Author's response to reviews

Title: Genetic loci linked to Type 1 Diabetes and Multiple Sclerosis families in Sardinia

Authors:

Maristella Pitzalis (marist@mcweb.unica.it)
Patrizia Zavattari (zavatta@mcweb.unica.it)
Raffaele Murru (rmurru@unica.it)
Elisabetta Deidda (eli.deidda@mcweb.unica.it)
Magdalena Zoledziewska (magdaz@mcweb.unica.it)
Daniela Murru (danielmurru@tiscali.it)
Loredana Moi (lmoi@mcweb.unica.it)
Costantino Motzo (costantino.motzo@tiscali.it)
Valeria Orru (v.orru@tiscali.it)
Gianna Costa (csm@unica.it)
Elisabetta Solla (csm@unica.it)
Elisabetta Fadda (csm@unica.it)
Lucia Schirru (csm@unica.it)
Maria Cristina Melis (csm@unica.it)
Marina Lai (csm@unica.it)
Cristina Mancosu (cmancosu@unica.it)
Stefania Tranquilli (csm@unica.it)
Stefania Cuccu (csm@unica.it)
Marcella Rolesu (csm@unica.it)
Maria Antonietta Secchi (csm@unica.it)
Daniela Corongiu (csm@unica.it)
Daniela Contu (dconti@mcweb.unica.it)
Rosanna Lampis (rlampis@mcweb.unica.it)
Annalisa Nucaro (anuc@tiscali.it)
Gavino Pala (gavinopala@virgilio.it)
Adolfo Pacifico (pacificoadolfo@katamail.com)
Mario Maioli (marimaio@uniss.it)
Paola Frongia (annapaolafrongia@aob.it)
Margherita Chessa (margheritachessa@aob.it)
Rossella Ricciardi (rossellaricciardi@aob.it)
Stanislao Lostia (slostia@hotmail.com)
Anna Maria Marinaro (amarinaro@pharms1.medicina.uniss.it)
Anna Franca Milia (pediatria.hsf@aslnuoro.it)
Novella Landis (noviland@tiscali.it)
Maria Antonietta Zedda (niettazedda@interfree.it)
Michael B. Whalen (whalenmb@hotmail.com)
Federico Santoni (santoni@crs4.it)
Maria Giovanna Marrosu (gmarrosu@unica.it)
Marcella Devoto (devoto@email.chop.edu)
Francesco Cucca (fcucca@uniss.it)
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Author's response to reviews: see over
We are grateful to the reviewers for their pertinent comments. Below, specific responses to their observations are reported point by point.

**Reviewer 1 (comments in italics)**

_I would be interested in the amount of the genome that is excluded at various lambda values (λ=2, =3) given the sample size in the MS, the diabetes and the combined samples._

We performed exclusion mapping using the GeneHunter programme (version 2.1, release 3), investigating sibling relative risks of 2 and 3 for each of the three traits, and present results for % of genome excluded, at the canonical -2 LOD level, for each. As expected, we do not have much exclusion power for the MS dataset, but for the others, in particular the combined autoimmunity set, we maintain that there was sufficient power to locate any major loci, if present. Our results indicate that, as expected, there are not likely to be any novel autosomal loci that carry a λs greater than 3 (the same effect of the HLA superlocus in T1D). We now present these exclusion results just after we present the positive linkage findings.

_There are only 12 families segregating both conditions. These families are germane to the possibility of common susceptibility alleles posed by the authors._

_I would like to have some background data of these families presented in text format (it doesn’t need a table). Were there any differences in age of onset, the sex ratio or, perhaps, even the presence of other autoimmune conditions?_

While the number of individuals with both diseases is 12, there are 17 families in which both diseases co-occur. We have now modified the online figure to make this clearer. Plus, we have added a section where we describe the sex ratio and age of onset in more detail.

_With respect to other autoimmune diseases, in the 17 families with both diseases, there was also one case of autoimmune thyroid disease. In T1D families, there were 9 patients who also had celiac disease and 23 individuals with autoimmune thyroid disease. In the MS families, we found 4 cases of autoimmune thyroid disease. However, M. Marrosu and colleagues are currently in the process of recalling and re-examining some family members of the MS pedigrees that have not been tested for other -and in diagnostic terms more insidious- autoimmune diseases, such as autoimmune thyroid disease and celiac disease. So the data concerning additional autoimmune diseases are still to be regarded as tentative, and we thought it premature to insert this preliminary information into the paper._

_I am interested in linkage at certain candidate loci. Chromosome 10q21 and 20p12 would be of interest in this limited subset of MS+T1D families. IL2R at chromosome 10p15 has been convincingly shown in recent genome-wide association studies of MS and T1D to be a mild risk allele. I would like to see if there is any increased linkage here. This should be relatively straightforward to accomplish and may warrant a line or two in the results section. Perhaps more to the point, if the reviewers genotyped the families for the IL2RA SNPs, the results would also be worth presenting. However, I realize this is beyond the scope of this publication._

Overall, the linkage peaks in our study and in previous work do not coincide with the association signals from the recent GWA scans. While there is still the possibility that these discrepancies can be explained by the insufficient map coverage of the SNP panels used in the first generation GWA scans, it is probable that past linkage evidence was in fact due to random fluctuation in allele sharing. Concerning the referee’s point, we do not see any indication of increased linkage in the chromosome regions containing the IL2RA and IL7RA genes, but given the size of our data set, the
size effects of these variants and the size of an effect that we would be able to detect with linkage, this is not surprising. For instance, linkage in the IL2RA gene region (carrying, as pointed out by the referee, an intermediate size effect) was not detected even in large sample sets of ASP T1D families from the same population (the UK) in which the IL2RA association was described. Indeed the original linkage signal on chromosome 10 (IDDM10) does not include the IL2RA gene, and was most likely a false positive result (BMC Genetics 2007 May 17;8:24). We have modified the text to give these results, pointing out that, as stated above, we may not have sufficient power to detect linkage signals from either of these genes. Still, the two types of analyses have different properties and it is worth examining a population, particularly an exceptional one such as Sardinia, with both methods. Indeed linkage is more informative in detecting rare but more penetrant variants while association is more effective in revealing common but less penetrant genes; so the non-coincidence of results does not necessarily mean that one or the other are wrong.

Reviewer 2 (comments in italics)

In the discussion, more details should be given for the overlap of linkage signals at chromosomes 1q, 10q & 18p with other linkage and association studies, in particular the recent genome wide association studies.

We have modified the text to discuss this. We also reported that given the size of our sample set and the size of the genetic effects detected in the GWA scans, it is not surprising that the signals in the GWA scans and in our linkage study do not overlap. Still, as we pointed out in our reply to the other reviewer, the two types of analyses have different properties and it is worth examining a population, particularly a special one such as Sardinia, with both methods. Indeed linkage is more informative in detecting rare but more penetrant variants while association is more effective in revealing common but less penetrant genes; so the non-coincidence of results does not necessarily mean that one or the other are wrong.

Method: Paragraph 1. The total no. of affected T1D varies in the same paragraph. Line 5 suggests 297 diabetics while line 11 suggests 289 diabetics.

The numbers do not correspond because, while 297 is the total number of patients with type 1 diabetes, in 8 families there was a single diabetes case, but also at least one multiple sclerosis case. Hence, these 8 families were considered in the combined analysis to detect linkage with shared autoimmunity loci but could not be used to detect linkage with T1D alone. We have modified the text to state that more clearly.

The online figure has also been corrected to show the various familial subsets better.

Discretionary Revisions (which the author can choose to ignore)

1. The linkage signals identified for T1D, MS or combined diseases were modest except for the known major T1D locus at HLA. It would be interesting to test for linkage at other regions by conditioning on the linkage result at HLA loci (e.g. using the method in Nature Genetics 1999, 21:213-215). This analysis may help to unravel novel loci which may interact or act synergistically with the HLA locus, which otherwise may be masked by the strong effect at HLA.

Conditioning the linkage data on the main HLA disease locus was indeed an option we considered at the study design stage and in fact it is something we have applied in early linkage studies (see for instance Nature Genetics. 1998 Jul; 19(3): 301-302, Nature Genetics. 1998 Jul; 19(3): 297-300,
However, after these studies we realized that with this approach some additional evidence of linkage was easily detected but was most likely a false positive result due to the increased number of tests performed. This reservation is particularly cogent considering the small sample size we analysed in this study (particularly for MS) and the reduction of power due to this subgrouping analysis. In the case of MS, another factor increasing our scepticism was the modest contribution of the HLA region to the familial clustering of disease which further complicates this type of analysis. In the balance, we thus decided not to perform the conditioning analyses.