Peer Review File

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Reviewer A

Comment 1: Please add the two global burden of diseases epidemiological articles as reference. [10.1016/j.eclinm.2023.102193, 10.1111/all.15807]
Reply 1: Thank you for the suggestion. More information on the global epidemiology of eczema has been added in the introductory paragraph. (Please see Page 6, Lines 127-130)
Changes in the text: According to the Global Burden of Disease Study in 2019, eczema has an age-standardized prevalence rate of 2.28%, with peak prevalence in children aged between five and nine (1). It accounts for 36.17% of immune-mediated inflammatory disease cases and is more commonly observed in individuals with higher socio-demographic status (1,2).

Comment 2: Please add in the introduction that proliferation of atopic eczema guidelines adapted to the national reality [10.1111/dth.13121] that contribute in term of applicability to increase the compliance of the patients lowered during covid [10.1111/dth.13508]
Reply 2: Thank you for the suggestion — the establishment of national guidelines, like that by Damiani *et al*, was added to illustrate the treatment spectrum of eczema. (Please see Page 6, Lines 130-133)
Changes in the text: The treatment modality of choice is individualized, primarily depending on patients' signs and symptoms (3). Damiani *et al* established an Italian guideline to maintain a standard of care for eczema control and guide practitioners in adopting the novel biologic — dupilumab for eczema (3).

Comment 3: Please explain how the environment conditions [10.23736/S2784-8671.22.07386-8, 10.1097/DER.0000000000000772], age [10.1111/jdv.17094], may act in terms of triggers by changing the microbes' interaction in the cutaneous microbiome. [10.3389/fmicb.2022.944365]
Reply 3: Thank you for the suggestion. The authors have added extra sentences to illustrate the role of environment and age on cutaneous microbiome interaction. (Please see Page 6, Lines 134-140)
Changes in the text: The pathogenesis of eczema is multifactorial with environmental factors and genetic predisposition; these patients are thought to have skin barrier defects accompanied by skin dysbiosis (4). Studies from the coronavirus disease 2019 (COVID-19) pandemic suggested mask-wearing may be associated with exacerbated eczema (5), possibly owing to the increased abundance of Malassezia fungi on skin (6,7). The composition of cutaneous microbiome not only varies between healthy and eczematous individuals, but also with age among the latter group (8).

Comment 4: Please mention how therapies can alter the risk to develop eczema [10.26355/eurrev_202206_28977, 10.26355/eurrev_202109_26652]
Reply 4: Thank you for the suggestion. At the end of the discussion section, the authors

mentioned the possibility of studying SNPs of IL-4 gene in future studies and for studying any association between SNPs and efficacy and adverse drug reaction profiles of dupilumab therapy. The authors hope that this coheres with the study's focus on the role of genetics in eczema. (Please see Page 15, Lines 418-422)
Changes in the text: This study was also limited to examining the SNPs of *TGFB1, IL10*, *IL6R* and *STAT3.* Future research may investigate the associations between SNPs of the IL-4 gene and variations in the efficacy and adverse drug reactions of dupilumab efficacy (9), and SNPs of the STAT6 gene and the usefulness of topical nanoparticle cream (10).

Comment 5: Please in the conclusion try to evaluate the new potential of topical creams with new nanotechnologies [10.1016/j.bioactmat.2019.11.003]
Reply 5: Thank you for the suggestion. As reported by Damiani *et al*, some STAT6-binding nanoparticle drugs are under development for use as topical cream, so I mentioned the possibility of studying STAT6 gene single nucleotide polymorphisms in future studies at the end of the discussion section. (Please see Page 15, Lines 418-422)
Changes in the text: This study was also limited to examining the SNPs of *TGFB1, IL10*, *IL6R* and *STAT3.* Future research may investigate the associations between SNPs of the IL-4 gene and variations in efficacy and adverse drug reactions of dupilumab efficacy (50) as well as between SNPs of STAT6 gene and the usefulness of topical nanoparticle cream (51).

Reviewer B

This is an interesting area of study in a population where more research is required. It aims to investigate the influence of a set of immune regulatory genes in the pathogenesis of eczema in Asian children which appears to differ from Caucasian children.
My main concerns with the study are related to the validity of the control group and the descriptions of the sub phenotypes and high and low risk groups. The issues are detailed below:

Introduction -
Comment 1: The sentence beginning IgE is... on line 79 needs some grammatical correction and is somewhat simplistic.
Reply 1: Thank you for the suggestion. This sentence has been rephrased; hopefully it is more concise and accurate. (Please see Page 6, Lines 143-146)
Changes to the text: IgE is produced by plasma cells as part of the adaptive immune response; this pathway is promoted by helper T (Th2) cell-mediated cytokines interleukin (IL)-4 and IL-13 (11).

Comment 2: The paragraph beginning on line 99 needs either the past or present tense to be used uniformly.
Reply 2: The authors are grateful for this reminder. This paragraph has been modified such that it is uniformly in present tense. (Please see Page 7, Lines 167-175)
Changes to the text: TGFβ-1 interacts with multiple cell types to inhibit cell proliferation and

apoptosis (12). IL-10, produced by B1 lymphocytes and found in normal skin (13), serves two main functions: (i) inhibiting synthesis of pro-inflammatory cytokines and (ii) inducing class switching in B cells (14). IL-6 is crucial for skin barrier repair and for forming complexes with *IL-6R*, which then migrate to the damaged epidermal layers for repairing permeability barriers through increased *STAT3* phosphorylation (15). STAT3 is a key transcription regulator with multiple roles in inflammation and immune responses; mutations of this gene are associated with immunological diseases such as hyper-IgE syndrome. STAT3 activation is also involved in IL-10 and IL-6 anti-inflammatory signalling (16).

Methods -

Comment 3: Could you replace the term 'genetically unrelated children' with unrelated children including no siblings?

Reply 3: Well noted, thank you for the comment. (Please see Page 8, Line 203)

Changes to the text: This study recruited unrelated children, including those without siblings, with physician-diagnosed eczema and non-allergic controls from both pediatric clinics of our university-affiliated teaching hospital and several community-based studies in Hong Kong (19-22).

Comment 4: Line 121 - explain recruitment from community-based studies?

Reply 4: Thank you for the enquiry. Details regarding recruitment from these community-based studies have been added to the manuscript. (Please see Page 8, Lines 206-210)

Changes in the text: The latter community-based studies were conducted in local schoolchildren primarily for elucidating the epidemiology of childhood obesity and metabolic syndrome (23-26). The participating subjects who suffered from physician-diagnosed eczema and those free from any allergic disease were also recruited into this study. Their blood samples were subjected to our SNP genotyping and specific IgE measurement.

Comment 5: Overall the subject selection need much better description. What determined whether a SPT or a plasma IgE was performed? How many of each were in the subjects and controls?

Reply 5: Thank you for the enquiry. Details have been added regarding the use of SPT versus plasma IgE in this study. (Please see Page 9, Lines 237-239)

Changes in the text: Allergen sensitization was assessed by either SPT with standardized crude extracts of *Dermatophagoides pteronyssinus*, cat dander, and mixed cockroaches (ALK Abelló, Round Rock, TX, USA) or by plasma allergen-specific IgE (sIgE) levels to the same allergens by fluorescent enzyme immunoassay (AutoCAP, Phadia AB, Uppsala, Sweden) as decided by the individual studies. In general, SPT was used in studies involving subjects recruited in hospitals while blood IgE assays were used in community-based studies.

Comment 6: It is concerning that over 50% of the controls, whilst not reporting a history of clinical disease, had positive sensitisations and were therefore considered atopic. In the age groups studied the controls still have the possibility of demonstrating subsequent symptoms.

Reply 6: Thank you for the comment; indeed this issue was not well elaborated in the original version. Details have been added in an attempt to account for the phenomenon. (Please see Page

14, Lines 390-418)
Changes in the text: For example, atopy could only be assessed in 946 cases and 553 controls in total. Among the latter, 97 controls were detected by SPT and 456 controls were identified by allergen-specific IgE assays. This study found high rate of atopy among the controls who were free from any asthma, rhinitis and eczema. Although this atopy prevalence was high in our controls, this finding was consistent with our earlier genetic studies of Hong Kong children with different allergic diseases (19,20,22,46,47). The reasons accounting for this high rate of atopy are unclear, but such may be related to high indoor exposure to house dust mites and other inhalant allergens (48,49). Besides, our controls were significantly older (difference in mean of 2.4 years) than the cases (***Table 2***), suggesting that their clinical status as controls — freed from allergic diseases — was more reliable at an older age.

Comment 7: Many references are made to the sub phenotypes - they need to be specifically defined in the Methods section.
Reply 7: Thank you for the reminder - indeed the correlation between eczema and parameters (eosinophil count, allergen sensitization, total IgE levels) was not well-illustrated in the original version. A paragraph has been added to describe this correlation. (Please see Page 8, Lines 220-224)
Changes in the text: The subphenotypes of eczema refer to factors which predict progression to eczema and its severity (27). Eosinophil count (eos%), expressed as the percentage of total peripheral blood leukocytes, positively correlates with serum total IgE concentration, early eczema onset and persistence of eczematous lesions (28). Allergen sensitization, measured by SPT or IgE levels, is often present in eczematous children (19,20,29,30).

Comment 8: Much more description of how the GMDR performed the classification into high risk and low risk populations is needed here and how many were in each group. Exactly what do you mean by high risk and low risk in this study?
Reply 8: Thank you for the enquiry. GMDR categorized subjects into different risk groups for having a certain phenotype among eczema cases and controls when they had different combinations of genotypes (i.e. 2-locus, 3-locus and so on). It would not tell the exact number of subjects with each risk group, but, as in Figure 1, the figures above the bars in each cell are the scores calculated from GMDR analysis. More details have been added to the text. (Please see Page 10, Lines 277-279)
Changes in the text: Subjects were classified by GMDR into low-risk and high-risk groups; their genotypes predicted the respective risks for having eczematous phenotypes and subphenotypes such as high eos% and raised total IgE levels. The results were stratified by subjects' eczema status.

Results -
Comment 9: Line 192 states that subjects with eczema had significantly higher LogIgE than controls yet Table 2 shows 1.8 +/- 0.7 for eczema and 2.7+/- 0.8 for controls?
Reply 9: Thank you for the comment. We apologize for the discrepancy in data. The LogIgE values in Table 2 have been amended.
Changes in the text: Not applicable; please see Table 2.

Comment 10: Line 197 - the p value quoted in the text is different to the table - P=0.001 compared to P=0.009

Reply 10: Thank you for the comment. We apologize for the discrepancy in data. The p value should be 0.009. (Please see Page 11, Line 301)

Changes in the text: *TGFB1*_rs1800469 was associated with both eczema (odds ratio [OR] 0.82, 95% confidence interval [CI] 0.73-0.92; P=0.001) (***Table 3***) and atopic eczema (OR 0.83, 95% CI 0.72-0.95; P=0.009), with the former association still significant after adjusting for coexisting asthma (P=0.005).

Comment 11: As mentioned in the Methods section the results would have been much easier to interpret if the sub phenotypes of eczema had been better delineated earlier.

Reply 11: This is well understood. A paragraph has been added in Page 8 Lines 217-221, in an attempt to better illustrate the correlation between eczema and the subphenotypes (eosinophil count, allergen sensitization etcetera).

Changes in the text: The subphenotypes of eczema refer to factors which predict progression to eczema and its severity (27). Eosinophil count (eos%), expressed as the percentage of total peripheral blood leukocytes, positively correlates with serum total IgE concentration, early eczema onset and persistence of eczematous lesions (28). Allergen sensitization, measured by SPT or IgE levels, is often present in eczematous children (19,20,29,30).

Comment 12: I think Fig S1 is superfluous.

Reply 12: Well received; Fig S1 has been deleted.

Changes to the text: The sentence concerning Fig S1, as well as the figure itself, have been deleted.

Discussion -

Comment 13: Whilst the findings of the study are interesting they are not well discussed in the context of previous publications. For example, the section relating to published studies investigating IL-6, Th-17 and IL-33 are not well-linked to the results of this study. Identify your main findings and then place them in context more closely.

Reply 13: We agree with this comment, and have deleted the paragraphs on IL-6, Th-17 and IL-33 in hope of achieving a more focused discussion. We also linked our findings of *IL6R* polymorphism to current literature. (Please see Page 12, Lines 334-335)

Changes in the text: In particular, the contribution of *IL6R* polymorphism in atopic dermatitis aligns with a recently published study involving Asian children (32).