Eur. J. Oncol., vol. 13, n. 3, pp. 187-192, 2008

Polymorphisms and haplotypes of XRCC1 and APE1 and risk of childhood leukaemia in China: a case-control analysis

Polimorfismo ed aplotipi di XRCC1 ed APE1 e rischio di leucemia infantile in Cina: un'analisi caso-controllo

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Summary

***Aims*. To explore the relationship between genetic polymorphism of DNA repair and susceptibility to childhood leukaemia. *Materials and methods*. A hospital-based case-control study, with 105 cases and 108 controls, was conducted, to analyze the distribution of X-ray radiation, polymorphisms of *XRCC1* and *APE1* in study subjects, and investigate the interaction between X-ray radiation and polymorphisms. *Results*. There was an increased risk of leukaemia in children exposed to X-ray radiation. *XRCC1 399* and *APE1 148* polymorphisms are related to susceptibility to acute leukaemia (*OR=2.05, 95%CI 1.14-3.70; OR=1.94, 95%CI 1.05-3.58*), while no gene-environment interaction effect was found. The analysis of haplotypes of polymorphisms in *XRCC1* showed that children carrying Hap2 and Hap4 had an increased risk of leukaemia (*OR=2.88, 95%CI 1.58-5.25; OR=3.76, 95%CI 1.17-12.08*). *Conclusion*. Polymorphisms of *XRCC1* and *APE1* involved in base excision repair might influence**

Riassunto

***Finalità*. Studiare le correlazioni tra polimorfismo genetico della riparazione del DNA e predisposizione alle leucemie infantili. *Materiali e metodi*. È stato condotto uno studio caso-controllo su base ospedaliera, con 105 casi e 108 controlli, per analizzare la distribuzione dell'esposizione a raggi X, del polimorfismo di *XRCC1* e *APE1* nei soggetti studiati, e studiare le interazioni tra esposizione a raggi X e polimorfismo. *Risultati*. C'è stato un aumento del rischio di leucemie nei bambini esposti a raggi X. I polimorfismi di *XRCC1 399* e di *APE1 148* sono correlati alla predisposizione a leucemia *(OR=2,05, 95%CI=1,14-3,70; OR=1,94, 95%CI=1,05-3,58)*, mentre non è stato trovato alcun effetto dell'interazione gene-ambiente. L'analisi degli aplotipi dei polimorfismi in *XRCC1* ha mostrato che i bambini portatori di Hap2 e Hap4 hanno un aumentato rischio di leucemia (*OR=2,88, 95%CI=1,58-5,25; OR=3,76, 95%CI=1,17-12,08*). *Conclusioni*. Il polimorfismo di *HRCC1* e *APE1*, coinvolto nella ri-**

Received/Pervenuto 22.4.2008 - Accepted/Accettato 28.5.2008
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the repair of DNA damage and the susceptibility to childhood leukaemia. Eur. J. Oncol., 13 (3), 187-192, 2008

Key words: **childhood leukaemia, genetic polymorphism, DNA repair**

parazione della escissione di basi, potrebbe influenzare la riparazione del danno al DNA e la predisposizione alle leucemie infantili. Eur. J. Oncol., 13 (3), 187-192, 2008

Parole chiave: **leucemie infantili, polimorfismo genetico, riparazione del DNA**

Introduction

Childhood leukaemia, the most common malignancy under the age of 15, accounts for about one third of all childhood cancer[1]. There are 250,000 new cases every year throughout the world and the incidence rate is about 8-9 per 100,000. Confirmed clinical and epidemiologic associations explain less than 10% of disease incidence, leaving 90% of cases with an unclear aetiology[2]. Epidemiologic studies show that children exposed to ionizing radiation or carcinogenic chemicals, including benzene, are prone to leukaemia, which indicates that environmental risk factors may play an important rôle in the development of childhood leukaemia[3]. However, in a similar way to most human diseases, the cause of childhood leukaemia involves not only environmental risk factors but also individual susceptibility.

It has been known that genes involved in DNA repair are critical in maintaining the integrity of genetic material as well as in protecting against mutations that could result in cancer[4]. Maintenance of genomic integrity in mammalian cells depends heavily on the presence of efficient DNA repair systems. Reduction in mammalian DNA repair capacity is associated with increasing birth defects, cancer and reduced lifespan. The human DNA repair system includes base excision repair (BER), nucleotide excision repair (NER), double strands break repair (DSBR), and mismatched repair (MMR). This system repairs damaged DNA caused by environmental carcinogenic factors. Single nucleotide polymorphisms (SNPs), existing widely in the human genome, may result in alteration of structure and activity of gene products, and influence the response to carcinogenic factors[5, 6]. Many researches have found that SNPs on some DNA repair genes are related to cancer predisposition in some individuals.

Radiation damages cellular DNA in many ways, requiring the concerted action of a number of DNA repair enzymes in the BER pathway where *XRCC1* (X-ray repair cross complementing group 1) and *APE1* (apurinic apyrimidinic endonuclease 1) protein play an important rôle in the process of repair[7]. We hypothesize that SNPs in *XRCC1* and *APE1* may influence radiation sensitivity and susceptibility to childhood leukaemia and have conducted a hospital-based case-control study to investigate the relationship between SNPs and genetic predisposition.

Materials and methods

Study population

A convenience sample of 105 inpatients, pathologically diagnosed with childhood leukaemia in two tertiary paediatric hospitals in Shanghai from October 2001 to October 2004, were included in the study including 94 ALL (acute lymphoblastic leukaemia) patients and 11 ANLL (acute nonlymphoblastic leukaemia) patients. Children from the same hospital without cancer or haematological diseases were selected as controls (n=108). All cases and controls were under 14 years old. Informed consent was obtained from all parents of the study subjects. Peripheral blood cells were collected, after serum had been separated and used for biochemistry tests in the hospital laboratories, and were stored at -70°C until the DNA samples were extracted from the blood clots with phenol-chloroform methods.

Data collection

A questionnaire was used to evaluate the exposure to possible risk factors including X-ray radiation,

house decoration, nearby gas stations, and parental occupational exposure to carcinogens. Trained interviewers contacted the parents and mothers were asked questions about exposure one year before diagnosis in the ward.

Genotyping

SNPs on *XRCC1* and *APE1* were detected with polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) techniques. The sequence of *XRCC1* and *APE1* was searched in GeneBank, and information about SNPs was obtained in the single nucleotide polymorphism data base (dbSNP). Primer Premier 5.0 was used to design the primers for PCR amplification. The polymorphic sequences flanking codon 194 (C/T, Arg→Trp), 280 (G/A, Arg→His) and 399 (G/A, Arg→Gln) in *XRCC1* and codon 148 (T/G, Asp→Glu) in *APE1* were amplified by PCR. The primers and reaction condition were as follows: *XRCC1* 194: F 5'-GCC CCG TCC CAG GTA-3', R 5'-AGC CCC AAG ACC CTT TCA CT-3', 94° 1 min→58° 50s→72° 50s, 35 cycles; *XRCC1* 280: F 5'-TGG GGC CTG GAT TGC TGG GTC TG-3', R 5'-CAG CAC CAC TAC CAC ACC CTG AAG G-3', 94° 1 min→69.5° 1 min→72° 35s, 35 cycles; *XRCC1* 399: F 5'-TTG TGC TTT CTC TGT GTC CA-3', R 5'-TCC TCC AGC CTT TTC TGA TA-3', 94° 1 min→56° 1 min→72° 1 min, 35 cycles; *APE1* 148: F 5'-CTG TTT CAT TTC TAT AGG CTA-3', R 5'-AGG AAC TTG CGA AAG GCT TC-3', 94° 40s→54° 40s→72° 35s, 35 cycles. PCR products were digested with specific restriction enzymes *Msp I*, *Rsa I*, *Msp I* and *Xsp I* in 37° for 16 hours, and resolved on agarose gels for electrophoresis. RFLP results were visualized using a UV transilluminator after ethidium bromide staining.

Statistical analysis

Statistical analysis was done in Statistical Package for Social Science (SPSS) release 11.5. Risk of childhood leukaemia was analyzed using the Chi square (χ^2) test. The associations between genotypes and disease were analyzed by calculating the crude Odds Ratios (COR) and 95% Confidence Intervals (95%CI). OR was also adjusted for X-ray radiation by unconditional logistic regression analysis.

Results

The case and control groups included 105 and 108 patients respectively, and the distribution of gender and age in the two groups has no significant difference (Table 1). The proportion of children exposed to more than one X-ray radiation in the case group were more than in the control group, which suggests that X-ray radiation may be one of the environmental risk factors.

Children carrying alleles of *XRCC1* 399Gln (OR=2.05, 95%CI 1.14-3.70), *APE1* 148Glu (OR=1.94, 95%CI 1.05-3.58) had a higher risk of leukaemia (Table 2). Whether adjusted for X-ray radiation or not, the OR is more than 1, which indicated that polymorphisms of *XRCC1* 399 and *APE1* 148 may be related to genetic susceptibility to childhood leukaemia.

To study the interaction between X-ray radiation and genetic polymorphisms, a stratification analysis was conducted (Table 3). The ORs between blocks were not significantly different, suggesting that gene-environment interactions might not exist.

Childhood ALL risk was analyzed using the χ^2 test: 94 ALL cases were selected among the total 105 children with acute leukaemia, and it was found that children carrying alleles of *XRCC1* 399Gln (OR=2.05, 95%CI 1.12-3.78) might have a higher risk of ALL (Table 4).

A haplotype is the combination of nearby SNPs in one chromosome, which has much more genetic

Table 1 - Gender, age and exposure to risk factors in the subjects

	Control (N = 108)	Case (N = 105)	χ^2	*p*
Gender				
Male	69	61		
Female	39	44	0.75	0.39
Age				
0-4	41	35		
5-9	35	43		
10-15	32	27	1.68	0.43
X-ray radiation exposure				
None	78	47		
≥1	30	58	16.5	0.00*

* $p<0.05$

Table 2 - *XRCC1* and *APE1* polymorphisms and childhood leukaemia (ALL and ANLL)

Polymorphisms	Control (N = 108)	Case (N = 105)	OR (95%CI)	ORadj (95%CI)[a]
XRCC1 Arg194Trp				
Arg/Arg	42	52		
Arg/Trp+Trp/Trp	66	53	0.65 (0.38-1.12)	0.62 (0.34-1.11)
XRCC1 Arg280His				
Arg/Arg	81	71		
Arg/His+His/His	27	34	1.44 (0.79-2.61)	1.49 (0.78-2.86)
XRCC1 Arg399Gln				
Arg/Arg	62	45		
Arg/Gln+Gln/Gln	46	60	1.80 (1.04-3.09)*	2.05 (1.14-3.70)*
APE1 Asp148Glu				
Asp/Asp	48	31		
Asp/Glu+Glu/Glu	60	74	1.91 (1.08-3.36)*	1.94 (1.05-3.58)*

[a] adjusted for X-ray radiation
* $p<0.05$

Table 3 - *XRCC1* and *APE1* polymorphisms and childhood leukaemia (ALL and ANLL), stratified by X-ray radiation

Genotypes	X-ray (none)			X-ray (≥1)		
	Control (N = 78)	Case (N = 47)	OR (95%CI)	Control (N = 30)	Case (N = 58)	OR (95%CI)
XRCC1 194						
Arg/Arg	30	25		12	27	
Arg/Trp+Trp/Trp	48	22	0.55 (0.26-1.14)	18	31	0.76 (0.31-1.87)
XRCC1 280						
Arg/Arg	58	34		23	37	
Arg/His+His/His	20	13	1.11 (0.49-2.51)	7	21	1.86 (0.68-5.08)
XRCC1 399						
Arg/Arg	43	20		19	25	
Arg/Gln+Gln/Gln	35	27	1.66 (0.80-3.44)	11	33	2.28 (0.92-5.64)
APE1 148						
Asp/Asp	36	16		12	15	
Asp/Glu+Glu/Glu	42	31	1.66 (0.78-3.52)	18	43	1.91 (0.75-4.88)

information than a SNP at single sites. We used PHASE 2.0.2, a software for haplotype analysis to calculate the distribution of 8 haplotypes in two groups. Compared with the group carrying Hap1(*XRCC1* 194 Arg, *XRCC1* 280 Arg and *XRCC1* 399Arg), children carrying Hap2 (*XRCC1* 194 Arg, *XRCC1* 280 Arg and *XRCC1* 399Gln) and Hap4 (*XRCC1* 194 Arg, *XRCC1* 280 His and *XRCC1* 399Gln) had an increased risk of leukaemia (OR=2.88, 95%CI 1.58-5.25; OR=3.76, 95%CI 1.17-12.08) (Table 5).

Discussion

It has been suggested that reduced DNA repair capacity may be a susceptibility factor for human cancer. Genetic variations in DNA repair genes may

Table 4 - *XRCC1* and *APE1* polymorphisms and childhood leukaemia (ALL), analyzed using the χ^2 test

Polymorphisms	Control N = 108)	Case (N = 94)	OR (95%CI)	ORadj (95%CI)[a]
XRCC1 Arg194Trp				
Arg/Arg	42	47		
Arg/Trp+Trp/Trp	66	47	0.64 (0.36-1.11)	0.61 (0.34-1.12)
XRCC1 Arg280His				
Arg/Arg	81	61		
Arg/His+His/His	27	33	1.62 (0.88-2.98)	1.71 (0.89-3.31)
XRCC1 Arg399Gln				
Arg/Arg	62	40		
Arg/Gln+Gln/Gln	46	54	1.82 (1.04-3.18)*	2.05 (1.12-3.78)*
APE1 Asp148Glu				
Asp/Asp	48	30		
Asp/Glu+Glu/Glu	60	64	1.71 (0.96-3.04)	1.71 (0.92-3.20)

[a] adjusted for X-ray radiation
* $p<0.05$

Table 5 - The haplotypes of *XRCC1* and childhood leukaemia

Haplotypes[a]	Case	Control	OR (95%CI)	*p*
Hap1(111)	83	104	1.00	
Hap2(112)	46	20	2.88 (1.58-5.25)	0.0004
Hap3(121)	6	4	1.88 (0.51-6.88)	0.33
Hap4(122)	12	4	3.76 (1.17-12.08)	0.02
Hap5(211)	34	45	0.95 (0.56-1.61)	0.84
Hap6(212)	8	20	0.50 (0.21-1.20)	0.11
Hap7(221)	11	10	1.38 (0.56-3.40)	0.48
Hap8(222)	10	9	1.39 (0.54-3.58)	0.49

[a] Alleles from the left to the right are *XRCC1* Arg194Trp, *XRCC1* Arg280His and *XRCC1* Arg399Gln: 1 stands for the wild type, 2 for the mutant type

lead to inter-individual variation in DNA repair capacity and modify the associations between exogenous and endogenous carcinogens and cancer risk. DNA damage caused by X-ray radiation, such as base lesion, oxidative damage and strand breaks, may be an important molecular event in the initiation of leukaemia.

The *XRCC1* protein is involved in the BER pathway, which is responsible for the repair of a wide variety of non-bulky exogenous and endogenous base damage and single strand breaks[8]. Although *XRCC1* has no known enzymatic activity, there are three distinct domains that are sites of interaction with DNA polymerase, poly (ADP-ribose) polymerase and DNA ligase III. *XRCC1* also interacts with polynucleotide kinase and *APE1*. This suggests that *XRCC1* may act as a nucleating factor in BER by bringing different components together at the site of action to promote the efficiency of the repair machinery. A number of SNPs in *XRCC1* have been identified[9], with some altering BER proficiency and, in turn, conferring genetic predisposition to cancer[10-13]. Our results suggested *XRCC1* 399 mutant allele is related to higher risk of childhood leukaemia, in accordance with prior functional and epidemiological research[14].

The multifunctional *APE1* is responsible for the repair of AP (apurine/apyrimidine) sites in DNA[15]. It also functions as a redox factor facilitating the DNA-binding capability of numerous transcription factors[16]. Abasic sites represent ubiquitous DNA lesions that arise spontaneously or are induced by DNA-damaging agents. They block DNA replication and are considered cytotoxic and mutagenic. *APE1* plays a central rôle in BER in two distinct ways. First, it initiates repair of AP sites in DNA produced either spontaneously or after removal of uracil and alkylated bases by mono-functional DNA glycosylases. Second, it can act as a 3'-phosphoesterase to repair strand breaks either directly induced by reactive oxygen species or indirectly through the AP lyase reaction of DNA damage-specific glycosylases. Two functional studies found that variation at

codon 148 in *APE1* gene (*Asp*/*Glu*) leads to alteration of radiation sensitivity[17, 18]. In our study, there was a relationship between this polymorphism and the risk of leukaemia, which indicated that *APE1* might be of importance in the process of repairing DNA damage caused by X-ray radiation.

Our study results suggest that *XRCC1* and *APE1* polymorphisms might significantly influence the risk of leukaemia. Despite the fact that our results did not demonstrate an interaction between X-ray radiation and these polymorphisms, further studies with larger sample sizes are needed to conclusively answer this question. Meanwhile, we have only studied four polymorphisms in two BER genes, and more important repair genes in different pathways need to be explored in order to obtain a full-scale understanding of genetic and environmental factors that influence susceptibility to childhood leukaemia and prevent the disease.

Acknowledgements

We are very grateful to Drs. Myron A. Mehlman and Joel Forman (Department of Community and Preventative Medicine, Mount Sinai School of Medicine, New York, USA) for English editing on this article.

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