Author's response to reviews

Title: Prevalence of asthma and allergies in the Greek-Cypriot and Turkish-Cypriot communities in Cyprus: a bi-communal cross-sectional study.

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Author's response to reviews: see over
04 March 2013

Dr. Isabella Annesi-Maesano,
Associate Editor, BMC Public Health

Dear Dr Annesi-Maesano,

Re: Manuscript MS: 1199178007804063 entitled "Prevalence of asthma and allergies in the Greek-Cypriot and Turkish-Cypriot communities in Cyprus: a bi-communal cross-sectional study": Point-by-point reply to reviewer’s comments

We would like to thank you for offering us the opportunity to resubmit a revised version of the above manuscript. We would particularly like to thank the reviewers (Dr Karadag and Dr Videla) for taking the time to evaluate our manuscript. We are grateful for their constructive comments and suggestions, which helped clarify some important issues and improve the paper.

We were happy to see that the reviewer recognized the importance of the findings of this first collaborative bi-communal study in Cyprus investigating with a standardized protocol the prevalence of asthma and allergies among children from two communities that have lived apart for nearly 40 years. With lack of recent and consistent data from the Turkish-Cypriot community, we speculated that the prevalence of asthma and allergies would be lower on the north of the island. However, contrary to our original hypothesis, we found similar if not higher burden of asthma and rhinoconjunctivitis (but not eczema) among T/C children, not explained by the less “westernized” lifestyle. We are certain that, like the reviewers, these findings would be of interest to the readers of BMC Public Health.

We have now addressed all the recommendations and issues raised by the reviewers. Please find our point-by-point responses as well as relevant action. Corresponding changes are also highlighted in the manuscript. We also found your suggestion about including Venn diagrams showing the
interrelationship between the different study outcomes very useful. Thus, we have added a figure (Figure 1) to the manuscript while as a result previous Figure 1 has been re-named Figure 2. Also, following your suggestion, we have now also refrained from using acronyms (specifically G/C and T/C) in the legends of the Tables.

As a result of the reviewers’ suggestions we added three articles to the reference list (and hence previous numbering has changed). Other than this, we have only made some minor editing changes to the rest of the text.

We look forward to receiving your response.

Yours sincerely,

Panayiotis Yiallouros, M.D., PhD
Point–by point reply to the comments of Dr Bulent Karadag

Major Revisions

1) Methods: In terms of investigating the effect of possible confounding factors, it might be interesting to include all variables such as doctor diagnosis of asthma, etc. In contrast to lower rate of parental history of allergy, the prevalence of allergic symptoms were higher in the T/C community. So including the diagnosis of allergy and lifetime asthma and allergic symptoms would strengthen the findings.

Reply: We agree with the reviewer on this point. It is very interesting that the prevalence of asthmatic and allergic symptoms is higher in the T/C community, despite the much lower reported prevalence of family history of allergy in this community. As we mention in the Discussion, we believe that this is more likely to be a result of underestimation among T/C family members, perhaps due to difference in diagnostic labeling or reduced access to specialized care in the previous generation. As we couldn’t rule out with certainly whether there are discrepancies in the understanding and/or use of diagnostic labeling between the two communities in our days, we had originally decided to refrain from comparing diagnostic outcomes, and focused only on reported symptoms. Nevertheless, following the reviewers suggestion, we have now extended our investigation to asthma, eczema and hay fever diagnosis as well as ever wheeze. It appears that with the exception of diagnosis of eczema (which was also the only outcome for which higher frequency of symptoms were reported among the G/C community), consistently higher prevalence of ever wheeze, diagnosis of asthma and hay-fever was observed in the T/C community in both age groups. The full results of this analysis are now presented in Table 1 below. In the interest of keeping the manuscript focused on symptoms outcomes, we did not include the full Table in the manuscript but comment in the text of the manuscript on the extra analysis (in Methods, Definition of Outcome Variables) and the results (in Results, Prevalence of asthma and allergic symptoms in the G/C and T/C communities, last paragraph), as these reinforce our original speculation that family history of allergy may have been underestimated among T/C parents.
Table 1. The prevalence of asthma and allergic diagnostic outcomes investigated among 7-8 and 13-14 year-old participating children from the G/C and T/C communities along with unadjusted and adjusted odds ratios (and 95% CI) comparing the two communities.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>G/C</th>
<th>T/C</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR† (95% CI)</th>
<th>Adjusted OR‡ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prevalence (95% CI)</td>
<td>Prevalence (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever Wheeze</td>
<td>25.31 (23.54, 27.18)</td>
<td>26.86 (25.11, 28.69)</td>
<td>0.92 (0.81, 1.05)</td>
<td>0.82 (0.76, 0.99)</td>
<td>0.92 (0.76, 1.12)</td>
</tr>
<tr>
<td></td>
<td>17.84 (16.29, 19.50)</td>
<td>19.29 (17.75, 20.93)</td>
<td>0.91 (0.78, 1.06)</td>
<td>0.86 (0.69, 1.07)</td>
<td>0.95 (0.77, 1.19)</td>
</tr>
<tr>
<td>Diagnosis Asthma</td>
<td>13.93 (12.53, 15.45)</td>
<td>4.16 (3.42, 5.05)</td>
<td>3.72 (2.95, 4.74)</td>
<td>2.29 (1.64, 3.23)</td>
<td>2.59 (1.87, 3.61)</td>
</tr>
<tr>
<td>Diagnosis Eczema</td>
<td>5.40 (4.52, 6.44)</td>
<td>(10.41, 13.00)</td>
<td>0.43 (0.34, 0.54)</td>
<td>0.39 (0.28, 0.54)</td>
<td>0.48 (0.35, 0.65)</td>
</tr>
<tr>
<td>Diagnosis Hay-fever</td>
<td>7.69 (6.70, 8.82)</td>
<td>10.69 (9.65, 11.82)</td>
<td>0.70 (0.58, 0.84)</td>
<td>0.76 (0.59, 0.97)</td>
<td>0.82 (0.64, 1.05)</td>
</tr>
<tr>
<td></td>
<td>11.28 (10.08, 12.60)</td>
<td>15.37 (14.15, 16.68)</td>
<td>0.70 (0.60, 0.82)</td>
<td>0.73 (0.59, 0.90)</td>
<td>0.79 (0.64, 0.98)</td>
</tr>
<tr>
<td>Diagnosis Eczema</td>
<td>3.42 (2.77, 4.23)</td>
<td>0.64 (0.41, 0.98)</td>
<td>5.53 (3.46, 9.28)</td>
<td>5.58 (3.06, 10.64)</td>
<td>6.05 (3.34, 11.46)</td>
</tr>
<tr>
<td>Diagnosis Hay-fever</td>
<td>2.85 (2.25, 3.59)</td>
<td>10.18 (9.17, 11.28)</td>
<td>0.26 (0.20, 0.34)</td>
<td>0.23 (0.16, 0.32)</td>
<td>0.25 (0.18, 0.35)</td>
</tr>
</tbody>
</table>

† For 7-8 years old: Adjusted for sex, parent’s nationality, parent’s level of education, area of residence, family history of allergy, number of siblings, passive smoking, animals at home, maternal smoking during pregnancy, mode of delivery, birth weight, exclusive breastfeeding duration and bedroom sharing.

‡ For 13-14 years old: Adjusted for sex, parent’s nationality, parent’s level of education, area of residence, family history of allergy, number of siblings, passive and active smoking and animals at home.

‡ Adjusted for all the above participant characteristics except family history of allergy.
2) Another interesting point is the looking at the difference of the prevalence of atopic disorders among the Cypriot and the immigrant groups. These data may also be presented in the Results.

Reply: Stratifying the analysis in terms of the ethnic background of the participants is certainly interesting as, at least to some extent, it would shed additional light to whether the observed differences between the communities are the result of different genetic composition of the study samples from each community. In all analyses presented in the manuscript, we have adjusted for parents’ nationality (i.e. defined as “both parent of Cypriot origin (T/C or G/C)” Vs “at least one parent not of Cypriot origin). However, bounded by the study protocol as approved by the United Nations Development Program (UNDP-ACT) and the research teams from both communities, we cannot release separate data by ethnic background in the T/C community as, politically, this is a very sensitive issue. In order to overcome this difficulty, we have now performed a sensitivity analysis by repeating the models restricting the analysis to those children whose parents are both of Cypriot (T/C or G/C) origin, without presenting total sample numbers. The results of this analysis are now presented in a new Table - named Table 3; thus, all the Tables that appear in the previous version of the manuscript have now been thereafter renumbered accordingly.

Inferences regarding the observed differences between the two communities remain largely unchanged when non-Cypriot children are excluded from the analysis. The prevalence in the T/C community moves downwards in the case of all respiratory outcomes, thus the gap between the two communities appears smaller and bigger in the case of eczema. This is suggestive of a higher prevalence among the children in the T/C community whose parents are not both T/C (i.e. immigrant groups). Nevertheless, and while confidence intervals become wider due to the reduced sample size, the prevalence still remains higher for all study outcomes (other than eczema) in the T/C rather than the G/C community. These results are now described alongside the newly added Table in the updated version of the manuscript (in Results, Prevalence of asthma and allergic symptoms in the G/C and T/C communities, pp 12. last paragraph).

3) In terms of developing logistic regression models, which methods were used (stepwise, etc.)?

Reply: We thank the reviewer for this comment as it gave us the opportunity to clarify further the statistical methods, especially since the second reviewer (Dr Videla) has also commented on this. The main factor under investigation in this study was membership in either the G/C or T/C community. Thus, stepwise models were not used since the purpose here was not to identify a set of risk/protective factors but to explore differences in the prevalence outcomes between the two communities while taking into account the socio-demographic composition and the frequency of potential risk factors in these communities in order to investigate the extent to which any differences in these between the two
communities account for the observed differences in the study outcomes. A hierarchical approach was used whereby the crude estimates (model 1) were adjusted for socio-demographic characteristics and a priori selected main risk factors for asthma/allergy excluding (model 2) and including (model 3) family history of allergy for which a substantial (and doubtful) discrepancy was observed between the two communities. In order to explore whether any of the investigated factors are differentially related to the study outcomes in the two communities, at the final stage models were extended to include an interaction term between each factor and community. The statistical significance was tested in Likelihood Ratio Tests (LRT) for effect modification comparing models with and without the interaction terms. The Methods, Statistical analyses section of the manuscript was now been re-written to reflect this process more accurately.

4) It seems that different risk factors play role in G/C and T/C communities. It may also be presented separately to understand the differences in both communities.

Reply: Indeed, we agree with the reviewer that it is important to examine the role of each risk factor in the two communities separately. However, presenting the stratified analysis would not add value to the paper, especially since the risk factors identified were similar, with the very few exceptions that are already mentioned in the manuscript. For each risk factor, we examined evidence of effect modification by community by including interaction terms in the models. In general, while the observed magnitude of association with some of the risk factors in the stratified analysis appeared different between the two communities, there were only a very few cases were there was any statistical evidence of effect modification. The risk factors for which a different strength of association was observed between the two communities are now presented more clearly in the Results section (pp.14, pp.15-16) of the article, reporting separately the ORs in the two communities.

5) Limitations may be discussed at the 2nd or 3rd paragraph of the Discussion. Although the authors think that the lower participation rate is not problematic in G/C group, due to the cultural differences it might result a lower participation of allergic children into the study.

Reply: We have now moved the paragraph discussing the Limitations of the study from the end of the Discussion section to the 2nd paragraph as suggested by the reviewer. We have also explained that, although the socio-demographic composition of the G/C sample supports its representativeness, we cannot entirely rule out that the findings are not affected by the lower participation among G/C children. Differential selection bias (whereby allergic G/C children were less likely to participate in the study than T/C allergic children) would result in an underestimation of the true prevalence in the G/C
community. This has now been added in the Limitations (Discussion, pp. 18, first paragraph). Nevertheless, we do not see any reason why the incentive to participate would be differential amongst allergic children in the two communities, in which case differences might be even larger than those recorded.

6) Also the different findings in age groups should be discussed.

Reply: We thank the reviewer for his interest in the differences in the two age-groups. These findings were already discussed at several points of the Discussion and we generally feel that further expansion on these would make the text more extensive and difficult for the general reader to follow. However, in response to the reviewer’s suggestion, we have now added a short summary of the main observed differences between the younger and older age groups (Discussion, pp. 20, 2nd para), before discussing each of these in more detail. These were the association of (a) number of siblings with the study outcomes (which was restricted to the younger children), (b) area of residence (with lower prevalence of wheeze in rural areas but only among older children) and (c) exposure to tobacco smoke (with consistently higher risk for all study outcomes in older, but not younger children).

Minor Revisions

Table 5 can be omitted

Reply: We feel that the issue of a potentially differential association between family history of allergy and study outcomes between the two communities is central to the article and it would be better if Table 5 (with the full results of this analysis) remain in the manuscript. The frequency of family history was markedly lower in the T/C community compared to the G/C community, and this is the reason that all the previous analyses were repeated to include and exclude family history from the models. This led us to suspect that there might have been underestimation and thus misclassification in the case of T/C, in which case we would expect that the strength of association between the study outcomes and family history would be weaker in T/C. Indeed, the analysis showed that at least in the case of the younger age-group, the magnitude of association with family history was consistently larger in the G/C community. However, this was not the case with the older age-group and, overall, there was no statistical evidence that the association of family history with the study outcomes differed between the two communities. We have now explained this logic more clearly in the Results, Association of family history of allergy with asthma and allergic symptoms section.
Major Compulsory Revisions

1. Title page: Add the word “children of Greek- Cypriot and Turkish-Cypriot communities”
Reply: We would like to thank the reviewer for his suggestion. The title has been modified accordingly and it now reads “Prevalence of asthma and allergies in children from the Greek-Cypriot and Turkish - Cypriot communities in Cyprus: a bi-communal cross-sectional study.”

2. Abstract: the conclusions should state clearly that: 1) the prevalence of respiratory outcomes in the G/C group was lower that the T/C group 2) The results seem to contradict the hygiene hypothesis as the most educated and urban located population had the lower prevalence
Reply: Following the reviewer’s suggestion we have now rephrased the Results section of the abstract to clearly state that in contrast to our speculation the prevalence rates of respiratory outcomes (but not eczema) in the G/C community were lower than the T/C community in both age-groups. Nevertheless, we are reluctant to conclude that our results contradict the hygiene hypothesis with regards to all the factors that have been historically projected with it. While many presumably protective factors (as projected in the hygiene hypothesis) were indeed more prevalent in the T/C community (such as less urban environment and bedroom sharing), the opposite was observed for other factors such as nursery attendance in the first year of life which was more common in the G/C community. Moreover, only a very small number of families kept farm animals at home even in the T/C community, while we didn’t have information on other important aspects, such as specifically living in a farming environment.

3. Methods section: Definition of predictor variables: please explain more clearly if the child or the parent reported the risk factors, specially in terms of smoking exposure. It seems obvious that the parent described them, but the last paragraph inclines the reader to think that the risk factors were answered by the parents only for the 7 – 8 year old children.
Reply: In Methods, Study population and design, we have now more clearly stated that according to the ISAAC protocol the questionnaires were completed by the parents of the younger children and it was self-completed by the older children, which is why no information was available on several factors in this age-group including maternal smoking habits during pregnancy (the older children only provided information about their own smoking habits), birth weight, duration of exclusive
breastfeeding and nursery attendance in first year of life.

2. Statistical analysis: Rephrase the analysis strategy in a more straightforward manner: i.e: “A multiple logistic regression (stepwise?) analysis was applied to estimate the association between risk factors and outcomes”. I believe adjustment is a better term that effect modification in order to describe the analysis process.

Reply:  We thank the reviewer for this comment as it gave us the opportunity to clarify any confusion around this issue, especially since the first reviewer (Dr Karadag) has also commented on this—see point 3 above. The main factor under investigation in this study was membership in either the G/C or T/C community. Thus, stepwise models were not used since the purpose here was not to identify a set of risk/protective factors but to explore differences in the prevalence outcomes between the two communities while taking into account the socio-demographic composition and the frequency of potential risk factors in these communities in order to investigate the extent to which any differences in these between the two communities account for the observed differences in the study outcomes. A hierarchical approach was used whereby the crude estimates (model 1) were adjusted for socio-demographic characteristics and a priori selected main risk factors for asthma/allergy excluding (model 2) and including (model 3) family history of allergy for which a substantial (and doubtful) discrepancy was observed between the two communities. In order to explore whether any of the investigated factors are differentially related to the study outcomes in the two communities, at the final stage models were extended to include an interaction term between each factor and community. The statistical significance was tested in Likelihood Ratio Tests (LRT) for effect modification comparing models with and without the interaction terms. The Methods, Statistical analyses section of the manuscript was now been re-written to reflect this process more accurately.

5. Results: Population characteristics: The first phrase would be put at the end of the paragraph for clarity. First enumerate all the protective factors and the prevalence and at the end conclude saying that protective factors were more prevalent in the T/C community

Reply:  This has now been moved following the reviewer’s suggestion.

Minor Essential Revisions

1. Background section: First paragraph: Use “innate advantage” instead of “nativity”

Reply:  “Nativity” has been changed to “innate advantage”.

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2. Methods section: Study population: include information on validation of the Greek and Turkish versions of the ISAAC

Reply: We have now included a short description of existing evidence regarding the validity of the Greek and Turkish versions of the ISAAC questionnaire in the Methods/Definition of Outcome Variables of the manuscript. Specifically, we discuss that while “The Greek version of the ISAAC questionnaire has not been officially validated, the translation used in this study is the only available Greek version of the ISAAC core questionnaire which has been adopted by the ISAAC study and has been used by two ISAAC centers in Greece (Athens, Thessaloniki). Furthermore, it has been used as a standard tool ever since in several studies in Greece including the PANACEA epidemiological study (Priftis et al, 2007) while it has been recently shown that reported symptoms correlate well with objective measures of allergy (skin prick testing) (Papadopoulou et al, 2011). With regards to the Turkish version, this was piloted three months before the ISAAC study where the parental self-administered questionnaire was evaluated by face-to-face interview with parents and was found to be concordant (Kuyuku et al, 2006)”.

3. Methods section: Definition of predictor variables: instead of “profile the communities” in “order to describe the prevalence of risk factors”

Reply: This has been done.

Discretionary Revisions

1. Discussion: I believe the main interest of the study is describing the results of a natural experiment in which two communities with some degree of common racial background and living in closer areas – with different lifestyles - have different prevalence of allergy and asthma, and the results seem to contradict the hygiene hypothesis. As this results are challenging, I believe the authors should propose possible explanations for the phenomenon, and search the previous literature for similar results. Maybe the racial background has any influence in modulating the response to the risk (or protective) factors.

Reply: We agree with the reviewer that the genetic background of the populations in the two communities (or indeed the interaction of genetic susceptibility with environmental risk (or protective) factors) may be responsible for the observed differences. We have now expanded our discussion on this issue in the Discussion (pp. 20, first paragraph) and provided two additional references.