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SHORT COMMUNICATION

Lack of replication of celiac disease risk variants reported in a Spanish population using an independent Spanish sample

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Celiac disease (CD) is an inflammatory condition affecting small bowel and triggered by gluten (or related proteins) ingestion in genetic susceptible individuals. Polymorphisms in three genes, SERPINE2, PPP6C and PBX3, have recently been associated with CD in the Spanish population. However, this association could not be replicated in the UK population using imputed data. As this second study analyzed a different population, we aimed at reevaluating the role of those polymorphisms using an independent Spanish sample. We genotyped three single nucleotide polymorphisms: rs6747096 in SERPINE2, rs458046 in PPP6C and rs7040561 in PBX3, in 417 CD patients, 527 ethnically matched healthy controls and parents of 304 CD patients. A case–control study using the χ^2-test and a familial study using the transmission disequilibrium test were performed. No association was detected in those analyses. Therefore, our results seem to discard the role of the previously described polymorphisms in SERPINE2, PPP6C and PBX3 in CD susceptibility.
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Keywords: *SERPINE2; PPP6C; PBX3; CD susceptibility*

Introduction

Celiac disease (CD) is a complex disease mediated by immune processes triggered after ingestion of gluten or related proteins in genetically susceptible individuals.[1] The genetic basis of this disease is being slowly unraveled by advances in experimental techniques. Combining two different gene-search approaches, Castellanos-Rubio *et al*.[2] reported new CD risk variants in the Spanish Basque population. They combined information provided by whole-genome expression profiling experiments and linkage studies (that is, functional and positional information) and found significant association with four single nucleotide polymorphisms (SNPs) located in the *SERPINE2* (serine protease inhibitor, clade E, member 2), *PPP6C* (protein phosphatase 6, catalytic subunit) and *PBX3* (pre-B-cell leukemia homeobox 3) genes.

The *SERPINE2* gene maps in the CD linkage region 2q33–q35 and *PPP6C* and *PBX3* in 9q33–q34. *SERPINE2* is involved in extracellular matrix production and it has been widely studied in relation to COPD (chronic obstructive pulmonary disease).[3] *PPP6C* encodes a protein phosphatase which seems to be involved in cell-cycle regulation.[4] Finally, PBX3 is a transcription factor implicated in basic developmental functions, including some related to immune cells.[5]

The association between those genes and CD susceptibility described by Castellanos-Rubio *et al*. was questioned by Hunt *et al*.,[6] who used data from a genome-wide association study performed in the British population to impute data corresponding to the previously associated SNPs. No replication was obtained and the authors suggested different possible explanations. Therefore, the debate is open because for every SNP the imputation is not 100% accurate, an issue recently discussed.[7] As an explanation of the observed discrepancies between the two studies could be owed to population or clinical heterogeneity, we aimed at evaluating the role of those SNPs in an independent Spanish sample of pediatric typical CD patients.

Results and discussion

Allele frequencies obtained in our case–control study are shown in Table 1 together with the previously published data.[2,6] No significant differences emerged when comparing our CD patients and controls. Additionally, we performed a transmission disequilibrium test (TDT) with data from 304 trios and no significant association was observed either.

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Table 1 Allelic frequencies of the three studied SNPs

Gene	*SNP*	*Alleles*	*Spanish (Castellanos-rubio et al)*			*UK GWAS*			*Spanish replication sample*				
									Case–control			*TDT*	
		1/2	*1 in cases*	*1 in controls*	*P*[a]	*1 in cases*	*1 in controls*	*P*	*1 in cases*	*1 in controls*	*P*	*1T:1U*	*P*
SERPINE2	rs6747096	G/A	0.16	0.29	0.008	0.21	0.18	0.016[b]	0.24	0.22	0.56	106:100	0.36
PPP6C	rs458046	T/A	0.52	0.39	0.043	0.43	0.43	0.21	0.47	0.46	0.84	148:141	0.36
PBX3	rs7040561	T/A	0.15	0.06	0.021	0.16	0.14	0.12	0.10	0.11	0.57	50:69	0.05

Abbreviations: SNP, single nucleotide polymorphism; TDT, transmission disequilibrium test.
We studied 417 unrelated Spanish CD patients (diagnosed following the European Society for Paediatric Gastroenterology and Nutrition (ESPGAN)[10] criteria), 527 ethnically matched healthy controls (mainly blood donors and hospital staff) and parents of 304 out of those CD patients. A total of 97% CD patients are HLA-DQ2- and/or HLA-DQ8-positive. Samples were consecutively collected in two centres of the same region (Hospital La Paz and Hospital Clínico San Carlos, Madrid). A written informed consent was obtained from all the participants and the Ethics Committee of the Hospital Clínico San Carlos approved this study. All samples were genotyped by TaqMan technology following the manufacturer's recommendations (Applied Biosystems Inc., Foster City, CA, USA) for the three SNPs previously reported as involved in disease susceptibility: rs6747096 in *SERPINE2*, rs458046 in *PPP6C* and rs7040561 in *PBX3*. We did not include the other significantly associated SNP rs459311 in *PPP6C*, due to its high correlation with the already included rs458046 ($r^2 = 0.97$). Case–control comparisons were performed using the χ^2-test; and a TDT was used in the familial analyses. Statistical power calculations were carried out with the EpiInfo v5 software.
[a]*P* values correspond to the corrected values after using Bonferroni correction.
[b]Note that this borderline significant result is in the opposite direction than the originally described.

As the two SNPs analyzed in the 9q region are in moderate linkage disequilibrium ($D' = 0.5$, $r^2 = 0.037$, in our control sample), haplotypic analysis was performed, but significance was not improved (data not shown).

Therefore, our results seem to confirm the lack of association with CD of the studied SNPs in the *SERPINE2*, *PPP6C* and *PBX3* genes, concordantly with the data reported by Hunt *et al*.[6] This negative result is probably not due to lack of statistical power, because our study shows more than 99.9% statistical power (calculated with EpiInfo v.6.02, World Health Organization, Geneva, Switzerland) to detect the effects (odds ratios, ORs) initially described (at $P = 0.01$). The risks originally reported could be overestimated, but we can reach 80% statistical power to detect modest ORs (0.7, 1.3 and 1.5 in the *SERPINE2*, *PPP6C* and *PBX3* genes, respectively). Nonetheless, smaller-size effects cannot be formally discarded. Differences in the clinical features of the patients studied probably do not affect; we used a uniform Spanish population composed of only pediatric (mean age = 6.0 years (range, 9 months to 17 years)) and typical CD patients, to minimize differences with the previously studied Spanish group. Interestingly, our TDT study confirms the lack of association precluding population stratification, a problem that can be present in case–control studies and, therefore, might affect the Castellanos-Rubio *et al*. study.[2] When some stratification is present in the studied population, a different proportion of individuals belonging to diverse ethnic subgroups in cases and controls may lead to significant differences in allelic frequencies between cases and controls, but this problem is avoided with the TDT. Therefore, a TDT study in the Basque population would be very interesting, as it could definitively solve this issue. Provided the positive association is confirmed, a possible specific effect in the Basque population could be discovered.

Although the *SERPINE2* polymorphism is not associated with CD, this does not mean that the widely replicated CD linkage region 2q33 (*CELIAC3*) is not a true susceptibility locus. This region also contains the candidate *CTLA4*/*ICOS* genes, approximately 20 Mb apart, extensively studied in relation to CD due to their role in T-lymphocyte activity regulation, and recently associated with CD susceptibility in a population with proven linkage.[8] The *PPP6C* and *PBX3* loci are located in 9q. This region showed linkage to CD in the North American population[9] but, in contrast to 2q33, no replication has been reported. Further studies are necessary to confirm this possible linkage region and, if replicated, to look for the etiologic genes.

Conflict of interest

The authors declare no conflict of interest.

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