTTTTCGACGAGTTA	1980
var3	-----	
var1	-----	
var2	-----	
genome	AGATCCTGCGATTATTGCCTCATTACGAGAGTGTCCGCGATCGATGATTCATTGATAAG	2040
var3	-----	
var1	-----	
var2	-----	
genome	ATATTGTATCGGAAATATCCCGATAATTACATCTAAAATTATTCTATTCAATTTTTTTTT	2100
var3	-----	
var1	-----	
var2	-----	
genome	TTTTTCGTTTCAAATTATCGTGTTTTCTTACTAATTAATTCGATTGATAAAGCGATTGAT	2160
var3	-----	
var1	-----	
var2	-----	
genome	AACGTCATAAATAAAAAGATCTTGACGTATATTTCTTCTAGAATAATGCGTCGTGTTATT	2220
var3	-----	
var1	-----	
var2	-----	
genome	ATTCAATTAGTTTTTTTCTTTCATTTTCGCATCCCTTGATATGCATGACAATTGAGTTGGA	2280
var3	-----	
var1	-----	
var2	-----	
genome	AATTCCAACCTCGAAAAAAAAAAGAAATGAAACAAGAACCGGTTAATATGTTTTTTTTTT	2340
var3	-----	
var1	-----	
var2	-----	
genome	TTAAAGTTGTTTCCGTTTAAACAATCACGTAGATATACAATAACAAATACCATACCTGCAG	2400
var3	-----	
var1	-----	
var2	-----	
genome	AAATGATAATTTTTTCTTTTTTCTTTTATGGAAACATTTTATTATCGATCATTTTAT	2460
var3	-----	
var1	-----	
var2	-----	
genome	TATTGGATTATTATCAATCGAACTATCGATTAATCGAGATAACGATGAAAACCAGATAA	2520
var3	-----	
var1	-----	
var2	-----	
genome	TTTTGCGACACGGAGGAAAAAATCGAAAAAATTTTCCATCCGATTCCATCCTTTATTA	2580
var3	-----	
var1	-----	
var2	-----	
genome	----- AAATCGATACAATTAGCCAAGAAAAATCAGTTCCATTA	154
	----- AAATCGATACAATTAGCCAAGAAAAATCAGTTCCATTA	154
	----- AAATCGATACAATTAGCCAAGAAAAATCAGTTCCATTA	154

genome	TCCAAAATTCGTGTTATTTTCAGAAATCGATACAATTAGCCAAGAAAATCAGTTCCATTA	2640

var3	ATTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGCCGAAGGATATATCTTTCTTCT	214
var1	ATTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGCCGAAGGATATATCTTTCTTCT	214
var2	ATTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGCCGAAGGATATATCTTTCTTCT	214
genome	ATTCGAGAGACGATTTTCTTAAATTAGTCAAAGATGTGCCGAAGGATATATCTTTCTTCT	2700

var3	CCTCGAGCA-----	223
var1	CCTCGAGCA-----	223
var2	CCTCGAGCA-----	223
genome	CCTCGAGCACTAAGTATGATCTTAAATAACTTGTTAACAGGAATTATGGATCTATAGAAA	2760

var3	-----	
var1	-----	
var2	-----	
genome	GGAATAGTACGTAAGAAAAACCGGTTGACTCGTGACATTGCGAAAAACAAATATATTGTT	2820
var3	-----	
var1	-----	
var2	-----	
genome	ACACGACCTTGTAATTGCACTTTATTCGGATTATTAAGAAAAACAAAATTACAAAAT	2880
var3	-----	
var1	-----	
var2	-----	
genome	TGCGTTTGAAAAAAAAAACGAAAAAGAACGCCGTGAAGCATCACGGTAAATAACTAC	2940
var3	-----	
var1	-----	
var2	-----	
genome	AGTTGACTTCGTAAATCTTTGAATATTTGAAGAACAAATTATTATGACTATTAATCAAT	3000
var3	-----	
var1	-----	
var2	-----	
genome	TGTAATAGATGCAAAATATTGTTGCGTTAGATAAACATCTTAAAGTTTGTAATATCTTCTA	3060
var3	-----	
var1	-----	
var2	-----	
genome	CCAATTTTATCGTTCAAACCTTGATTATCAGTGAACGTGAGATCATTCAATTGATCGCAGC	3120
var3	-----	
var1	-----	
var2	-----	
genome	ATAAAAAGATATACTTAATATAAGTATTTCAATTCAAATTTAAATCTGCGAATAACTTGT	3180
var3	-----	
var1	-----	
var2	-----	
genome	AAATTGCAAAAATTTAACTCGAAAAAGTTTTTTAGTAAAAAGAAATTGAAAAAGAAA	3240
var3	-----	
var1	-----	
var2	-----	
genome	AAAAAGAACACGATTTTCGCATGAATTTCTAGAAAAATGATATGATAATTTCTTCTCG	3300

var3 -----
var1 -----
var2 -----
genome CTTAGAATTTCTCTATTATTACTTTTTCAAAAATTTTACTTTTTTAAATTCTTGCAAAGC 3360

var3 -----
var1 -----
var2 -----
genome TCGTTCTCGCTATCCTCGTATATTATCAGATGAATCTGATAATTATATAGAAACATTTTC 3420

var3 -----
var1 -----
var2 -----
genome TTTTTCTTATCGTTACAATGACTCGCGTTGACCTCTGGTTTGTCAAACATCAGTTTTTTC 3480

var3 -----
var1 -----
var2 -----
genome TCCACGTAATAAGGATCGAATAACTTACAGGGTTCTTTTTCCAAACTTTTTATGAAATCT 3540

var3 -----
var1 -----
var2 -----
genome TTGAGATCTTGAAGAAATCGGTGTTCTTCAAAAACCACCGAAATTGTCCGTATTCGGC 3600

var3 -----
var1 -----
var2 -----
genome ACTAGCCTATCGCTAATTTGATTGATTTCTCCAAGGTGGGCAATCTGATCGTGGAGTGT 3660

var3 -----
var1 -----
var2 -----
genome CCAAATCGCACCTTATCTTTTTCCGTATTTTTTTTATTTCGATGATAATTCAGAGTTGGA 3720

var3 -----
var1 -----
var2 -----
genome TGTTCGGTGCTGTACGCGATTTGTAACCTACACGGCCGTCTCCAAGTGTGCACACTAGAC 3780

var3 -----
var1 -----
var2 -----
genome ATAGGCACGGAATACGCATTGCAAATATCTATGGTACGATTAAACAAAATATCTGTTATG 3840

var3 -----
var1 -----
var2 -----
genome TTCATTGTGCTGTTCAATCTAGTTATTCCTAATGAAAAAATTAATTCGAATCATACAA 3900

var3 -----
var1 -----
var2 -----
genome AAGATTCCGAAGATTTGAAAAAAAAAAGATTGAAAGAAGTGATTTCTTGCCCTCGTTT 3960

var3 -----

var1	-----	
var2	-----	
genome	CCAATATCTCCGCGAGAATTCCATTTGCGCCGTAAGTCTCTATGTATTTAGGTGTGCTCAA	4020
var3	-----	
var1	-----	
var2	-----	
genome	TCCAACGACAACGTTTTTCATCTCCTGGACCATTCTGTCTCTTGATAGCTTTGATGGTTGG	4080
var3	-----	
var1	-----	
var2	-----	
genome	TGCCATTTCTCTTCCAATTTTATCCTATTAATGCTCTCCTCGATCTCTTCTGTGCTGGA	4140
var3	-----	
var1	-----	
var2	-----	
genome	CGTCGAGATCTCAAGCTTAAAAAGATTGAAAAGATCACCTCGCTCAAATCGTCATCGAA	4200
var3	-----	
var1	-----	
var2	-----	
genome	AGACGCGTCATTTCATCGCGCTGATTTCTTTGCATCGATTTTCATTTTGATTAACATTGGT	4260
var3	-----	
var1	-----	
var2	-----	
genome	TACGAAGTCACAATTCGATGACCGAGTTTCCACTTCTCGATGTAACGACTCGTCGTCCT	4320
var3	-----	
var1	-----	
var2	-----	
genome	CGCCTCCTTATTATTATCGACGAAATCTTTTTTTACAAATTCCTGATGATTTTGACGACA	4380
var3	-----	
var1	-----	
var2	-----	
genome	ATACGATCGAAACTTGAAAAGTCTTGAACGTGAAAATGTAAGGTAAACATTCTTTTTCC	4440
var3	-----	
var1	-----	
var2	-----	
genome	TCCACGAAATTATTCTATAACCTCATTTTATATTATATACCTTACCGTTTCAATTTTTTC	4500
var3	-----	
var1	-----	
var2	-----	
genome	TTTTTGTAATTGATGTTAGTTGTCGGCACGATGTAGGAGAATATATGCCTCGAATCGTGA	4560
var3	-----	
var1	-----	
var2	-----	
genome	GATTCCATAAAGGCAGACACGTTTTTCTACCTTCGACTATCCATCCAAACCAATCGAGA	4620
var3	-----	
var1	-----	
var2	-----	

genome	ATCACATCCTCCGAAAGATAATTGTGTATACCGTCGGCGAGACCATCGGCCAAAGCGAAG	4680
var3	-----	
var1	-----	
var2	-----	
genome	TAACTATTCGCAACTGAAATTGCTTGTACAAAATTTTCCATTGTTCTTAATTATCTGAAA	4740
var3	-----	
var1	-----	
var2	-----	
genome	AAGTAGGATAAAATTTATGGCAAAATGTTTCCAAGAAATGATGTTTCTTACGTCAAACCAC	4800
var3	-----	
var1	-----	
var2	-----	
genome	AAAAACCACATTGAACTAGACATAACTAGACATGGGAATTCGAGGTAAAGAAAAGGGAAA	4860
var3	-----GTAGAATGGGAGAAA	238
var1	-----GTAGAATGGGAGAAA	238
var2	-----GTAGAATGGGAGAAA	238
genome	TCCTCTGAGTAATTCAAATTGTCACTGAACGGGTTCTTCTTTCAGGTAGAATGGGAGAAA	4920

var3	CTGAACGTTTGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCAAACAG	298
var1	CTGAACGTTTGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCAAACAG	298
var2	CTGAACGTTTGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCAAACAG	298
genome	CTGAACGTTTGAATGCCGAGCGACCAAATCAAGCTTTGTGTATGCCTGAATTGCAAACAG	4980

var3	TTCCTCTATTAGAAAATGAGCCATCTGTTATTTATTATCCAACCTTGACACGGATCAAAA	358
var1	TTCCTCTATTAGAAAATGAGCCATCTGTTATTTATTATCCAACCTTGACACGGATCAAAA	358
var2	TTCCTCTATTAGAAAATGAGCCACCTGTTATTTATTATCCAACCTTGACACGGATCAAAA	358
genome	TTCCTCTATTAGAAAATGAGCCATCTGTTATTTATTATCCAACCTTGACACGGATCAAAA	5040

var3	GATGCGGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTC	418
var1	GATGCGGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTC	418
var2	GATGCGGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTC	418
genome	GATGCGGAGGTTGTTGTACACACTCACTCCTCTCTTGTCAACCAACGGCTACGGAAATTC	5100

var3	GAAATTTTCGAG-----	429
var1	GAAATTTTCGAG-----	429
var2	GAAATTTTCGAG-----	429
genome	GAAATTTTCGAGGTAAAGTCACATTTGATTGAATGTTATCGATAAATATATATATGTATG	5160

var3	-----ATTCTTGTGACAATTCTTGAAT	451
var1	-----ATTCTTGTGACAATTCTTGAAT	451
var2	-----ATTCTTGTGACAATTCTTGAAT	451
genome	TTATTCCAATTAATCACGTTATTAATTAATTTAAAGATTCTTGTGACAATTCTTGAAT	5220

var3	CCAGTGGTAAATTAAATATCAAGGCAAACGTATCGTACCGATAGAGGAGCACACGCAAT	511
var1	CCAGTGGTAAATTAAATATCAAGGCAAACGTATCGTACCGATAGAGGAGCACACGCAAT	511
var2	CCAGTGGTAAATTAAATATCAAGGCAAACGTATCGTACCGATAGAGGAGCACACGCAAT	511
genome	CCAGTGGTAAATTAAATATCAAGGCAAACGTATCGTACCGATAGAGGAGCACACGCAAT	5280

var3	GCACGTGTGACTGCAAAATAAAGGAGACG-----	540
var1	GCACGTGTGACTGCAAAATAAAGGAGACG-----	540
var2	GCACGTGTGACTGCAAAATAAAGGAGACG-----	540
genome	GCACGTGTGACTGCAAAATAAAGGAGACGTTAATTTGAGAACAGTCGATAATTGAGATCT	5340

var3 -----
var1 -----
var2 -----
genome TCCTTTTAAACTATATAAGTATAAGTATAAGTCGGTTCGCAATCTGCAAATCTTAATGT 5400

var3 -----
var1 -----
var2 -----
genome ATAATTTGCGATGAATTAGCTGCAATGAATTTTATTTTCGATAATTTAATTAGTTTAAAT 5460

var3 -----
var1 -----
var2 -----
genome CGGAAGATTTGAATTTTCAATTTTCAAAATGATTAAATTTTAAATAATGTAACGTGTGA 5520

var3 -----GACTGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGT 589
var1 -----GACTGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGT 589
var2 -----GACTGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGT 589
genome TATTTTTCAGGACTGTAACAAGAAACAGTCTTATGTACCAGAGGAATGTACATGTGCGT 5580

var3 GCAACAACGTCGATGAACAGAGAAAGTGTAAATGAAAGCAACATAAAAAATGTGGCACCCAG 649
var1 GCAACAACGTCGATGAACAGAAAAAGTGTAAATGAAAGCAACATAAAAAATGTGGCACCCAG 649
var2 GCAACAACGTCGATGAACAGAAAAAGTGTAAATGAAAGCAACATAAAAAATGTGGCACCCAG 649
genome GCAACAACGTCGATGAACAGAAAAAGTGTAAATGAAAGCAACATAAAAAATGTGGCACCCAG 5640

var3 ATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACTGGTTTTTATTTTCGACC 709
var1 ATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACTGGTTTTTATTTTCGACC 709
var2 ATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACTGGTTTTTATTTTCGACC 709
genome ATCTTTGTAGCTGTTTTTGCAGAGAAACACAGGAATGTTCAACTGGTTTTTATTTTCGACC 5700

var3 AAAATTCAT- ----- 718
var1 AAAATTCATGCAG- ----- 722
var2 AAAATTCATGCAGGTGTGAACGGAACAACAAGGATTTGTAAATACGGGAATAACATCTTTC 769
genome AAAATTCATGCAGGTGTGAACGGAACAACAAGGATTTGTAAATACGGGAATAACATCTTTC 5760

var3 -----
var1 -----
var2 CTTCTTTGCTGCATTTCTATTATCTTGTATATCTT- ----- 804
genome CTTCTTTGCTGCATTTCTATTATCTTGTATATCTTGTAAAGCTGTGCATGATGCATCCAAT 5820

var3 -----
var1 -----
var2 -----
genome CTTTTTTTTTGTTTTTTATTTTAATATTTTACCTTTTTTTTTCTTTTTCTTAATATT 5880

var3 -----
var1 -----
var2 -----
genome TTATTTTTCCGCTTATATCGCATAAATTAACATAGCTTATTTGCTTTATATTCATTATTG 5940

var3 -----
var1 -----
var2 -----
genome CATTAAATCAAATTTTCTTCATTAACATTTTTCTTAAATGCTTTACTTACTTTTATATT 6000

var3 -----

var1 -----
var2 -----
genome TTATTCGACTGATATAATTAAAAACAGTTAAAATAATGATACTACTTTCTCATACATGAAT 6060

var3 -----
var1 -----
var2 -----
genome TCATGAATGCACACTGTGTGTTTATTAACAAAATATAAATTTTCCATTATGAGAATAACA 6120

var3 -----ATGCCTGCAAGTACCGCTATCTA 741
var1 -----ATGCCTGCAAGTACCGCTATCTA 745
var2 -----ATGCCTGCAAGTACCGCTATCTA 827
genome TATTATCTTAAACTCTGAGTATTTAAAAATATTTTATGCCTGCAAGTACCGCTATCTA 6180

var3 GAACATGGTTTACATCCACAAAAGGTTCTGATTATAGATTTCGGACAAACACAAAGACCAG 801
var1 GAACATGGTTTACATCCACAAAAGGTTCTGATTATAGATTTCGGACAAACACAAAGACCAG 805
var2 GAACATGGTTTACATCCACAAAAGGTTCTGATTATAGATTTCGGACAAACACAAAGACCAG 887
genome GAACATGGTTTACATCCACAAAAGGTTCTGATTATAGATTTCGGACAAACACAAAGACCAG 6240

var3 ATAATGTACCACCGGTAAATAATTGCCCTAGATTCAGATGATCCTAGAAGAAAACCTAAAC 861
var1 ATAATGTACCACCGGTAATAATTGCCCTAGATTCAGATGATCCTAGAAGAAAACCTAAAC 865
var2 ATAATGTACCACCGGTAATAATTGCCCTAGATTCAGATGATCCTAGAAGAAAACCTAAAC 947
genome ATAATGTACCACCGGTAATAATTGCCCTAGATTCAGATGATCCTAGAAGAAAACCTAAAC 6300

var3 CAGATCCAGAATAA 875
var1 CAGATCCAGAATAA 879
var2 CAGATCCAGAATAA 961
genome CAGATCCAGAATAA 6314

Figure S4: sIgE reactivity of non-HBV-allergic control patients. sIgE immunoreactivity of non-HBV-allergic patients (n = 10) with recombinant C1q and PVF1 as well as native Api m 1 (phospholipase A2) in ELISA.

