

University of Dundee

DOCTOR OF PHILOSOPHY

Skin barrier dysfunction in common genetic disorders

Chen, Huijia

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Skin barrier dysfunction in common genetic disorders

Huijia Chen

2011

University of Dundee

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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 University 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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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 <i>in situ</i> hybridisation |
| <i>FLG</i> | 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 |
| <i>IVL</i> | 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 |
| <i>LOR</i> | 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 |
| <i>P. acnes</i> | <i>Propionibacterium acnes</i> |
| 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 |
| <i>SPINK5</i> | serine protease inhibitor Kazal type 5 gene |
| SPRR | small proline-rich proteins |
| SPT | skin prick test |
| SSCP | single stranded conformation polymorphism |
| <i>STS</i> | 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)