The molecular allergology of subtropical grass pollen

Thina Kailaivasan[1], Janet M. Davies[1,2]*

[1]School of Biomedical Sciences, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane QLD, Australia

[2]Metro North Hospital and Health Service, Brisbane QLD, Australia

*corresponding author

Associate Professor Janet Davies

Centre for Children's Health Research

Queensland University of Technology

62 Graham Road, South Brisbane QLD 4101, Australia

j36.davies@qut.edu.au

1. Abstract

Grass pollens are amongst the most important aeroallergen sources world-wide triggering allergic rhinoconjunctivitis and asthma in sensitised patients. Much of what we know about the allergen components of grasses is informed by research on pollen of temperate (Pooideae) species that are abundant in the Northern Hemisphere. However, climate changes are altering the biogeographical distribution as well as timing and allergenicity of grass pollen. This provides an impetus for better understanding of the contribution of subtropical subfamilies of grasses to pollen allergy globally. Pollen of Chloridoideae (e.g. *Cynodon dactylon*; Bermuda grass) and Panicoideae (e.g. *Paspalum notatum*; Bahia grass or *Sorghum halepense*; Johnson grass) subfamilies are clinically important in subtropical zones of Australia, Asia, India, Africa, and America. These grasses differ ecologically and phylogenetically from temperate grasses and, importantly their allergen composition is qualitatively different. For example, subtropical grass pollens appear to lack the major group 5 grass pollen allergen family. In this Chapter we will summarize current knowledge of the epidemiology and immunology of subtropical Chloridoideae and Pancoideae pollen allergens, describing the biochemical characteristics of known isoforms and variants as well as current knowledge of the properties and structures of recombinant subtropical pollen allergens. Whilst only one subtropical allergen component; Cyn d 1 of Bermuda grass pollen, is available commercially for diagnostic use, as a natural purified form, a number of allergens of Panicoideae grass pollen; Zea m 1, Zea m 3 and Zea m 13 of maize, Pas n 1 and Pas n 13 of Bahia, or Sor h 1, Sor h 2, Sor h 13 and Sor h 23 of Johnson grass, have been discovered. Research effort is directed towards making available subtropical grass pollen allergen components as innovative treatment and diagnostic options that more specifically address the needs of patients from warmer regions of the globe.

Key words

Grass pollen, allergic rhinitis, subtropical grass pollen, Bermuda grass pollen, Bahia grass pollen

Epidemiology and immune recognition of subtropical grass

There is an inverse biogeographical distribution of temperate and subtropical grass pollen with the subtropical species being more abundant closer to the equator (Esch, 2004). The size of the world's population living in subtropical climates is increasing globally and the subtropical zones are widening (Gupta, 2002; Seidel et al.,, 2008). In southern United States of America (USA), such as Florida, Texas, Louisiana and Mississippi, the population increased by 18.3% to 52.3 million between 2000 and 2010 (US Census Bureau). The biomass of subtropical grasses (Morgan et al.,, 2011) and the biogeographical distribution of subtropical grasses is predicted to expand with climate change (Gupta, 2002), increasing the exposure to subtropical GP allergens (Kongpanichkul et al.,, 1995) and intensifying the burden of allergic respiratory diseases (Beggs, 2009; ZiskaandBeggs, 2011). The epidemiology of subtropical grasses pollens and their contribution to allergic rhinoconjunctivitis and asthma in subtropical regions has previously been reviewed (Davies, 2014).Here a comparison between temperate and subtropical grass pollen biogeography, epidemiology and immunology is summarized.

In 1972, Hensel and Griffith (HenselandGriffith, 1972) examined sensitisation frequencies in the 429 patients from Louisiana, a subtropical region in USA; Bahia (*Paspalum notatum)* GP was the most frequently recognized GP but that patterns of sensitization included of SPT positivity to Bahia, Dallis (*Paspalum dilatatum*), Johnson (*Sorghum halepense*), Bermuda (*Cynodon dactylon*) and *Phleum pratense* GP. Application of the Prauss Kausner test (CohenandZelaya-Quesada, 2002) with serum of five Bahia GP-allergic patients to non-allergic

recipients, showed the highest SPT reactivity to Bahia than other species of GP (HenselandGriffith, 1972).

In a survey of sensitisation of 345 children with allergic diseases of military personnel in Lackland, Texas, Bahia GP showed the highest frequency (38%) of positive SPT of a panel of 51 common aeroallergens (CalabriaandDice, 2007). Interestingly, the frequency of sensitivity to pollens of other grasses Bermuda (35.9%), Ryegrass (34.8%) and Timothy (34.2%), were only slightly lower. The authors considered the study cohort to be from a "mobile" population and it included children up to 18 years of age. There was an increase in prevalence of allergic sensitivity with age, therefore it was unclear whether sensitisation to GP occurred whilst the children lived and were exposed to grasses of Texas.

Examination of serum IgE reactivity to GP in allergy patients from Europe and North America revealed high correlations between specific IgE to various GP in single point tests (AnderssonandLidholm, 2003; Johansen et al.,, 2009). However, the degree of correlation between specific IgE concentrations to subtropical GP and temperate GP was not as high as amongst temperate GP. Most of the patient sera examined in those studies were sourced from patients in regions primarily exposed to temperate GP.

IgE cross-inhibition assays can be used to indicate the avidity of interaction based on the inhibitor concentration at which 50% (IC50) of IgE reactivity with the target allergen is blocked, and the degree of specificity of response (maximum IgE cross inhibition) (Aalberse, 2007). IgE cross reactivity between subtropical and temperate GP is incomplete and mostly non-reciprocal, depending on the origin of the patient and their exposure to subtropical and/or temperate GP allergens (Davies, Dang, et al.,, 2011; Davies et al.,, 2012; Weber, 2003; WhiteandBernstein, 2003). The capacity for the immune system to differentiate between

allergens of temperate and subtropical GP at the T and B cell level has important implications for the specificity of GP allergy diagnosis and the likely efficacy of GP allergen specific immunotherapy (Burton et al.,, 2002; Etto et al.,, 2012; Eusebius et al.,, 2002; Nony et al.,, 2015).

In a cross-inhibition study from Minnesota, USA, with pooled sera of five donors highly allergic to northern GP, Bermuda GP was unable to inhibit 50% of IgE with June (*Koeleria macrantha*), orchard (*Dactylis glomerata*), meadow fescue (*Festuca pratensis*) or Ryegrass (*Lolium perenne*) GP. In the converse experiments, four orders of magnitude more Timothy GP extract was required to achieve 50% inhibition of IgE with Bermuda GP (LeifermanandGleich, 1976).

Similarly, northern GP showed reciprocal cross-inhibition of IgE reactivity with an array of temperate GP in studies with pooled sera from US army volunteers (Martin et al.,, 1985). However, *C. dactylon* and *P. notatum* GP extracts showed limited capacity to inhibit pooled serum IgE reactivity with pollens of *L. perenne* and *P. pratense* GP extracts in radio allergosorbant assays. Pollen of northern grasses inhibited most but not all IgE reactivity with *P. notatum* grass, suggesting "unique allergenicity" for *P. notatum* compared with a temperate GP. Although the origin of the subjects and their primary exposure to temperate or subtropical grasses was not described, distinct IgE reactivity between subtropical and temperate GP were evident.

Small numbers of patients from Florida showed no *in vivo* cross-reactivity between pollen of *P. pratense* and *P. notatum* in nasal provocation tests in patients with allergic sensitivity to either *P. pratense* or *P. notatum* (Phillips et al.,, 1989).

Recently, Ramirez et al. (Ramirez et al.,, 2015) used an allergen challenge chamber challenge in Texas to investigate sensitivity to Timothy GP where subtropical grasses predominate without natural Timothy GP exposure. Of the 22 participants, SPT and specific IgE to Timothy and subtropical Bahia, Johnson and Bermuda GP correlated with symptom scores following exposure to Timothy GP in an allergen challenge chamber. The authors did not observe differences between symptom scores of participants who were locals of Texas and those who had lived outside of Texas for >5 years. However, at excessively high allergen exposure in the chamber (over 3000 grains per meter cubed over three hours) those participants who were local Texans showed slower kinetics of symptom escalation than those who had been exposed to Timothy GP outside Texas suggesting differences in allergic sensitivity between the two sub-groups of participants (Ramirez, et al., 2015).

In different states of Australia, Davies et al. (Davies, Dang, et al., 2011; Davies, et al., 2012) showed that levels of specific IgE reactivity with subtropical and temperate GP differ depending on the biogeographical origin and grass exposure patterns of the patient. Moreover, the finding of specific IgE recognition of subtropical species of GP in patients from subtropical Queensland was validated in other laboratories (Nony, et al., 2015).

Biology and Biochemistry of Subtropical GP Allergens

GP allergen families include proteins with particular biochemical structures and functions within the pollen from which they are derived. The allergens discovered within subtropical GP include the group 1; β-expansin, group 13; polygalacturonase, and others described below. Notably, the highly allergenic group 5 allergen of temperate GP does not occur in subtropical grass pollens. Table A.1 summarizes the subtropical GP allergens identified to date for which there is evidence of patient IgE reactivity or allergenicity. Proteomic analysis of

Bermuda GP revealed eight allergens that shared similarities to known pollen allergen families based on mass spectrometry and databases comparisons (Kao et al.,, 2005). Analysis of the complete proteome, transcriptome and allergome of Johnson GP, demonstrated that there are more gene transcripts present within the pollen that encode for allergen-like proteins, and more detectible isoforms translated into proteins and packaged within pollen, than there are IgE-binding proteins detected with serum of relevant, clinically-affected allergy patients. Thus the allergen status of putative allergens identified by molecular biological techniques must be verified (Pomes A, 2018) .

<Table A.1>

Major allergens: Group 1

The major Group 1 GP allergens, β-expansins constitute up to 10% of total pollen (Drew et al.,, 2011). Functionally, these non-proteolytic glycoproteins are involved in the loosening of the cell walls to facilitate invasion of the pollen tube (Cosgrove et al.,, 1997). In molecular and biochemical studies of the maize (*Zea mays*) allergen Zea m 1, it was proposed that β-expansins loosens plant cell walls by disrupting noncovalent junctions between the matrix polysaccharide glucuronoarabinoxylan, that binds cellulose (Wang et al.,, 2016). Cyn d 1, from Bermuda GP, was the first group 1 allergen to be characterized as a 32 kDa protein with high N-terminal sequence homology to the well characterized group 1 allergen of Ryegrass, Lol p 1 (Shen et al.,, 1988; Matthiesen et al.,, 1991). Group 1 allergens of subtropical grass pollens show the highest frequency of IgE reactivity by immunoblotting in GP allergic patients (Davies, 2014), ranging from IgE reactivity to Cyn d 1 in 76% of 21 patients from Taiwanese and 100% of 44 patients from New South Wales, Australia (Shen, et al., 1988; FordandBaldo, 1987). A

33kDa acidic (pI 6.59) allergen purified biochemically from Bahia GP showed IgE reactivity with sera from patients in Florida USA (Ghobrial et al.,, 2002). The Bahia GP Group 1allergen of 29 kDa was subsequently cloned, Pas n 1 (Davies et al.,, 2008; White et al.,, 2009). By ELISA recombinant Pas n 1 showed IgE with 47 of 55 (85%) patients from the temperate climate city of Melbourne Australia (Davies, et al., 2008) and by ImmunoCAP purified Pas n 1 showed IgE reactivity with 91.2% of 182 GP-allergic (Timbrell et al.,, 2014). Sor h 1 was identified (Avjioglu et al.,, 1993) and latter characterized as 30 kDa protein that showed by ELISA IgE reactivity with 76% in sera of 64 patients from Queensland, Australia (Campbell et al.,, 2015).

Other important allergen families: Group 13 allergens

The next prominent group of allergens in subtropical GP are the Group 13 allergens. Zea m 13 was the first subtropical group 13 allergen identified, as a 50-60 kDa protein that had 42% IgE reactivity in 24 grass-pollen allergic patients (Petersen et al.,, 2001). Digestion of Zea m 13 with endoproteinase Lys-C and isolation of a 35 kDa protein fragment revealed complete identity with polygalacturonase (Petersen, et al., 2001). These enzymes degrade cell wall pectin (Swoboda et al.,, 2004).The group 13 allergens have subsequently been identified in Bahia and Johnson GP, both as 55 kDa proteins with high N-peptide sequence homology to that of Zea m 13 (Campbell, et al., 2015; Davies, Voskamp, et al.,, 2011). Two isoforms of native Sor h 13 with observed molecular weights of 55 and 54 kDa were purified from Johnson GP (Campbell, et al., 2015). Whilst eight gene transcripts encoding polygalacturonase were present in Johnson GP transcriptome, two detected in the proteome corresponded to the purified isoforms designated Sor h 13.0101 and Sor h 13.0201 by mass spectrometry analysis (Campbell, et al., 2015) .

Pan-allergens: Group 7 and 12

Group 12 allergens are profilins, ubiquitous proteins found in all eukaryotic organisms that are pan-allergen responsible for cross reactivity between pollen, latex and plant foods (Matricardi et al.,, 2016). Profilins are 12-16 kDa actin-binding protein involved in the generation of the cytoskeleton (Gunning et al.,, 2015). There were three transcripts encoding profilins in Johnson GP that were present in the proteome however, there were no IgE reactive spots consistent with profilin detected on two dimensional immunoblotting with sera pooled from GP-allergic patients from a subtropical region (Campbell, et al., 2015). To date, the frequency of IgE reactivity with profilins of subtropical grass pollens has not been established for clinically relevant patient cohorts.

Group 7 allergens have been classified as polcalcins, due to the presence of two calcium binding domains. Cyn d 7 was cloned from Bermuda GP and plaque blots of recombinant Cyn d 7 were found to be IgE reactive in 10% of 30 patients from a temperate region of Melbourne. Cyn d 7 showed protein sequence similarity with Bet v 4 (66%) from Birch and Bra r 1 (30%) from oilseed rape, two known calcium binding allergens (Smith et al.,, 1997; Suphioglu et al.,, 1997). Four of 68 cDNA transcripts encoding polcalcins were expressed as proteins in the Johnson GP proteome but IgE reactivity was not detected with pooled GP-allergic sera (Campbell, et al., 2015).

Minor allergen groups: Group 2, 4, 23 and 24

Group 4 allergens are metallo-flavoprotein or berberine-bridge orthologues that function as an oxidase in biosynthesis of pollen-specific secondary metabolites (Huang, Peng, et al.,, 2012). Purified Cyn d 4 was IgE reactive in 5 of 10 patients with seasonal allergic rhinitis patients and Bermuda-GP allergy from Florida (Su et al.,, 1996). One cDNA transcript of the

Johnson GP transcriptome matched known GP group 4 allergens, but IgE reactivity was not observed by with pooled sera of GP-allergic patients from a subtropical region (Campbell, et al., 2015).

Cyn d 24 is exclusively reported in Bermuda GP. This allergen shares homology with pathogenesis-related protein, PR-1, ranging from 45.2% amino acid identity with maize to 49.6% with barley (*Hordeum vulgare*) (Chow et al.,, 2005). These are stress proteins produced by plants following bacterial or fungal infections, flooding or freezing temperatures (Stintzi et al.,, 1993). The frequency of IgE reactivity with Cyn d 24 was 29% of 21 Bermuda GP-allergic asthma patients from Taiwan (Horng-Der et al.,, 1988).

The group 2 allergens are 12 kDa proteins with pI of 4.9 and 5.9 for two isoforms. Whilst being similar to they are distinct from the group 1 allergen carboxy-terminal domain but clustered separately from group 1 allergen in a dendrogram (Campbell, et al., 2015). Zea m 2 has been mentioned in several studies, and shares similarity with Zea m 3, but its IgE reactivity has not been evaluated (Oldenburg et al.,, 2011; Petersen et al.,, 2006). In studies of the temperate Timothy GP, the corresponding group 2 and 3 allergens, Phl p 2 and Phl p 3, have high IgE cross-reactivity and homology to the C terminal domain of beta-expansins i.e. group 1 allergens (Matricardi, et al., 2016; Devanaboyina et al.,, 2014). Three isoforms of Sor h 2 were identified and found to bind IgE reactivity of GP-allergic sera from patients in subtropical Queensland, Australia (Campbell, et al., 2015).

The group 23 allergen, Sor h 23 was observed as a 29 kDa proteins with isoforms of pI of 5.7 and 6.9. Sor h 23 was found to be larger than its closest homolog, Cyn d 23 (22.2 kDa, 6.22 pI) suggesting they are very different (Campbell, et al., 2015). Whilst Cyn d 23 is listed as an allergen in the WHO/IUIS database, its existence is only discussed (Yang, 2005) and there is a

lack of studies on its frequency of IgE reactivity with relevant patient sera and its biochemical function.

8. Subtropical grass allergen structures

Zea m 1

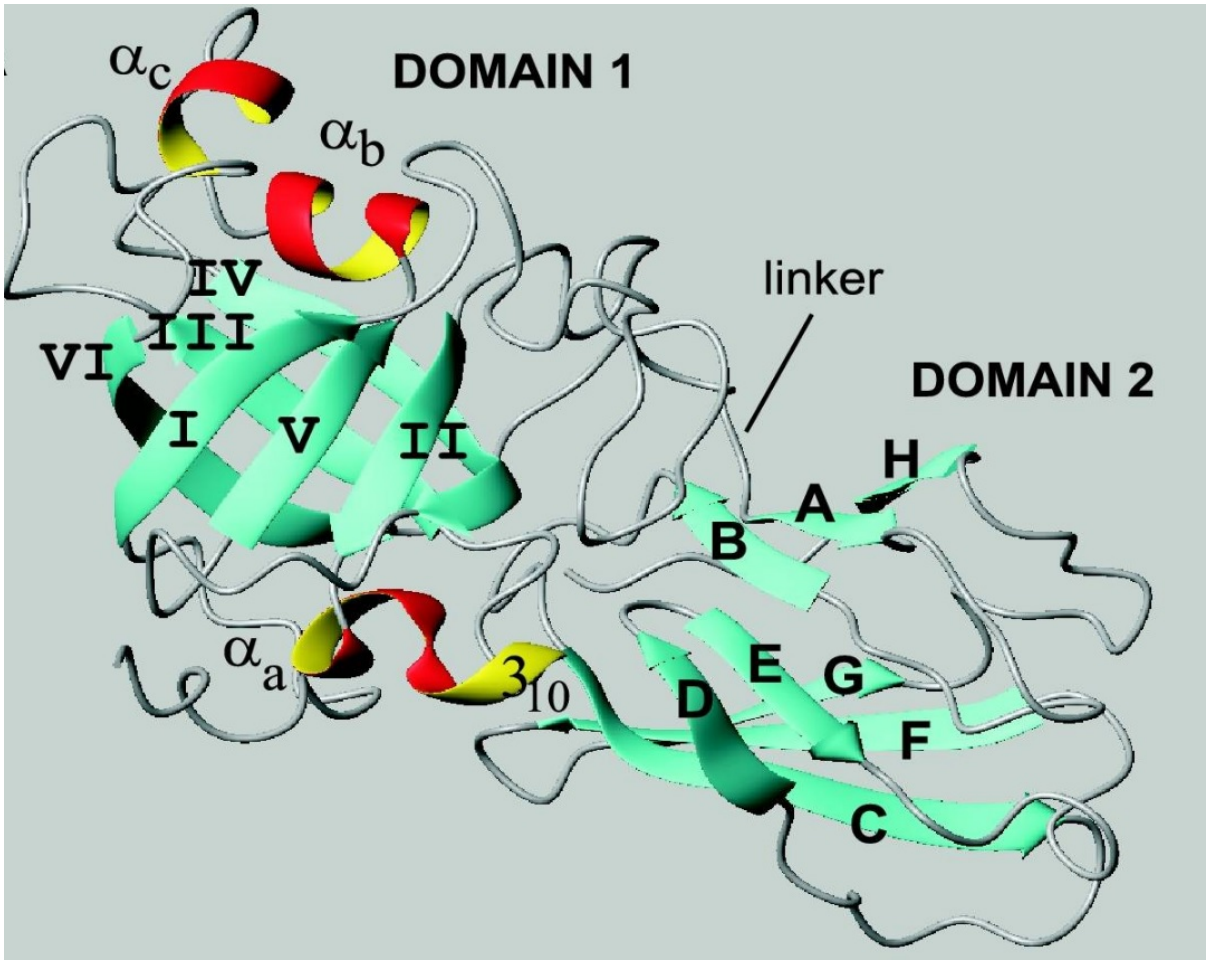

Figure 1 Ribbon model of Zea m 1 showing the two globular domains and their anti-parrallel beta sheet fold. Adapted from Yennawar et al. 2006 (Yennawar et al.,, 2006)

The crystal structure of the β-expansin group 1 pollen allergen Zea m 1 isoform d (Figure 1), from purified maize pollen, was elucidated by x-ray crystallography to 2.75-A resolution (Yennawar, et al., 2006). Termed EXPB1 (Genbank AAO45608), it consists of two domains packed closely and has a groove with potential to bind a glycan backbone of approximately

10 sugar residues. The N-terminal domain 1, has sequence similarity of 20% to the catalytic domain of family-45 glycoside hydrolases GH45. The C-terminal domain 2, has sequence similarity between <10% to 35% identity to group 2 and 3 allergens, which are expansin-like proteins (Yennawar, et al., 2006).

Cyn d 4

The crystal structure of purified natural Cyn d 4 (Figure 2), was reported at 2.15 A resolution (Huang, Peng, et al., 2012). It has structural similarity to the vanillyl alcohol oxidase (VAO) superfamily with two distinct domains; an FAD-binding domain and substrate-binding domain. The FAD-binding domain includes the N-terminal and C-terminal residues and folds into two subdomains; one with 5 β-strands surrounded by five α-helices (subdomain 1), and another with 4 central β-strands sandwiched by 3 α-helices (subdomain 2). The substrate binding domain consists of seven β-strands surrounded by 5 α-helices. Two N-glycosylation sites were also observed. Cyn d 4 also contains an FAD cofactor that is covalently linked to His88 and Cys152, making it a bicovalent flavoprotein. There is also a deep, large cavity where most residues are hydrophobic. Taken together, these indicate the involvement of Cyn d 4 as an oxidase involved in biosynthesis. Cyn d 4 contains a large number of solvent-exposed, positively-charged residues, which result in its basic pI of close to 10. When compared with models of group 4 allergens, two conserved patches were identified as putative antibody or IgE binding regions while five conserved clusters identified could comprise of cross-reactive epitopes (Huang, Peng, et al., 2012).

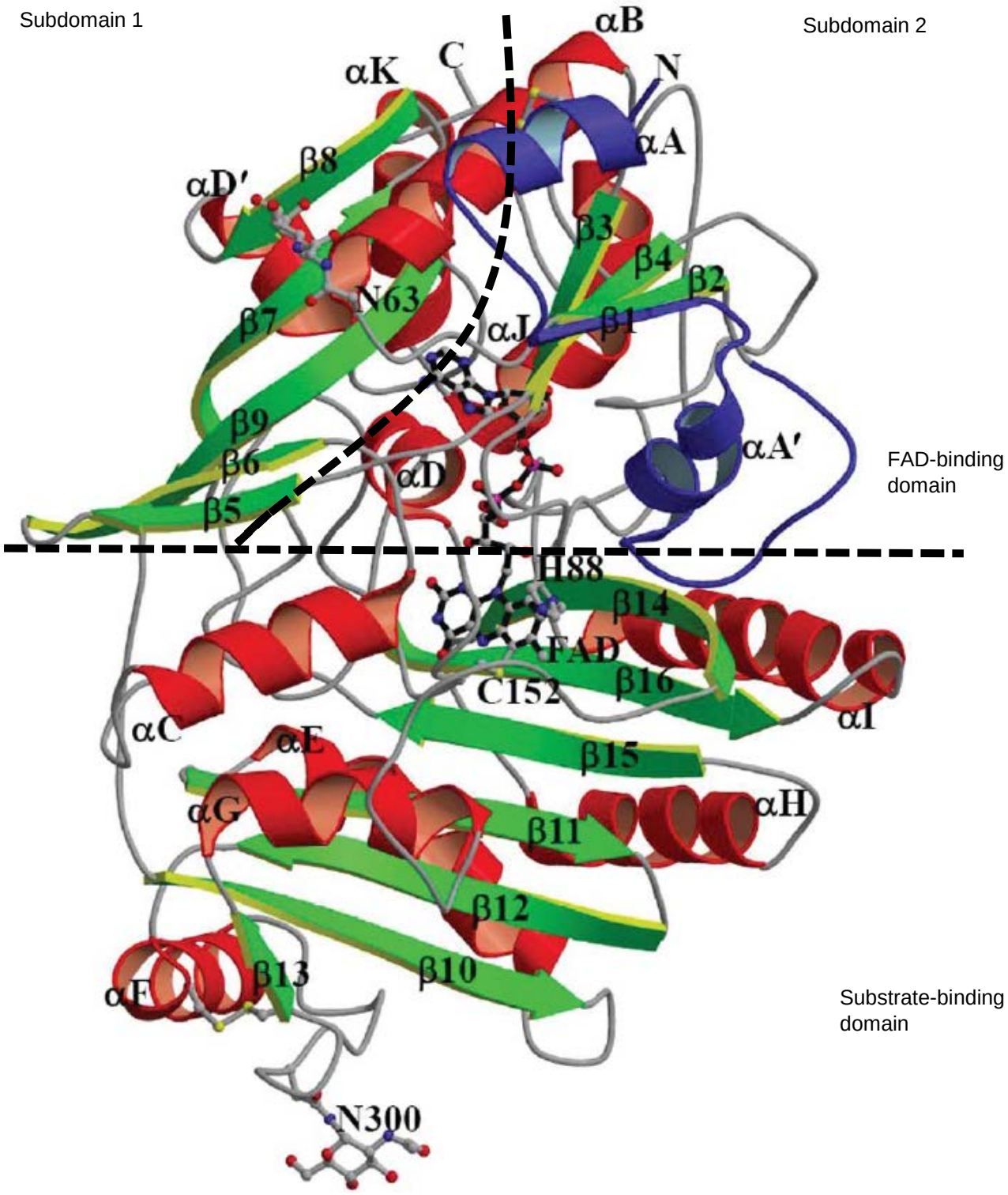

Figure A.2 Stereoview of Cyn d 4 crystal structure displayed as ribbon and stick figure, N-terminal segment in blue. Adapted from Huang et al. 2012 (Huang, Peng, et al., 2012).

Isoallergens and isoforms

Allergens can exist as isoallergens or isoforms with varying molecular weights and isoelectric points (pI) (Pomes A, 2018). Isoallergens show sequence differences and can arise from separate gene loci, as exemplified by two Zea m 1 isoforms (GenBank accession nos. AY104999 and AY104125) having higher sequence similarity with Lol p 1 and Phl p 1 than with two other Zea m 1 isoforms (AY197352 and AY197353) (Li et al.,, 2003). While all four Zea m 1 isoforms exhibited similar biochemical activity (cell wall extension), there were differences in protein abundance and reactivity with monoclonal antibodies (Li, et al., 2003). This implies structural and potentially, immunological differences between isoforms. Table A2 summarizes the known isoforms of known subtropical grass pollen allergens.

<Table A.2>

The number of isoforms and isoallergens of subtropical grasses listed in Table A.2 were compiled from the WHO/IUIS Allergen Nomenclature database. Cyn d 1 has two identified isoallergens; Cyn d 1.01 with 7 variants and Cyn d 1.02 with 4 variants. Cyn d 1 was first described as a 32 kDa protein with a pI of 6.2. Chang et al were the first to identify between four to ten isoforms with pI ranging from 5.6 to 7.3, classified by acidity (acidic, basic, neutral) and with the basic and neutral isoforms having lower molecular weight (Chang et al.,). More importantly, the basic and neutral isoforms were found to have higher RAST inhibition compared to the acidic isoforms for inhibition of pooled patient IgE reactivity with the basic isoform fraction, suggesting unique allergic determinants in the basic and neutral isoforms. However, the acidic isoform fraction showed higher level of IgE reactivity with pooled serum than the neutral isoform fraction.

Screening of the Bermuda cDNA library for unidentified Cyn d 1 isoallergens identified four new cDNA clones encoding acidic proteins that share 86% identity with the basic isoforms identified by Chang et al (Au et al.,, 2002). Further comparisons of these two groups of revealed different net charges of -2 for the acidic isoallergen and +8 for basic isoallergen, different signal peptides of 18 amino acid residues for acidic and 26 for basic, as well as the presence of additional GA residues at the C-terminus in some basic isoallergens that are not present in the acidic isoallergens.

Two isoforms of Sor h 1 were discovered separately; one basic Sor h 1.02 and acidic Sor h 1.01, but their IgE reactivity has not been directly compared (Avjioglu, et al., 1993; Campbell, et al., 2015).

Currently, there is a lack of information regarding the clinical implications of multiple isoallergens from subtropical GP for instance isoforms of Cyn d 1, Zea m 1 and Sor h 1. However, studies on other inhalant allergens have demonstrated immunological differences between their respective isoallergens (Christensen et al.,, 2010; Hartl et al.,, 1999). Further research on immunoreactivity of subtropical GP isoallergens is needed to improve our understanding of the importance of particular isoforms for diagnositic accuracy or therapeutic benefit.

Diagnostics

Clinical utilization of component-resolved diagnostics (CRD) complements skin prick tests and serum-specific IgE testing with whole extracts (Scala et al.,, 2010). When compared to skin prick tests, CRD is more specific in identifying the primary sensitizing allergen or disease-causing allergen source, which is essential for precise selection of immunotherapy (Gonzalez-Mancebo et al.,, 2017; Saltabayeva et al.,, 2017).

nCyn d 1 was the first subtropical GP allergen to be commercially available for CRD, specifically the ImmunoCAP system (Matthiesen, et al., 1991; ThermoFisher, 2012). The utility of this assay system was recently demonstrated by a cross-sectional study in southern China that used CRD to investigate sensitization profiles towards purified allergens from Bermuda, Timothy and *Humulus scandens* in 346 patients with allergic rhinitis and/or asthma (Luo et al.,, 2017) (Luo et al.,, 2016). nCyn d 1 has also been included as one of the 112 allergens on the ImmunoCAP ISAC multiplex array (ThermoFisher, 2012). rCyn d 12 was also tested as part of the ISAC array in the first extensive cross-sectional study of 23, 077 Italian patients, but it is not commercially available (Scala, et al., 2010).

An issue is that nCyn d 1 is glycosylated near the amino terminus, thus testing of IgE reactivity with nCyn d 1 in populations not exposed to Bermuda grass can give positive results that are difficult to reconcile clinically (Cabauatan et al.,, 2014). These positive reactions could be low affinity cross-reactivity of IgE primarily specific to temperate GP allergens, or due to non-specific, clinically-irrelevant binding to the cross-reactive carbohydrate component. Note that in the Philippines, a tropical region with a low population frequency of reactivity with GP (Davies), there was no difference in IgE reactivity to nCyn d 1 in patients with symptomatic allergic rhinitis and those reporting no allergic rhinitis, and the IgE binding to nCyn d 1 could be reduced by inhibition with CCD-containing nPhl p4 or by deglycosylation (Cabauatan, et al., 2014).

In Australia where GP allergy frequency is high, specific IgE reactivity was detected with recombinant Pas n 1 (Davies, et al., 2008), indicating that the carbohydrate component is not involved in IgE binding to this rPas n 1. Moreover, in separate studies in Florida USA, deglycosylation of the group 1 allergen of Bahia GP did not diminish IgE binding (Ghobrial, et

al., 2002), indicating that in geographically relevant locations CCD is not the reason for detection of IgE binding with group 1 allergens of subtropical grass pollens.

The potential for nPas n 1 in the diagnosis of patients with allergic rhinitis due to Bahia GP allergy was shown with biotinylated nPas n 1 coated onto a streptavidin ImmunoCAP (Timbrell, et al., 2014). Serum specific IgE towards nPas n 1 of 182 GP-allergic patients, with clinical history of allergic rhinitis, was highly correlated with BaGP SPT (r = 0.795) and BaGP IgE (r = 0.951). Furthermore, this assay showed high sensitivity detecting of Pas n 1 specific IgE (92.4%, cut-off at 0.225kU/L), high specificity (93.1%) and low inter-assay co-efficient of variation (6.92%) (Timbrell, et al., 2014). However, it was observed that 8% of other allergy patients also showed IgE reactivity with nPas n 1 suggesting that some positive responses in this assay format could be due to cross-reactive carbohydrate moieties (Timbrell, et al., 2014), indicating the need for recombinant subtropical grass pollen group 1 allergen components for diagnosis.

Treatment

Allergen immunotherapy (AIT) is a commonly administered and effective treatment for controlling allergic rhinitis due to GP (Walker et al.,, 1995; Bousquet et al.,, 1998). While subcutaneous and sublingual immunotherapy options are safe and effective, for reducing clinically symptoms and medication use in adults and children with allergic rhinitis and asthma (Dhami, Kakourou, et al.,, 2017) (Dhami, Nurmatov, et al.,, 2017), they both require repeated doses to achieve long-term efficacy and have rare but potentially harmful side effects (Klimek et al.,, 2016).

New treatment methods are being trialled including synthetic peptide immuno-regulatory epitopes (SPIRE) (LarchéandKay, 2004). This treatment method requires lower doses and a shorter treatment course compared to current SCIT or SLIT and due to its short length, has lowered chance to cross-link IgE on mast cells and basophils (Creticos, 2014). SPIRE for grass allergy is being developed, comprising of T-cell epitope peptides from the subtropical Cyn d 1 and a four temperate GP group 5 allergens; Lol p 5 (Ryegrass), Phl p 5 (Timothy), Dac g 5 (orchard) and Hol l 5 (velvet). In a multicentre phase II clinical trial involving 282 patients with a randomized, double-blind, placebo-controlled design, following 14 weeks of treatment with this GP SPIRE significantly reduced rhinoconjunctivitis symptoms in an Environmental Exposure Unit, and exposure to a natural pollen season in Canada (Ellis et al.,, 2017). An optional follow-up study on this cohort after a second natural pollen season showed a sustained treatment effect after cessation of dosing (Ellis et al.,, 2015).

A DNA vaccine candidate for subtropical GP has been designed by inserting cDNA of Cyn d 1 into the vector pcDNA3 (Huang, Chu, et al.,, 2012) . A model of BALB/C mice sensitized by intraperitoneal injection twice to rCyn d 1, was immunized by intramuscular injection of recombinant plasmid Cyn d 1 (pCyn d 1). This induced Th1 responses, characterized by increased allergen specific IgG and interferon-γ levels from CD4+ and CD8+ as well as suppression of specific-IgE in serum (Huang, Chu, et al., 2012). A similar study on neonatal BALB/C mice vaccinated with pCyn d 1 and sensitized twice with intraperitoneal rCyn d 1 showed a similar suppression of specific IgE response, induction of Th1 response and IL-4 secretion in spleen (Huang et al.,, 2014).

Summary and conclusions

The differences in frequency of SPT, levels of IgE reactivity and patterns of cross-reactivity reported for subtropical grass pollen allergens of Bahia, Bermuda and Johnson GP are likely to be clinically relevant for patients in subtropical regions. Others report the ratio of allergen specific IgE to total IgE (Di Lorenzo et al.,, 2009), the number allergen components recognized within Timothy GP (Darsow et al.,, 2014), and the recognition of particular allergen component (Savi et al.,, 2013), to indicate severity or progression of allergic respiratory disease (Hatzler et al.,, 2012). With increasing capacity to measure levels of specific IgE to allergen components, implementation of more quantitative and molecularly defined allergy testing is likely to be clinically helpful for subtropical grass pollen allergen components. Further research with quantitative methods is needed to determine the rates and levels of sensitisation to standardized recombinant allergen components such as Cyn d 1, Pas n 1, Sor h 1, Cyn d 4, Pas n 13 and Sor h 13 in relevant patients with allergic respiratory diseases from subtropical regions to evaluate the importance of particular subtroptical GP allergen component isoforms and to drive the advancement of more defined and specific diagnosis and treatment options for subtropical GP allergy.

Acknowledgements

TK receives an International Post Graduate Research Scholarship with an Excellence Top Up Scholarship from Queensland University of Technology. JMD receives grant funding for related research from the National Health and Medical Research Council of Australia, Australian Research Council, the National Health and Medical Research Foundation and Queensland University of Technology.

Conflict of interest statement

JMD leads the NHMRC AusPollen Partnership Project (GNT 1116107) with matching cash and in kind co-sponsorship from The Australasian Society for Clinical Immunology and Allergy, Asthma Australia, Bureau of Meteorology, Commonwealth Scientific and Industrial Research Organisation, Stallergenes Australia, Meteorology Switzerland. She is an investigator of the ARC Discovery (DP170101630), undertakes research for the Bureau of Meteorology on a Thunderstorm Asthma Pollen Forecasting Project. JMD received funds from The Victorian State government of Australia Department of Health and Human Services to report on a review of literature and public health implications of thunderstorm asthma and from the Bureau of Meteorology for implementation of the Victorian Thunderstorm Asthma Pollen Surveillance (VicTAPS) project. She has received grants from the NHMRC, National Foundation for Medical Research Innovation, the Allergy and Immunology Foundation of Australasia, Asthma Australia, Queensland University of Technology, Asthma Australia, The University of Queensland and a contracted research grants from Stallergenes (France), Abionic (Switzerland), in-kind provision of materials from ThermoFisher (Sweden) and services from Sullivan Nicolaides Pathology (QLD, Australia). She is an inventor on patents assigned to QUT granted in Australia, allowed in USA and three applications pending examination in Australia, USA and Europe. Assoc. Prof. Davies's institute has received Honorarium payments and travel expenses for education sessions and conference presentations from Stallergenes Australia, GlaxoSmithKliene, Wymedical, and Meda Pharmaceuticals.

Table A.1 Biochemical families, biological function crystal structure and post-translational modifications of known subtropical grass pollen allergens.

Biochemical family	**Biological function**	**Protein structure available**	**Name**	**Post-translational modifications**	**References**
β-expansin (Group 1) 29-31kDa	Cell-wall loosening to allow cell enlargement during plant growth (Wang, et al., 2016) (Cosgrove, et al., 1997) .	Zea m 1	Pas n 1 Cyn d 1 Sor h 1 Zea m 1	N-glycosylation	(Davies, et al., 2008) (Matthiesen, et al., 1991) (Avjioglu, et al., 1993) (Campbell, et al., 2015) (Li, et al., 2003)
Expansin-like protein/ C-terminal β-expansin (Group 2); 12 kDa	Similar to β-expansin	N/A	Sor h 2		(Campbell, et al., 2015)
Metallo-flavoprotein/glycoprotein (Group 4); 60 kDa, pI 9.3	Berberine bridge enzyme orthologue (Davies, 2014) Oxidase involved in biosynthesis of pollen-specific apolar secondary metabolite	Cyn d 4	Cyn d 4		(Huang, Peng, et al., 2012)
Polcalcin (Group 7); 12kDa	Two calcium-binding domains (EF-hands)	Cyn d 7 (Smith, et al., 1997)	Cyn d 7		(Suphioglu, et al., 1997)
Profilin (Group 12); 14.1 kDa	Actin-binding protein	N/A	Cyn d 12 Zea m 12		(Asturias et al.,, 1997) (Kovar et al.,, 2000)

Polygalacturonase (Group 13); 54-55 kDa	Pectin-degrading enzymes (Swoboda, et al., 2004)	N/A	Pas n 13 Sor h 13 Zea m 13	N-glycosylation	(Davies, Voskamp, et al., 2011) (Campbell, et al., 2015) (Petersen, et al., 2006)
Pathogenesis-related protein (Group 24); 21 kDa	Proteins released by plants when faced with environmental stress (Stintzi, et al., 1993)	N/A	Cyn d 24	N-glycosylation	(Chow, et al., 2005) (Stintzi, et al., 1993)

Table A.2 Subtropical grass pollen allergens purification method, recombinant expression systems, variants and protein/nucleotide accession numbers.

Allergen	**Frequency of IgE reactivity**	**Natural allergens (purification methods)**	**Recombinant allergens (expression system)**	**Isoallergen / variants (IUIS nomenclature)**	**Accession numbers (allergen.org)**			**References**
					Genbank Nucleotide	Genbank Protein	Uniprot	
Pas n 1	85% of 55 to 92% of 182 GP-allergic patients	nPas n 1 - ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography	rPas n 1– expressed in *E. coli* transformed with pET-28a-Pas n 1 vector.	Pas n 1.0101	EU327342	ACA23876	B8PYF3	(Ghobrial, et al., 2002) (Drew, et al., 2011) (Davies, et al., 2008) (Davies et al.,, 2005)
Cyn d 1	76% of 21 GP-allergic patients, 100% in 44	nCyn d 1	rCyn d 1 - vector pTrc 99A	Cyn d 1.0101 Cyn d 1.0102	S83343 N/A	AAB50734 N/A	O04701 N/A	(Shen, et al., 1988)

	Bermuda GP SPT positive patients	- concanavalin A-Sepharose affinity chromatography, and carboxymethyl-Sepharose chromatography	expressed in *E. coli* and yeast expression vector pHIL-S1 expressed in *Pichia pastoris*	Cyn d 1.0103 Cyn d 1.0104 Cyn d 1.0105 Cyn d 1.0106 Cyn d 1.0107 Cyn d 1.0201 Cyn d 1.0202 Cyn d 1.0203 Cyn d 1.0204	N/A N/A N/A N/A N/A AF177030 AF177378 AF177380 AF159703	N/A N/A N/A N/A N/A AAK96255 AAL14077 AAL14079 AAF80379	N/A N/A N/A N/A N/A Q947S7 Q947S6 Q947S4 Q9FVM0	(FordandBaldo, 1987) (Matthiesen, et al., 1991) (Chang et al.,, 1999) (Smith et al.,, 1996)
Sor h 1	76% of 64 GP-allergic patients	nSor h 1 – ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography		Sor h 1.0101 Sor h 1.0201	KF887425 KF887426	AIL01316 AIL01317	C5WMS3 A0A077B4J2	(Avjioglu, et al., 1993) (Campbell, et al., 2015)
Zea m 1	Positive for maize pollen exposed individuals	nZea m 1 – acetate buffer extraction, Carboxymethyl-sepharose chromatography, CM-silica HPLC		Zea m 1.0101 N/A N/A N/A N/A	L14271 N/A N/A N/A N/A	AAA33496 AY104999 AY104125 AY197352* AY197353** *	Q07154 N/A N/A N/A N/A	(Wu et al.,, 2001) (Petersen, et al., 2006) (Li, et al., 2003) these four not named in IUIS *designated EXPB9, gene distinct form EXPB1 **identical sequence to Zea m 1.0101

								(EXPB1) but longer
Sor h 2	IgE reactive by immunoblotting in Johnson GP positive patient	nSor h 2 – ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography	rSor h 2 expressed in *E.coli* transformed with pET-28a plasmid. (Davies, unpublished)	Sor h 2.0101 Sor h 2.0201	KF887427 KF887428	AIL01318 AIL01319	A0A077B7S9 A0A077B2S0	(Campbell, et al., 2015)
Cyn d 4	5 of 10 patients with seasonal allergic rhinitis	nCyn d 4 – ammonium sulphate precipitation, Sephadex G-25, ion-exchange, blue gel affinity, and reverse-phase high-performance liquid chromatography		N/A	AY451241	AAS02108	Q5QJ60	(Su, et al., 1996) (Huang, Peng, et al., 2012)
Cyn d 7	10% of 30 GP-allergic patients with AR by IgE immunoblotting		rCyn d 7 – λgtll cDNA library of mature Bermuda anther screened for IgE binding of pooled GP-allergic sera	Cyn d 7.0101	X91256	CAA62634	P94092	(Suphioglu, et al., 1997)
Cyn d 12	20% in 30 GP-allergic patients		rCyn d 12– *E. coli* BL21 (DE3) using pKN172 vector	Cyn d 12.0101	Y08390	CAA69670	O04725	(Asturias, et al., 1997) (Kao, et al., 2005)
Zea m 12			rZea m 12 – vector pET-23a expressed in *E.*	Zea m 12.0101 Zea m 12.0102 Zea m 12.0103 Zea m 12.0104	X73279 X73280 X73281 AF032370	CAA51718 CAA51719 CAA51720 AAB86960	P35081 P35082 P35083 O22655	(Kovar, et al., 2000)

			coli strain BL21(DE3)	Zea m 12.0105	AF201459	AAG35601	Q9FR39	
Pas n 13	48% of 71 GP-allergic patients	nPas n 13 - ammonium sulphate precipitation, hydrophobic interaction chromatography and size exclusion chromatography		N/A	N/A	N/A	N/A	(Davies, Voskamp, et al., 2011)
Sor h 13	43.8% of 64 GP-allergic patients	nSor h 13 – ammonium sulphate precipitation, hydrophobic interaction and size exclusion chromatography		Sor h 13.0101 Sor h 13.0201	KF887429 KF887430	AIL01320 AIL01321	A0A077B155 A0A077B569	(Campbell, et al., 2015)
Zea m 13	IgE reactive by immunoblotting in patients with maize pollen exposure		rZea m 13 – cDNA cloned into λ-ZapXR bacteriophage, plaque-purified	N/A	N/A	N/A	Q1ZYQ5	(Petersen, et al., 2006)
Cyn d 24	29% of 21 asthmatic, Bermuda GP-allergic patients	nCyn d 24 – CM-TSK column and reverse HPLC		Cyn d 24.0101	AY720896	AAU15051	Q647J6	(Chow, et al., 2005) (Horng-Der, et al., 1988)

References

Esch, R. E. (2004). Grass pollen allergens. Clinical allergy and immunology, 18, 185-205. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15042916

Gupta, A. (2002). Geoindicators for tropical urbanization. Environmental Geology, 42, 736–742. Retrieved from

Seidel, D. J., Fu, Q., Randel, W. J., & Riechler, T. J. (2008). Widening of the tropical belt in a changing climate. Nature Geoscience, 1, 21-24. Retrieved from

Morgan, J. A., LeCain, D. R., Pendall, E., Blumenthal, D. M., Kimball, B. A., Carrillo, Y., . . . West, M. (2011). C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. Nature, 476(7359), 202-205. doi: nature10274 [pii]10.1038/nature10274

Kongpanichkul, A., Vichyanond, P., & Tuchinda, M. (1995). Allergen skin test reactivities among asthmatic Thai children. Journal of Medical Association of Thailand, 80, 69-74. Retrieved from

Beggs, P. J. (2009). Climate change and plant food allergens. J Allergy Clin Immunol, 123(1), 271-272; author reply 272. doi: S0091-6749(08)01871-X [pii]10.1016/j.jaci.2008.10.025

Ziska, L. H., & Beggs, P. J. (2011). Anthropogenic climate change and allergen exposure: The role of plant biology. J Allergy Clin Immunol. doi: S0091-6749(11)01665-4 [pii]10.1016/j.jaci.2011.10.032

Davies, J. M. (2014). Grass pollen allergens globally: the contribution of subtropical grasses to burden of allergic respiratory diseases. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 44(6), 790-801. doi: 10.1111/cea.12317

Hensel, A. E., Jr., & Griffith, R. C. (1972). Clinical experiences with Paspalum notatum (Bahia grass): a new grass antigen. South Med J, 65(6), 690-693. Retrieved from

Cohen, S. G., & Zelaya-Quesada, M. (2002). Portier, Richet, and the discovery of anaphylaxis: a centennial. J Allergy Clin Immunol, 110(2), 331-336. Retrieved from

Calabria, C. W., & Dice, J. (2007). Aeroallergen sensitization rates in military children with rhinitis symptoms. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, 99(2), 161-169. doi: 10.1016/S1081-1206(10)60640-0

Andersson, K., & Lidholm, J. (2003). Characteristics and immunobiology of grass pollen allergens. International archives of allergy and immunology, 130(2), 87-107. doi: 10.1159/000069013

Johansen, N., Weber, R. W., Ipsen, H., Barber, D., Broge, L., & Hejl, C. (2009). Extensive IgE cross-reactivity towards the Pooideae grasses substantiated for a large number of grass-pollen-sensitized subjects. International archives of allergy and immunology, 150(4), 325-334. doi: 000226233 [pii]10.1159/000226233

Aalberse, R. C. (2007). Assessment of allergen cross-reactivity. Clin Mol Allergy, 5, 2. doi: 1476-7961-5-2 [pii]10.1186/1476-7961-5-2

Davies, J. M., Dang, T. D., Voskamp, A., Drew, A. C., Biondo, M., Phung, M., . . . O'Hehir, R. E. (2011). Functional immunoglobulin E cross-reactivity between Pas n 1 of Bahia grass pollen and other group 1 grass pollen allergens. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 41(2), 281-291. doi: 10.1111/j.1365-2222.2010.03670.x

Davies, J. M., Li, H., Green, M., Towers, M., & Upham, J. W. (2012). Subtropical grass pollen allergens are important for allergic respiratory diseases in subtropical regions. Clinical and translational allergy, 2(1), 4. doi: 10.1186/2045-7022-2-4

Weber, R. W. (2003). Patterns of pollen cross-allergenicity. J Allergy Clin Immunol, 112(2), 229-239. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12897724

White, J. F., & Bernstein, D. I. (2003). Key pollen allergens in North America. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, 91(5), 425-435; quiz 435-426, 492. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14692424

Burton, M. D., Papalia, L., Eusebius, N. P., O'Hehir, R. E., & Rolland, J. M. (2002). Characterization of the human T cell response to rye grass pollen allergens Lol p 1 and Lol p 5. Allergy, 57(12), 1136-1144. Retrieved from

Etto, T., de, B. C., Prickett, S., Gardner, L. M., Voskamp, A., Davies, J. M., . . . Rolland, J. M. (2012). Unique and cross-reactive T cell epitope peptides of the major Bahia grass pollen allergen, Pas n 1. International archives of allergy and immunology, 159(4), 355-366. doi: 10.1159/000338290

Eusebius, N. P., Papalia, L., Suphioglu, C., McLellan, S. C., Varney, M., Rolland, J. M., & O'Hehir, R. E. (2002). Oligoclonal analysis of the atopic T cell response to the group 1 allergen of Cynodon dactylon (bermuda grass) pollen: pre- and post-allergen-specific immunotherapy. International archives of allergy and immunology, 127(3), 234-244. Retrieved from http://www.online.karger.com/library/karger/renderer/dataset.exe?jcode=IAA&action=render&rendertype=fulltext&uid=IAA.iaa27234

Nony, E., Timbrell, V., Hrabina, M., Boutron, M., Solley, G., Moingeon, P., & Davies, J. M. (2015). Specific IgE recognition of pollen allergens from subtropic grasses in patients from the subtropics. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, 114(3), 214-220.e212. doi: 10.1016/j.anai.2014.12.005

Leiferman, K. M., & Gleich, G. J. (1976). The cross-reactivity of IgE antibodies with pollen allergens. I. Analyses of various species of grass pollens. J Allergy Clin Immunol, 58(1 PT. 2), 129-139. Retrieved from

Martin, B. G., Mansfield, L. E., & Nelson, H. S. (1985). Cross-allergenicity among the grasses. Ann Allergy, 54(2), 99-104. Retrieved from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=3970392

Phillips, J. W., Bucholtz, G. A., Fernandez-Caldas, E., Bukantz, S. C., & Lockey, R. F. (1989). Bahia grass pollen, a significant aeroallergen: evidence for the lack of clinical cross-reactivity with timothy grass pollen. Ann Allergy, 63(6 Pt 1), 503-507. Retrieved from

Ramirez, D. A., Andrews, C. P., Rather, C. G., & Jacobs, R. L. (2015). Responsiveness to timothy grass pollen in individuals without known natural exposure in an allergen challenge chamber. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology, 114(3), 226-232. doi: 10.1016/j.anai.2014.11.006

Kao, S. H., Su, S. N., Huang, S. W., Tsai, J. J., & Chow, L. P. (2005). Sub-proteome analysis of novel IgE-binding proteins from Bermuda grass pollen. Proteomics, 5(14), 3805-3813. doi: 10.1002/pmic.200401229

Pomes A, D. J., Gadermaier G, Hilger C, Holzhauser T, Lidholm J, Lopata A, Mueller GA, Nandy A, Radauer A, Chan SK, Jappe U, Kleine-Tebbe J, Thomas WR, Chapman MD, van Hage M, van Ree R, Vieths S, Raulf M, Goodman R. (2018). WHO/IUIS Allergen Nomenclature: providing a common language. Molecular Immunology(Special issue). Retrieved from

Drew, A. C., Davies, J. M., Dang, T. D., Rolland, J. M., & O'Hehir, R. E. (2011). Purification of the major group 1 allergen from Bahia grass

pollen, Pas n 1. International archives of allergy and immunology, 154(4), 295-298. doi: 10.1159/000321821

Cosgrove, D. J., Bedinger, P., & Durachko, D. M. (1997). Group I allergens of grass pollen as cell wall-loosening agents. Proceedings of the National Academy of Sciences, 94(12), 6559-6564. Retrieved from http://www.pnas.org/content/94/12/6559.abstract

Wang, T., Chen, Y., Tabuchi, A., Cosgrove, D. J., & Hong, M. (2016). The Target of beta-Expansin EXPB1 in Maize Cell Walls from Binding and Solid-State NMR Studies. Plant physiology, 172(4), 2107-2119. doi: 10.1104/pp.16.01311

Shen, H. D., Wang, S. R., Tang, R. B., Chang, Z. N., Su, S. N., & Han, S. H. (1988). Identification of allergens and antigens of Bermuda grass (Cynodon dactylon) pollen by immunoblot analysis. Clinical allergy, 18(4), 401-409. Retrieved from

Matthiesen, F., Schumacher, M. J., & Lowenstein, H. (1991). Characterization of the major allergen of Cynodon dactylon (Bermuda grass) pollen, Cyn d I. J Allergy Clin Immunol, 88(5), 763-774. Retrieved from

Ford, S. A., & Baldo, B. A. (1987). Identification of Bermuda grass (Cynodon dactylon)--pollen allergens by electroblotting. J Allergy Clin Immunol, 79(5), 711-720. Retrieved from

Ghobrial, G., Naser, S. A., Sweeney, M., & White, R. (2002). Identification and characterization of the allergenic proteins of Bahia grass (Paspalum notatum) pollen. International archives of allergy and immunology, 128(4), 304-309. Retrieved from

Davies, J. M., Mittag, D., Dang, T. D., Symons, K., Voskamp, A., Rolland, J. M., & O'Hehir, R. E. (2008). Molecular cloning, expression and immunological characterisation of Pas n 1, the major allergen of Bahia grass Paspalum notatum pollen. Molecular Immunology, 46(2), 286-293. doi: https://doi.org/10.1016/j.molimm.2008.08.267

White, J. M., Majidi, A., Nasar, S. A., Sweeney, M., & White, R. S. (2009). Characterisation of the Group I Allergen of Bahia Grass Pollen. Open Allergy Journal, 2, 27-29. Retrieved from

Timbrell, V. L., Riebelt, L., Simmonds, C., Solley, G., Smith, W. B., McLean-Tooke, A., . . . Davies, J. M. (2014). An immunodiagnostic assay for quantitation of specific IgE to the major pollen allergen component, Pas n 1, of the subtropical Bahia grass. International archives of allergy and immunology, 165(4), 219-228. doi: 10.1159/000369341

Avjioglu, A., Creaney, J., Smith, P. M., Taylor, P., Singh, M. B., & Knox, R. B. (1993). Cloning and characterization of the major allergen of Sorghum halepense, a subtropical grass. Molecular Biology and Immunology of Allergens, 161-164. Retrieved from

Campbell, B. C., Gilding, E. K., Timbrell, V., Guru, P., Loo, D., Zennaro, D., . . . Davies, J. M. (2015). Total transcriptome, proteome, and allergome of Johnson grass pollen, which is important for allergic rhinitis in subtropical regions. J Allergy Clin Immunol, 135(1), 133-142. doi: 10.1016/j.jaci.2014.06.034

Petersen, A., Suck, R., Hagen, S., Cromwell, O., Fiebig, H., & Becker, W. M. (2001). Group 13 grass allergens: structural variability between different grass species and analysis of proteolytic stability. J Allergy Clin Immunol, 107(5), 856-862. doi: 10.1067/mai.2001.114114

Swoboda, I., Grote, M., Verdino, P., Keller, W., Singh, M. B., De Weerd, N., . . . Spitzauer, S. (2004). Molecular characterization of polygalacturonases as grass pollen-specific marker allergens: expulsion from pollen via submicronic respirable particles. Journal of immunology (Baltimore, Md. : 1950), 172(10), 6490-6500. Retrieved from

Davies, J. M., Voskamp, A., Dang, T. D., Pettit, B., Loo, D., Petersen, A., . . . O'Hehir, R. E. (2011). The dominant 55 kDa allergen of the subtropical Bahia grass (Paspalum notatum) pollen is a group 13

pollen allergen, Pas n 13. Mol Immunol, 48(6-7), 931-940. doi: 10.1016/j.molimm.2010.12.013

Matricardi, P. M., Kleine-Tebbe, J., Hoffmann, H. J., Valenta, R., Hilger, C., Hofmaier, S., . . . Ollert, M. (2016). EAACI Molecular Allergology User's Guide. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology, 27 Suppl 23, 1-250. doi: 10.1111/pai.12563

Gunning, P. W., Ghoshdastider, U., Whitaker, S., Popp, D., & Robinson, R. C. (2015). The evolution of compositionally and functionally distinct actin filaments. Journal of cell science, 128(11), 2009-2019. doi: 10.1242/jcs.165563

Smith, P. M., Xu, H., Swoboda, I., & Singh, M. B. (1997). Identification of a Ca2+ binding protein as a new Bermuda grass pollen allergen Cyn d 7: IgE cross-reactivity with oilseed rape pollen allergen Bra r 1. International archives of allergy and immunology, 114(3), 265-271. Retrieved from

Suphioglu, C., Ferreira, F., & Knox, R. B. (1997). Molecular cloning and immunological characterisation of Cyn d 7, a novel calcium-binding allergen from Bermuda grass pollen. FEBS letters, 402(2-3), 167-172. Retrieved from

Huang, T. H., Peng, H. J., Su, S. N., & Liaw, S. H. (2012). Various cross-reactivity of the grass pollen group 4 allergens: crystallographic study of the Bermuda grass isoallergen Cyn d 4. Acta crystallographica. Section D, Biological crystallography, 68(Pt 10), 1303-1310. doi: 10.1107/s0907444912027552

Su, S. N., Shu, P., Lau, G. X., Yang, S. Y., Huang, S. W., & Lee, Y. C. (1996). Immunologic and physicochemical studies of Bermuda grass pollen antigen BG60. J Allergy Clin Immunol, 98(3), 486-494. Retrieved from

Chow, L. P., Chiu, L. L., Khoo, K. H., Peng, H. J., Yang, S. Y., Huang, S. W., & Su, S. N. (2005). Purification and structural analysis of the novel glycoprotein allergen Cyn d 24, a pathogenesis-related

protein PR-1, from Bermuda grass pollen. The FEBS journal, 272(24), 6218-6227. doi: 10.1111/j.1742-4658.2005.05000.x

Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoglu, S., Kauffmann, S., Geoffroy, P., . . . Fritig, B. (1993). Plant 'pathogenesis-related' proteins and their role in defense against pathogens. Biochimie, 75(8), 687-706. Retrieved from

Horng-Der, S., Wang, S. R., Tang, R. B., Chang, Z.-N., Su, S. N., & Han, S. H. (1988). Identification of allergens and antigens of Bermuda grass (Cynodon dactylon) pollen by immunoblot analysis. [Article]. Clinical Allergy: Journal of the British Allergy Society, 18(4), 401-409. Retrieved from https://gateway.library.qut.edu.au/login?url=https://search.ebscohost.com/login.aspx?direct=true&AuthType=ip,sso&db=afh&AN=16211225&site=ehost-live&scope=site

Oldenburg, M., Petersen, A., & Baur, X. (2011). Maize pollen is an important allergen in occupationally exposed workers. Journal of Occupational Medicine and Toxicology (London, England), 6, 32-32. doi: 10.1186/1745-6673-6-32

Petersen, A., Dresselhaus, T., Grobe, K., & Becker, W. M. (2006). Proteome analysis of maize pollen for allergy-relevant components. Proteomics, 6(23), 6317-6325. doi: 10.1002/pmic.200600173

Devanaboyina, S. C., Cornelius, C., Lupinek, C., Fauland, K., Dall'Antonia, F., Nandy, A., . . . Keller, W. (2014). High-resolution crystal structure and IgE recognition of the major grass pollen allergen Phl p 3. Allergy, 69(12), 1617-1628. doi: 10.1111/all.12511

Yang, S.-Y., You,Y.-J. and Chang,Z.-N. (2005). *Molecular cloning of a low molecular weight allergen of Bermuda grass pollen*.

Yennawar, N. H., Li, L. C., Dudzinski, D. M., Tabuchi, A., & Cosgrove, D. J. (2006). Crystal structure and activities of EXPB1 (Zea m 1), a beta-expansin and group-1 pollen allergen from maize.

Proceedings of the National Academy of Sciences of the United States of America, 103(40), 14664-14671. doi: 10.1073/pnas.0605979103

Li, L. C., Bedinger, P. A., Volk, C., Jones, A. D., & Cosgrove, D. J. (2003). Purification and characterization of four beta-expansins (Zea m 1 isoforms) from maize pollen. Plant physiology, 132(4), 2073-2085. Retrieved from

Chang, Z.-N., Liu, C.-C., Tam, M. F., Peng, H.-J., Tsai, J.-J., & Han, S.-H. (1995). Characterization of the isoforms of the group I allergen of Cynodon dactylon. Journal of Allergy and Clinical Immunology, 95(6), 1206-1214. doi: 10.1016/S0091-6749(95)70077-3

Au, L. C., Lin, S. T., Peng, H. J., Liang, C. C., Lee, S. S., Liao, C. D., & Chang, Z. N. (2002). Molecular cloning and sequence analysis of full-length cDNAs encoding new group of Cyn d 1 isoallergens. Allergy, 57(3), 215-220. Retrieved from

Christensen, L. H., Riise, E., Bang, L., Zhang, C., & Lund, K. (2010). Isoallergen Variations Contribute to the Overall Complexity of Effector Cell Degranulation: Effect Mediated through Differentiated IgE Affinity. The Journal of Immunology, 184(9), 4966-4972. doi: 10.4049/jimmunol.0904038

Hartl, A., Kiesslich, J., Weiss, R., Bernhaupt, A., Mostbock, S., Scheiblhofer, S., . . . Thalhamer, J. (1999). Isoforms of the major allergen of birch pollen induce different immune responses after genetic immunization. International archives of allergy and immunology, 120(1), 17-29. doi: 10.1159/000024216

Scala, E., Alessandri, C., Bernardi, M. L., Ferrara, R., Palazzo, P., Pomponi, D., . . . Mari, A. (2010). Cross-sectional survey on immunoglobulin E reactivity in 23,077 subjects using an allergenic molecule-based microarray detection system. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 40(6), 911-921. doi: 10.1111/j.1365-2222.2010.03470.x

Gonzalez-Mancebo, E., Dominguez-Ortega, J., Blanco-Bermejo, S., Gonzalez-Seco, E., Trujillo, M. J., & de la Torre, F. (2017). Comparison of two diagnostic techniques, skin-prick test and component resolved diagnosis in the follow-up of a cohort of paediatric patients with pollinosis. Multicentre pilot study in a highly exposed allergenic area. Allergologia et immunopathologia, 45(2), 121-126. doi: 10.1016/j.aller.2016.04.005

Saltabayeva, U., Garib, V., Morenko, M., Rosenson, R., Ispayeva, Z., Gatauova, M., . . . Valenta, R. (2017). Greater Real-Life Diagnostic Efficacy of Allergen Molecule-Based Diagnosis for Prescription of Immunotherapy in an Area with Multiple Pollen Exposure. International archives of allergy and immunology, 173(2), 93-98. doi: 10.1159/000477442

ThermoFisher (Singer-songwriter). (2012). Allergen Components, ImmunoCAP Allergen Information. On *http://www.inter-2t.phadia.com/en/Products/Allergy-testing-products/ImmunoCAP-Allergens/Allergen-components-list/*. Sweden.

Luo, W., Pan, G., Huang, H., Zheng, P., Wei, N., Zhang, Y., . . . Sun, B. (2017). A Component-resolved Diagnostic Approach for a Study on Grass Pollen Allergens in Chinese Southerners with Allergic Rhinitis and/or Asthma. Journal of visualized experiments : JoVE(124). doi: 10.3791/55723

Luo, W., Huang, H., Zheng, P., Wei, N., Luo, J., Sun, B., & Zeng, G. (2016). Major grass pollen allergens and components detected in a southern Chinese cohort of patients with allergic rhinitis and/or asthma. Mol Immunol, 78, 105-112. doi: 10.1016/j.molimm.2016.08.013

Cabauatan, C. R., Lupinek, C., Scheiblhofer, S., Weiss, R., Focke-Tejkl, M., Bhalla, P. L., . . . Valenta, R. (2014). Allergen microarray detects high prevalence of asymptomatic IgE sensitizations to tropical pollen-derived carbohydrates. J Allergy Clin Immunol, 133(3), 910-914.e915. doi: 10.1016/j.jaci.2013.10.004

Walker, S. M., Varney, V. A., Gaga, M., Jacobson, M. R., & Durham, S. R. (1995). Grass pollen immunotherapy: efficacy and safety during

a 4-year follow-up study. Allergy, 50(5), 405-413. doi: 10.1111/j.1398-9995.1995.tb01170.x

Bousquet, J., Lockey, R., & Malling, H.-J. (1998). Allergen immunotherapy: Therapeutic vaccines for allergic diseases A WHO position paper. Journal of Allergy and Clinical Immunology, 102(4), 558-562. doi: https://doi.org/10.1016/S0091-6749(98)70271-4

Dhami, S., Kakourou, A., Asamoah, F., Agache, I., Lau, S., Jutel, M., . . . Sheikh, A. (2017). Allergen immunotherapy for allergic asthma: A systematic review and meta-analysis. Allergy, 72(12), 1825-1848. doi: 10.1111/all.13208

Dhami, S., Nurmatov, U., Arasi, S., Khan, T., Asaria, M., Zaman, H., . . . Sheikh, A. (2017). Allergen immunotherapy for allergic rhinoconjunctivitis: A systematic review and meta-analysis. Allergy, 72(11), 1597-1631. doi: 10.1111/all.13201

Klimek, L., Pfaar, O., & Worm, M. (2016). New opportunities for allergen immunotherapy using synthetic peptide immuno-regulatory epitopes (SPIREs). Expert review of clinical immunology, 12(10), 1123-1135. doi: 10.1080/1744666x.2016.1189825

Larché, M., & Kay, A. B. (2004). The effects of T cell peptides in patients sensitive to cats. Clinical & Experimental Allergy Reviews, 4, 252-257. doi: 10.1111/j.1472-9725.2004.00062.x

Creticos, P. S. (2014). Advances in synthetic peptide immuno-regulatory epitopes. The World Allergy Organization journal, 7(1), 30. doi: 10.1186/1939-4551-7-30

Ellis, A. K., Frankish, C. W., O'Hehir, R. E., Armstrong, K., Steacy, L., Larché, M., & Hafner, R. P. (2017). Treatment with grass allergen peptides improves symptoms of grass pollen–induced allergic rhinoconjunctivitis. Journal of Allergy and Clinical Immunology, 140(2), 486-496. doi: 10.1016/j.jaci.2016.11.043

Ellis, A., Frankish, C. W., Armstrong, K., Larché, M., Steacy, L., Hafner, R., & O'Hehir, R. (2015). Persistent Treatment Effect with Grass Synthetic Peptide Immuno-Regulatory Epitopes in Grass Allergy Symptoms in an Environmental Exposure Unit Challenge after a Second Season of Natural Pollen Exposure. Journal of Allergy and Clinical Immunology, 135(2), AB158. doi: 10.1016/j.jaci.2014.12.1457

Huang, C. F., Chu, C. H., Wu, C. C., Chang, Z. N., Chue, F. L., & Peng, H. J. (2012). Induction of specific Th1 responses and suppression of IgE antibody formation by vaccination with plasmid DNA encoding Cyn d 1. International archives of allergy and immunology, 158(2), 142-150. doi: 10.1159/000331140

Huang, C. F., Wu, C. C., Chu, D. M., Chang, Z. N., Chue, F. L., & Peng, H. J. (2014). Neonatal vaccination with plasmid DNA encoding Cyn d 1 effectively prevents allergic responses in mice. American journal of rhinology & allergy, 28(3), e144-147. doi: 10.2500/ajra.2014.28.4020

Di Lorenzo, G., Mansueto, P., Pacor, M. L., Rizzo, M., Castello, F., Martinelli, N., . . . Ditto, A. M. (2009). Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy. [Research Support, Non-U.S. Gov't]. J Allergy Clin Immunol, 123(5), 1103-1110, 1110 e1101-1104. doi: 10.1016/j.jaci.2009.02.012

Darsow, U., Brockow, K., Pfab, F., Jakob, T., Petersson, C. J., Borres, M. P., . . . Huss-Marp, J. (2014). Allergens. Heterogeneity of molecular sensitization profiles in grass pollen allergy--implications for immunotherapy? Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 44(5), 778-786. doi: 10.1111/cea.12303

Savi, E., Peveri, S., Incorvaia, C., Dell'Albani, I., Marcucci, F., Di Cara, G., & Frati, F. (2013). Association between a low IgE response to Phl p 5 and absence of asthma in patients with grass pollen allergy. Clin Mol Allergy, 11(1), 3. doi: 10.1186/1476-7961-11-3

Hatzler, L., Panetta, V., Lau, S., Wagner, P., Bergmann, R. L., Illi, S., . . . Matricardi, P. M. (2012). Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. J Allergy Clin Immunol, 130(4), 894-901 e895. doi: 10.1016/j.jaci.2012.05.053

Asturias, J. A., Arilla, M. C., Gomez-Bayon, N., Martinez, J., Martinez, A., & Palacios, R. (1997). Cloning and high level expression of Cynodon dactylon (Bermuda grass) pollen profilin (Cyn d 12) in Escherichia coli: purification and characterization of the allergen. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 27(11), 1307-1313. Retrieved from

Kovar, D. R., Drobak, B. K., & Staiger, C. J. (2000). Maize profilin isoforms are functionally distinct. The Plant cell, 12(4), 583-598. Retrieved from

Davies, J. M., Bright, M. L., Rolland, J. M., & O'Hehir R, E. (2005). Bahia grass pollen specific IgE is common in seasonal rhinitis patients but has limited cross-reactivity with Ryegrass. Allergy, 60(2), 251-255. Retrieved from

Chang, Z. N., Peng, H. J., Lee, W. C., Chen, T. S., Chua, K. Y., Tsai, L. C., . . . Han, S. H. (1999). Sequence polymorphism of the group 1 allergen of Bermuda grass pollen. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 29(4), 488-496. Retrieved from

Smith, P. M., Suphioglu, C., Griffith, I. J., Theriault, K., Knox, R. B., & Singh, M. B. (1996). Cloning and expression in yeast Pichia pastoris of a biologically active form of Cyn d 1, the major allergen of Bermuda grass pollen. J Allergy Clin Immunol, 98(2), 331-343. Retrieved from

Wu, Y., Meeley, R. B., & Cosgrove, D. J. (2001). Analysis and expression of the alpha-expansin and beta-expansin gene families in maize. Plant physiology, 126(1), 222-232. Retrieved from