**Supplementary material for:**

**Palm reading and water divining: A cross-sectional study of the accuracy of palmar hyper-linearity and trans-epidermal water loss to identify individuals with a filaggrin gene null mutation**

Lowe AJ, PhD1,2, Lee B, MBBS1, Orchard D, FACD3, King E, MNP3, Abramson MJ, PhD 4, Allen KJ, PhD 2, Hui J, PhD 5, Southey MC, PhD 6, Lodge CJ, PhD 1,2\*, Dharmage SC, PhD 1,2\*.

\*Equal senior author.

1. Allergy and Lung Health Unit, Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia
2. Murdoch Children’s Research Institute, Melbourne, Australia
3. Department of Dermatology, Royal Children’s Hospital, Melbourne, Australia.
4. School of Public Health & Preventive Medicine, Monash University, Melbourne, Australia.
5. School of Population Health, the University of Western Australia, Perth, Australia.
6. Department of Clinical Pathology, The Melbourne Medical School, The University of Melbourne, & Melbourne Australia & Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Melbourne, Australia, & Cancer Epidemiology Division, Cancer Council Victoria, Victoria, Australia

## Supplementary Methods:

The Melbourne Atopy Cohort Study (MACS) is a single-centre study of children with a family history of allergic disease1. A total of 620 infants were recruited before birth, between 1990 and 1994 in Melbourne, Australia. Infants were eligible if one or more of their first-degree family members had asthma, allergic rhinitis, eczema, or severe food allergy. The study was originally a randomized trial of three infant formulas2. Written informed consent was obtained from all mothers at the time of enrolment. When the original MACS participants were approximately 18 years of age, they, and their family members were invited to attend a clinical follow-up. All participants at the 18-year follow-up provided written consent. The project was approved by the Human Research Ethics Committee of the Mercy Hospital for Women (up to 12 years, reference numbers R07/20 and R88/06) and Royal Children’s Hospital (18 years, reference number 28035).

Genetic testing of the *FLG* gene mutations werecarried out on blood or saliva samples collected at the 18-year follow-up. Saliva samples were collected using OraGene collection kit (DNA Genotek Inc.) with the DNA extracted using the standardized techniques. The five most common *FLG*-null mutations in Caucasian populations (R501x, S3247X, 2282del4, R2447X, 3702delG) were genotyped using the Taqman platform by PathWest Laboratory Medicine of Western Australia.

To count the number of palm lines, a midline was first marked out from middle of wrist to the base of thumb. Vertical lines, were counted if they were more than 2 cm and crossed the midline. The horizontal lines were counted if they were more prominent than the vertical lines, more than 2cm in length and crossed the midline.

Interactions between palmar hyperlinearity and TEWL, as well as sex and current AD were tested using likelihood ratio tests. For the continuous measurements of TEWL and vertical and horizontal lines, potential non-linearity of associations was assessed using Stata’s “fracpoly” command.

## Supplementary results:

In total, there was one homozygote *FLG*-null mutation identified (R501X) and one compound heterozygote identified (R501X and 2282del4). The most common *FLG*-null mutation was 2282del4 (n=15), followed by R501X (n=14), R2447X (n=5), S3247X (4). No participant was found to have a null mutation in 3702delG

Adjusting the hyper-linearity or line count for TEWL did not materially alter the results. While there was no evidence of non-linearity in the associations between horizontal lines or TEWL and *FLG* null mutations, the proportion of participants with *FLG* null mutations did not increase after 14 vertical lines, and possibly decreased.

There was some evidence that the association with any hyperlinearity and *FLG-*null mutations was stronger in females (OR=13.3, 95%CI=2.8-63.5) than males (3.9, 95%CI=1.4-11.0, p for interaction = 0.15). There was also some evidence that the per vertical line association with *FLG* null was stronger in those with current eczema (OR=1.1, 95%CI=1.0-1.2) than in those without current eczema (1.04, 99%CI=0.99-1.10, p interaction =0.16, note, strata specific associations listed here could not be estimated allowing for family clustering due to small numbers)

## References

1 Lowe AJ, Lodge CJ, Allen KJ *et al.* Cohort Profile: Melbourne Atopy Cohort study (MACS). *International journal of epidemiology* 2017; **46**: 25-6.

2 Lowe AJ, Hosking CS, Bennett CM *et al.* Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: A randomized controlled trial. *The Journal of allergy and clinical immunology* 2011; **128**: 360-5.