RESEARCH REPORT

A prospective study of polymorphisms of DNA repair genes XRCC1, XPD23 and APE/ref-1 and risk of stroke in Linxian, China

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Background: Stroke is the leading cause of death in Linxian, China. Although there is evidence of DNA damage in experimental stroke, no data exist on DNA repair and stroke in human populations.
Aim: To assess the risk of stroke conferred by polymorphisms in the DNA repair genes, XRCC1, XPD23 and APE/ref-1 in a cohort of individuals originally assembled as subjects in two cancer prevention trials in Linxian, China.
Methods: The subjects for this prospective study were sampled from a cohort of 4005 eligible subjects who were alive and cancer free in 1991 and had blood samples available for DNA extraction. Using real-time Taqman analyses, all incident cases of stroke (n = 118) that developed from May 1996, and an age- and a sex-stratified random sample (n = 454) drawn from all eligible subjects were genotyped. Cox proportional hazards models were used to estimate relative risks (RRs) and 95% CIs.
Results: No association was observed between polymorphisms in APE/ref-1 codon 148 and XRCC1*6 codon 194, and stroke. Polymorphisms in XRCC1*10 codon 399 were associated with a significantly reduced risk of stroke (RR 0.59, 95% CI 0.36 to 0.96, p = 0.033), whereas XPD23 codon 312 was associated with a significantly increased risk of stroke (RR 2.18, 95% CI 1.14 to 4.17, p = 0.010).
Conclusions: Polymorphisms in DNA repair genes may be important in the aetiology of stroke. These data should stimulate research on DNA damage and repair in stroke.

The brain depends on high rates of oxidative metabolism to provide energy. This metabolic activity produces reactive oxygen species as a by product that has been implicated in brain injuries such as stroke.[1] Chronic exposure to higher concentrations of reactive oxygen species may necessitate constant DNA repair activity. Therefore, people with DNA repair gene variants that result in lower repair activity may be at higher risk of neurological disease. The genetics of stroke is an area of increasing research interest,[2] and the National Institute of Neurological Disorders and Stroke has promoted research on DNA damage and repair in stroke.[3] However, to date, no relationship between DNA repair and stroke in human population studies has been reported, nor are there any reports on the association between DNA repair genes and other neurological diseases such as Alzheimer's or Parkinson's in epidemiological studies.

Overall, stroke is the major cause of death in urban and rural areas of China.[4] Although DNA repair genes (XRCC1, XPD23 and APE/ref-1) and risk of stroke is a new area of investigation, several studies have reported a link between such genes and different types of cancer.[5–8] We studied the relationships among polymorphisms in the DNA repair genes XRCC1 (*x* ray repair cross-complementary group 1), XPD (xerodermda pigmentosum complementary group D, also known as excision repair cross-complementing repair deficiency) and APE (apurinic/apyrimidinic nuclease also known as multifunctional DNA repair enzyme) in the population of Linxian, a rural county in north-central China, where stroke is the leading cause of death.[9]

METHODS

Study population

The subjects in this study were drawn from the individuals who were alive and disease free at the end of the two Nutrition Intervention Trials (the Dysplasia Trial and the General Population Trial), which we conducted in Linxian between 1985 and 1991.[10–14] Samples were collected at the start of the cohort and the end points were assessed as they occurred. All phases of this study were approved by the appropriate institutional review boards at the US National Cancer Institute (Bethesda, Maryland, USA) and the Cancer Institute of the Chinese Academy of Medical Sciences (Beijing, People's Republic of China), and all subjects provided informed consent.

The Linxian intervention trials ended in May 1991, at which time approximately 6000 individuals were alive and disease free. Of the 6000 individuals, we selected all the individuals from whom DNA was extracted (yields of $\geqslant$1.5 μg; n = 4005). From this eligible group (n = 4005), we genotyped all incident cases of stroke (n = 118) that occurred between May 1991 and May 1996. Detailed methods of the blood collection have been published elsewhere.[14 15] In accordance with a stratified case–cohort design,[16] we selected an age-stratified ($\leqslant$50, 51–60, >60 years) and a sex-stratified subcohort (n = 454) of all eligible cohort members (n = 4005) to serve as the reference group. The sampling fractions were specified so that the cases were frequency matched by age and sex to the subcohort.

Criteria for diagnosis of stroke

During the follow-up period, study personnel contacted village health workers monthly to assess the vital status of all the subjects in the cohort. The initial diagnoses of stroke were made by village doctors. The final classification of stroke was made by a panel of senior Chinese diagnosticians, some of whom were members of the study team. They reviewed the clinical histories and medical records provided by the initial doctors, as well as any additional materials available in the local hospitals. Thus, this adjudicating panel of diagnosticians was not blinded to the

Table 1 Characteristics of case–cohort subjects, Nutrition Intervention Trials, Linxian, China*

	Subcohort†	Stroke cases
Number	454	118
Median (IQR) age (years)	54 (50–60)	60 (54–64)
Male (%)	55.5	52.5
Smoking (%)	39.3	31.4
Male smoking (%)	70.7	66.1
Female smoking (%)	0.50	0.0
Alcohol use (%)	26.4	17.0‡
Male drinking (%)	40.6	32.1
Female drinking (%)	9.0	3.2
Median (IQR) systolic blood pressure (mm Hg)	142 (127–160)	169 (154–188)‡
Median (IQR) diastolic blood pressure (mm Hg)	86 (78–95)	97 (86–108)‡
Median (IQR) BMI, (kg/m^2)	21.4 (20.0–22.8)	20.9 (19.7–22.9)
General Population Trial (%)	63.7	50.0‡
Dysplasia Trial (%)	36.3	50.0

BMI, body mass index; IQR, interquartile range.
*The Nutrition Intervention Trials consisted of two studies, the General Population Trial and the Dysplasia Trial.
†The subcohort of 454 subjects was drawn as an age- and a sex-stratified random sample from 4005 subjects in the General Population Trial and Dysplasia Trial.
‡Significant ($p<0.05$) differences between subcohort and stroke cases.

diagnoses made by the initial doctors. When there was discordance among the diagnosticians, it was settled by consensus.

The final classification of stroke was made by senior Chinese diagnosticians who reviewed the clinical histories and medical records provided by the village doctors, as well as any additional material from the local hospitals. Because CT and MRI were not available in this rural area, haemorrhagic and ischaemic strokes could not be separated. The diagnostic criteria for stroke used in this study were based on the standard practice in China in the early 1990s,[17 18] and have been used in previous analysis examining the associations of stroke with specific nutrient supplements[9 19 20] and serum nutrients.[21]

Genotyping

Genomic DNA was genotyped using real-time TaqMan analysis (PE Biosystems, Foster City, California, USA), with methods modified from those described previously.[22] PCR primers and dual-labelled allele discrimination probes were designed using PrimerExpress V.1.0 (PE Biosystems). Probes that had a predicted melting temperature near 68°C, with the polymorphic base near the centre were selected. Flanking PCR primers were selected based on the calculated penalty score, melting temperature, length and amplimer size. Oligonucleotide sequences for the analyses were

- XRCC1*6 codon 194
- forward primer: GAGGATGAGAGCGCCAACTCT
- reverse primer: ACGTTGTCCGAGCTCACCTG
- T allele probe: CTCTTCTTCAGC**T**GGATCAACAAGA
- C allele probe: TCTTCTTCAGC**C**GGATCAACAAG
- XRCC1*10, codon 399
- forward primer: GTAAGGAGTGGGTGCTGGACTGT
- reverse primer: GTCTGACTCCCCTCCAGATTCC
- A allele probe: CTGCCCTCCC**A**GAGGTAAGGCCTC
- G allele probe: CTGCCCTCCC**G**GAGGTAAGGCC
- XPD23, codon 312
- PCR forward: GTACCGGCGTCTGGTGGA
- PCR reverse: GGATGGAGCCAGGCACTG
- G allele probe: CTGCCCGACGAAGTGCTGCAG
- A allele probe: CTGCCCAACGAAGTGCTGCAGG
- APE/ref-1, codon 148
- forward primer: TCTATCTCTGCCCCACCTCTTG

Table 2 Genotype frequency (%), relative risks and 95% CIs for genetic polymorphisms in DNA repair genes

	Subcohort			Stroke cases				
	Wt	Ht	Vt†	Wt	Ht	Vt	RR (95% CI)‡	p Value§
APE/ref-1 A/A, A/T, T/T	33.1	65.7	1.2	33.0	65.1	1.9	0.93 (0.55 to 1.56)	0.77
XRCC1*6 T/T, T/C, C/C	50.6	45.2	4.2	45.6	48.3	6.1	1.35 (0.83 to 2.20)	0.23
XRCC1*10 A/A, A/G, G/G	45.9	46.2	7.9	58.3	36.5	5.2	0.59 (0.36 to 0.96)	0.033
XPD23 G/G, G/A/A/A	86.1	13.9	0.0	75.2	24.8	0.0	2.18 (1.14 to 4.17)	0.010

Ht, heterozygotic variant type; Vt, homozygotic variant type; Wt, wild type.
†The Hardy–Weinberg equilibrium assumption was tested for each genotype using a χ^2 test. p Values were as follows: APE/ref-1, <0.001; XRCC1*6, 0.002; XRCC1*10, 0.10; and XPD23, 0.13
‡Calculated using a Cox model adjusted for sex and age with additional adjustment for smoking, drinking, BMI, systolic and diastolic blood pressure, and trial. The RR is for the combination of Ht and Vt compared with Wt.
§p Values are from 1 degree-of-freedom score tests comparing a combined category of Ht and Vt with the Wt in the base model. We collapsed the genotypes because of the small numbers of subjects that were homozygotic variants.

- reverse primer: ACGAGTCAAATTCAGCCACAATC
- A allele probe: TCATGCTCCTC**A**TCGCCTATAGA
- T allele probe: FAMTCATGCTCCTCCTCGCCTATAGATAMRA.

Genotyping reactions (10 µl) contained approximately 40 ng of genomic DNA, 1× TaqMan Master Mix, dual-labelled probes (100 nM each) and PCR primers (900 nM each). Reactions were performed in 96-well MicroAmp Optical reaction plates and caps (PE Biosystems). Plates were incubated at 50℃ for 2 min, 95℃ for 10 min, followed by 40 cycles of 95℃ for 15 s and 62℃ for 1 min. An annealing temperature of 64℃ was used for the exon 6 assay. Reaction data were analysed with Sequence Detection System V.1.6.3. All laboratory personnel were blinded to disease status of the samples and a blinded repeat genotyping of 96 of the DNA samples yielded about 100% concordance for the polymorphisms.

Statistical analysis

Descriptive statistics and group comparisons were completed using SAS V.8.2. We used the Peanuts module in Epicure (Hirosoft International, Seattle, Washington, USA) to estimate the relative risks (RRs) and 95% CIs from the age- and sex-stratified case–cohort Cox proportional hazards models.[11 12 14 16 23] Additional stratum-specific age variables were included to adjust for within-stratum age variation. All Cox proportional hazards models also included the following covariates: (1) smoking defined as a dichotomous variable, never versus ever (>6 months of smoking); (2) drinking alcohol defined as a dichotomous variable, none versus any in the previous 12 months; (3) body mass index (kg/m^2); (4, 5) diastolic and systolic blood pressure (mm Hg); and (6) trial (the General Population or the Dysplasia Trial). Nested models were compared using score tests. All the reported p values are two sided; we defined significance as $p<0.05$. We tested the proportional hazards assumptions for each main effect (genotype) using a time-dependent covariate (genotype×follow-up time), and detected no deviations from model assumptions.

Because individuals with homozygotic variants were uncommon for all the genotypes examined, for risk estimation we combined homozygotic variants and heterozygotic individuals into one group. Therefore, all genotype analyses compared homozygotic wild-type individuals with subjects who were heterozygotic or homozygotic variants. Hardy–Weinberg equilibrium tests (χ^2) were calculated using only the randomly selected subjects in the subcohort. Linkage disequilibrium was tested using Tajima's D statistic.

Table 3 Adjusted relative risks and 95% CI for the associations between combined genotypes and stroke

	Subcohort (%)	Stroke (%)	RR (95% CI)*	p Value†
XRCC1*10 = Vt; XPD23 = Wt	46.9	25.7	1.0 (reference)	0.0013
XRCC1*10 = Wt; XPD23 = Wt	40.0	48.5	2.37 (1.32 to 4.26)	
XRCC1*10 = Vt; XPD23 = Vt	7.2	15.8	4.20 (1.77 to 10.02)	
XRCC1*10 = Wt; XPD23 = Vt	5.9	9.9	3.14 (1.14 to 8.67)	

Vt, homozygotic variant type; Wt, wild type.
*Calculated using a Cox model adjusted for sex and age with additional adjustment for smoking, drinking, body mass index, systolic and diastolic blood pressure, and trial.
†p Value is from the 3 degree-of-freedom score test of association for the addition of main effect terms to the base model.

RESULTS

Table 1 contains a summary of the baseline characteristics of the subjects as measured in 1985. Compared with the subcohort, patients with stroke were older ($p<0.001$), less likely to drink ($p<0.026$) and had significantly higher systolic and diastolic blood pressure (both $p<0.001$). Table 2 contrasts the genotype frequencies in the cases with those in the subcohort for each of the four polymorphisms. Using the individuals in the randomly selected subcohort, we tested whether genotype frequencies corresponded to what would be expected under the Hardy–Weinberg equilibrium. Significant deviations were detected for APE/ref-1 ($p<0.001$) and XRCC1*6 ($p<0.002$); no such deviations were found for XPD ($p = 0.13$) and XRCC1*10 ($p = 0.10$). Since both the XRCC1 and XPD genes are located at chromosome 19q13, we tested for linkage disequilibrium using Tajima's D statistic. We found Tajima's D to be +0.15, indicating that these polymorphisms were not linked.

Using the study subcohort, we also estimated the correlation between the genotypes and selected covariates. Although the magnitude of all Pearson correlation coefficients was small, some were statistically significant. For XRCC1*6, there was a significant positive correlation with sex ($r = 0.12$, $p = 0.0098$); for XRCC1*10, there was a significant negative correlation with sex ($r = -0.12$, $p = 0.013$) and cigarette smoking ($r = -0.1$, $p = 0.046$). For XPD23, there was a significant negative correlation with alcohol drinking ($r = -0.11$, $p = 0.023$).

Table 2 contains the estimates of RRs and their 95% CIs for the association of the genotypes with incident stroke for each polymorphism. We found that neither the presence of variant APE/ref-1 allele (A/T or T/T) nor the presence of the variant XRCC1*6 allele (T/C or C/C) increased the risk of stroke when compared with the wild-type genotypes (A/A and T/T, respectively). However, compared with the wild-type individuals, subjects with a variant XRCC1*10 allele (A/G or G/G) were found to have a significantly reduced risk of stroke (RR 0.59, 95% CI 0.36 to 0.96). In contrast, those with the XPD23 allele (G/A or A/A) had a significantly increased risk of stroke (RR 2.18, 95% CI 1.14 to 4.17).

Table 3 contains estimates of the joint effects of the XRCC1*10 and XPD23 polymorphisms. The reference groups were defined to be the subjects who were variant at XRCC1*10 and wild type at XPD23. This group contained the largest number of subjects, and had the lowest mortality from stroke. Compared with this reference group, the risks in each of the other genotypes were increased from approximately two- to fourfold. These risk estimates based on categorising individuals by their XRCC1*10 and XPD23 polymorphisms did not significantly differ ($p = 0.08$) from those predicted from the individual estimates in table 2.

DISCUSSION

DNA repair enzyme gene polymorphisms may alter the function or efficiency of DNA repair and may contribute to stroke susceptibility.[3] DNA repair systems are safeguards to maintain genomic integrity in the face of environmental stressors, cumulative effects of age and general DNA replication errors. XRCC1 and APE are thought to play a role in the base excision repair pathway that removes ''non-bulky'' base adducts produced by methylation, oxidation, reduction or fragmentation of bases by ionising radiation or oxidative damage.[24] XPD is thought to be involved in nucleotide excision repair, which repairs ''bulky'' lesions such as pyrimidine dimers, other photoproducts and larger chemical adducts.[25 26]

In this prospective study, we observed 118 incident strokes over a 5-year period in a cohort of 4005 individuals. We found that people with one or more XPD23 variant alleles had a risk of

stroke approximately twice as high (RR 2.18, 95% CI 1.14 to 41.7) as those with the wild-type genotype. In contrast, the individuals with a variant XRCC1*10 allele had approximately 40% fewer strokes (RR 0.59, 95% CI 0.36 to 0.96) than those with the wild-type genotype. When classified with respect to both polymorphisms, 47% of the population with variant XRCC1*10 and the wild-type XPD23 had the lowest risk of stroke. The increased risk for individuals with one of the other three genotypes ranged from a little over twofold to a little over fourfold (table 3). We found no association between the risk of stroke and polymorphisms in the APE/ref-1 or XRCC1*6 genes.

The nutritional status of our subjects may have made them particularly susceptible to genetic inefficiencies in DNA repair. We and others have documented numerous chronic nutritional deficiencies in this population.[9 10 14 21 27–29] Recently, we have measured pre-intervention levels of folate, B_{12} and homocysteine in a random sample of 3000 individuals from our Linxian cohort, and found that 90% of this population had marginal folate status (serum folate <6 ng/l) and 75% had marginal B_{12} status (serum B_{12} <200 pg/ml).[15 29] Consistent with these low serum levels, the serum homocysteine levels were approximately 2–3 times higher than those typically found in women and men in Western populations.[15 29] High levels of homocysteine have been found to be associated with increased DNA damage and reduced DNA repair,[30 31] and increased rates of stroke both in China [32 33] and in Western populations.[34 35] Since the nutrient deficiencies we describe make this Chinese population particularly sensitive for detecting the effect of inefficiencies in DNA repair on stroke, the magnitude of the effects of the polymorphisms in Linxian may be greater than the effects in Western populations where these nutrient deficiencies are rare. Nevertheless, it is quite possible that DNA repair polymorphisms may affect the incidence of stroke in the West as well, possibly owing to the suboptimal intake of dietary folate and B_{12} vitamins, or independent of nutritional factors. Although there is little direct evidence of a relationship between DNA repair capacity and stroke, recent studies from animal models of expression of proteins in DNA repair pathways suggest that it is important in repairing damage after transient ischaemic events.[36 37]

The design and execution of our study assure that certain sources of error were avoided. The subjects were from a well-defined population cohort with ethnic homogeneity. The serum for the genotype measurement and the covariate data was collected at the start of the cohort; this prevents the recall biases that can exist in retrospective studies. Local doctors made end point classification in a timely manner, and the documentation and diagnoses were reviewed by a single panel of expert clinicians.

Our study also had limitations. Although the diagnostic criteria for stroke were consistent, because CT and MRI were not available in this rural area we were prevented from subclassifying the strokes into ischaemic or haemorrhagic categories. Therefore, we cannot address the issue of whether the association of these polymorphisms with stroke might differ by subtype. This probably limits the generalisability of our results, since the ratio between ischaemic and haemorrhagic stroke differs in China and in the West, with more haemorrhagic strokes being diagnosed in China than in the West. Also, this inability to distinguish between the different stroke pathologies probably introduced a conservative bias to our findings, minimising the risk estimates since these estimates must be averages of what are possibly two different associations.

In any case, any misclassification that exists is not related to the DNA polymorphism status, and could not result in false-positive findings. Our study is also limited by its relatively small number of stroke cases. Although this does not affect the validity of the significant associations we report with XRCC1*10 and XPD23 polymorphisms, it does limit our ability to detect interactions between these two polymorphisms, or to conclude that APE/ref-1 or XRCC1*6 polymorphisms have no important effect on stroke.

In summary, we found that subjects with a variant XRCC1*10 had a decreased risk of stroke, and those with a variant XPD23 had an increased risk. This is the first population-based study to suggest that DNA repair, and by extension DNA damage, may play a role in the pathogenesis of stroke in humans. Although the size of this study is too small to be conclusive in any way, it should provide additional impetus for research on DNA damage and stroke in general, and on the relationship of polymorphisms in DNA repair enzymes and stroke in particular. There are no immediate policy implications of this study, because these data need to be confirmed in other studies. In the future, however, it is possible that new research could lead to drugs that target DNA repair genes as a new therapeutic strategy for stroke. In addition, lifestyle factors such as nutritional agents that may stimulate DNA repair capacity might be useful in preventing stroke.

What this paper adds

- To date, no data on the relationship between DNA repair and stroke in human population studies have been reported, nor are there any reports on the association between DNA repair genes and other neurological diseases such as Alzheimer's or Parkinson's in epidemiological studies.
- Our results suggest that polymorphisms in DNA repair genes may be important in the aetiology of stroke.

Policy implications

- There are no immediate policy implications because these data need to be confirmed in other studies.
- In the future, it is possible that drugs that target DNA repair genes could be tested for stroke.
- Lifestyle factors such as nutritional factors that could possibly stimulate DNA repair capacity might be useful in preventing stroke.

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REFERENCES

1 **Chan P**. Mitochondrial dysfunction and oxidative stress as determinants of cell death/survival in stroke. *Ann NY Acad Sci* 2005;**1042**:203–9.
2 **Alberts M**. Stroke genetics update. *Stroke* 2003;**34**:342–4.
3 **Chopp M**, Chan P, Hsu C, *et al*. DNA damage and repair in central nervous system injury. National Institute of Neurological Disorders and Stroke Workshop Summary. *Stroke* 1996;**27**:363–9.
4 **Wu Z**, Yao C, Zhao D, *et al*. Sino-MONICA project: a collaborative study on trends and determinants in cardiovascular diseases in China, Part i: morbidity and mortality monitoring. *Circulation* 2001;**103**:462–8.
5 **Matakidou A**, Eisen T, Fleischmann C, *et al*. Evaluation of xeroderma pigmentosum XPA, XPC, XPD, XPF, XPB, XPG, and DDB2 genes in familial early-onset lung cancer predisposition. *Int J Cancer* 2006;**119**:964–7.
6 **Mechanic L**, Millikan R, Player J, *et al*. Polymorphisms in nucleotide excision repair genes, smoking and breast cancer in African Americans and whites: a population-based case-control study. *Carcinogenesis* 2006;**27**:1377–85.
7 **Li C**, Liu Z, Wang L-E, *et al*. Genetic variants of the ADPRT, XRCC1 and APE1 genes and risk of cutaneous melanoma. *Carcinogenesis* 2006;**27**:1894–901.
8 **Skjelbred C**, Saebo M, Wallin H, *et al*. Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norweigian cohort: a case control study. *BMC Cancer* 2006;**6**:67.
9 **Mark S**, Wang W, Fraumeni J, *et al*. Do nutritional supplements lower the risk of stroke or hypertension? *Epidemiology* 1998;**9**:9–15.
10 **Li J**, Taylor P, Li B, *et al*. Nutrition intervention trials in Lixian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with esophageal dysplasia. *J Natl Cancer Inst* 1993;**85**:1492–8.
11 **Mark S**, Liu S, Li Y, *et al*. The effect of vitamin and mineral supplementation on esophageal cytology: results from the Linxian Dysplasia Trial. *Int J Cancer* 1994;**57**:162–6.
12 **Blot W**, Li Y, Taylor P, *et al*. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993;**85**:1483–92.
13 **Dawsey S**, Wang G, Taylor P, *et al*. Effects of vitamin/mineral supplementation on prevalence of histological dysplasia and early cancer of the esophagus and stomach: results from the Dysplasia Trial in Lixian, China. *Cancer Epidemiol Biomarkers Prev* 1994;**3**:167–72.
14 **Li B**, Taylor P, Li Y, *et al*. Linxian nutrition intervention trials. Design, methods, participant characteristics, and compliance. *Ann Epidemiol* 1993;**3**:577–85.
15 **Stolzenberg-Solomon R**, Qiao Y-L, Abnet C, *et al*. Esophageal and gastric cardia cancer risk and folate- and vitamin B12-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomarkers Prev* 2003;**12**:1222–6.
16 **Prentice R**. A case-cohort design for epidemiologic cohort studies and disease prevention trials. *Biometrika* 1986;**73**:1–11.
17 **Liu H**. *Treatment and rescue of cerebral vascular disease*. Zhengzhou, China: Henan College of Medical Sciences Publishing House, 2000.
18 **Li L**. *Internal medicine*. Beijing, China: People's Medicine Publishing House, 1988.
19 **Mark S**, Wang W, Fraumeni JJ, *et al*. The effect of nutritional supplementation on stroke mortality and blood presure: results from the Linxian Nutritional Trials. In: Neve J, Chappuis P, Lamand M, eds. *Therapeutic uses of trace elements*. New York: Plenum Press, 1996:395–402.
20 **Mark S**, Wang W, Fraumeni J, *et al*. Lowered risk of hypertension and cerebrovascular disease after vitamin/mineral supplementation: the Linxian Nutrition Intervention Trial. *Am J Epidemiol* 1996;**143**:658–64.
21 **Wei W-Q**, Abnet C, Qiao Y-L, *et al*. Prospective study of serum selenium concentrations and esophageal and gastric cardia cancer, heart disease, stroke, and total death. *Am J Clin Nutr* 2004;**79**:80–5.
22 **Ratnasinghe D**, Yao S-X, Tangrea J, *et al*. Polymorphisms of the DNA repair gene XRCC1 and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:119–23.
23 **Mark S**, Katki H. Influence function based variance estimation and missing data issues in case-cohort studies. *Lifetime Data Anal* 2001;**7**:331–44.
24 **Yu Z**, Chen J, Ford B, *et al*. Human DNA repair systems: an overview. *Environ Mol Mutagen* 1999;**33**:3–20.
25 **Goode E**, Ulrich C, Potter J. Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;**11**:1513–30.
26 **Hou S-M**, Falt S, Angelini S, *et al*. The XPD variant alleles are associated with increased aromatic DNA adduct level and lung cancer risk. *Carcinogenesis* 2002;**23**:599–603.
27 **Zou X**, Taylor P, Mark S, *et al*. Seasonal variation of food composition and selected nutrient intake in Lixian, a high risk area for esophageal cancer in China. *Int J Vitam Nutr Res* 2002;**72**:375–82.
28 **Yang C**, Sun Y, Yang Q, *et al*. Vitamin A and other deficiencies in Linxian, a high esophageal cancer incidence area in Northern China. *J Natl Cancer Inst* 1984;**73**:1449–53.
29 **Mark S**, Qiao Y-L, Selhub J, *et al*. Serum cysteine and riboflavin are inversely related to to incident esophageal and grastric cardia cancers. AACR abstract. *Proc Am Assoc Cancer Res* 2001.
30 **Kruman I**, Kumaravel T, Lohani A, *et al*. Folic acid deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of alzheimer's disease. *J Neurosci* 2002;**22**:1752–62.
31 **Choi S**, Mason J. Folate and carcinogenesis: an integrated scheme. *J Nutr* 2000;**130**:129–32.
32 **Hankey G**, Eikelboom J. Homocysteine and stroke. *Curr Opin Oncol* 2001;**14**:95–102.
33 **Li Z**, Sun L, Zhang H, *et al*. Elevated plasma homocysteine was associated with hemorraghic and ischemic stroke, but methylenetetrahydrofolate reductase gene C677T polymorphism was a risk factor for thrombotic stroke: a multicenter case-control study in China. *Stroke* 2003;**34**:2085–90.
34 **Giles W**, Croft J, Greenlund K, *et al*. Total homocysteine concentration and the likelihood of nonfatal stroke: results from the Third National Health and Nutrition Examination Survey, 1988–1994. *Stroke* 1998;**29**:2473–7.
35 **Perry I**, Refsum H, Morris R, *et al*. Prospective study of serum total homocysteine concentration and risk of stroke in middle-aged British men. *Lancet* 1995;**346**:1395–8.
36 **Kawase M**, Fujimura M, Morita-Fujimura Y, *et al*. Reduction of apurinic/apyrimidinic endonuclease expression after transient global cerebral ischemia in rats. *Stroke* 1999;**30**:441–9.
37 **Fujimura M**, Morita-Fujimura Y, Sugawara T, *et al*. Early decrease of XRCC1, a DNA base excision repair protein may contribute to DNA fragmentation after transient focal cerebreal ischemia in mice. *Stroke* 1999;**30**:2456–63.