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Myeloma Article

Synergy of two human endogenous retroviruses in multiple myeloma

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ABSTRACT

Multiple myeloma (MM) is a severe, incurable neoplasm of the plasma cells. In this study we have used genetic epidemiology to associate the risk of MM with endogenous retroviral loci in humans. We used SNP analysis on a Sequenom® platform and statistical analysis in SPSS. Markers near two endogenous retroviral loci, HERV-Fc1 on chromosome X and HERV-K on chromosome 1, were associated with MM. Moreover, there was strong gene–gene interaction in relation to risk of MM. We take this as indirect confirmation of the association.

1. Introduction

Multiple myeloma (MM) is a severe, incurable neoplasm of the plasma cells, the ultimately differentiated antibody producing B cell in the immune response. Genetic factors as part of the pathogenesis in MM were recently described by scans of SNPs (Genome-wide association studies i.e. GWASes) [1–4]. These findings are supported by epidemiological studies that have shown a three-fold higher incidence of the preceding condition monoclonal gammopathy of undetermined significance (MGUS) among African-Americans as compared to Caucasians and an increased risk of MGUS among first-degree relatives of MM and MGUS patients [5,6].

Several indications associate risk of MM to mechanisms involving B-cell responses such as infection, inflammation and autoimmunity [7–11]. During normal development of a B cell, rearrangement of the immunoglobulin heavy chain (IgH) locus at 14q32 is essential for the diversity of the immune response. The normal process results in double-strand DNA breaks and a highly effective immune response is therefore obtained at the cost of potential mistakes caused by unrepaired DNA strand breaks. In myeloma cells mistakes in translocation of the immunoglobulin heavy chain (IgH) gene are seen in 50–70% of patients and thereby imply that the gene rearrangement in the immune response or a chronic inflammatory drive are important underlying mechanisms [12]. Chronic inflammation involving B cells can be caused by exogenous as well as endogenous stimulation of the immune system. The epidemiological studies on exogenous virus infection and risk of multiple MM are inconclusive [13–18]. A possible endogenous "driver" of inflammation in man may be endogenous viruses which are inherited in a Mendelian manner. No evidence is available on endogenous viruses and risk of MM but studies on lymphoma indicate that endogenous viruses may be involved in activation of proto-oncogenes—a process that may emulate the initiation of cancer by exogenous retroviruses [19–21].

Endogenous viruses are retroviral sequences embedded in the host germ-line DNA, presumably as a consequence of past infections. The viral loci are now transmitted as Mendelian entities. The human genome contains in the order of such 100000 such loci distributed on all chromosomes but most sequences are grossly defective, and only 51 loci are able, with one or two mutations, to encode a viral protein [Nexø BA et al., manuscript submitted]. Very few seem complete. However, recombination between endogenous viruses happens in animals [22] and in these it leads to replication competent and sometimes pathogenic entities.

Here, in a Danish population-based collection of patients with multiple myeloma, we identify two susceptibility markers near

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endogenous retrovirus genes associated with risk of MM. One of these loci has previously been implicated in Multiple Sclerosis [23].

2. Materials and methods

2.1. Patients

Patients diagnosed with MM and treated with high dose treatment (HDT) and hematopoietic stem cell support from August 1994 to August 2004 were recruited from four participating centres in Denmark. Three hundred forty-eight patients were included in the study [6]. 197 were males. PBMC was purified from 292 leukapheresis products by buffy coat preparation. From 56 patients 10 times 10 μm sections were collected from paraffin embedded bone marrow samples. Material was not available for 19 patients undergoing HDT and these patients were not included in the study. The study was approved by the Danish Ethical Committee (01-158/03).

2.2. Controls

500 control samples were collected among medical students of Danish extraction at Aarhus University. In addition 43 blood donors recruited at Aarhus were used as controls. 180 were males.

2.3. DNA purification

DNA from patients was purified from PBMC by a salting out method [27] or from paraffin embedded tissue by phenol extraction as described elsewhere [26]. DNA from controls was purified from fresh blood or PBMCs.

2.4. SNP typing

SNPs were genotyped using a Sequenom® platform (San Diego, CA) using conditions as previously described [23,24]. The SNPs investigated are listed in Nexø BA et al. (manuscript submitted). They are characterized by being less than 10 kb from an endogenous retroviral locus with the potential (given one or two mutations) to encode a viral protein.

2.5. Statistics

Data were stored and processed in SPSS (IBM, Armonk, NY). For association of SNPs with disease we used the Crosstabs function and compared genotype frequencies. In case of X-linked markers we used gender separation and combined the p-values using Fisher's method. For synergy we recoded controls as 0 and cases as 1 and used ANOVA. We gender-separated the data and again used Fisher's method to combine the p-values. The p-value for the product terms was used as measure of synergy. We used Bonferroni correction to correct for the multiplicity of testing, i.e. multiplied the p-value with the number of SNPs tested. The resulting value is called p_B.

2.6. Figure

Fig. 1 was developed in Excel (Microsoft, Redmond, USA).

3. Results

3.1. Studies of association

We performed statistical tests on 157 SNPs located near endogenous retroviral loci in the materials. The remaining 67 were rejected, mainly on the basis of the quality of the scoring on the Sequenom or because the results were monotonous. Also, the quality of SNPs in the DNA from tissue sections was generally poor and about 75% of the results were no-calls. However, when SNPs from this source were acceptable, they were included.

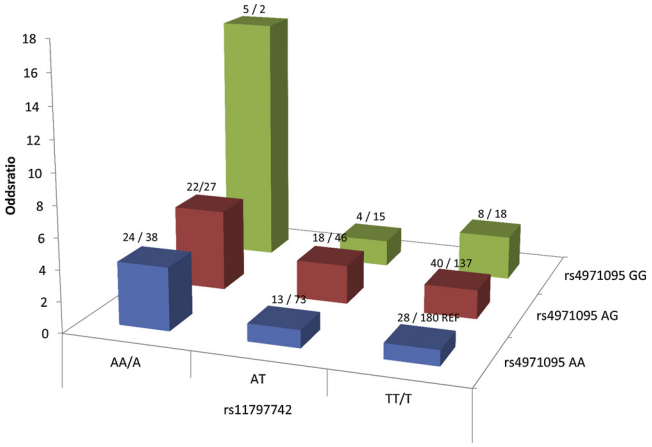

Fig. 1. Odds-ratios for MM and occurrences of cases and controls in relation to genotypes for the two markers rs11797742 and rs4971095. Males were calculated as homozygotes for rs11797742 although the really are hemizygotes.

Among the 157 SNPs one was significant also after the Bonferroni correction and one was almost so. The first one was rs11797742 (position 97,839,702 on the X-chromosome), which is located near HERV-Fc1 (position 97,841,482–97,849,424) ($OR_{Females} = 1.71$ CI95 = (1.21–2.41), $OR_{Males} = 2.36$; CI95 = (1.41–3.97), p-value = 3.0×10^{-5}, p_B-value = 0.004). The NCBI database lists this locus as HERV-Fc2; however this is clearly an error [28]. The second and almost significant SNP was rs4971095 (position 155,636,433 on chromosome 1), which is located near a HERV-K virus (position 155,626,666–155,635,845) (OR = 1.51; CI95 = (1.20–1.90), p-value = 0.0005, p_B-value = 0.079).

3.2. Studies of synergy

We have not at present materials to repeat the results above. Instead we looked for internal consistency in the data. To look for synergy between HERV-K on chromosome 1 and HERV-Fc1 on chromosome X, we calculated the interaction term for the SNPs in an ANOVA after gender separation. Here, we took advantage of the fact that the different measures in an ANOVA are statistically independent, and therefore statistical interaction reflects synergy. The results are shown in Table 1. When combining the two multiplication-term p-values from each gender using Fisher's method we arrived at a p-value of 8.8×10^{-7}. Thus, the markers showed strong evidence of interaction, i.e. the data had higher-order structure, presumably reflecting the underlying biology. This is illustrated in Fig. 1 where the one double homozygote has a very high OR. In this figure we have combined male and female results even though males are technically hemizygote, not homozygote for rs11797742.

4. Discussion

We have chosen to study the involvement of endogenous retroviruses in human disease by genetic epidemiology rather than virology. The reasons for this are three-fold: (1) We avoid the worrisome issue of viruses that are merely passengers in disease. If the viruses are merely passengers they will not register in genetics as associated with disease. (2) We can directly identify the viral loci involved, whereas virology cannot identify the identity of the virus, only its type short of actual cloning and sequencing the isolated virus. (3) We focused our attention on the approximately 50