

## Impact of polymorphic variation at 7p15.3, 3p22.1 and 2p23.3 loci on risk of multiple myeloma

Multiple myeloma (MM) is the second most common haematological cancer after non-Hodgkin lymphoma (Raab et al, 2009), with a worldwide age-standardized rate of 1-5/ 100 000 new cases every year. In Europe the incidence is slightly higher, with 4-6/100 000 and 3-2/100 000 new cases, respectively in men and women (Ferlay et al, 2010). MM is characterized by the proliferation of a single clone of plasma cells located in the bone marrow that, in the most advanced stages, can migrate to extra-medullary districts.

Several lines of evidence suggest that genetic factors are involved in MM pathogenesis (Altieri et al, 2006), however the genetic basis of the disease is largely unknown. Recently the first genome-wide association study (GWAS) of MM based on a meta-analysis of German and UK datasets reported that variation at 2p23.3 (rs6746082), 7p15.3 (rs4487645) and 3p22.1 (rs1052501) influences MM risk (Broderick et al, 2011). Because of the capricious nature of association studies, multiple replications in independent populations are highly desirable to lend credibility to the findings of a study. In addition, replication in different populations helps to generalize findings from the GWASs.

Therefore we genotyped the single nucleotide polymorphisms (SNPs) rs6746082, rs4487645 and rs1052501 in 1139 MM cases and 1352 controls ascertained through the International Multiple Myeloma rESEarch (IMMEnSE) consortium with the aim to validate the reported associations. Briefly, cases were defined by a confirmed diagnosis of MM according to the International Myeloma Working Group (2003) criteria, while region-specific subpopulations of controls were selected from the general population and hospitalized subjects with different diagnoses excluding cancer (details are given in supplementary material). For each subject, informed consent was obtained and the study was approved by the relevant ethical committees. The IMMEnSE bio-bank is set up at German Cancer Research Centre (DKFZ) in Heidelberg, where, with the exception of the Danish controls, genotyping was conducted using TaqMan technology (ABI; Applied Biosystems, Foster City, CA, USA) and adequate quality control procedures. Genotypes for Danish controls obtained in the context of previously published GWASs were made available for this analysis (see supplementary material for details). There was no evidence of departure from Hardy–Weinberg equilibrium (HWE) among controls in the overall sample set and in each specific subpopulation ( $P > 0.02$ ). The mean call rate of SNP genotyping was 98.0% (97.8–98.5%) and was uniform between cases and controls and in all the different subpopulations. Concordance of genotypes between duplicate samples was >99%.

The main effects of the genetic polymorphisms on MM were assessed by unconditional logistic regression adjusted for age, gender and region of origin. The genotype frequencies for each of the three SNPs showed a relationship with MM risk consistent with previous observations. Cochran–Mantel–Haenszel (M-H) test and the Breslow–Day (B-D) test were used to verify the heterogeneity within the subpopulations of the IMMEnSE consortium for all the SNPs. There was no evidence of significant heterogeneity for the genotype and allelic distributions among the seven centres of the IMMEnSE consortium for the SNPs rs4487645 and rs6746082, while some heterogeneity was found for the SNP rs1052501 (M-H  $P_{\text{genotype}} = 0.030$ ,  $P_{\text{allele}} = 0.054$ . B-D  $P_{\text{genotype}} = 0.029$ ,  $P_{\text{allele}} = 0.052$ ).

The strongest association was shown by rs4487645 at 7p15.3, with an almost 1-4-fold increased risk for the C allele (odds ratio [OR] = 1.37, 95% confidence interval [C.I.]: 1.21–1.56,  $P = 7.96 \times 10^{-7}$ ,  $P$ -trend =  $2.00 \times 10^{-4}$ ).

The second strongest association was observed for the A allele of the SNP rs6746082 (OR = 1.22, 95% C.I.: 1.05–1.41,  $P = 0.008$ ,  $P$ -trend = 0.011), while a non-significant association with the OR going in the same direction reported in the GWAS was found for the SNP rs1052501 (OR = 1.12, 95% C. I.: 0.98–1.30,  $P = 0.098$ ,  $P$ -trend = 0.53) (Table I). In order to estimate the consistency and strength of the association of these three genetic loci with MM risk, we conducted a meta-analysis under the assumption of a fixed effect model with the two previous GWASs. All the three SNPs showed a statistically significant association with MM risk at genome-wide level ( $P < 10^{-7}$ ), without statistically significant evidence for heterogeneity among the findings.

The association of the rs4487645 with MM risk emerged as the strongest from both this study and the GWAS (Broderick et al, 2011). This SNP belongs to the DNAH11 (dynein, axonemal, heavy chain 11) gene, and the linkage disequilibrium block where it maps includes the CDCA7L (cell division cycle associated 7-like) gene, which encodes a MYC-interacting T protein that could provide clues to the functional basis of this association, as already pointed out by Broderick et al (2011). The relevance of the MYC pathway in MM is strongly supported by the recent evidence of the presence of a MYC signature characterizing the progression from monoclonal gammopathy of undetermined significance (MGUS) to MM (Chng et al, 2011). Moreover we recently observed an association between a polymorphism in the 8q24 region, thought to have a functional role on MYC regulation, and MM risk (Campa et al, 2012). All together, these findings strongly point towards a crucial role of the MYC pathway in MM onset. In light of these evidences, targeting MYC in MM therapy appears to be particularly appropriate. Indeed, as recently shown, the selective targeting of the MYC pathway with inhibitors of the bromodomain and extraterminal (BET) proteins has proven to be particularly effective in MM cell lines and animal models, resulting in a significant inhibition of cell growth (Delmore et al, 2011). In addition, the identification of high risk myeloma settings for which new combination therapies can be designed to significantly improve patient outcome is an open challenge (Richardson et al, 2011). The clear establishment of genetic risk factors could offer new clues for the classification of high-risk MM patients for which ad hoc combination therapies could be therefore developed. In this sense, the present study provides further evidence for the role of genetic variation at 7p15-3 (rs4487645), 3p22.1 (rs1052501) and 2p23.3 (rs6746082) as determinants of MM risk. With a population of over 1100 case-control pairs, the IMMEnSE consortium has provided a mechanism for this validation ensuring adequate statistical power to replicate the associations.

Further work is needed to refine and validate results emerging from GWASs in order to clearly establish additional genetic risk factors. This will lead to further improvement of our understanding of MM pathogenesis and the discovery of new potential targets for MM therapies.

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