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RESEARCH

Hodgkin Disease Therapy Induced Second Malignancy Susceptibility 6q21 Functional Variants in Roma and Hungarian Population Samples

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Abstract Patients treated successfully for pediatric Hodgkin's lymphoma are known to develop secondary malignancies; care is already taken in treatment to prevent this adverse effect. Recent GWAS study identified rs4946728 and rs1040411 noncoding SNPs located between PRDM1 and ATG1 genes on chromosome 6q21 as risk factors for secondary malignancies in patients formerly treated with radiotherapy for pediatric Hodgkin disease. We investigated the allele frequencies of these two SNPs in biobanked, randomly selected DNA of average, apparently healthy Hungarians (n =277) and in samples of Roma (n =279) population living Hungary. The risk allele frequency for rs4946728 was 79.4 % in Hungarian and 83.5 % in Roma samples, while for rs1040411 it was 56.4 % in Hungarian and 55.8 % in Roma samples. These values are quite similar in the two populations, and are rather high. The values are higher than those frequencies observed in the controls (rs4946728: 59.1 % and rs1040411: 39.6 %, $p <$ 0.05), and are in the range of the cases (86 % and 68.2 %, respectively) of the above original GWAS study. Our findings suggest, that beside the already taken precautions, genetic characterization of Hungarian pediatric Hodgkin patients seems to be advantageous prior to the treatment of their disease.

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Introduction

Patients treated successfully with radiotherapy for their pediatric Hodgkin disease are at risk to develop secondary malignancies by a reported cumulative incidence rate of 18.4 % by 30 years after treatment [1–3]. Recent GWAS study identified the rs4946728 and rs1040411 noncoding SNPs located between PRDM1 and ATG1 genes, residing on chromosome 6q21, as risk factors for secondary malignancies in patients formerly treated with radiotherapy for their pediatric Hodgkin's malignant lymphoma [4]. The exact mechanism of action and explanation for the susceptibility nature of these variants in not known. The PRDM1 gene encodes a zinc finger transcriptional repressor involved in a complex spectrum of major cellular processes like proliferation, differentiation, immunity and apoptosis [5–10]. Decreased basal excretion of PRDM1 due to these SNPs is suggestive for impaired induction of the PRDM1 protein after radiation exposure [4].

Despite of the fact that the Hungarians are a unique nation in Europe due to the trans-Ural origin of their founder nation, the Magyars, modern genetic approaches sere cumulative growing evidence that they were slowly mingled after their settling in the Carpathian basin and the genetic profile of the contemporary Hungarians are quite similar to the neighboring European populations [11–13]. This does not apply to the Roma (Gipsy, Romany) people living in Hungary, their genetic determinants are quite different as they remained relative closed population [14–16].

The goal of the current study was to determine the allele profiles of these 2 SNPs utilizing anonymous DNA samples pooled in our biobanks.

Patients and methods

Patients

To test the previously, by Best et al, reported association of PRDM1 variants, we have recruited a total of 582 DNA pooled from apparently healthy Hungarian and Roma individuals in our biobank, the Biobanks.hu (BBMRI.hu) system, deposited originally by the anonymous option. DNA samples from a total of 289 Hungarians (166 males and 123 females, mean age: 37±1.00, range: 18–64 year) and 293 Roma subjects (124 males and 169 females, mean age: 55±1.02, range: 13–90 year) were tested.

In addition, discovery, replication and combined cases and control groups taken from the study of Best et al at Nature Medicine 2011 were enrolled to our analyses [4]. The discovery set consisted of 96 SMN cases (19 males, 77 females) and 82 SMN-free controls (31 males, 51 females). All cases and controls were individuals of European descent diagnosed with Hodgkin's lymphoma as children (median age: 15.6, range: 8–20) and treated with 25–44 Gy radiation therapy with or without alkylating chemotherapy. Cases developed SMNs with a mean latency of 20.0 years (s.d. = 5.8 years, range: 6–34). Controls were followed for at least 27 years (median: 32 years, range: 27–38) to ensure that the maximal contamination of controls by future cases was<2 %. To replicate their findings, an independent set of 62 cases with SMNs (6 males, 56 females) and 71 SMN-free controls (14 males, 57 females) were enrolled, all treated for Hodgkin's lymphoma in childhood with 25–44 Gy mediastinal radiation therapy. The replication cases and controls were self-identified as white, non-Hispanic. The genotypes and allele frequencies of the PRDM1 variants were also analyzed in combined set of discovery and replication collection of patients.

Methods

The SNP allelic variants were determined using two predesigned TaqMan SNP Genotyping Assays (Lifetechnologies, Foster City, CA, USA). Primers and probe sequences are available upon request. PCR conditions were as follows: 10 min initial denaturation at 95 °C followed by 40 cycles of 15 s at 95 °C and 60 s at 60 °C. The amplifications were carried out with TaqMan Universal PCR Master Mix (Lifetechnologies, Foster City, CA, USA) by Chromo4 (Bio-Rad, Hercules, CA, USA) machines.

Results

Table 1 shows the distribution of the PRDM1 gene rs4946728 and rs1040411 genotypes as well as the risk allele frequencies of the variants in the sample groups. The genotype and allele distributions of both two alterations were in Hardy-Weinberg equilibrium in the subgroups. The profile of allelic variants for both of rs4946728 and rs1040411, including the risk-associated minor allele frequencies did not significantly differ between the Hungarian and Roma samples.

As Table 1 shows, the homozygous carriers of the PRDM1 rs4946728 risk C allele were much frequent in the Hungarian and Roma subgroups compared to the discovery and combined controls presented by Best et al. In the Roma subgroup, the heterozygous carriers of the PRDM1 rs4946728 risk C allele were significantly prevalent compared to all three control groups. In addition, considerable accumulation of the PRDM1 rs4946728 risk C allele was observed in both the Hungarian and Roma populations compared to the discovery and combined controls (79.4 and 83.5 % vs. 59.1 and 63.7 %; $p<0.05$).

The homozygous carriers of the PRDM1 rs1040411 risk A allele were significantly frequent in the Hungarian and Roma subgroups compared to the discovery and combined controls. Statistically significant accumulation of the PRDM1 rs1040411 risk A allele was detected in the Hungarian and Roma populations compared to the discovery controls (56.4 and 55.8 % vs. 39.6 %; $p<0.05$). Besides, in the Hungarian subgroup, the frequency of the rs1040411 risk A allele was higher than in combined controls, as well (56.4 % vs. 44.1 %; $p<0.05$).

As Table 1 shows, the risk alleles of PRDM1 variants (rs4946728 C; rs1040411 A) did not show accumulation in neither the Hungarian nor the Roma groups compared to the discovery, replication and combined cases. The allele frequencies observed among the "Cases" groups by Best et al. did not also significantly differ from those of our sample groups for either PRDM1 variant.

Discussion

The use of susceptibility variants in prevention of side effects of drugs or treatments represent a unique and still growing area in clinical diagnostics part of the personalized medicine component of the contemporary medical care [17, 18]. Even if there are well established associations, like the association of polygenic diseases, including stroke, inflammatory bowel diseases, or ischemic hearth disease with certain susceptibility variants, they are still not recommended by all of the leading genetic authorities due to their limited clinical utility and validity [19, 20]. There are others, like pharmacogenetic or pharmacogenomic tests that are widely recommended, but their significance is still not recognized by the clinicians, as most doctors still not embraced the genetic revolution, as a survey of the American Medical Association revealed that only 10 % of the responders thought they had enough knowledge to use gene tests in prescribing medicines, although they

Table 1 Distribution of the PRDM1 rs4946728 and rs1040411 genotypes in Hungarian and Roma population samples, with cases and controls from Best et al

	rs4946728						rs1040411					
	Hungarian samples $n=277$			Roma samples $n=279$			Hungarian samples $n=274$			Roma samples $n=276$		
Genotype	A/A $n=7^{abc}$	A/C $n=100$	C/C $n=170^{ac}$	A/A $n=7^{abc}$	A/C $n=78^{abc}$	C/C $n=194^{ac}$	A/A $n=84^{ac}$	A/G $n=141$	G/G $n=49^{acd}$	A/A $n=84^{ac}$	A/G $n=140$	G/G $n=52^{acde}$
Risk allele frequency	C 79.4%^{ac}			C 83.5%^{ac}			A 56.4%^{ac}			A 55.8%^{a}		
Discovery cases [f]												
Genotype	A/A $n=2$		A/C $n=23$		C/C $n=71$		A/A $n=42$		A/G $n=47$		G/G $n=7$	
Risk allele frequency	C 86 %						A 68.2 %					
Replication cases [f]												
Genotype	A/A $n=1$		A/C $n=17$		C/C $n=43$		A/A $n=22$		A/G $n=30$		G/G $n=9$	
Risk allele frequency	C 84.4 %						A 60.6 %					
Combined cases [f]												
Genotype	A/A $n=3$		A/C $n=40$		C/C $n=114$		A/A $n=64$		A/G $n=77$		G/G $n=16$	
Risk allele frequency	C 85.3 %						A 65.3 %					
Discovery controls [f]												
Genotype	A/A $n=12$		A/C $n=43$		C/C $n=27$		A/A $n=10$		A/G $n=45$		G/G $n=27$	
Risk allele frequency	C 59.1 %						A 39.6 %					
Replication controls [f]												
Genotype	A/A $n=6$		A/C $n=32$		C/C $n=33$		A/A $n=18$		A/G $n=33$		G/G $n=19$	
Risk allele frequency	C 69.1 %						A 49.3 %					
Combined controls [f]												
Genotype	A/A $n=18$		A/C $n=75$		C/C $n=60$		A/A $n=28$		A/G $n=78$		G/G $n=46$	
Risk allele frequency	C 63.7 %						A 44.1 %					

[a] $p<0.05$ vs. discovery controls

[b] $p<0.05$ vs. replication controls

[c] $p<0.05$ vs. combined controls

[d] $p<0.05$ vs. discovery cases

[e] $p<0.05$ vs. combined cases

[f] Calculated from genotype count data of Table 1 of Timothy Best et al. Variants at 6q21 implicate PRDM1 in the etiology of therapy-induced second malignancies after Hodgkin's lymphoma at Nature Medicine 2011 [4]

nearly all though such tests were useful [21]. Taken together, the spectrum of the available and offered test rapidly increases and certainly will have revolutionary significance in the future.

Development of secondary malignancies after primary treatment of Hodgkin's malignant lymphoma is well known amongst the medical practitioners; [2, 3, 22–25] however, no exact data about the incidence rate in Hungary has been published. The recently identified SNPs associated with the development of the secondary malignancies is a very recent and clinically promising observation [4], prior to its clinical use confirmatory and national population genetic studies are needed. In the current work we observed no difference between the allele profiles of the 2 examined SNPs between the Hungarian and Roma people. This might be only somewhat surprising result, since the even if there are major differences in genetic determinants in several genes and SNPs between these two populations, some common features already have been identified [14, 15, 26].

The novelty of the unanticipated finding is the difference between our allele distributions, and those available in the public database domains. We do not know the exact explanation of this difference. The average people living in Hungary do not differ in large majority in their SNP profile from the neighboring nations; however, some differences can occur, of course. The present data suggest that these SNPs belong to such category.

The future pragmatic aspect of the previous and current observations in this topic is related with the practical question whether to screen the Hodgkin patients for these variants prior to their treatment or not, take into account their genetic structure in the treatment design or not, especially when the doses of the radiotherapy is calculated; a dose-dependent effect is suggested by the initial observations. As the frequency of the secondary malignancies is high, the question should not be neglected, therefore further epidemiology and clinical observations are needed to establish firm recommendations.

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