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Skin barrier dysfunction in common genetic disorders

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CHAPTER 3

THE FILAGGRIN GENE IN OTHER ALLERGIC CONDITIONS AND GENODERMATOSES

3.1 INTRODUCTION

3.1.1 Secondary atopic conditions related to atopic dermatitis

In some populations, up to 50% of children with moderate-to-severe AD carry *FLG* mutations and defects in *FLG* also predispose AD patients to asthma, allergic rhinitis and related allergic phenotypes (Marenholz *et al.*, 2006; Palmer *et al.*, 2006; Smith *et al.*, 2006; Schuttelaar *et al.*, 2009). Asthma (OMIM #600807) is characterised by coughing, wheezing and bronchial hyperresponsiveness (Illig and Wjst, 2002), while allergic rhinitis (OMIM #607154) is the inflammation of upper respiratory mucosal membranes caused by allergen exposure. Food allergy to common allergens like peanut has also been observed in at least one third of children with AD (Brown *et al.*, 2011). Collectively, AD, asthma allergic rhinitis and other atopic diathesis are grouped as hypersensitive, complex traits with multiple genetic and environmental aetiological factors.

Many AD patients are also plagued by inflammatory infections due to recurrent skin colonisation by *Staphylococcus aureus* (Hanifin and Rogge, 1977). Given the highly significant association of *FLG*-null mutations with AD, it is surprising that the effect of *FLG*-null mutations on recurrent infections in AD patients has not been well investigated. Interestingly, one study has found that patients with *FLG*-null mutations have an increased risk of eczema herpeticum (Gao *et al.*, 2009). Appreciation of the co-regulation

and inter-dependence of both epidermal permeability and antimicrobial barrier function in the stratum corneum have just begun to emerge (reviewed in Cork *et al.*, 2009).

3.1.1.1 Peanut allergy

One of the atopic conditions that is associated with *FLG*-null mutations is early-childhood food allergy (Bock *et al.*, 2007). In particular, allergy to peanut may result in severe immune response and lead to death if treatment is not rapidly admininstered (Bock *et al.*, 2007). The prevalance of peanut allergy has increased steadily in developed countries such as the United Kingdom and Canda over the past decades (reviewed in; Burks, 2008; Sicherer *et al.*, 2010), and is believed to have stabilised to a prevalence of 1.2 to 1.6% in pre-school and school children (Ben-Shoshan *et al.*, 2009; Sicherer *et al.*, 2010). In comparison, past studies in Asia reported a very low prevalence of peanut allergy and hypersensitivity in Asia (Hill *et al.*, 1999), although there is also a rising trend seen in urbanised countries such as Singapore (Chiang *et al.*, 2007).

The annual consumption of peanut in China and United States is similar (Beyer *et al.*, 2001) and it has been suggested that the difference in peanut allergy rates in Asia and western countries can be attributed to the preparation methods of peanuts, which can affect their allergenicity. Specifically, western populations frequently eat roasted peanuts, which have been demonstrated to possess a higher allergenicity compared to the boiled/raw peanuts consumed

in Asia (Beyer *et al.*, 2001). In addition, peanut allergy is strongly heritable, with a much stronger concordance rate (64%) in monozygotic twins compared to dizygotic twins (7%) (Sicherer *et al.*, 2000). Therefore, it is possible that the variation in peanut allergy rates might also be caused by discrete genetic differences between western and Asian populations.

Recently, a study by Brown *et al*. confirmed the loss-of-function variants in *FLG* as a significant risk factor for peanut allergy in food challenge-positive patients (P=3.0 X 10^{-6}; OR=5.3; 95% CI=2.8-12.2), and this association was replicated in a large Canadian study (P=5.4 X 10^{-5}; OR=1.9; 95% CI=1.4-2.6). The association of *FLG*-null mutations with peanut allergy remains significant (P=0.008) after controlling for the confounding effection of co-existent AD in these patients (Brown *et al.*, 2011). In Asian countries such as Singapore, peanut sensitisation has been linked to a family history of AD, a young age of presentation and polysensitisation to other foods, including shellfish, cow's milk and wheat (Chiang *et al.*, 2007). However, the genetic basis of food allergy, including peanut sensitisation in Asian remained poorly characterised.

3.1.2 Dry skin and acne vulgaris

Acne vulgaris (acne) is a very common skin condition, affecting 88-94% of Singaporean adolescents (Tan *et al.*, 2007; Yosipovitch *et al.*, 2007). Familial studies have suggested that significant genetic factors interplay with environmental influences such as diet and personal hygiene to cause acne (Goulden *et al.*, 1999; Bataille *et al.*, 2002). Acne breakouts affect the areas

of skin richly supplied with sebaceous follicles, including the face, the upper part of the chest, and the back. The clinical features of acne include seborrhoea, comedone formation, inflammatory pustles, nodules, and cysts, with resultant scarring. Acne patients are believed to have a propensity for follicular epidermal hyperproliferation with subsequent occlusion of the follicle; this can lead to inflammation when hormonal imbalances, excess sebum production and hypersensitivity to *Propionibacterium acnes* also occur (Purdy and de Berker, 2006). *P. acnes* is an anaerobic bacteria and can promote inflammation through a variety of mechanisms – studies have shown that *P. acnes* produce lipases which liberate pro-inflammatory fatty acids from sebum to activate the toll-like receptor 2 on monocytes and neutrophils. Subsequently, an inflammatory cascade is triggered to release interleukins 12 and 8 and tumor necrosis factor (Kim *et al.*, 2002).

In **Chapter 2**, the crucial role of filaggrin in the maintenance of skin barrier moisture was demonstrated but its influence on other complex skin disorders like acne is still currently unclear. Previous immunohistochemistry studies showed increased filaggrin expression in the sebaceous duct and infundibulum of acne skin (Kurokawa *et al.*, 1988); *P. acnes* strains also increase the expression of filaggrin and other differentiation-specific markers in normal human epidermal keratinocytes *in vitro* and in the suprabasal layers of human skin explants (Jarrousse *et al.*, 2007). Similarly, inflammatory cytokines resulted in increased filaggrin expression in sebaceous gland explants (Guy and Kealey, 1998). Currently, it is not known if inflammation represents a primary or secondary phenomenon acne development; the basis of change in

filaggrin expression in acne skin has also not been determined. Recently, a study conducted on 284 European patients presenting at a dermatology clinic showed an association of *FLG*-null mutations with dry skin; at the same time, it was also observed that the *FLG*-null mutations were negatively associated with a group of patients with self-recalled acne (OR=0.3; 95% CI=0.1-1.0). This suggested that inheritance of *FLG*-null alleles might lead to decreased incidence of acne; however, this association did not reach statistical significance (P=0.08) and requires further investigation (Sergeant *et al.*, 2009).

3.1.3 Aims of this chapter

- Examine the association of *FLG*-null mutations with recurrent skin infection in Singaporean Chinese patients with AD.

- Investigate the association *FLG*-null mutations with peanut sensitisation in a pilot study of 27 peanut sensitised Singaporean Chinese children.

- Explore the potential contribution of *FLG*-null mutations towards the pathogensis of acne vulgaris in an adult Singaporean Chinese cohort.

3.2 MATERIALS AND METHODS

3.2.1 Study populations

3.2.1.1 Childhood-adolescent Singaporean Chinese atopic dermatitis cohort (sub-group)

For further study of the association of *FLG*-null mutations with recurrent infections in the presence of AD, patients recruited from the NSC (n=354) as described in **Chapter 2.2.2** were included in this sub-group study. The patient or parent/guardian completed both interviewer- and self-administered questionnaires. Data collected included demographic characteristics, age of onset of AD, personal history of physician-diagnosed atopic diseases such as asthma or allergic rhinitis, prior episodes of disease flares and skin infections in the last year that required antibiotics and details of treatment usage. In addition, the medical records of each patient were retrospectively reviewed to corroborate the information. A disease flare was defined as an episode of disease exacerbation requiring an escalation in therapy or hospital admission; an infective episode was defined as an episode of skin infection with clinical signs such as crusting, oozing, and/or purulent discharge that necessitated systemic or topical antibiotic therapy.

AD patients (n=71) from the National University Hospital were not included in this study by the collaborator's choice.

3.2.1.2 Peanut sensitisation pilot study

Twenty-seven Singaporean Chinese peanut sensitised children were collected as part of an ongoing investigation approved by the ethics committee of the Kendang Kerbau Women's and Children's Hospital, Singapore. Analysis was carried out on patients who were referred to the skin prick test (SPT) laboratory from the allergy and pulmonary outpatient clinics during a 3-year period between 2003 and 2006; patients were included in the analysis if they had a history of suspected food hypersensitivity and if they had clear evidence of food-allergen sensitisation [as ascertained by at least one positive SPT on the food allergen panel as described (Chiang *et al.*, 2010)]. All SPTs were performed in the respiratory laboratory by one of three experienced technicians using the Greer-Pick SPT device (Greer Laboratories, Lenoir, NC, USA) applied to the forearm or the back (for children aged under 2 years) and subsequently evaluated for weal size after 15-20 minutes. Results were regarded as positive if the mean weal diameter greater than the negative control. Histamine (10 mg/ml) was used as a positive control (Vanto, 1983).

3.2.1.3 Adult Singaporean Chinese acne vulgaris cohort

A total of 287 Singaporean Chinese patients presenting with acne to the National Skin Centre, a major dermatology outpatient facility in Singapore, were recruited. The patients have a mean age of 22 years (SD 4.8), range 14-50 (27% <20 years of age and 95% were under 30 years) and 76.3% were male. Acne symptoms were reported for a mean of 5.6 years (SD 4.2), range from <1 to 32 years. Patients with polycystic ovarian syndrome were excluded from this study. Acne severity was assessed using the Global Severity Assessment Score (Lehmann *et al.*, 2002) – 100 patients had (34.8%) had mild acne, 129 (44.9%) had moderate acne and 58 (20.3%) had severe acne. Population controls (n=440) with unknown acne status from the Singapore Bio-Bank were used as detailed in **Chapter 2.2.2**.

All studies were approved by the local domain specific ethical review board in accordance with the declaration of Helsinki and all participants gave written informed consent.

3.2.2. Screening for Asian specific *FLG*-null mutations

All 287 acne cases, 27 children from the peanut sensitisation pilot study, and 440 population controls were screened for all 22 population-specific *FLG*-null mutations as described in **Chapter 2.2.4**.

Two novel *FLG*-null mutations, c.6834del5 and c.8157delC were detected in the process of screening for c.6950del8 and p.S2706X mutations respectively and confirmed by alternative methods.

All sequencing methods were devised and validated by Dr. Rebecca Haines and myself; *FLG*-null mutation screening of all samples were completed with the assistance of Dr. Rebecca Haines, Ms Christabelle Goh and Dr. John Common from the Institute of Medical Biology, Singapore.

3.2.3 Statistical analysis

For the acne case-control cohort and peanut sensitisation pilot study, statistical analysis was performed using Fisher's exact test and logistic regression analyses as described in **Chapter 2.2.6.** Power calculations were performed using Quanto version 1.2.4 (University of Southern California, http://hydra.usc.edu.gxe/). Analyses were performed in collaboration with Dr. Sara Brown from the University of Dundee, UK.

To test for the influence of *FLG*-null mutations on recurrent infections within the sub-group of AD patients recruited from NSC, AD patients were stratified into AD_{FLG} and $AD_{NON\text{-}FLG}$ groups. The Fisher's exact test was used to investigate all genotype-phenotype associations. After adjusting for age and gender, odd ratios were calculated using logistic regression models that were fitted for a case-control type of comparison within this cohort of AD patients. For continuous variables, such as the average number of disease flares in a

year, ANOVA was conducted to compare across the genotype groups. Data were analysed using SAS version 9.1 (SAS Institute Inc, Cary, NC, USA). This analysis was performed by Ms Sophie Cai from the National Skin Centre, Singapore.

In all cases, only patients with complete data for all screened *FLG*-null mutation were included in the combined *FLG*-null genotype analysis.

3.3 RESULTS AND DISCUSSION

3.3.1 *FLG*-null mutations increase the susceptibility of recurrent infections in the presence of atopic dermatitis

A subset of patient (n=324) from the childhood-adolescent AD cohort described in **Chapter 2.2.2** was further analysed for recurrent infections. Detailed demographic characteristics, genotype and clinical features are shown in **Table 3.1**. A total of 21.6% patients belonged to the AD_{FLG} group – 3.7% carried two *FLG*-null mutations while 17.9% carried a single *FLG*-null mutation. In this AD cohort, *FLG* mutations were not detected in 78.4% of the patients (denoted as $AD_{NON\text{-}FLG}$).

The majority of patients (94.4%) had moderate-severe AD with a mean objective SCORAD of 37.0 (0-77; **Table 3.1**). 75% of patients carrying two *FLG*-null mutations had severe AD, whereas 48.3% of patients with one *FLG* mutation and 36.2% of $AD_{NON\text{-}FLG}$ patients were diagnosed with severe AD. Collectively, AD_{FLG} was significantly associated with severe AD (P=0.013; OR=1.98, 95% CI=1.14 – 3.43), early onset of AD before two years of age (P<0.0001; OR=5.02, 95% CI=2.77-9.11), and severe IV (P=0.002; OR=3.88, 95% CI=1.68-8.96) as compared to $AD_{NON\text{-}FLG}$ (**Table 3.2**).

Table 3.1 Additional clinical features of a subgroup of patients in the childhood-adolescent AD cohort

Demographic and clinical features	Number	Percentage (%)
Sex		
Male	222	68.5
Female	102	31.5
Onset of AD		
< 2 years	133	41.1
≥ 2 years	191	58.9
FLG genotype[1]		
AA	254	78.4
Aa	58	17.9
aa	12	3.7
AD severity[2]		
Severe	129	39.8
Moderate	177	54.6
Mild	18	5.6
Ichthyosis vulgaris (IV)	151	46.6
Severe	25	7.7
Moderate	36	11.1
Mild	90	27.8
Recurrent infections requiring antibiotics >4 times in past year	33	10.2
Asthma	94	30.7
Allergic rhinitis	213	65.7
Palmar hyperlinearity	131	40.4
Keratosis pilaris	22	6.8

Table 3.2 Association of *FLG*-null mutations with recurrent infections in Singaporean Chinese with AD

Skin phenotype and clinical feature		*FLG* genotype			Combined *FLG*-null genotype		
	n	AA	Aa	aa	*P* value	OR	95% CI
AD severity							
Severe	128	92	28	9	0.013	1.98	1.14 – 3.43
Moderate	178	148	26	5	0.015	0.52	0.30 – 0.89
Mild	18	14	4	0	0.896	1.08	0.34 – 3.49
Early-onset AD (<2 years of age)	134	85	37	12	<0.0001	5.02	2.77 – 9.11
Recurrent infections requiring antibiotics >4 times in past year	33	9	18	6	<0.0001	12.51	4.98 – 31.45
Mean no. of flares in past year	227	0.3	1.3	1.1	<0.0001	-	-
Treatment in past year							
Topical steroids							
Highly potent	24	7	14	3	<0.0001	11.06	4.13 – 29.62
Potent	150	98	41	11	<0.0001	9.49	3.27 – 27.55
Mild	198	158	35	5	<0.0001	0.17	0.07 – 0.40
Oral prednisolone	55	26	23	6	<0.0001	7.04	3.39 – 14.64
Systemic immunosuppressants	10	4	4	2	0.016	5.72	1.43 – 22.87
Topical calcineurin inhibitors	28	12	11	5	<0.0001	5.38	2.33 – 12.44

Table 3.1 also details the treatment history of AD patients in the past 12 months. Of note, 33 patients (10.2%) had recurrent skin infections requiring antibiotics greater than 4 times in the past year. AD_{FLG} was strongly associated with recurrent infections requiring either topical or oral antibiotics >4 times (P<0.0001; OR=12.5, 95% CI=4.98 – 31.5) in the past year as well as with increased number of disease flares (P<0.0001). AD_{FLG} was also associated with treatment using highly potent topical steroids (P<0.0001), topical calcineurin inhibitors (P<0.0001), systemic prednisolone (P<0.0001) and immunosuppressants (P=0.016). As it was possible that patients with severe AD were more likely to develop recurrent infections requiring antibiotics, we further investigated the association of AD_{FLG} with recurrent infections in different AD severity groups. The association with AD_{FLG} remained significant even within the severe and moderate AD groups; the OR for developing recurrent infections was 37.4 (95% CI=3.73 – 374.3) if a patient carrying 2 *FLG*-null mutations had severe AD.

A key novel finding of this subgroup analysis was that AD_{FLG} is associated with a significantly increased risk of recurrent bacterial skin infections requiring antibiotics >4 times within the past year (P<0.0001). This remained significant after analysis within subgroups of different AD severity and even in the subsequent 1-year follow-up of patients (results not shown). This suggested that the increased risk of recurrent infections is attributed to the presence of at least 1 *FLG*-null mutation in AD patients. In addition, we found that AD_{FLG} patients had significantly more disease flares in a year. Consequently, AD_{FLG} patients were more likely to require more aggressive

therapy with highly potent topical steroids, systemic prednisolone and corticosteroid sparing immunosuppressive agents.

These results highlight the importance of the epidermal skin barrier as an integral part of the cutaneous antimicrobial barrier. In particular, it underscores the role that filaggrin plays in bacterial colonisation and secondary infections in AD skin. Filaggrin is crucial in maintaining not only the physical barrier against microbes, but also the acid mantle of the skin after it is hydrolysed to hydrophilic amino acids, in particular *trans*-UCA and PCA (reviewed in Cork *et al.*, 2009). A previous study showed that AD patients with *FLG*-null mutations had significantly higher skin pH (Jungersted *et al.*, 2010). Increased pH has been shown to facilitate bacterial growth of *Staphyloccus aureus* as well as increased serine protease activity, which further aggravates the barrier defect in AD (reviewed in O'Regan and Irvine, 2008).

It is important to acknowledge that these associations do not necessarily prove an aetiological link with *FLG* genotype. Infections may be a result of disease severity *per se*, or from the treatment used in severe AD, such as the systemic immunosuppressants. However, we note that a recent study did not find an increased risk of infectious diseases in Asian AD patients treated on long-term cyclosporine therapy (Kim *et al.*, 2010).

In conclusion, this study showed for the first time that *FLG*-null mutations are associated with increased risk of recurrent bacterial skin infections and disease

flares in Singaporean Chinese AD patients. This suggests a worse prognosis for AD_{FLG} patients and preventive treatment of these patients should commence as early as possible.

3.3.2 *FLG*-null mutations are not significantly associated with peanut sensitisation in 27 Singaporean Chinese children

This is the first report of *FLG*-null mutations in an Asian population of peanut sensitised patients. *FLG*-null mutations were detected in 4 out of 27 (14.8%) of our peanut sensitised patients compared to a prevalance of 7.3% in the Singaporean Chinese population. Three patients were heterozygous for the p.S406X mutation, one patient was heterozygous for the 3321delA mutation (**Table 3.3**). However, in this pilot study, *FLG*-null mutations are not significantly associated with peanut sensitisation in Singaporean Chinese children (Fisher's exact test P=0.180; OR=2.3; 95% CI=0.76-7.20).

In contrast to a similar underlying genetic basis leading to AD in both Europe and Asia as discussed in **Chapter 2**, the lack of association of *FLG*-null mutations to peanut sensitisation in this pilot study suggested that the effect of peanut allergen sensitisation through increased transcutaneous exposure (due to a skin barrier defect) might be less important in Asian peanut sensitisation cases. Nevertheless, we appreciate that the barrier defect caused by *FLG*-null mutations might still be of fundamental importance in the development of peanut sensitisation because this condition is associated with AD; early prevention of AD might be helpful in ameliorating other atopic symptoms.

Table 3.3 *FLG*-null mutations do not associate significantly with peanut sensitisation in 27 peanut-sensitised children in Singapore

***FLG* mutation**	**Cohort**	***FLG* Genotype[1]**				***P* value**
		AA	**Aa**	**aa**	**Total**	
c.S406X	Controls	438	2	0	440	**0.002**
	Cases	24	3	0	27	
c.2282del4	Controls	436	1	0	437	1.000
	Cases	27	0	0	27	
c.3321delA	Controls	430	10	0	440	0.484
	Cases	26	1	0	27	
p.S1302X	Controls	434	4	0	438	1.000
	Cases	27	0	0	27	
S1515X	Controls	438	2	0	440	1.000
	Cases	27	0	0	27	
c.6950del8	Controls	436	4	0	440	1.000
	Cases	27	0	0	27	
c.7945delA	Controls	439	1	0	440	1.000
	Cases	27	0	0	27	
p.S2706X	Controls	431	7	0	438	1.000
	Cases	27	0	0	27	
p.R4307X	Controls	438	1	0	439	1.000
	Cases	27	0	0	27	
***FLG*-null combined genotype**	Controls	403	29	1	433	**0.180**
	Cases	23	4	0	27	

[1] – 'aa' in the *FLG*-null combined genotype refers to patients who carry two *FLG*-null mutations. These patients may be homozygous (carrying the same *FLG* mutation in both alleles) or compound heterozygotes (carrying a different *FLG* mutation in each allele).

Among the limitations of this study is the small number of patients (n=27) available for this pilot study, which might be inadequate to detect weak effects of *FLG*-null mutations as a predisposing factor to peanut allergy in an Asian population. Moreover, not all patients underwent peanut challenges to determine their clinical reactivity. The double-blind, placebo-controlled food challenge is considered the gold standard for the definitive diagnosis of peanut allergy (Kim *et al.*, 2010), but this is not always possible to execute. In future, large prospective longitudinal cohort studies in Asia are needed for the elucidation of the prevalence of peanut allergy in this region to provide adequate characterisation of the genetic and environmental effects responsible for the aetiology of peanut allergy.

3.3.3 *FLG*-null mutations do not protect against acne vulgaris

In the acne vulgaris cohort, a total of 12 known *FLG*-null mutations were detected – p.S406X, c.1249insG, c.2282del4, c.3321delA, p.S1302X, p.S1515X, c.6950del8, p.Q2417X, p.E2422X, c.7945delA, p.S2706X and p.R4307X. Two previously unreported mutations c.6834del5 and c.8157delC were also detected (**Table 3.4**). 8.2% of the Singaporean Chinese acne cases carried one or more *FLG*-null mutations and this was not significantly different from the 7.3% prevalence of *FLG* mutations in the population controls (Fisher's exact test P=0.783, OR=1.2; 95% CI=0.7-2.1; **Table 3.4**). Assuming a population prevalence of 88% for acne (Tan et al., 2007), this case-control cohort size would give a power of 99% to detect an odds ratio of 0.3 (Sergeant *et al.*, 2009) for the combined *FLG*-null genotype, with a two-

Table 3.4 *FLG*-null mutations do not confer decreased susceptibility to acne vulgaris

***FLG* mutation**	**Acne vulgaris cases & unselected population controls**	***FLG* Genotype[1]**			**Total**	***P value***
		AA	**Aa**	**aa**		
p.S406X	Cases	279	0	0	279	0.524
	Controls	438	2	0	440	
c.1249insG	Cases	276	4	0	280	0.023
	Controls	440	0	0	440	
c.2282del4	Cases	275	0	0	275	1.000
	Controls	436	1	0	437	
c.3321delA	Cases	277	4	0	281	0.582
	Controls	439	10	0	440	
p.S1302X	Cases	272	0	0	272	0.304
	Controls	434	4	0	438	
p.S1515X	Cases	275	1	0	276	1.000
	Controls	438	2	0	440	
c.6834del5*	Cases	280	1	0	281	0.390
	Controls	440	0	0	440	
c.6950del8	Cases	278	3	0	281	1.000
	Controls	436	4	0	440	
p.Q2417X	Cases	278	2	0	280	0.151
	Controls	440	0	0	440	
p.E2422X	Cases	278	2	0	280	0.151
	Controls	440	0	0	440	
c.7945delA	Cases	274	2	0	276	0.562
	Controls	439	1	0	440	
p.S2706X	Cases	276	1	0	280	0.160
	Controls	431	7	0	438	
c.8157delC*	Cases	278	1	0	279	0.388
	Controls	440	0	0	440	
p.R4307X	Cases	278	1	0	279	0.488
	Controls	438	1	0	439	
Combined *FLG* null genotype	Cases	235	21	0	256	**0.783**
	Controls	402	31	1	434	

* = Previously unreported *FLG*-null mutation

sides P=0.05. Therefore, it is unlikely that the lack of association of *FLG*-null mutations with acne was due to lack of power of the study. Furthermore, the comprehensive screening of all 22 reported *FLG*-null mutations from this

carefully characterised Singaporean Chinese population meant that incomplete ascertainment of the *FLG* genotype was hardly possible unless the mutation is very rare. Our findings indicate that filaggrin haploinsufficiency is unlikely to have a protective effect in acne vulgaris; it is likely that the overexpression of filaggrin is not important for the pathogenesis of this condition but might be a secondary phenomenon due to pathologic changes in keratinocyte differentiation.

3.4 CONCLUSIONS

In conclusion, the harbouring of *FLG*-null mutations by AD patients could result in the atopic march, including asthma, allergic rhinitis, peanut allergy, and in this study, the increased susceptibility for recurrent skin infections in the presence of AD.

However, we did not detect an association of *FLG*-null mutations with peanut sensitisation when a pilot cohort of 27 peanut sensitised Singaporean Chinese children was studied. Peanut allergy is a multi-factorial condition caused by the complex interaction of environmental and genetic factors, therefore this lack of association with *FLG*-null mutations might be due to distinct ethnic differences between Asia and Europe. The mechanism of peanut sensitisation remains unclear; therefore a larger cohort of well-characterised peanut allergy patients will be necessary to assess the genetic and environmental diversity in Asia.

FLG-null mutations were also excluded as a protective factor in the pathogenesis of acne vulgaris; its increased expression in acne skin is likely to be a secondary consequence in the Singaporean Chinese population.