Letters

Comments

Distribution of HERV-LTR elements in *HLA-DQB1* alleles: comments on the article by Bieda et al.

To the Editor: We read with interest the recent article by Bieda et al. [1], which indicates that the retroviral long terminal repeat adjacent to the *HLA DQB1* gene (*DQ-LTR13*) marks additional genetic risk for Type 1 diabetes (IDDM) on high risk DQ haplotypes.

We recently reported the detailed polymorphism of the 5'-flanking region of the *HLA-DQB1* alleles [2]. In this study, we analysed the presence of *LTR13* (along with the presence of *LTR3* and *LTR5*, also located in the close vicinity of *DQB1*) in a case-control study and a large panel of homozygous B-cell lines. Interestingly, we found that every single LTR typing could be explained by linkage disequilibrium with *DQB1* alleles, independently of the haplotypes carrying them. Regarding *LTR13*, positivity was always associated with *DQB1**0302, *0303 and *0402 alleles. Although family studies should be carried out to validate the linkages, 345 individuals and 42 B-cell lines followed entirely the LTR pattern established and therefore we believe that *LTR13* has no additive value over *HLA-DQB1* in predisposition to autoimmune diseases.

Given the complete linkage disequilibrium between *LTR13* and *DQB1**0302, *0303 and *0402 alleles, the conclusion of Bieda et al. that *LTR13*, within the HLA region, is an "additional and independent" genetic risk marker in IDDM, is not correct. Nevertheless, given the fact that the associations of *DQB1**0302, *DQB1**0303 and *DQB1**0402 with IDDM are well-established and that only these three alleles are *LTR13*-positive, and considering that *LTR* elements are known to regulate gene expression, an interesting hypothesis would have been the analysis of the possible involvement of *LTR13* in the association of *DQB1**0302, *0303 and *0402 with IDDM.

The human genome contains between 30 and 50 proviral copies of human endogenous retroviruses (*HERV*) and up to 25 000 copies of their solitary long terminal repeats (*LTRs*). Insertions and deletions of these retroviral sequences have probably played an important role in generating the HLA diversity [3]. Of these, three *HERV-LTR* sequences, i.e. *LTR13* along with *LTR3* and *LTR5*, are inserted in the vicinity of the *DQB1* locus as mentioned above [4, 5]. In our study [2], *LTR3* positivity was always associated with certain *DQB1* alleles and sequencing analysis of the 5'-flanking region in homozygous B cell lines showed the absence of *LTR3* and a massive deletion

of 5 635 base-pairs in certain *DQB1* alleles. Regarding *LTR5*, it showed identical distribution to *LTR3*, consistently with a previously suggested *LTR3-LTR5* tandem arrangement.

In their study, Bieda et al. mention their previous reports suggesting that *LTR3* might have an additive effect in the contribution of the *HLA class II* region to predisposition to rheumatoid arthritis (RA) and IDDM [6, 7]. With our work we showed that the undetected deletion in the flanking region of some *DQB1* alleles and a lack of stratification for HLA typing explains previously reported associations of the *LTR3* element with RA and Type 1 diabetes (IDDM).

Unfortunately, Bieda et al. do not mention our findings on the distribution of *LTR* elements in *HLA-DQB1* alleles. The comment they make on our article is reduced to page 444 line 12 "a significant association for *DQB1**0601-*LTR13*⁺ with RA has been reported". In our article we clearly state that positivity for *LTR* elements did not increase the risk of developing RA by itself. The only exception would be the *LTR3*-positive *DQB1**0601 allele (seven cases against one control). However, the numbers are too small to be interpretable and would require confirmation in other patients' and controls' data. Moreover, a role of *LTR3* in RA susceptibility would be inconsistent with the fact that both *DQB1**0302-*DR4* and *DQB1**0301-*DR4* haplotypes are associated with RA, the former being *LTR3*- and *LTR5*-positive while the *DQB1* 5'-flanking region of the latter only contains a deletion.

We would like to emphasize that careful analysis of polymorphisms can allow the identification of stable *HLA* configurations, thereby avoiding potential confounder effects of an adjacent locus when reporting associations of particular markers with disease predisposition. Likewise, a thorough knowledge of allele distributions among loci could lead to a simplified typing of *HLA* haplotypes rather than particular loci. Such information would not only be valuable for phylogeny, population and disease association studies but even more when looking for *HLA* matching prior to transplantation.

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Acknowledgements. This work was supported by grant SAF00-213 from Plan Nacional de I+D (CICYT).

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Author's Reply

***DQ-LTR13* modifies Type 1 diabetes (IDDM) susceptibility on high risk *DQ* haplotypes: reply to the comments of Pascual et al.**

To the Editor: Although we refer to Pascual's study in our original paper [1], these authors like to reiterate their earlier conclusion that *DQ-LTR13* is not associated with “autoimmunity” [2]. However the two studies are completely different and therefore not comparable.

Pascual et al. [3] analysed 145 patients with rheumatoid arthritis (RA), 200 healthy controls and 42 selected homozygous cell lines. In contrast we investigated 284 complete families comprising of both parents and at least one child affected by Type 1 diabetes (IDDM) which enabled us to analyse the segregation of 1136 haplotypes with *DQ-LTR13*, *DQA1* and *DQB1* alleles and their transmission to patients [1]. Thus our data quality is completely different in comparison to Pascual's study of unrelated individuals which does not allow the assignment of linkage between *DQ-LTR13* and *DQ* in heterozygous individuals. Furthermore we addressed IDDM in Germany and Belgium, whereas Pascual et al studied RA in Spain. Although Pascual et al announce the importance of family studies in their original contribution, they have not obtained any additional data so far. We can therefore only encourage them to carry out transmission distortion tests (TDT) in trio families because only the complete assignment of parental co-transmission or non-transmission can answer the question of an independent *DQ-LTR13* association with a complex disease such as RA.

We confirm our results leading to the conclusion that *DQ-LTR13* is an “additional and independent genetic risk marker” in IDDM. The combined transmission of *DQ-LTR13*

with selected *DQA1/DQB1* haplotypes and the calculation of the relative risk (RR) clearly indicated that the risk mediated by the *DQ-LTR13* is additional to those conferred by *DQ2* and *DQ8*, respectively. The term “independent” referred to the linkage disequilibrium (LD) between the previously reported *DQ-LTR3* [4] and *DQ-LTR13*. This LD was so distinct that both could be considered as independent genetic risk markers.

We differentiated between the transmission of *DQ8/LTR13+* vs. *DQ8/LTR13-* and *DQ2/LTR13+* vs. *DQ2/LTR13-* haplotypes in a transmission distortion test and obtained a significant preferential transmission of *DQ8/LTR13+* compared with *DQ8/LTR13-*. *DQ2* in contrast was significantly more often transmitted without *DQ-LTR13* [1]. This transmission pattern obviously depends on these two haplotypes as we could show a distinct pattern for all not-*DQ2*/not-*DQ8* haplotypes that differs for the presence of *DQ-LTR13*.

Pascual et al. find *DQ-LTR13* positivity “always” associated with *DQB1**0302, *0303 and 0402 alleles. This is in contrast to our results of some *DQB1**0302/*LTR13*-negative as well as *DQB1**0201/*LTR13*-positive alleles. The allele *DQB1**0302 is more often associated with presence of *DQ-LTR13* than with its absence, but *DQ-LTR13* is not confined to the three *DQB1* alleles mentioned above, but also found in *DQB1**0201(5%), *DQB1**0301(7%), *DQB1**0501(7%), *DQB1**0502(8%), *DQB1**0602(4%) and *DQB1**0603(5%) [6]. The discrepancy of the two reports is therefore most probably due to the smaller sample of haplotypes studied in the Spanish data set.

The main interest of our study was to characterize *DQ-LTR13* as a possible genetic risk marker to IDDM. Previous reports suggesting that “*DQ-LTR3* might have an additive effect in the contribution of HLA class II alleles to predisposition to rheumatoid arthritis (RA) and IDDM” [5, 6] represent an earlier state of knowledge and have to be seen in the light of our current data. As clearly pointed out in our publication, the effect of *DQ-LTR13* is independent from *DQ-LTR3*. The massive deletion in the 5'-flanking region of the *DQB1* gene and its association with the absence of *DQ-LTR3* in certain *DQB1* alleles described by Pascual et al. has therefore no influence on the results obtained for *DQ-LTR13* as it is located outside this deletion.

Due to the complexity of the two diseases and the different populations studied the two reports do not necessarily contradict each other. Our earlier work of a *DQ-LTR3* association in German patients with rheumatoid arthritis [5] is currently ex-