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POLYMORPHISMS IN GENES CODING FOR MAJOR DNA REPAIR PROTEINS *XPC, XPD* AND *XRCC3* MAY MODULATE THE RISK OF CEREBROVASCULAR INCIDENTS IN THE BULGARIAN POPULATION

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Abstract: Eukaryotic cells are subjected daily to high levels of genotoxic damage. In young and healthy individuals the damage is normally promptly repaired. With age, the efficiency of DNA repair declines and the levels of unrepaired damage begin to grow, accelerating replicative quiescence and/or cell death in aged tissues. Carriership of variant alleles of genes coding for proteins responsible for DNA damage identification and repair may be associated with subtly deficient DNA repair and reduced capacity for cell and tissue renewal. The latter may increase the risk for development of degenerative disease.

This study analyses the association between carriership of variant alleles of common polymorphisms in genes coding for proteins functioning in nucleotide excision repair (*XPC* ins83, *XPD* (*ERCC2*) Lys751Gln), repair of breaks by homologous recombination (*XRCC3* Thr241Met), damage-associated signalling and assessment of levels of damage (*TP53* Pro72Arg) and the risk for cerebrovascular incidents in the Bulgarian population. There were statistically significant deviations from the Hardy-Weinberg equilibrium between the group of patients with cerebrovascular incidents and the controls for the markers *XPC* ins83, *XPD* Lys751Gln and *XRCC3* Thr241Met. Concomitant carriership of the pro-apoptotic *TP53* 72Arg-allele together with ‚high-risk' variant alleles of the other three polymorphisms included in the study was significantly more common in patients with a history of cerebrovascular incidents than in healthy age-matched controls. Carriership of variant alleles of the polymorphisms *XPC* ins83, *XPD* Lys751Gln and *XRCC3* Thr241Met and the pro-apoptotic 72Arg allele may constitute a risk factor for cerebrovascular incidents in the Bulgarian population.

INTRODUCTION

Cells in eukaryotic tissues are subjected daily to a significant amount of genotoxic damage. It is normally promptly repaired before the next replication cycle begins. There is very low (but, at the same time, inevitable) risk that damage may be missed or ignored by the cellular repair machinery. Cells that have sustained unrepaired damage beyond a certain critical threshold may be forced into replicative senescence and/or routed to the programmed cell death pathway in order to prevent the propagation of genetic errors.

The mechanisms for repair of genomic damage operate very efficiently in young and healthy individuals. With age, the efficiency of identification and repair of DNA damage declines and the level of unrepaired damage begins to grow. This may increase the risk of cancerous transformation or may cause unwarranted activation of the protective mechanisms of induction of replicative senescence and programmed cell death. The latter is associated with increased cell attrition rates and subsequent loss of capacity for cell and tissue renewal (degenerative disease).

Profound deficiencies in the capacity for identification and repair of DNA damage are usually characterised by early-onset cancer-proneness and/or degenerative disease (severe neurological deficits, early cataracts, premature loss of adipose tissue, skin and hair abnormalities) (Lehmann, 2001). Mild (subclinical) DNA repair deficiency may be conferred by carriership of variant alleles of genes coding for proteins responsible for DNA repair and maintenance of genomic integrity. Genetic polymorphism associated with mild phenotype modification is quite common in genes of DNA damage identification and repair, although the frequencies of the variant alleles may vary between different populations. Unlike severe genetic defects that usually cause rapid loss of the disease alleles from the gene pool, carriership of common polymorphisms in genes of DNA repair rarely has noticeable effects in young and healthy individuals and is, therefore, subjected to relatively low levels of selection bias. Nevertheless, subtle inherited deficiencies of DNA damage and repair machinery may increase the risk for common diseases in later age (Khalil et al., 2012). Genetic predisposition to reduced capacity to identify and repair genotoxic damage may increase the risk of degenerative disease and cancer in later age and/or predispose the carrier to specific complications that may occur in the course of the disease and/or following specific treatments (Petkova et al., 2013).

Vascular disease is very common and its prevalence rises with advancing age. Evidence of carotid atherosclerosis may be found in over 50 % of individuals aged 50 or more (Joakimsen et al., 1999; Mozaffarian et al., 2015). Atherosclerotic vascular disease (extracranial as well as intracranial) is a recognised risk factor for ischaemic stroke and brain haemorrhage. The prevalence of cerebral amyloid angiopathy (CAA - associated with increased risk for brain haemorrhage) is also

age-dependent (in those over 70, the prevalence of CAA may vary between 25 and 60 %) (Keage et al., 2009). The incidence of cerebrovascular incidents (brain haemorrhage and ischemic stroke) is between 2 and 5 % in individuals over 50 and may grow to 15 % in the age group of > 70 (Mozaffarian et al., 2015).

The maintenance of the integrity of the endothelium of cerebral blood vessels is crucial for the risk of cerebrovascular incidents. As most tissues of epithelial origin, vascular endothelium is subject to regular replacement. Apart from the normal ‚wear and tear' associated with the hydrodynamic pressure on the vessel wall, there are many other sources of damage to the endothelium, among which genotoxic damage is prominent. The main sources of genotoxic damage in endothelial cells are the oxidative stress generated from normal cellular metabolism and the ‚oxidative bursts' generated by immunocompetent cells in the circulation. Increased levels of genotoxic damage may result in delayed cell replacement and/or increased rates of cell death, creating localised areas of critically low shear stress within the vessel wall where the risk of occurrence of an integrity breach may be very high. Loss of vessel wall integrity may result in haemorrhage and/or recruitment of thrombocytes and immune cells and at the site of vascular injury, increasing the risk of vasoocclusion and localised inflammatory response. The immediate post-incident phase is characterised by a further rapid increase in the levels of reactive oxygen species and rapid death of cells at the lesion site (within minutes and hours after the incident) and in the lesion penumbra (peaking 24 hours after the incident) (Allen & Bayraktutan, 2009). The increase in the levels of oxidative stress is accounted for by the inefficient oxygen utilisation by damaged cells as well as by the localised and systemic inflammatory response to the damage (Liao et al., 2013). The additional oxidative stress puts extra strain onto the cellular machinery for repair of DNA damage. This stress may not be optimally managed, especially in aged cells and tissues and/or when the capacity to repair genotoxic damage is below the average. As a result, mass cell death may occur at the lesion site within minutes and hours of the incident. Apart from the localised effects at the lesion site, increased levels of unrepaired genotoxic damage may also exert ‚long-distance' effects in specific cell populations that are exquisitely vulnerable to oxidative stress, such as selected neuronal populations in the hippocampus, the basal ganglia, and the Purkinje cells in the cerebellum) (Baron et al., 2014). The latter may be at least part of the cause of the temporary (reversible) neurological disturbances in the post-stroke period as well as the progressive (irreversible) neurological decline that may sometimes develop after cerebrovascular incidents. Decreased capacity to repair DNA damage may increase the risk of vascular disease and acute vascular events and the risk for immediate and delayed damage after vascular incidents.

The aim of the present study was to elicit a potential link between carriership of polymorphic alleles conferring subtle decrease in the capacity to identify and repair genotoxic damage and alleles conferring increased propensity to apoptosis

in damaged cells, and the risk of cerebrovascular incidents in the Bulgarian population. Based on our previous studies upon the applicability of markers for individual capacity for DNA repair in the Bulgarian population (Chelenkova et al., 2014a, 2014b) we selected the markers *XPC* ins83, *XPD* Lys751Gln (both in genes coding for major proteins of nucleotide excision repair); *XRCC3* Thr241Met (in a gene coding for one of the major proteins of homologous recombination) and *TP53* Pro72Arg (in the *TP53* gene, coding for p53, a protein playing a major role in the decision-making about whether the damage may be repaired or that the cell ought to be routed towards the apoptotic pathway). The insertion allele of the *XPC*ins83 polymorphism, the 751Gln allele of the *XPD* (*ERCC2*) Lys751Gln polymorphism (rs13181) and the 241Met *XRCC3* Thr241Met polymorphism (rs861539) confer lower-than-average capacity for detection and repair of DNA damage (Khan et al., 2000; Smith et al., 2008; Wlodarczyk & Nowicka, 2012). The 72Arg allele of the *TP53* Pro72Arg polymorphism (rs 1042522) is associated with increased propensity to route cells that have sustained damage to the apoptotic pathway rather than inducing cell cycle arrest and attempting DNA repair (Thomas et al., 1999).

MATERIALS AND METHODS

Seventy unrelated individuals with a history of cerebrovascular incidents (ischemic strokes and brain haemorrhages) aged 20-70, referred by the Clinic of Neurology at University Hospital „Alexandrovska" - Sofia comprised the patients' group. The control group included 95 age-matched unrelated clinically healthy volunteers. Informed consent was obtained from all patients and volunteers prior to inclusion in the study. DNA was extracted by STS-one tube kit (STS Ltd.) from 200 µl of peripheral blood. The polymorphisms *XPC* ins83, *XPD* Lys751Gln, *XRCC3* Thr241Met and *TP53* Pro72Arg were analysed according to (Krüger et al., 2005; López -Cima et al., 2007). Statistical analysis was carried out with Arlequin 3.5.1.3 (Excoffier & Lischer, 2010).

RESULTS AND DISCUSSION

The frequencies of the wildtype and variant alleles, the observed and expected heterozygosity and inbreeding indices were calculated for the two study groups. The raw data is presented in table 1.

Table 1. Allelic frequencies, heterozygosity and inbreeding indices of the genetic markers used in the present study. P_{wta} - frequency of the wildtype allele, P_{va} - frequency of the variant allele, H_{exp} - expected heterozygosity, H_{obs} - observed heterozygosity, F_{is} - inbreeding coefficient (Weir & Cockerham, 1984

Marker (wildtype allele/variant allele)	$P_{wta/}$ P_{va} control group	$P_{wta/}$ P_{va} patients' group	H_{exp}/H_{obs} control group	H_{exp}/H_{obs} patients' group	F_{is} control group	F_{is} patients' group
XPC ins83 (del/ins)	0,602/0,398	0,580/0,420	0,470/0,340	0,490/0,490	0,287	-0,004
XPD Lys751Gln (Lys/Gln)	0,601/0,399	0,656/0,344	0,480/0,540	0,450/0,590	-0,111	-0,309
XRCC3 Thr 241Met (Thr/Met)	0,546/0,454	0,520/0,480	0,500/0,590	0,510/0,880	-0,186	-0,754
TP53 Pro72Arg (Pro/Arg)	0,316/0,684	0,321/0,679	0,430/0,560	0,440/0,410	-0,018	0,058

Significant deviations from the Hardy-Weinberg equilibrium ($P<0.05$) were observed for the marker *XPC* ins83 (deviation observed in the control group) and for the markers *XPD* Lys751Gln and *XRCC3* Thr241Met (deviations observed in the patients' group).

There was a significant excess of *XPC* del/del homozygotes in the control group compared to the patients' group, despite the fact that the difference in the frequencies of the del and ins allele between the two groups was not significant. This reflected in the observed vs. expected heterozygosity ($H_{obs} << H_{exp}$) and the inbreeding index (Fi_s) in the control group (strongly positive).

There was a small but statistically significant difference in the prevalence in the variant allele of the XPD Lys751Gln polymorphism between the patients' group and the control group, with the variant allele (conferring subtle deficiency of DNA repair) being actually more common in the control group than in the patients' group. It is possible that this distribution occurred by pure chance, but the probability for this is quite low ($P=0.015$).

There was an excess of *XRCC3* Thr/Met heterozygotes in the patients' group (indicated by the difference of observed vs. expected heterozygosity ($H_{obs} >> H_{exp}$) and the inbreeding index (F_{is}) in the patients' group (strongly negative).

Despite the fact that the prevalence of the *TP53* 72Pro allele was lower than the 72Arg allele, a slight excess of 72Pro/Arg heterozygotes was observed in the

control group compared to the patients‘ group, whereas the 72Arg/Arg (‚pro-apoptotic‘) genotype was more prevalent in the patients‘ group than in the control group.

For two of the studied markers (*XPC* ins83 and *XRCC3* Thr241Met) carriers of at least one variant allele were significantly more common in the patients‘ group than in the control group. *XPC* is one of the proteins in the dimer that scans untranscribed regions for damage and recruits the other proteins of nucleotide excision repair to the lesion site. Decreased capacity to identify and repair DNA damage conferred by the insertion allele of *XPC* ins83 may increase the risk of vascular events by interfering with the capacity for self-renewal of the cells of the endothelial wall (by means of induction of replicative arrest until the damage is repaired and/or signalling for induction of the apoptotic pathway in the presence of damage that could not be repaired).

XPD is one of the two helicases that unwind DNA in the vicinity of lesion sites in order to provide unimpeded access of the repair machinery. In could be expected that reduced efficiency of identification and repair of DNA damage due to carriership of a polymorphic allele conferring subtly decreased enzyme activity may contribute to the risk of vascular events by increasing the rate of accumulation of unrepaired damage in DNA. Apparently, this is not the case for the *XPD* Lys751Gln polymorphism in the Bulgarian population. There was, nevertheless, an excess of 751Lys/Gln heterozygotes and, respectively, a correspondent deviation of the value of F_{is} towards higher negative values in the patients‘ group compared to the control group, despite the decreased frequency of the variant allele in the patients‘ group. In the research area of genetic predisposition it is not uncommon to observe that carriership of heterozygous genotypes may exhibit stronger association with increased or decreased risk for specific diseases and conditions than carriership of homozygous genotypes. A similar genotype-phenotype relationship has been described for a number of genes coding for protein of DNA repair and maintenance of genomic integrity (Conde et al., 2009; Zhang et al., 2012, and others). This may be at least partly due to the fact that the frequency of the variant allele/s may be low and, respectively, some of the homozygous genotypes may be too rare for detailed studies of their effects on the phenotype. On the other hand, there may be a specific interaction/s (on gene, transcript or protein level/s) between the two variant forms that may constitute potent phenotype modifier/s. Thus, carriership of the heterozygous *XPD* 751Lys/Gln genotype may be specifically associated with increased risk of vascular incidents in the Bulgarian population, but more studies in larger groups may be needed in order to clarify the specificities of the potential association.

The main types of lesions caused by oxidative stress are oxidised bases in DNA and double-strand breaks. The latter is considered very toxic to the cell. Unlike other types of damage, double-strand breaks are generally not tolerated in normal (nontransformed) cells and are rapidly repaired (usually - by the

mechanism of homologous recombination) or else the cell is routed to apoptosis. Decreased capacity to repair double-strand breaks conferred by carriership of the 241Met allele of the *XRCC3* Thr241Met polymorphism may increase the risk of vascular events by accelerating the death of the cells carrying unrepaired double-strand breaks. As with the *XPD* Lys751Gln polymorphism discussed above, the heterozygous Thr241Met genotype may exhibit stronger association with increased risk of vascular incidents in the Bulgarian population than either of the homozygous genotypes, but more studies may be needed to confirm the initial findings.

Carriership of the 72Arg allele the *TP53* Pro72Arg polymorphism on its own may not play a significant role in the constitution of the risk of cerebrovascular incidents. Nevertheless, concurrent carriership of the 72Arg allele of *TP53* together with variant allele/s of other genes coding for proteins of DNA repair may contribute to the increase the risk of vascular events by lowering the threshold of genotoxic damage beyond which damaged cells are targeted to the programmed cell death pathway.

It is possible that an increased risk of immediate and delayed cell death at the lesion site, in ischemic penumbra and the susceptible cell populations elsewhere in the brain conferred by deficient DNA repair/increased pro-apoptotic tendency may be associated with an increased risk of neurological decline after the incident (specifically, memory impairment/post-stroke dementia due to hippocampal damage). Again, further studies in larger groups may be needed to clarify the genotype-phenotype relationships between subtle genetic deficiency of DNA repair and risk for neurological decline secondary to vascular incidents.

It is possible that carriers of variant allele/s of genes coding for key proteins of DNA damage identification and repair/proteins responsible for maintenance of the integrity of the genome) may benefit from more intensive measures for prevention of vascular disease. These may include traditional approaches (lifestyle alterations (changes in the diet, quitting harmful habits such as smoking, etc.) and therapeutic interventions targeted at maintaining arterial pressure, blood glucose and cholesterol levels within normal range)); as well as therapies decreasing oxidative burden (antioxidant therapies). The latter may also potentially be used to augment conservative treatment in the acute period after the vascular incident/s and during the extended recovery period.

CONCLUSIONS

Subtle deficiency of DNA repair conferred by carriership of variant alleles of genes coding for key proteins of DNA damage identification and repair (homozygous ins/ins genotype by the marker *XPC* ins83, heterozygous genotypes by the markers *XPD* Lys751Gln and *XRCC3* Thr241Met), with or without the homozygous ‚pro-apoptotic' *TP53* 72Arg/Arg genotype may increase the risk of

cerebrovascular incidents in the Bulgarian population. There may be the potential benefits of antioxidant therapy for asymptomatic carriers of ‚high-risk' genotypes (for the purposes of prevention of vascular incidents) and individuals that have already had vascular incident/s (in order to accelerate recovery and/or prevent neurological decline). Further studies may be needed in order to characterise the association between decreased capacity for DNA repair; the risk of vascular damage and post-incident neurological decline.

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