Sequence variation at the MTHFD1L-AKAP12 and FOPNL loci does not influence multiple myeloma survival in Sweden

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Multiple myeloma (MM) is the second most common hematologic malignancy. The disease is defined by an uninhibited, clonal growth of plasma cells in the bone marrow1. It is preceded by monoclonal gammopathy of unknown significance (MGUS)2, a common condition defined as a clonal growth of plasma cells that does not yet satisfy the criteria for MM, but progresses to MM at a rate of ~1% per year3.

Increasing evidence supports that the biology of MM is influenced by inborn genetic variation. MM and MGUS show familial clustering, and genome-wide association studies have identified DNA sequence variants that influence MM risk4–8. Additionally, two recent studies indicate that genetic variation could also influence MM survival9,10.

In the first of these, Johnson et al.9 describe an association between overall survival in multiple myeloma (MM-OS) and rs12374648, located between the MTHFDIL and AKAP12 genes at chromosome 6q25.19. The protein encoded by MTHFDIL is involved in folate metabolism11, and AKAP12 is related to cell growth12. The association with MM-OS was detected in a meta-analysis of 3256 cases from four clinical trials: two from IMMEnSE and the one from Utah (n = 772) and one from Utah, n = 315) (combined P = 0.044; HR = 1.34, 95% CI 1.01–1.78). Yet, the positive replication result was driven by a P-value of 0.004 with unrealistically large effect size (HR = 9.73) in a subset of 109 patients from Spain in IMMEnSE, whereas the other six subsets (Italy, Poland, Portugal, Denmark, Edmonton in IMMEnSE and the one from Utah) did not show any evidence of association (Supplementary Table 7 in ref.10).

Given that the MTHFDIL association was not replicated after discovery analysis and that the FOPLN association was based on small sample sizes, it remains a possibility that these two associations are false discoveries due to a winners curse effect. We therefore looked for further support of the MTHFD1L-AKAP12 and FOPLN loci in a Swedish study population. For this, we retrieved clinical data for 871 patients diagnosed with MM between 2005 and 2015 from the Swedish Multiple Myeloma Registry (Sahlgrenska Hospital, Gothenburg) (Table 1), which records clinical data on MM patients in Sweden and has about 90% inclusion rate compared to the Swedish Cancer Registry. The patients had been previously genotyped in genome-wide association studies using population-based samples from the Swedish...
National Myeloma Biobank (Skåne University Hospital, Lund)\(^6,7\). The clinical data and samples were obtained subject to informed consent and ethical approval (Lund University, dnr 2013/540), and in accordance with the principles of the Declaration of Helsinki. The samples were genotyped using Illumina microarrays and imputed with phased reference haplotypes from 1000 Genomes\(^6,14\).

To test for association between genotypes and MM-OS, we used log rank test implemented in R (v.2.8) with adjustment for age, sex, and International Staging System (ISS) score. Survival was calculated from the date treatment started until the date of death, or until 5 April 2016 (median follow-up time 39.5 months).

In our analysis, we did not see any evidence of association with MM-OS for either rs12374648 ($P = 0.84$; HR = 0.97, 95% CI = 0.81–1.2) or rs72773978 ($P = 0.93$; HR = 0.98, 95% CI = 0.7–1.4) (Fig. 1). For completeness, we also tested for associations between MM-OS and all

| Table 1 Clinical characteristics of the study population |
|--------------------------------------------------------|
| Number of cases                                       | 871 |
| Gender                                                 |     |
| Male                                                    | 531 |
| Female                                                  | 340 |
| Median age at diagnosis                                | 68  |
| Median follow-up (months)                              | 39.48 |
| Deceased during follow-up                              |     |
| Yes                                                     | 393 |
| No                                                      | 478 |
| ISS                                                     |     |
| I                                                       | 179 |
| II                                                      | 339 |
| III                                                     | 234 |
| Unknown                                                 | 119 |
| Heavy chain paraprotein                                |     |
| IgA                                                     | 191 |
| IgG                                                     | 536 |
| IgD                                                     | 6  |
| IgM                                                     | 6  |
| Not detected                                           | 132 |
| Light chain paraprotein                                |     |
| Lambda                                                  | 240 |
| Kappa                                                   | 446 |
| Not detected or not done                                | 185 |
| Median plasma cells in bone marrow (%)                 | 22  |
| Treatment received                                     |     |
| Proteasome inhibitor                                    | 427 (49.02%) |
| Immunomodulatory (IMiD)                                 | 228 (26.18%) |
| Chemotherapy                                            | 678 (77.84%) |
| Autologous stem cell transplantation (ASCT)             | 283 (32.49%) |
| Other or no treatment                                   | 112 (12.86%) |
| Anemia (%)                                              | 26.18 |
| Hypercalcemia (%)                                       | 8.04 |
| Renal failure (%)                                       | 13.6 |

We also tested for associations between MM-OS and all
variants with minor allele frequency (MAF) >5% located within 1 Mb of MTHFD1L-AKAP12 (6,515 variants) or FOPNL (3,892 variants) but could not identify any significant association with any of these variants. Thus, we could not replicate the associations between MM-OS and MTHFD1L-AKAP12 and FOPNL in a population-based series, nor identify any other alleles associations with MM-OS at these loci.

Our results, in conjunction with the small sample sizes, lack of robust replication in the original studies, and the fact that the original studies do not replicate each other, indicate that the reported associations are false positives. As for alternative explanations, a first possibility could be limited power of our data set. Yet, our sample is comparable in size (n = 871) to the largest of the reported individual sample sets, including UK-My9 (n = 1,163) and UK-My11 (n = 871) where rs12374648 at MTHFD1L was detected, and substantially larger than the data sets where rs72773978 at FOPNL was detected. Power calculations indicate that our sample set has about 71% chance to detect an effect with HR = 1.34 (the effect size of rs12374648 and the replication effect size of rs72773978), and about 99% chance to detect an effect with HR = 2.65 (the discovery effect size of rs72773978), in our sample set. A second possibility could be differences in geographic origin. However, this also seems unlikely given that the two reported variants are common, both in our data (MAF 21.5 and 4.7%) and in the different populations of 1000 Genomes. Finally, a third possibility could be differences in clinical characteristics between the study populations. One difference is that our material is population-based, whereas the studies by Johnsson et al. and Ziv et al. are based on patients recruited into clinical trials. As a result, our population is older (average 68 years vs 54–66 years), and has not been selected for patients without comorbidity, as is common in clinical trials. A higher incidence of comorbidity could dilute effects of DNA sequence variation on survival, and differences in age and comorbidity will carry differences in treatment. For example, some of the reported populations contain a high proportion of patients who received autologous stem cell transplantation (ASCT; 100% in the German and US sample sets in Johnson et al.), whereas our study population contains 32.5% transplanted patients.

In summary, our results together with the limitations of the original studies indicate that the reported associations between the MTHFD1L and FOPNL loci and MM survival are false positives due to a winner’s curse effect. While there could be alternative explanations, these seem unlikely in comparison. Our results motivate the collection of larger data sets to understand the impact of genetic variation on clinical outcome in MM.

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Conflict of interest
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