The fine art of vascular wall maintenance. Carriership of XPC, TP53 and APOE polymorphisms may be a risk factor for cerebral vascular accidents in the Bulgarian population

Pavlina Chelenkova, Rumena Petkova, Teodora Chamo, Sashka Zhelyakova, Ivaylo Tournev and Stoyan Chakarov

Department of Biochemistry, Faculty of Biology, Sofia University ‘St. Kliment Ohridski’, Sofia, Bulgaria; Faculty of Medicine, Sofia University ‘St. Kliment Ohridski’, Sofia, Bulgaria; Clinic of Neurology, Medical University Hospital ‘Alexandrovska’, Medical University of Sofia, Sofia, Bulgaria

ABSTRACT

Efficient maintenance of the integrity of the endothelium of cerebral blood vessels is crucially important, especially when the vessel walls are subjected to greater-than-normal levels of stress. Persistently high levels of genotoxic stress may result from lower capacity to detect and repair DNA damage conferred by carriership of variants of key genes of DNA repair/maintenance of genomic integrity. Adult Bulgarian patients with a history of cerebral vascular accidents (CVAs) and age-matched healthy controls were analyzed for 11 markers, including 7 DNA polymorphisms in genes coding for proteins of DNA repair and maintenance of genomic integrity, 3 hypercoagulability markers and 1 marker for susceptibility for cerebral amyloidosis. Homozygous carriership of the del allele of the polymorphism XPCins83 was associated with decreased risk of CVAs (RR = 0.446, 95% CI: 0.225–0.886, p = 0.021). In individuals carrying the ‘protective’ del/del XPCins83 genotype, carriership of the ‘pro-apoptotic’ genotype Arg/Arg in the TP53 locus was associated with increased risk of CVAs (RR = 1.845, 95% CI: 1.049–3.244, p = 0.034). Carriers of the Lys751Gln genotype at the ERCC2 locus were at increased risk of CVAs (RR = 2.055, 95% CI: 1.09–3.876, p = 0.025). Carriership of the E2/E3 genotype at the APOE locus decreased the risk of CVAs in Bulgarian males (RR = 0.279, 95% CI: 0.090–0.873, p = 0.028). Male Bulgarian carriers of the APOE4 allele were at increased risk of CVAs. Carriership of the common prothrombotic mutations Factor V Leiden, PT G20210A, MTHFR C677T and PAI1 4G/5G had no significant effect on the risk of CVAs in Bulgarian adults.

Abbreviations: AD: Alzheimer’s disease; ROS: reactive oxygen species; UTR: untranslated region; APOE: Apolipoprotein E; BER: base excision repair; CAA: cerebral amyloid angiopathy; CVA: cerebral vascular accident; ERCC: excision repair cross-complementation group; FVL: Factor V Leiden; HDL: high density lipoprotein; LDL: low density lipoprotein; MTHFR: 5,10-methylenetetrahydrofolate reductase; NER: nucleotide excision repair; PAI1: plasminogen activator inhibitor1; PT: prothrombin; RR: relative risk; TP53: tumour protein p53; XPC: xeroderma pigmentosum complementation group C; XPD: xeroderma pigmentosum complementation group D; XRCC: X-ray repair cross-complementing protein

Introduction

There's birth, there's death and in between there's maintenance.
Tom Robbins, Fierce Invalids Home from Hot Climates (2000)

Cerebral vascular accidents (CVAs, commonly known as stroke) are the third most common cause of death worldwide. Lifelong risk of CVAs may be as high as 1:6 and about half of the patients are younger than 65 years at the time of their first vascular accident [1]. Cerebral vascular disease usually predates the onset of the first vascular accident by years and decades. Advanced age, African ethnic origin and male sex are universal, non-modifiable risk factors for stroke [2]. Genetic risk factors for stroke have been identified only for a proportion of cases [3]. Modifiable risk factors for stroke include hypertension, type 2 diabetes, smoking, coronary heart disease, atrial fibrillation and...
left ventricular hypertrophy [2]. Other known risk factors for stroke may include hypercoagulability; impaired lipid profile and atherosclerotic and/or amyloid involvement of cerebral blood vessels [4,5]. Cerebral amyloid angiopathy (CAA), already shown to be a major risk factor for Alzheimer’s disease (AD), was also identified as a culprit in ischemic CVAs as well as in brain haemorrhages [6,7].

CVAs are typically more common in males than in age-matched females. The chances for survival after CVA are generally lower for males than for females but female survivors are usually left with more disability than males [8,9]. The gender bias in the risk of stroke may result, at least partly, from a variety of potentially modifiable factors that are more likely to be observed in males than in females. Males are more likely to smoke at any age, are less likely to adhere to a strict therapeutic regimen and generally have higher stroke may result, at least partly, from a variety of potentially modifiable factors that are more likely to be observed in males than in females. Males are more likely to smoke at any age, are less likely to adhere to a strict therapeutic regimen and generally have higher levels of total and LDL cholesterol and lower levels of HDL cholesterol than age-matched females [10], although these differences tend to decrease with age.

Despite the fact that increased risk of stroke may run in families and the detailed risk profiles outlining the modifiable risk factors, it is still difficult to determine the individual risk of stroke. In most cases the risk estimates may safely predict the risk for a group but not for each and every individual in a group.

The capacity of the endothelium to maintain its integrity under increased hydrodynamic stress may constitute yet another type of factor in the assessment of the risk for vascular accidents. Whether the endothelium of the blood vessel would maintain its integrity under adverse conditions (increased levels of oxidative stress, chronic inflammation, accumulation of atherosclerotic plaque and/or amyloid) may be difficult to predict for each and every patient. This factor is stochastic in its nature but it is, nevertheless, determined at least partly by genetic factors, including the above-mentioned inherent defects of lipid metabolism and hypercoagulability. The role of the capacity to manage genotoxic damage has only recently begun to gain the attention of researchers and clinicians as a major factor in human disease.

The sources of oxidative damage in living cells are many and varied, but for the purposes of classification, they fall into two large groups: endogenous (product of normal cell metabolism) and exogenous (resulting from environmental influences such as chemicals, ionizing radiation, etc.). Endogenous oxidative damage is very common, as reactive oxygen species (ROS) are normal by-products of oxidative phosphorylation in living cells. In young and healthy people, genotoxic damage is promptly repaired by the cellular DNA repair machinery. Cells that have sustained damage to their DNA are normally excluded from division until the levels of damage are assessed and DNA is repaired. If the damage is assessed to be too extensive and cannot be repaired, the damaged cell may be routed to programmed cell death. In the latter case, a replacement may be called forth by activating the stem cell niche to produce new differentiated cell/s. With age, however, the capacity to identify and repair DNA damage declines and unrepaired damage begins to accumulate. This may result in increased risk of carcinogenic transformation (if cells with damaged DNA are allowed to divide) or in degenerative disease (if the stem cell niches are unable to provide adequate rates of replacement of damaged cells). The development of degenerative disease may greatly accelerate if the influx or ROS generated by oxidative phosphorylation is too great (e.g. in carriers of mitochondrial haplogroups with ineffective oxygen utilization) and/or when the mechanisms that identify and manage genotoxic (specifically, oxidative) damage are operating with lower-than-normal efficiency.

In sites of vascular ischemia ROS are generated in ample amounts by damaged cells and immune system cells are recruited at the lesion site. Post-mortem studies of post-stroke murine and human brains showed that enzymatic activities of BER (excision of modified bases, excision of apurine/apyrimidine sites) were greatly decreased compared to age-matched individuals with cause of death other than stroke [11].

Several polymorphisms in human genes coding for proteins of DNA repair that are associated with a subtle decrease of the capacity to manage oxidative damage have been described. Polymorphisms in the human OGG1 gene, POLB, the NEIL gene family and XRCC2 and XRCC3 were identified as the potential culprit in common diseases of middle and advanced age such as cancer and type 2 diabetes [12,13]. Thus, a potential link between metabolic syndrome and cancer was proposed [14,15]. Carriership of polymorphic variants of genes coding for proteins functioning in repair of oxidative lesions (hOGG1 and XRCC1) as well as proteins functioning in NER (XPD (ERCC2)) were later demonstrated to be a risk factor for stroke, with variant alleles of the XRCC1 gene being associated also with stroke volume [16–20].

In individuals with genetic backgrounds predisposing to less efficient recognition and repair of DNA damage, persistent genotoxic attack resulting from plaque accumulation in the vascular wall, the accompanying chronic inflammation and, eventually,
ischemia may result in increased endothelial attrition and/or delayed cell replacement. This may generate localized areas within the vessel wall where the shear stress becomes critically low and the risk for occurrence of an integrity breach may be greatly increased. The latter may result in platelet activation and formation of thrombi at the vascular injury site, producing acute ischemia. Under ischemic conditions, the levels of production of ROS increase even further, augmented by the oxidative bursts generated by immune cells recruited at the site of the injury. Increased levels of genotoxic damage cause mass cell death at the lesion site and the stroke penumbra, peaking at 24 h post-accident [21]. Cells that are located at significant distance from the injury site (e.g. the Purkinje cells in the cerebellum, the hippocampus and the basal ganglia) may also be susceptible to cell death after a vascular accident [22]. This may occur over several weeks or months after the accident, resulting in post-stroke cognitive decline and/or poor recovery of motor skills. Again, those with less-than-average capacity to manage genotoxic damage may be more prone to delayed and/or long-distance effects than individuals with normal or superior capacity for repair of genotoxic damage.

The primary aim of this study was to assess whether individual capacity to identify and repair DNA damage plays a role in the constitution of risk for CVAs in the Bulgarian population. Seven genetic polymorphisms in genes coding for key proteins of DNA repair and maintenance of genomic integrity have been selected, namely TP53 Pro72Arg, XPC 83ins, ERCC1 C8092A, XRCC3 Thr241Met, XRCC1 Arg399Gln, XPD Lys751Gln and MTHFR C677T. Among these polymorphisms there were markers specific for BER (XRCC1 Arg399Gln) and repair of double-strand breaks (XRCC3 Thr241Met) as representatives of the most typical lesions caused by ROS. NER-specific polymorphisms (XPCins83, XPD Lys751Gln and ERCC1 C8092A) were also included, as most types of oxidative damage may potentially be repaired by NER. The panel included the TP53 polymorphism Pro72Arg as a marker of the capacity for maintenance of genomic integrity. In order to differentiate the effects of other genetic factors that may increase the risk of stroke, an analysis of the potential effects of the common prothrombotic markers FV Leiden, PT G20210A, PAI1 4G/5G and the APOE locus (responsible for variations in the lipid profile and the propensity for cerebral amyloidosis) was also carried out. The MTHFR C677T polymorphism has initially been described as a genetic factor for thrombosis and vascular disease but is also a key enzymatic activity in the detoxification of genotoxic agents [23,24]. Thus, we included the MTHFR C677T polymorphism in the study as a marker for individual capacity for management of genotoxic damage as well as a marker for risk of thrombosis.

Materials and methods

The initial study groups comprised 73 diabetes-free individuals (41 males, 32 females) with a history of CVAs (ischemic strokes) and 201 clinically healthy volunteers (123 males, 78 females), all of Bulgarian ethnic origin. All patients with CVAs were referred by the Clinic of Neurology at Medical University Hospital ‘Alexandrovska’ (Sofia). Written informed consent was obtained from all patients and volunteers prior to inclusion in the study. In both study groups, the target male-to-female ratio was 1:1; therefore, the initial groups have been restructured in order to achieve a male-to-female ratio maximally close to 1:1. After initial selection, four patients with CVAs were excluded from the study, two because of distant relatedness to other patients in the group and two because of ages below 20 or above 65 at the time of first CVA. Thus, the patients’ group eventually comprised 70 individuals, of which 38 were male and 32 were female. The control group was selected out of the initial group of healthy volunteers so that there was maximal match in the age distribution to the patients’ group (in order to minimize the effect of advanced age). Finally, the control group comprised 93 clinically healthy individuals (45 males, 48 females).

Postprandial peripheral blood was collected by venepuncture. DNA was extracted by STS-one tube kit (SciTechS, Ltd., Sofia, Bulgaria) from 200 μL of whole blood. We analyzed the polymorphisms TP53 Pro72Arg (rs1042522); XPCins83 (83 bp insertion/5 bp deletion in intron 9 of the XPC gene); C8092A (rs3212986) in the 3’-UTR of the ERCC1 gene; Thr241Met (rs861539) in the XRCC3 gene; Arg399Gln (rs25487) in the XRCC1 gene; XPD Lys751Gln (rs13181); the Leiden mutation in the FV gene (Arg506Gln, rs1800595); the G20210A (rs1799963) mutation in the 3’-UTR of the prothrombin gene; Ala222Val (C677T; rs1801133) in the MTHFR gene; the 4G/5G (rs1799889) polymorphism in the PAI1 gene and the E2/E3/E4 polymorphism in the APOE gene as described in [25–34]. The length of the amplicons was 136–871 bp. Two samples (one in each study group) were excluded from the analysis for the APOE triallelic system, probably due to the presence of rare alleles (E1, E5, etc.) that were undetectable by the assay used in the
study. Control assays of the same samples were carried out using primers for the human beta-globin locus [35] (HGB1: AAGGAACGTGGCTAGACACA and CATTGCGGCGGTCCACA, yielding 930 bp fragments and HGB7: ATGGCTGAAGCAGGTCG and CCGATGGGCTTATCG, yielding 908 bp fragments. Statistical analysis was carried out with Arlequin 3.5.1.3 [36].

Results and discussion

The frequencies of the wild-type and variant alleles for the 11 markers included in the study, the observed and expected heterozygosity and the inbreeding index for each marker were calculated for the two study groups. The results are presented in Table 1 for the control group and in Table 2 for the group of patients.

The panel of seven markers of individual capacity for DNA repair has been specifically selected to reflect the overall state of the basic mechanisms of DNA damage detection and repair: XP_Cins83, for the early stages of global genomic repair by NER; ERCC1 C 8092 A and XPD Lys751Gln, for the late stages of NER; XRCC1 Arg399Gln, for BER; XRCC3 Thr241Met, repair of double-strand breaks; TP53 Pro72Arg, maintenance of genomic integrity; and MTHFR C677T, detoxification of potentially genotoxic agents. Carriership of APOE variant alleles as a major risk factor for the development of CAA was also studied in conjunction with the capacity to manage genomic damage as an additional risk factor. Our previous works with several of these polymorphisms [37–39] have shown that there was some level of association of the carriership of their variant alleles with the risk of stroke in the Bulgarian population but the potential impact of a combination genotype has not been fully assessed until now. In the present study, we also factored in the risk of potential bias due to effects of other common genetic risk factors for thrombosis and vascular disease (FV Leiden, PT G20210A, MTHFR C677T, PAI1 4G/5G). Only results with significance level \( p \leq 0.05 \) are discussed in detail below.

Markers of individual repair capacity

TP53 Pro72Arg

The frequencies of the two allelic variants of the TP53 Pro72Arg polymorphism were apparently similar in the
two study groups (Tables 1 and 2) and was following the pattern of clinal distribution described earlier [40,41]. The homozygous ‘pro-apoptotic’ Arg/Arg genotype was, overall, the most common genotype in both study populations (0.46 in the control group vs. 0.42 in the patients’ group), followed by the heterozygous 72Pro/Arg genotype (0.42 in the control group vs. 0.44 in the patients’ group). The ‘pro-repair’ Pro/Pro genotype was observed with a frequency of 0.12 in the control group and 0.11 in the patients’ group, with expected frequency of the Pro/Pro genotype in both groups about 0.11. Thus, from the locus-by-locus analysis, it could be concluded that the TP53 Pro72Arg polymorphism did not have any perceptible effect on the risk for CVAs in our study groups. Nevertheless, analysis of the distribution of compound genotypes indicated that this polymorphism may, in fact, be a weak risk modifier. In patients with CVA carrying the ‘protective’ del/del XPC genotype (see below), concomitant carriership of the homozygous Arg/Arg genotype of the TP53 locus was quite common (0.35), whereas in the control group concomitant carriership of the del/del:Arg/Arg genotype was less common (0.22). In other words, carriers of the del/del genotype in the control group were more likely to carry also 72Pro allele-containing genotypes, whereas del/del homozygotes in the patients’ group were more likely to carry an Arg/Arg genotype. Thus, homozygous carriership of the ‘pro-apoptotic’ homozygous genotype by the TP53 Pro72Arg locus may be a risk factor for CVAs in the Bulgarian population (RR = 1.845, 95% Cl:1.049–3.244), p = 0.034, albeit it confers smaller risk of CVA than carriership of the insertion allele of the XPCins83 polymorphism. The role of the carriership of the Arg allele of the TP53 Pro72Arg polymorphism in the susceptibility to neuronal damage after brain ischemia has already been demonstrated [42]. Recently, it has been shown that carriership of the 72Pro allele was associated with improved outcome after CVAs with regard to functional recovery and that it exerted a beneficial effect in patients with transient ischemic attacks, a CVA-related condition that greatly increases the risk of stroke [43]. It is possible that in the Bulgarian population this polymorphism is inferior to XPCins83 with regard to its significance as a genetic risk factor for CVAs.

**XPCins83**

In the control group, the allelic frequency for the deletion (del, repair-proficient) allele was 0.657 and that for the insertion (ins, repair-deficient) allele was 0.343. In the patients’ group, the del allele was less common (0.587) and the ins allele was more common (0.413) than in the control group. There was a significant difference in the observed and expected distribution of XPCins83 genotypes within groups as well as between the study groups. Specifically, there was a statistically significant (77%) difference between the expected and the observed heterozygosity of the XPCins83 marker in the control group ($H_{exp} = 0.454$, $H_{obs} = 0.257$ at $p = 0.0007$). The proportion of clinically healthy individuals carrying the del/del genotype was 0.52, which was 21% higher than what could be expected given the allelic frequencies (0.43). For comparison, the difference between $H_{exp}$ and $H_{obs}$ in the patients’ group was only about 7%. The inbreeding coefficient in the control group was strongly positive ($F_{is} = 0.435$), whereas the $F_{is}$ in the patients’ group was weakly negative ($–0.039$). The del/del genotype was most common in the control group (0.40), whereas the heterozygous ins/del genotype was most common in the patients’ group (0.50). Apparently, in the control group there was an excess of del/del homozygotes at the expense of del/ins heterozygotes. The RR for the carriers of the ‘protective’ homozygous del/del genotype was 0.446 (95% Cl:0.225–0.886), $p = 0.021$.

**XPD Lys751Gln**

There was an excess of Lys751Gln heterozygotes in both groups, with the trend being more pronounced in the patients’ group. The excess of heterozygotes was 12% more than expected in the control group and 29% in the patients’ group than what could be expected by chance, given the allelic frequencies. This trend reflected on the value of $F_{is}$. The latter was weakly negative in the control group (–0.120) and strongly negative (–0.280) in the patients’ group. The RR for the carriers of the heterozygous genotype was 2.055 (95% Cl:1.09–3.876), at $p = 0.025$.

The distribution of the allelic frequencies and the different genotypes for the polymorphisms ERCC1 C8092A, XRCC1 Arg399Gln, XRCC3 Thr241Met and MTHFR C677T in the studied groups was unremarkable. Other authors have reported that upregulation of the expression of several proteins of repair by base excision (APE1, OGG1, XRCC1) improved long-term functional recovery after stroke [44]. It is possible that these polymorphisms do not play significant roles in the constitution of the risk for CVAs in the Bulgarian population.

**Common prothrombotic factors**

Carriership of the Leiden mutation was quite common in both groups. The frequency of the A allele in the
control group was 0.05, which corresponds to the frequency previously reported for the Bulgarian population [32,45]. The frequency of the A allele was slightly higher in the patients’ group (0.06). Homozygous carriers of the Leiden mutation were not identified in either of the study populations. Heterozygotes for the Leiden mutation were seen at a rate of 0.10 in the control group and 0.13 in the patients’ group.

The frequency of the A allele of the PT G20210A mutation was 0.016 in the control group and 0.043 in the patients’ group (2.68 times more frequently observed in patients with CVA than in healthy controls). Heterozygotes for the PT G20210A mutation were not identified in either group. Data published elsewhere suggest that the PT G20210A mutation is a stronger risk factor for stroke than Factor V Leiden [46]. Still, as the frequency of the mutated allele is quite low, these results could not be considered significant. Heterozygous carriers were seen at a rate of 0.03 in the control group and 0.085 in the patients’ group. Compound heterozygotes (carriers of both Factor V Leiden and PT G20210A) were not observed in the control group, but were identified in the patients’ group at a rate of 0.028. The experimental findings about the distribution of the alleles of the PAI1 4G/5G polymorphisms in our study groups were not statistically significant (p = 0.109), although the prevalence of homozygotes for the 5G allele in the patients’ group was unexpectedly low (0.015) at expected 5G/5G prevalence of 0.11.

**APOE E2/E3/E4 polymorphic system**

The frequency of the ‘baseline’ E3 allele (i.e. not associated with any perceptible effects on the phenotype) was lower in the control group than in the patients’ group (0.812 vs. 0.871). The frequency of the ‘low-risk’ E2 allele was twice as high in the control group as in the patients’ group (0.091 vs. 0.043). The frequency of the ‘high-risk’ E4 allele was higher in the control group than in the patients’ group (0.097 vs. 0.086). Heterozygotes by either of the variant alleles were not seen in the control group, whereas a single E2/E2 carrier and two E4/E4 carriers were identified in the group of patients. The latter makes up for 2.8% of the patients’ group, whereas the expected percentage of E4/E4 homozygotes was less than 1%, given the allelic frequencies. The rare E2/E4 genotype was not identified in either group. There was a slight excess of heterozygotes in the control group (H_{obs} = 0.376, vs. expected 0.325) and, respectively, F_{is} in the control group was negative (−0.159). In the patients’ group, heterozygotes of any type were significantly less common than in the control group (0.171) at expected heterozygocity rates of 0.232, resulting in a positive F_{is} of 0.266. Carriers of a single E4 allele were, overall, more common in the control group than in the patients’ group (0.193 vs. 0.114). Carriers of a single E2 allele (E2/E3 genotype) constituted 0.183 in the control group and 0.057 in the control group (3.2-fold difference in the prevalence of E2/E3 genotype in the control group). Carriership of the E2/E3 genotype may be associated with decreased risk for CVAs in the Bulgarian population (RR = 0.279 (95% CI:0.090–0.873), p = 0.028).

The distribution of APOE genotypes in our study groups also showed specificities with regard to male-to-female ratio. The two study groups were specifically selected to have a male-to-female ratio as close as possible to 1:1. In the patients’ group, the male-to-female ratio among the E4 allele carriers was 3:1, whereas in the control group the prevalence of males and females with a single E4 allele was very close to 1:1. The excess of E4 heterozygotes in the control group was apparently due to a larger proportion of E4-carrying clinically healthy females. This may mean that the CVA risk for a male carrier of E4 allele may be higher than the risk for a female E4 carrier.

The sex-dependent effects of carriership of variant APOE alleles have been extensively studied in relation to the risk of AD. Generally, it has been agreed that female E4 carriers are more likely to develop both early-onset and late-onset AD (analyzed in detail in [47,48]). Our results confirm that the effects of the carriership of the ‘high-risk’ E4 allele may be modulated by biological gender. It could be speculated that the contribution of the carriership of the E4 allele to the risk for CVAs in males is due to modulation of the inherent gender-associated risk for disorders in lipid metabolism.

It was already mentioned that the E2/E3 genotype was >3 times more common in the control group than in the patients’ group. There was also a peculiar male-to-female ratio among the E2/E3 carriers, with male E2/E3 carriers being 4 times less common in the patients’ group than females, whereas in the control group male E2/E3 carriers were two times less common than females (a twofold difference). It could be speculated that in the Bulgarian population the risk for CVAs is lower for a male E2 carrier than for a female carrier. Again, this effect may result from the modulation of the a priori higher risk of vascular disease in males due to impaired metabolism.
The beneficial effects of E2 carriehership apparently extend only to carriers of a single allele. Only a single E2/E2 homozygote was identified in both our study groups, a male with early-onset vascular disease (<50 at the age of first stroke).

Common composite genotypes in the studied populations

The most common composite genotype (TP53 Pro72Arg, XPCins83, ERCC1 C8092A, XRCC3 Thr241Met, XRCC1 Arg399Gln, XPD Lys751Gln, MTHFR C677T, FVLeiden, PT G20210A, PAI1 4G/5G, APOE) in the control group and the patients’ group differed only by the genotype in the XPCins 83 locus:

- Control group: Arg/Arg; del/del; C/C; Thr/Met; Arg/Gln; Lys/Gln; C/T; G/G; G/G; 4G/5G; E3/E3; vs.
- Patients’ group: Arg/Arg; ins/del; C/C; Thr/Met; Arg/Gln; Lys/Gln; C/T; G/G; G/G; 4G/5G; E3/E3.

Potential genotype–phenotype correlations

It could be speculated that homozygous carriehership of the del allele of the XPCins83 polymorphism exerts some protective effect against CVAs in the Bulgarian population. XPC, as part of the XPC-hHR23B complex is one of the first proteins to arrive at the site of lesions in DNA, signalling to the nucleotide excision repair machinery that there is unrepaircd damage [48]. This mechanism of activation of NER, however, plays a role only in the repair of untranscribed DNA. There are cell types (long-lived cells such as differentiated neurons and memory B-cells) that selectively suppress the repair of untranscribed DNA, focusing all repair capacity on transcribed DNA, focusing all repair capacity on transcribed DNA [49,50]. Therefore, unrepaircd damage in DNA would matter significantly only if it happened to be regions undergoing active transcription. Repair of damage in untranscribed regions of the genome, however, may matter significantly in cell types that are naturally subjected to a rapid turnover. The endothelelm of the vascular wall is normally replaced rapidly and is, therefore, strongly dependent on prompt repair of damage in untranscribed DNA. Subtle deficiencies in the recognition and/or repair of damage in untranscribed regions may not have significant effects on the phenotype in younger individuals but may result in accumulation of unrepaired lesions as age advances, when the capacity for repair of genotoxic damage naturally declines. Presence of unrepaired damage in the genome of a rapidly cycling cell may result in induction of mechanisms of programmed cell death and, potentially, activation of the endothelial stem cell niche in order to provide replacement cells. Adult stem cells, however, are also subject to aging and may be unable to keep up the pace of cellular replacement, especially if there is additional genotoxic load and/or subtle but lifelong deficiency in the mechanisms for management of genotoxic damage. Thus, the vascular wall in individuals with subtle deficiencies of DNA repair may be prone to destabilization. Unrecognised/untreated or poorly treated hypertension, increased oxidative stress due to persistent hyperglycaemia and/or presence of atherosclerotic or amyloid plaque may provide additional stress on a vascular wall that is already failing in its integrity.

The heterozygous ins/del genotype in the XPC locus was significantly more frequent in the patients’ group than in the control group. No statistically significant effects were observed for the distribution of the homozygous ins/ins genotype. Similarly, statistically significant effects of heterozygous carriehership of variant alleles on the risk of CVAs have also been identified for the polymorphism XPD Lys751Gln. Notably, association between heterozygosity for the latter polymorphism and senile cataract has already been demonstrated [51]. Presumably, higher-grade deficiency of NER (conferred by carriehership of two variant allele copies) may be perceived and compensated for (e.g. by means of upregulation of expression of key repair proteins, etc.) or effects similar to negative allele complementation may also play a role.

Carriehership of a single E2 allele of the APOE polymorphism may confer increased protection from CVA in the Bulgarian population, the effects being specifically relevant to male carriers. Carriehership of the A4 in the APOE locus may modulate the risk of CVAs in male carriers of APOE4 in the Bulgarian population. Considering that carriehership of variant APOE alleles is associated with modulation of the lipid metabolism profile (E4, unfavourable; E2, favourable), it is likely that APOE exerts its effects in the Bulgarian population by modulating the effects of some of the major factors for vascular disease that exhibit sex-dependent differences.

Conclusions

Carriehership of homozygous del/del XPCins83 genotype may be associated with decreased risk of CVAs in the Bulgarian population. Carriehership of variant ‘repair-deficient’ genotypes (heterozygous XPCins83 ins/del, XPD Lys751Gln) or ‘pro-apoptotic’ (TP53 Arg/Arg) genotypes may increase the risk for vascular disease and
vascular accidents, as they contribute to destabilization of the endothelial layer of the vascular wall by interfering with normal cellular turnover. This effect may potentially increase as age advances and/or when the individual develops diseases and conditions associated with increased level of endogenous oxidative damage (e.g. metabolic syndrome/type 2 diabetes). Carriership of a single E2 allele in the APOE locus may protect from CVAs, whereas carriership of a single E4 allele may increase the risk of CVAs in the Bulgarian population, both effects pertaining predominantly to male carriers. No significant association between carrier status and the risk of CVAs in the Bulgarian population was found for the common prothrombotic mutations Factor V Leiden, PT G20210A, MTHFR C677T and PAI1 4G/5G.

Acknowledgements
The authors wish to thank the patients and the volunteers that took part in the study.

Disclosure statement
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding
This research was supported by the National Science Fund, Ministry of Education and Science of Republic of Bulgaria under grants number DFNI-B01/2 and DMU-03-112.

ORCID
Stoyan Chakarov http://orcid.org/0000-0002-0712-9793

References
[1] Wolf PA, D’Agostino RB, Belanger AJ, et al. Probability of stroke: a risk profile from the Framingham Study. Stroke. 1991;22:312–318.
[2] Wolf PA. Stroke risk profiles. Stroke. 2009;40:573–574.
[3] Francis J, Raghunathan S, Khanna P. The role of genetics in stroke. Postgrad Med J. 2007;83:590–595.
[4] Mari D, Coppola R, Provenzano R. Hemostasis factors and aging. Exp Gerontol. 2008;43:66–73.
[5] Tuikkala P, Hartikainen S, Korhonen MJ, et al. Serum total cholesterol levels and all-cause mortality in a home-dwelling elderly population: a six-year follow-up. Scand J Prim Health Care. 2010;28:121–127.
[6] Thal DR, Ghebremedhin E, Rüb U, et al. Two types of sporadic cerebral amyloid angiopathy. J Neuropathol Exp Neurol. 2002;61:282–293.
[7] Yamada M. Predicting cerebral amyloid angiopathy-related intracerebral hemorrhages and other cerebrovascular disorders in Alzheimer’s disease. Front Neurol. 2012;3:64. DOI: 10.3389/fneur.2012.00064
[8] Petrea RE, Beiser AS, Seshadri S, et al. Gender differences in stroke incidence and poststroke disability in the Framingham heart study. Stroke. 2009;40:1032–1037.
[9] Spychalca MS, Honarpisheh P, McCullough LD. Sex differences in neuroinflammation and neuroprotection in ischemic stroke. J Neurosci Res. 2017;95:462–471.
[10] Kiel DP, Baron JA, P plymate SR, et al. Sex hormones and lipoproteins in men. Am J Med. 1989;87:35–39.
[11] Ghosh S, Canugovi C, Yoon JS, et al. Partial loss of the DNA repair scaffolding protein, Xrc1c, results in increased brain damage and reduced recovery from ischemic stroke in mice. Neurobiol Aging. 2015;36:2319–2330.
[12] Figueroa JD, Malats N, Rothman N, et al. Evaluation of genetic variation in the double-strand break repair pathway and bladder cancer risk. Carcinogenesis. 2007;28:1788–1793.
[13] Salmanoglu M, Kucukardali Y, Kucukodaci Z, et al. Prevalence of the DNA repair enzyme NEIL1 gene mutation in patients with type 2 diabetes in the Turkish population. J Endocrinol Invest. 2012;35:401–406.
[14] Petkova R, Tummala H, Zhelev N. Nothing in excess - lessons learned from the expression of high-mobility group proteins type A in non-cancer and cancer cells. Biotechnol Biotechnol Equip. 2011;25:2572–2575.
[15] Molenaar RJ, van Noorden CJ. Type 2 diabetes and cancer as redox diseases?. Lancet. 2014;384:853. DOI: 10.1016/S0140-6736(14)61485-9
[16] Mahabir S, Abnet CC, Qiao YL, et al. A prospective study of polymorphisms of DNA repair genes XRCC1, XPD23 and APE/ref-1 and risk of stroke in Linxian, China. J Epidemiol Community Health. 2007;61:737–741.
[17] He W, Huang P, Liu D, et al. Polymorphism of the XRCC1 gene as a modifier of the cerebral response in ischemic stroke. BMC Med Genet. 2006;7:78. DOI: 10.1186/1471-2350-7-78
[18] Orhan G, Elkama A, Mungan SÖ, et al. The impact of detoxifying and repair gene polymorphisms on oxidative stress in ischemic stroke. Neurosci. 2016;37:955–961.
[19] Dutra AV, Lin HF, Juo SH, et al. Analysis of the XRCC1 gene as a modifier of the cerebral response in ischemic stroke. BMC Med Genet. 2006;7:78. DOI: 10.1186/1471-2350-7-78
[20] Shyu HY, Shieh JC, Ji-Ho L, et al. Polymorphisms of DNA repair pathway genes and cigarette smoking in relation to susceptibility to large artery atherosclerotic stroke among ethnic Chinese in Taiwan. JAT. 2012;19:316–325.
[21] Allen CL, Bayraktutan U. Oxidative stress and its role in the pathogenesis of ischaemic stroke. Int J Stroke. 2009;4:461–470.
[22] Baron JC, Yamauchi H, Fujioka M, et al. Selective neuronal loss in ischemic stroke and cerebrovascular disease. J Cereb Blood Flow Metab. 2014;34:2–18.

[23] Froiss P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet. 1995;10:111–113.

[24] Nebert DW, McKinnon RA, Puga A. Human drug-metabolizing enzyme polymorphisms: effects on risk of toxicity and cancer. DNA Cell Biol. 1996;15:273–280.

[25] Mansfield MW, Strickland MH, Grant PJ. Plasminogen activator inhibitor-1 promoter polymorphism and coronary artery disease in non-insulin-dependent diabetes. Thromb Haemost. 1995;74:842–847.

[26] Poort SR, Rosendaal F, Reitsma PH, et al. A common polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. A case-control study. Circulation. 1996;94:1812–1814.

[27] Schmitz C, Lindpaintner K, Verhoef P, et al. Genetic polymorphism of methylenetetrahydrofolate reductase and colorectal cancer. Gut. 1996;38:968–973.

[28] Khan SG, Metter EJ, Tarone RE, et al. A new xeroderma pigmentosum group C poly(AT) insertion/deletion polymorphism. Carcinogenesis. 2000;21:1821–1825.

[29] Boyanovsky B, Russev M, Ganev V, et al. Prevention of factor V Leiden and prothrombin 20210 A variant in Bulgarian patients with pulmonary thromboembolism and deep venous thrombosis. Blood Coagul Fibrinolysis. 2001;12:639–642.

[30] Krüger S, Bier A, Engel C, et al. The p53 codon 72 variation is associated with the age of onset of hereditary non-polyposis colorectal cancer (HNPCC). J Med Genet. 2005;42:769–773.

[31] López-Cima MF, González-Arriaga P, García-Castro L, et al. Polymorphisms in XPC, XPD, XRCC1, and XRCC3 DNA repair genes and lung cancer risk in a population of northern Spain. BMC Cancer. 2007;7:162 DOI: 10.1111/j.1432-1033.1997.00669.x

[32] Chakarov S, Stoilov P, Alexandrov A, et al. Repair pattern in the beta-globin gene cluster of human fibroblasts after ultraviolet irradiation . Eur J Biochem. 1997;248:669–675.

[33] Excoffier L, Lischer HE. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol Resour. 2010;10:564–567.

[34] Chelenkova P, Petkova R, Chamova T, et al. Homozygous carriership of the wildtype allele of the XPcINS83 polymorphism may be an independent protective factor against cerebrovascular incidents in the Bulgarian population. Compt Rend Acad Bulg Sci. 2014;67:261–266.

[35] Chelenkova P, Petkova R, Chamova T, et al. Carriership of the variant alleles of APOE (E2, E4) may be associated with gender-dependent modulation of the risk for cerebrovascular incidents in the Bulgarian population. Compt Rend Acad Bulg Sci. 2016;69:1587–1594.

[36] Chicheva Z, Chelenkova P, Petkova R, et al. Children of the Sun, children of the Moon - a mini-panel for assessment of inter-individual variation between the capacity of healthy individuals to repair everyday genotoxic insults. Biotechnol Biotechnol Equip. 2012;26:3142–3147.

[37] Li P, Stetler RA, Leak RK, et al. Oxidative stress and the risk factor for vascular disease: a common mutation of the Sun, children of the Moon - a mini-panel for assessment of inter-individual variation between the capacity of healthy individuals to repair everyday genotoxic insults. Biotechnol Biotechnol Equip. 2012;26:3142–3147.

[38] Chelenkova P, Petkova R, Chamova T, et al. Polymorphisms in genes coding for major DNA repair proteins XPC, XPD and XRCC3 may modulate the risk of cerebrovascular incidents in the Bulgarian population. Ann Univ Sofia Fac Biol. 2016;101:116–124.

[39] Beckman G, Birgander R, Sjölander A, et al. Is p53 polymorphism maintained by natural selection?. Hum Hered. 1994;44:266–270.

[40] Dominguez-Sanchez JC, Delgado-Esteban M, Rodriguez-Hernandez I, et al. The human Tp53 Arg72Pro polymorphism explains different functional prognosis in stroke. J Exp Med. 2011;208:429–437.

[41] Rodriguez C, Sobrino T, Agulla J, et al. Neovascularization and functional recovery after intracerebral hemorrhage is conditioned by the Tp53 Arg72Pro single-nucleotide polymorphism. Cell Death Differ. 2017;24:144–154.

[42] Li P, Stetler RA, Leak RK, et al. Oxidative stress and DNA damage after cerebral ischemia: Potential therapeutic targets to repair the genome and improve stroke recovery. Neuropharmacology. 2018;134:208–217.

[43] Petkova R, Chakarov S, Horvath A, et al. Coexistence of a common prothrombotic risk factor and haemophilia in Bulgarian haemophilic population. Balk J Med Genet. 2001;4:37–39.

[44] Favaretto E, Sartori M, Conti E, et al. G1691A factor V mutation explains different functional prognosis in stroke. J Exp Med. 2011;208:429–437.

[45] Sugawara K. XPC: its product and biological roles. Adv Exp Med Biol. 2008;637:47–56.

[46] Nouspikel T, Hanawalt PC. DNA repair in terminally differentiated cells. DNA Repair (Amst). 2002;1:59–75.

[47] Nounspikel T, Hanawalt PC. DNA repair in terminally differentiated cells. DNA Repair (Amst). 2002;1:59–75.

[48] Chang Y, Lloret A. Why women have more Alzheimer’s disease than men: gender and mitochondrial toxicity of amyloid-beta peptide. JAD. 2010; 20:S527–S533.

[49] Sugawara K. XPC: its product and biological roles. Adv Exp Med Biol. 2008;637:47–56.

[50] Unal M, Güven M, Batar B, et al. Polymorphisms of DNA repair genes XPD and XRCC1 and risk of cataract development. Exp Eye Res. 2007;85:328–334.