DOCTOR OF PHILOSOPHY

Skin barrier dysfunction in common genetic disorders

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DECLARATION

I declare that I am the sole author of this thesis and that all references cited have been consulted by me personally. The work, of which this thesis is a record, has been done by myself, unless otherwise acknowledged. This work has not been previously submitted for a higher degree.

The thesis research is funded by the Agency for Science, Technology and Research (A*STAR) Graduate Academy, in a joint partnership between A*STAR, Singapore and the Unversity of Dundee.

Signed……

Huijia Chen

Date……

STATEMENT

I certify that Huijia Chen has fulfilled the conditions of the University of Dundee and that she is qualified to submit the accompanying thesis in the application for the degree of Doctor of Philosophy.

Signed……

Professor E.Birgitte Lane

Date……

Signed……

Professor W.H.Irwin McLean

Date……

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In March 2007, I returned to the McLean Laboratory for two years to start my Ph.D studies, working under the excellent mentorship of Aileen and Irwin. I will also like to thank everyone else in the human genetics laboratory for making me so at home in Dundee – with special heart-felt thanks to Frances for being constantly around to give advice on anything; Linda and Liz for their motherly, cheerful chatter; Yiwei, Liao and Toshi for their feisty banter and Sara for her kindness and constant enthusiasm to teach.

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LIST OF ABBREVIATIONS

6-FAM	6-carboxyfluorescein
AA	alopecia areata
AD	atopic dermatitis
AGC	protein kinase A/protein kinase G/protein kinase C
ALSPAC	Avon Longitudinal Study of Parents and Children
BH	bleomycin hydrolase
CAMK	Ca^{2+}/calmodulin-dependent protein kinase
CCM	chemical cleavage of mismatch
CDSN	corneodesmosin gene
CE	cornified envelope
CK1	casein kinase 1
CK2	casein kinase 2
CSGE	conformation sensitive gel electrophoresis
DGGE	denaturing gradient gel electrophoresis
DHPLC	denaturing high-performance liquid chromatography
DMSO	dimethyl sulphoxide
DNA-PK	DNA-dependent protein kinase
dNTP	deoxyribonucleotide triphosphate
DRR	distal regulatory region
EBS	epidermolysis bullosa simplex
EDC	epidermal differentiation complex
EDTA	ethylenediaminetetraacetic acid

EFAD	essential fatty acid deficiency
FGS	future generation sequencing
FISH	fluorescent *in situ* hybridisation
FLG	filaggrin gene
GSK3	glycogen synthase kinase 3
GWAS	genome-wide association studies
H&E	haematoxylin & eosin
HA	heteroduplex analysis
HEX	hexachloro-fluorescein
HMG-coA	hydroxymethylglutaryl coA reductase
HTSS	hypotrichosis simplex of the scalp
IL	interleukin
IV	ichthyosis vulgaris
IVL	involucrin gene
KLK5	kallikrein 5
KORA	Co-operative Health Research in the Region of Augsburg
LCE	late cornified envelope proteins
LD	linkage disequilibrium
LEKTI	lymphoepithelial kazal-type related inhibitor
LOR	loricrin gene
MAF	minor allele frequency
$MgCl_2$	magnesium chloride
MHC	major histocompatibility complex
NGS	next generation sequencing
NMF	natural moisturising factor

NOD	nucleotide-binding oligomerisation domain
NPV	negative predictive value
NSC	National Skin Centre
NUH	National University Hospital
oSCORAD	objective SCORAD
P. acnes	*Propionibacterium acnes*
PAD	peptidylarginine deiminase
PAMP	pathogen associated microbial patterns
PC	pachyonychia congenita
PCA	pyrrolidone carboxylic acid
PCR	polymerase chain reaction
PCSK6	proprotein convertase subtilisin/kexin type 6
PEP1	profilaggrin endopeptidase
PPAR	peroxisome-proliferator-activated receptor
PPase	protein phosphatase
PPV	positive predictive value
PRR (Chapter 1)	pattern recognition receptor
PRR (Chapter 2)	proximal regulatory region
PS	psoriasis
PTC	premature-termination-codon
PTT	protein truncation test
RDEB	recessive dystrophic epidermolysis bullosa
SCCE	stratum corneum chymotryptic enzyme
SCORAD	SCORing Atopic Dermatitis
SCTE	stratum corneum tryptic enzyme

SD	standard deviation
SNP	single nucleotide polymorphism
SPINK5	serine protease inhibitor Kazal type 5 gene
SPRR	small proline-rich proteins
SPT	skin prick test
SSCP	single stranded conformation polymorphism
STS	steriod sulphatase gene
TEWL	trans-epidermal water loss
TGM	transglutaminase
Th2	T-helper type 2
TLR	Toll-like receptor
UCA	urocanic acid
UTR	untranslated region
XLI	X-linked ichthyosis

ABSTRACT

One of the most important roles of the skin is the formation of an effective barrier to prevent desiccation as well as to keep out foreign pathogens and allergens. This is a tightly regulated process and involves many structural proteins, lipids, enzymes and biochemical components. One of the proteins that has an indispensable role in barrier formation is filaggrin, which is encoded by the filaggrin gene (*FLG*) that lies within a cluster of epidermal genes known as the epidermal differentiation complex (EDC) on chromosome 1q21. Recent studies in Europe have shown that null mutations in *FLG* lead to the loss of the filaggrin protein; this is the underlying genetic cause of ichthyosis vulgaris (IV) and is a significant predisposing factor for atopic dermatitis (AD) and other atopic conditions such as asthma, allergic rhinitis and food allergy. In this thesis, the critical role of *FLG*-null mutations was examined and confirmed as a strong predisposing factor for AD in Singaporean Chinese patients. In addition, AD patients with *FLG* mutations also showed an increased susceptibility for recurrent skin infections. Interestingly, a diverse and wide spectrum of *FLG*-null mutations was identified in the Singaporean Chinese population, as opposed to the dominance of a few common *FLG* mutations in Europe. This result highlighted discrete genetic variations between different ethnic groups. *FLG*-null mutations were also shown to have significant gene modifying effects on other skin barrier genes such as steroid sulphatase gene (*STS*) to exacerbate the phenotype of X-linked ichthyosis (XLI). Next, the effect of *FLG*-null mutations on other complex conditions such as acne vulgaris and childhood

peanut sensitisation was investigated but no significant association of *FLG* mutations with these diseases were observed in the Singaporean Chinese population. Lastly, a study was attempted to search for a candidate gene for psoriasis within the EDC, through the use of fine mapping techniques. With the advent of faster and cheaper next generation sequencing (NGS) in the near future, the quest for susceptibility factors in complex traits will increase in effectiveness and speed.

(334 words)