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Original Articles

Genetic variation in the major mitotic checkpoint genes associated with chromosomal aberrations in healthy humans

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ABSTRACT

Non-specific chromosomal aberrations (CAs) are microscopically detected in about 1% of lymphocytes drawn from healthy persons. Causes of CAs in general population are not known but they may be related to risk of cancer. In view of the importance of the mitotic checkpoint machinery on maintaining chromosomal integrity we selected 9 variants in main checkpoint related genes (BUB1B, BUB3, MAD2L1, CENPF, ESPL1/separase, NEK2, PTTG1/securin, ZWILCH and ZWINT) for a genotyping study on samples from healthy individuals (N = 330 to 729) whose lymphocytes had an increased number of CAs compared to persons with a low number of CAs. Genetic variation in individual genes played a minor importance, consistent with the high conservation and selection pressure of the checkpoint system. However, gene pairs were significantly associated with CAs: PTTG1-ZWILCH and PTTG1-ZWINT. MAD2L1 and PTTG1 were the most common partners in any of the two-way interactions. The results suggest that interactions at the level of cohesin (PTTG1) and kinetochore function (ZWINT, ZWILCH and MAD2L1) contribute to the frequency of CAs, suggesting that gene variants at different checkpoint functions appeared to be required for the formation of CAs.

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Introduction

Chromosomal aberrations (CAs) are markers of cancer risk and many specific clonal CAs are critical events in malignant transformation [1–4]. Non-specific CAs include missing, fragmented or fused chromosomal segments which are not clonal and may remain in lymphocytes for their life-time [5]. They are analyzed by microscopic scoring of metaphase nuclei from cultured lymphocytes and scored as chromosome-type aberrations (CSAs) and chromatid-type aberrations (CTAs). CSAs are thought to arise as a result of direct DNA damage or replication of a carcinogen-damaged DNA template; replication error may also lead to CTAs [5]. An alternative mechanism for CA formation is telomere erosion and the resulting erroneous joining of non-homologous chromosomes [6–9]. A further mechanism for CAs may be aneuploidy and chromosomal instability as a result of aberrant mitosis. Accurate chromosome segregation between two daughter cells during mitosis is supervised by a highly conserved signaling machinery termed the mitotic checkpoint which delays anaphase until all chromosomes are properly attached to the mitotic spindle [10]. Errors may interfere in many steps of the complex checkpoint control, including incorrect attachment of the kinetochore to microtubules and cohesion defects [10,11].

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In view of the a priori importance of mitotic checkpoint machinery on maintaining chromosomal integrity and the lacking data on potential role of gene variants influencing non-specific CAs we selected 9 main checkpoint related genes and their putative functional SNPs for a genotyping study on samples from individuals whose lymphocytes had an increased number of CAs ('cases') and compared them to persons with a low number of CAs ('controls'). In addition to individual variants, we tested for gene–gene interactions because mitotic checkpoint is a clockwork of many genes and interactions would be expected. The genes under study were BUB1B (mitotic checkpoint serine/threonine kinase B), BUB3 (mitotic checkpoint protein), MAD2L1 (mitotic arrest deficient-like 1), CENPF (centromere protein F), ESPL1 (extra spindle poles-like 1, separase), PTTG1 (pituitary tumor-transforming gene 1, securin), NEK2 (never in mitosis gene A-related kinase 2), ZWILCH (zwilch kinetochore protein) and ZWINT (ZW10 interactor). These genes encode proteins with important functions in mitosis [10,12]. BUB1B, BUB3 and MAD2L1 delay the onset of anaphase until all chromosomes are properly aligned at the metaphase plate; they are parts of the mitotic checkpoint complex [11]. CENPF encodes a protein that associates with the centromere-kinetochore complex. ESPL1/separase cleaves cohesin at the onset of anaphase while PTTG1/securin blocks the separase function. NEK2 is essential in centrosome separation by maintaining a stable attachment of microtubules to the kinetochore. The ZWINT and ZWILCH genes encode proteins that are involved in kinetochore function and released in preparation for mitosis [11,13].

Methods

The subjects were identified from a large cohort of volunteers who have been assayed for CAs in various previous studies; for details see References [14,15]. The participants provided an informed consent. Cytogenetic analysis was performed on cultured lymphocytes, as previously described, by microscopically analyzing (two microscopists in each lab) in a double-blind fashion coded slides of 100 mitoses per person for the frequency of total CAs (CAtot), CTAs and CSAs [3,16,17]. The CAs were categorized into a high-frequency group, 'cases' (>2%) and a low-frequency group, 'controls' (≤2%); this arbitrary cut-off point was based on our previous experience [3,17]. For CTAs and CSAs, the cut-off was 1%.

Genotyping method of the present polymorphisms was based on allelic discrimination using the TaqMan technology as described by us [18,19]. The analyses included the following SNPs and numbers of tested individuals: for BUB1B rs1801376 (N = 330), for BUB3 rs3808960 (N = 330), for MAD2L1 rs903147 (N = 330), for CENPF rs438034 (N = 618), for ESPL1 rs6580941 (N = 656), for NEK2 rs701928 (N = 663), for PTTG1 rs1862392 (N = 729), for ZWILCH rs3087660 (N = 674) and for ZWINT rs2241666 (N = 662). The reason for the varying number of samples was the availability of DNA. The BUB1B rs1801376 A/G SNP is a coding Arg/Glu variant; the BUB3 rs3808960 G/T SNP is a promoter polymorphism; the MAD2L1 rs903147 A/C SNP is a promoter polymorphism; the CENPF rs438034 G/A SNP is a coding Arg/Gly variant; the ESPL1 rs6580941 C/T SNP is a promoter polymorphism; the NEK2 rs701928 T/A SNP is a promoter polymorphism; the PTTG1 rs1862392 T/A SNP is a promoter polymorphism; the ZWILCH rs3087660 A/G SNP is a 5′UTR polymorphism; the ZWINT rs2241666 G/A SNP is a coding Arg/Gly variant. All promoter polymorphisms are predicted to occupy transcription factor binding sites [18,19].

Odds ratios (ORs) from multivariable logistic regression analysis were calculated by considering simultaneous effects of putative confounders, occupational exposures, age, gender and smoking habits on the CA frequencies. The associations of these possible confounding variables have been described [14,15]. For each SNP, adjusted ORs were calculated regarding their effect on CAtot, CTA and CSA. Irrespective of whether or not a SNP appeared to be individually significant, all possible pairs of two SNPs were considered for the SNP–SNP interaction analysis. The tested genetic models were 'three genotypes' of types AA, AB and BB; for 'the dominant mode of inheritance', AB and BB were merged as one group, while AA and AB together represented the reference group for 'the recessive model'. Moreover, genotypes were converted into zero, one or two risk alleles for the additive 'allele number' model. Likelihood ratio (LR) tests were performed to assess whether considering SNP–SNP interaction yielded a significantly better fit of the data. In addition, LR test statistics were calculated for the global null hypothesis to prove the significance of the whole model. If both SNPs significantly interacted with each other for various modes of inheritance for the same pair of SNPs, the model with the lowest Akaike Information Criterion was chosen. For the best model for a variant pair, the corresponding ORs and the Wald estimates for their confidence intervals and p-values were calculated. To assess the contribution of all genetic components (both SNPs and interaction term) to the model, LR based p-values were computed and shown as 'the overall p-value'.

The studies were coordinated at the German Cancer Research Center (DKFZ) with samples and study design obtained with informed consent approved by the local Ethical Committee of the Jessenius Medical Faculty and Slovak Medical University. Sampling of peripheral blood was carried out according to the Helsinki Declaration.

Results

The number of genotyped individuals ranged from 330 to 729, as detailed in Methods. Results for individual genotypes are shown in Table 1. CAtot did not show any nominally significant associations for the variants in the 9 genes. For CSA, the rare homozygotes of ZWINT showed an OR of 0.53 and the LR test p-value was 0.03. For CTA, the trend for the ZWINT variant was similar (OR 0.56), but it was even stronger for BUB1B for which the rare homozygous variant reached an OR of 0.30 (LR test p-value 0.03).

Pair-wise interactions of the genetic models of each of the 9 genes were tested for association with CAs (Table 2). Only the most significant models with p-values <0.05, based on the interaction term analysis and the LR test, are shown in Table 2; note that only the best model for each pair is listed in Table 2. Of the 21 tests shown, 6 included CAtot with 8 variants present. ESPL1 was a partner in 3 pairs, and PTTG1 and ZWILCH in 2 pairs each. The interaction term was most significant (p = 0.002) for the BUB3-ESPL1 pair. For CSA, also 6 variant pairs interacted, involving 8 genes. MAD2L1, CENPF, PTTG1 and ZWILCH were partners in 2 associations each. The interaction term was most significant (p = 0.01) for the CENPF-ESPL1 and PTTG-ZWILCH pairs. For CTA, a total of 9 gene pairs reached a significant interaction in which all 9 genes were involved. MAD2L1 was a partner in 4 pairs, PTTG1 and ZWINT in 3 pairs each and ZWILCH was a partner in 2 pairs. The interaction term was most significant (p = 0.001) for the NEK2-ZWINT pair. For CTA an interaction of the cohesin maintaining (PTTG1/securin) and degrading (ESPL1/separase) functions was observed.

In Table 2 several of the gene combinations were found for multiple CA types, although the genetic model was not always the same. Remarkably, 2 gene pairs were significant for all 3 types of CAs: PTTG1-ZWILCH and PTTG1-ZWINT. Of the 4 remaining gene pairs for CAtot, 2 were also found in other CA types (1 in CSA and 1 in CTA). CSA and CTA shared 2 additional gene pair associations (BUB1B-MAD2L1, MAD2L1-ZWILCH). In Table 2 we show also the global null hypothesis test which proved the adequacy of the whole statistical model for almost all pairs and for most associations showed smaller p-values than the interaction term analysis.

The ORs and the significances of the models for each combination of genotypes in Table 2 are shown in Supplementary Table S1. We also show there tests for total SNP information (overall p-value). For CAtot, 2 overall p-values were nominally significant, for CSA 1 was significant and for CTA 5 were significant. The lowest p-value was always found among the gene combinations which showed the most significant interaction terms in Table 2.

Discussion

Analysis of genotype-based genetic models for 9 genes for 3 types of CAs creates obviously a mass significance problem. However, it is difficult to estimate how many completely independent tests were carried out because CAtot depends on CSA and CTA, and because genotypes and genetic models are not independent. Anyway it is clear that the smallest p-values of 0.03 in the single SNP analysis in Table 1 would not survive any correction for multiple testing. In the interaction analysis in Table 2 for each CA type at least 72 independent tests were done (9 SNPs and 2 alleles). This would translate to a Bonferroni corrected p-value of 0.0007 (0.05/72). None of the interaction term p-values would survive this correction

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