Reviewer's report

Title: The Genetic Study of three Population Microisolates in South Tyrol (MICROS): study design and epidemiological perspectives

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Review of the article of Pattaro Cristian et al ‘The Genetic Study of three Population Microisolates in South Tyrol (MICROS): study design and epidemiological perspectives’

1. Is the question posed by the authors new and well defined?
The authors have posed the question of the importance of isolated populations for revealing the genetic aetiology of Mendelian and complex diseases. The question of whether the use of these isolated populations will be significantly advance these goals is not therefore that new, as mentioned the authors themselves in the Introduction.

Genetic isolates are really exceptional resources for the detection of susceptibility genes for complex diseases because of the potential reduction in their genetic and clinical heterogeneity. However, the outcome of these mapping efforts is dependant upon the demographic history of a given genetic isolate. The authors comments regarding the relative significance of demographically older small genetic isolates in comparison with young and very large isolates, such as Sardinia, Netherlands or Finland, is reasonable. At the same time very few studies are consider the effects of the isolates demographical history and ethnic cultural traditions on the results obtained when searching for genes which have important roles in complex diseases.

Recently a group of Russian–US researchers suggested such experimental design of cross-population study of a specific complex clinical (psychiatric) phenotype in ethnically and demographically diverse small genetic isolates of the Caucasus, Daghestan highlanders (Bulayeva et al., 2000-2006). Unfortunately, the authors may be are not familiar with these publications and have not cited them, despite the design suggested by the authors being very similar.

Nevertheless, the authors comments regarding the significance of small demographically ancient isolates for the study of the genetic aetiology of complex diseases is well defined. They propose an experimental design that is used by very few research groups in the field of mapping genes of complex diseases and this study is therefore valuable for the furtherance this genetic field.

2. Are the methods appropriate and well described, and are sufficient details provided to replicate the work?

The methods used to study the 3 small geographically and historically diverse three German-speaking microisolates (genetic isolates) of South Tyrol are appropriate and well described. The methods as described could be used to replicate the study results.

Are there any differences between the villages in the German dialects used in that might provide information about isolation between them in marriages and gene flow?

3. Are the data sound and well controlled?

The authors describes results of extensive survey (the MICROS) study that was carried out on the study of three villages of the Val Venosta as a part of a larger, ongoing genetic research program to assess the genetics of diseases that affect the South Tyrolean population. The objective of the survey was to screen the populations for questionnaire-based information, pedigree structure, clinical measurements and to take blood samples for analyses. The article describes preliminary data, but this is important for understanding of following genetic linkage and association results. The data are well controlled. The study had appropriate ethical approval. Data were analyzed using appropriate statistical methods.

Because the study of population microisolates is part of the population genetics field of human genetics it is important to use population-genetics terminology. In the terminology of human population genetics it may be more appropriate to use not ‘micro-isolate’ when describing small genetically isolated villages in S.Tyrol but ‘primary genetic isolates’ as suggested J. Neel in 1992, as he named ‘secondary isolates’ a demographically younger and large by sizes isolates.

From the villages description in the article it is unclear how significant any inter-village genetic differences are and what is the within- and between-isolate genetic diversity rate? Such information, along with marriage structure, including consanguineous marriages that have occurred in any isolates, would be useful
to define the isolates in this initial article describing them and for any future article describing the genomic linkage and association results.

4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes, the manuscript adheres to the relevant standards for reporting and data deposition. However, some of the results need clearer descriptions regarding epidemiology of common diseases observed. If the villages are independent isolates the epidemiology it would be better to present for every of the villages, including familial clustering in the diseases.

The authors did not describe clearly what type of samples and/or information was collected for what type of propose future analyses?

What goals of the study in the following situations:
· where they are limited ‘for analysis issues’ to the pedigrees’ generations 15 to 5?
· the larger extended pedigrees are separated into smaller branches?
· why were the 1732 singletons collected?

The authors note that “The birthplace of participants, their parents and grandparents was recorded: 85% of subjects from Martello, 77% from Valcelunga and 59% from Stelvio were born in the same village where they were residing at the time of the interview. When extending the area to the Val Venosta, we observed that the percentage of people born in the valley was 89% (Stelvio) to 99% (Valcelunga). A similar pattern was observed for the parent’s and grandparent’s generations (Figure 2).” Are the authors sure that village Stelvio is genetic isolate? Only 59% of endogamous (within village) marriages even in last 2-3 generations in this village indicates a high rate of gene flow and the destruction of the isolate. What did you define as the isolate unit? Was this one particular village or the extended area of the Val Venosta?

The authors note that “Interestingly, 36 subjects reported heart defects (3%, 95%CI: 2.1-4.1%).” Did the authors mean congenital heart diseases and what type of heart disease? What about co-morbidity among family members that you analyzed for familial clustering? This might be clearer if the authors describe the epidemiology and familial clustering for each three villages separately?

5. Are the discussion and conclusions well balanced and adequately supported by the data? The Discussion and Conclusion in general are well balanced. I’m fully in agreement that such inter-disciplinary study in genetic isolates can be valuable for mapping genes of any diseases. In this manuscript some questions are unclear. For example, the authors wrote in the Conclusion “Linkage disequilibrium and historical data from the South Tyrol region, and the small isolated villages suggest that individuals may share a small number of disease alleles.” The authors should explain how the historical data can suggest that individuals may share a small number of disease alleles? The effects of historical events on the population gene pool is via marriages that define a genetic structure and/or its dynamics in the population.

I am also concerned about the association study planned by the authors in the isolates; if the isolates are really small and all they are really three independent and ancient genetic isolates, all the isolate members should be more or less genetic relatives. This means that you cannot ascertain within these isolates and collect data from unrelated hundreds subjects without stratification when performing any case-control and LD analysis as required in the association-analysis design.

6. Do the title and abstract accurately convey what has been found? Yes, the Abstract is adequate to the article. But in the Title I’d suggest describing this as a Demographical and Epidemiological study rather about genetic one.

7. Is the writing acceptable? Yes. The article is important for researchers working in the field of mapping genes of complex diseases. But some changes that will clarify of the study results, especially regarding the description of the genetic isolates and the within and between isolates differences in epidemiology, will increase understanding by readers of methodology and results of this significant article.