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Original Article

Identification of SNPs in displacement loop region of mitochondrial DNA as risk factors for rheumatoid arthritis

Xiaoyun Zhang[1*], Xueqing Gao[2*], Shang Li[2], Chenxing Peng[3], Zhanjun Guo[1], Ruixing Zhang[2], Yue Zhao[2], Jianhua Wu[4]

[1]Department of Rheumatology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, P.R. China; [2]Departments of Gastroenterology and Hepatology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, P.R. China; [3]Department of Rheumatology, The Second Hospital of Hebei Medical University, Shijiazhuang, P.R. China; [4]Hebei Key Lab of Laboratory Animal, Hebei Medical University, Shijiazhuang, P.R. China. [*]Equal contributors.

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Abstract: Rheumatoid arthritis (RA) is chronic inflammatory synovitis characterized by oxidative damage that associated with joint deformities and joint dysfunction. Single nucleotide polymorphisms (SNPs) in the displacement loop (D-loop) region of peripheral blood mitochondrial DNA (mtDNA) were sequenced in RA patients to evaluate the predictive value for RA in a case-control study. SNPs of nucleotides 146T/C, 150C/T, 195T/C, 16260C/T, and 16519T/C were found to be closely related to RA risk at statistical levels ($p < 0.05$). The major alleles of nucleotides 195T/C, 16260C/T and 16519C/T, the minor alleles of nucleotides 146T/C and 150C/T were associated with increased risk for RA. In conclusion, The SNPs of mtDNA D-loop may play roles in pathogenesis of RA.

Keywords: D-loop, RA, SNP, onset risk

Introduction

Rheumatoid arthritis (RA), one of the most common autoimmune diseases characterized by synovial hyperplasia and joint disability, is related to early death, systemic complications, and high socioeconomic costs [1]. The prevalence of RA is around 0.5-1% worldwide and the prevalence in females is four times that of males [2]. The incidence of RA in China has ranged from 3.9 to 6.3 million new cases per year with a higher prevalence found in city [1, 3, 4]. Many factors, including ROS overproduction, contribute to the pathogenesis of RA [5].

The human mitochondrial genome is a length of 16 kb closed loop molecule consists of two ribosomal RNAs and a complete 22 tRNAs [6]. Due to high ROS content, lack of histone protection, and limited repair ability, mtDNA is more susceptible to DNA damage and is more prone to mutation than nuclear DNA [7-9]. The noncoding region of mtDNA which is called D-Loop, contains the origin of the leading chain of replication and the main promoter of transcription, is important to regulate replication and expression of the mitochondrial genome [10]. Many diseases including Parkinson's disease [11], coronary artery disease [12], chronic kidney disease [13] and renal cell carcinoma [14] have relationships with SNPs of mitochondrial D-loop. Several studies have focused on the predictive value of SNPs in the mtDNA D-loop region for some chronic disease [11-14], but the predictive value of SNPs in the mtDNA D-loop region for RA has not been evaluated. In this study, approximately 1 kb gene fragment of D-loop was sequenced to evaluate the role of D-loop SNPs in the pathogenesis of RA.

Materials and methods

Tissue specimens

Blood samples from 85 patients including 16 males and 69 females, confirmed to RA based on the 2010 revised criteria of the American College of Rheumatology [15], were obtained

Table 1. Clinical data in RA patients and control

Items	patients (n = 85)	Control (n = 100)
Age (years)	53.92 ± 11.47	54.86 ± 10.22
Gender (M/F)	16/69	20/80
Other immunological disease (yes/no)	46/39	-
DAS28		-
≤ 2.6	2	-
> 2.6DAS28 ≤ 3.2	3	-
> 3.2DAS28 ≤ 5.1	19	-
> 5.1	61	-
Anti-CCP (+/-)	41/44	-
CRP (+/-)	63/22	-
RF (+/-)	63/22	-

from the Department of Rheumatology in the Second Hospital of Hebei Medical University between 2017-5 (May 2017) and 2017-12 (Dec 2017). The clinical manifestations of RA patients including age, sex, disease activity score (DAS28), and the laboratory test results such as anti-CCP, CRP and ESR were collected. Disease activity was assessed by DAS28. DAS28 less than or equal to 2.6 was defined as clinical remission, 2.6-3.2 as low activity, 3.2-5.1 as moderate activity and higher than 5.1 as high activity [http://www.das28.nl/das28/DAScalculat-ors/dasculators.html]. The blood of age and sex matched healthy controls was also collected at the same time. The study was performed in accordance with the 1964 Declaration of Helsinki, and also was approved by the Institutional Research Ethics Committee and the Human Tissue Research Committee of the Second Hospital of Hebei Medical University. All patients provided written informed consent for the collection of samples and subsequent analysis. Wizard Genomic DNA extraction kit (Promega, Madison, WI, USA) was used for mtDNA extraction. mtDNA was stored in -20°C after extraction.

PCR amplification and sequence analysis.

The upstream primer 5'-CCCCATGCTTACAAGCAAGT-3' (nucleotide 16190-16209) and downstream primer 5'-GCTTTGAGGAGGTAAGCTAC-3' (nucleotide 602-583) were used to amplify a 982 bp product in mtDNA D-Loop region by PCR (Master Mix Kit nucleotide) [16]. The products were purified prior to sequencing. Cycle sequencing was carried out by the dGTP BigDye® Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystem, Foster City, CA) and then the products were separated on the ABI PRISM Genetic Analyzer 3730XL (Applied Biosystems). The sequence polymorphisms of mtDNA D-loop were confirmed by repeated analysis from both strands.

Statistical analysis

Student' t-test and rank sum test were used to determine differences in SNP distribution within the D-Loop region and the number of SNPs per patient among groups. Fisher's exact test and Chi-square was used to detect differences in individual SNPs for each patient group. Due to the non-normality of data distribution, the Rank sum test was used to determine the differences of SNP distribution within the D-Loop region and the number of SNPs per patient among groups. Chi-square test was used to identify the differences of age and sex distributions among groups and to determine the relationship between the SNPs of mtDNA D-loop and RA. A p value of less than 0.05 was considered statistically significant. All of the statistical analyses were done with the SPSS 21.0 software (SPSS Inc, Chicago, IL).

Results

The age and gender distributions between RA patients and controls were compared by the Chi-square test, and no statistical difference was found to exist between these two groups (**Table 1**).

The differences of SNPs distribution in the mitochondrial D-loop region was compared subsequently, SNPs were detected in 112 sites, the average SNPs frequency (the sum of SNPs per person) was not statistically different between RA patients and the controls (data not shown). The 25 SNPs with minor allele frequency higher than 5% were used to analyze their association with RA risk (**Figure 1**).

Five SNPs, were found in the D-loop that were significantly different by Chi-square test between RA and controls ($p < 0.05$). The major alleles of nucleotides 195T/C, 16260C/T and 16519C/T, the minor alleles of nucleotides

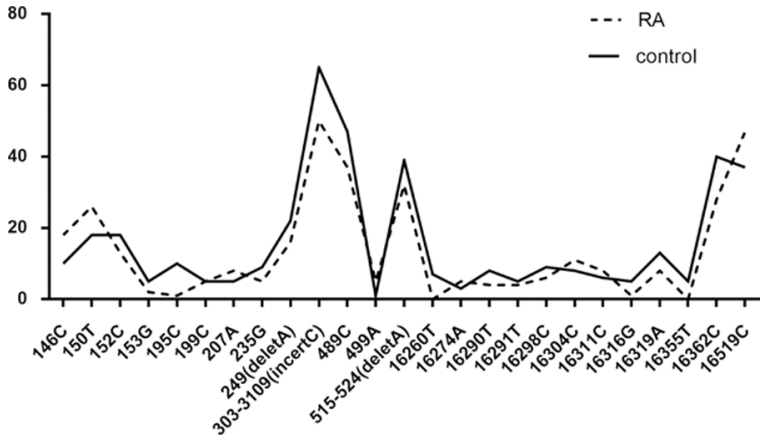

Figure 1. Distribution of D-loop SNPs at 25 sites (X axis) and their relative frequencies in percentage within each group (Y axis).

Table 2. RA Onset risk associated SNPs in RA

SNP	Genotype	RA (n = 85)	Control (n = 100)	*p*-value
16260				
	C/C	85 (100%)	93 (93%)	
	T/T	0 (0%)	7 (7%)	0.016
16519				
	T/T	38 (45%)	63 (63%)	
	C/C	47 (55%)	37 (37%)	0.013
146				
	T/T	67 (79%)	90 (90%)	
	C/C	18 (21%)	10 (10%)	0.035
150				
	C/C	59 (70%)	82 (82%)	
	T/T	26 (30%)	18 (18%)	0.045
195				
	T/T	84 (99%)	90 (90%)	
	C/C	1 (1%)	10 (10%)	0.011

146T/C and 150C/T were associated with an increased risk for RA (**Table 2**).

Discussion

The current study shows SNPs of nucleotides 146T/C, 150C/T, 195T/C, 16260C/T and 16519C/T that were associated with RA. Four of five RA risk associated SNPs were located in the hypervariable (HV) segment region of the D-loop, the site of 16260 located in HV-I, while the sites of 146, 150, 195 located in HV-II [https://mitomap.org/MITOMAP].

The D-loop of mtDNA plays an important role in regulating mitochondrial genome duplication and expression, so the SNPs in D-loop might inhibit the function of the electron transport chain that results in the release of a large amount of ROS thereby to interfere the process of oxidative phosphorylation and initiate the vicious cycle between D-loop SNP and ROS to induce nuclear genome damage and illness progress [17-20]. The allele 146C, 150T were more likely to develop chronic diseases such as Leber hereditary optic neuropathy [21, 22] and Parkinson's disease [23, 24], and the nucleotide 16260C/T was associated with schizophrenia [25]. In our previous study, the allele 16519C was identified as associated with the risk of gastric cancer due to its association with oxidative damage and ROS generation [26, 27]. A large amount of literature indicates that ROS can act as a secondary messenger in activating of nuclear factor kappa-B (NF-κB) to induce a variety of inflammatory mediators transcription so as to initiate the pathogenesis of RA [17, 28-32]. The mechanism of how normal alleles contributed to the pathogenesis of RA remains uncertain, and normal alleles in the mitochondrial D-loop associated with disease risk [26, 33] were not found. These alleles might link with candidate genes of diseases in mitochondrial coding region. Furthermore, complementary alleles might exhibit an inhibitory effect on the disease process. Subsequent functional studies are needed to validate the predictive values of these SNPs for RA.

Conclusion

Taken together, SNPs in D-loop play a role in the pathogenesis of rheumatic diseases.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhanjun Guo, Department of Rheumatology, The Fourth Hospital of Hebei Medical University, 12 Jiankang Road, Shijiazhuang 050011, P.R. China. Tel: 86-0311-860953-

42; Fax: 86-0311-86086919; E-mail: zjguo5886@aliyun.com

References

[1] Li R, Sun J, Ren LM, Wang HY, Liu WH, Zhang XW, Chen S, Mu R, He J, Zhao Y, Long L, Liu YY, Liu X, Lu XL, Li YH, Wang SY, Pan SS, Li C, Wang HY, Li ZG. Epidemiology of eight common rheumatic diseases in China: a large-scale cross-sectional survey in Beijing. Rheumatology (Oxford) 2012; 51: 721-9.

[2] Harris ED Jr. Pathogenesis of rheumatoid arthritis. Am J Med 1986; 80: 4-10.

[3] Langley PC, Mu R, Wu M, Dong P and Tang B. The impact of rheumatoid arthritis on the burden of disease in urban China. J Med Econ 2011; 14: 709-19.

[4] Xu C, Wang X, Mu R, Yang L, Zhang Y, Han S, Li X, Wang Y, Wang G, Zhu P, Jin H, Sun L, Chen H, Cui L, Zhang Z, Li Z, Li J, Zhang F, Lin J, Liu X, Hu S, Yang X, Lai B, Li X, Wang X, Su Y and Li Z. Societal costs of rheumatoid arthritis in China: a hospital-based cross-sectional study. Arthritis Care Res (Hoboken) 2014; 66: 523-31.

[5] Gajewski M, Rzodkiewicz P, Maśliński S. The human body as an energetic hybrid? New perspectives for chronic disease treatment? Reumatologia 2017; 55: 94-99.

[6] Shadel GS and Clayton DA. Mitochondrial DNA maintenance in vertebrates. Annu Rev Biochem 1997; 66: 409-35.

[7] Beal MF. Mitochondria, free radicals, and neurodegeneration. Curr Opin Neurobiol 1996; 6: 661-6.

[8] DiMauro S and Schon EA. Mitochondrial DNA mutations in human disease. Am J Med Genet 2001; 106: 18-26.

[9] Lightowlers RN, Chinnery PF, Turnbull DM and Howell N. Mammalian mitochondrial genetics: heredity, heteroplasmy and disease. Trends Genet 1997; 13: 450-5.

[10] Taanman JW. The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta 1999; 1410: 103-23.

[11] Wu HM, Li T, Wang ZF, Huang SS, Shao ZQ, Wang K, Zhong HQ, Chen SF, Zhang X and Zhu JH. Mitochondrial DNA variants modulate genetic susceptibility to Parkinson's disease in Han Chinese. Neurobiol Dis 2018; 114: 17-23.

[12] Golubenko MV, Nazarenko MS, Frolov AV, Sleptsov AA, Markov AV, Glushkova ME, Barbarash OL and Puzyrev VP. [Analysis of Heteroplasmy in the major noncoding region of mitochondrial DNA in the blood and atherosclerotic plaques of carotid arteries]. Genetika 2016; 52: 497-502.

[13] Bai Y, Guo Z, Xu J, Zhang J, Cui L, Zhang H, Zhang S and Ai X. Association of sequence polymorphism in the mitochondrial D-loop with chronic kidney disease. Ren Fail 2014; 36: 781-4.

[14] Zhang J, Guo Z, Bai Y, Cui L, Zhang S and Xu J. Identification of sequence polymorphisms in the displacement loop region of mitochondrial DNA as a risk factor for renal cell carcinoma. Biomed Rep 2013; 1: 563-6.

[15] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JM, Hobbs K, Huizinga TW, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Ménard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Symmons D, Tak PP, Upchurch KS, Vencovsky J, Wolfe F, Hawker G. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 2010; 69: 1580-8.

[16] Lee HC, Li SH, Lin JC, Wu CC, Yeh DC and Wei YH. Somatic mutations in the D-loop and decrease in the copy number of mitochondrial DNA in human hepatocellular carcinoma. Mutat Res 2004; 547: 71-8.

[17] Burska AN, Hunt L, Boissinot M, Strollo R, Ryan BJ, Vital E, Nissim A, Winyard PG, Emery P and Ponchel F. Autoantibodies to posttranslational modifications in rheumatoid arthritis. Mediators Inflamm 2014; 2014: 492873.

[18] Dement GA, Maloney SC and Reeves R. Nuclear HMGA1 nonhistone chromatin proteins directly influence mitochondrial transcription, maintenance, and function. Exp Cell Res 2007; 313: 77-87.

[19] Bandy B and Davison AJ. Mitochondrial mutations may increase oxidative stress: implications for carcinogenesis and aging? Free Radic Biol Med 1990; 8: 523-39.

[20] Hervouet E, Simonnet H and Godinot C. Mitochondria and reactive oxygen species in renal cancer. Biochimie 2007; 89: 1080-8.

[21] Howell N, Kubacka I, Halvorson S, Howell B, McCullough DA and Mackey D. Phylogenetic analysis of the mitochondrial genomes from Leber hereditary optic neuropathy pedigrees. Genetics 1995; 140: 285-302.

[22] Puomila A, Hamalainen P, Kivioja S, Savontaus ML, Koivumaki S, Huoponen K and Nikoskelainen E. Epidemiology and penetrance of Leber hereditary optic neuropathy in Finland. Eur J Hum Genet 2007; 15: 1079-89.

[23] Khusnutdinova E, Gilyazova I, Ruiz-Pesini E, Derbeneva O, Khusainova R, Khidiyatova I, Magzhanov R and Wallace DC. A mitochondrial etiology of neurodegenerative diseases: evidence from Parkinson's disease. Ann N Y Acad Sci 2008; 1147: 1-20.

[24] Vives-Bauza C, Andreu AL, Manfredi G, Beal MF, Janetzky B, Gruenewald TH and Lin MT. Sequence analysis of the entire mitochondrial genome in Parkinson's disease. Biochem Biophys Res Commun 2002; 290: 1593-601.
[25] Bandelt HJ, Olivieri A, Bravi C, Yao YG, Torroni A and Salas A. 'Distorted' mitochondrial DNA sequences in schizophrenic patients. Eur J Hum Genet 2007; 15: 400-2; author reply 402-4.
[26] Wang H, Wang Y, Zhao Q, Guo Z, Zhang F, Zhao Y and Zhang R. Identification of sequence polymorphisms in the D-Loop region of mitochondrial DNA as a risk factor for gastric cancer. Mitochondrial DNA A DNA Mapp Seq Anal 2016; 27: 1045-7.
[27] Wang Y, Zhao Y, Luo X, Guo Z and Zhang R. Cancer risk associated single nucleotide polymorphisms of mitochondrial D-loop and 8-hydroxy-2'-deoxyguanosine levels in gastric cancer. Biotechnology & Biotechnological Equipment 2017; 31: 363-366.
[28] Mateen S, Moin S, Shahzad S and Khan AQ. Level of inflammatory cytokines in rheumatoid arthritis patients: correlation with 25-hydroxy vitamin D and reactive oxygen species. PLoS One 2017; 12: e0178879.
[29] Filippin LI, Vercelino R, Marroni NP and Xavier RM. Redox signalling and the inflammatory response in rheumatoid arthritis. Clin Exp Immunol 2008; 152: 415-22.
[30] Mateen S, Moin S, Khan AQ, Zafar A and Fatima N. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PLoS One 2016; 11: e0152925.
[31] Sarban S, Kocyigit A, Yazar M and Isikan UE. Plasma total antioxidant capacity, lipid peroxidation, and erythrocyte antioxidant enzyme activities in patients with rheumatoid arthritis and osteoarthritis. Clin Biochem 2005; 38: 981-6.
[32] Shah D, Wanchu A and Bhatnagar A. Interaction between oxidative stress and chemokines: possible pathogenic role in systemic lupus erythematosus and rheumatoid arthritis. Immunobiology 2011; 216: 1010-7.
[33] Mousavizadeh K, Rajabi P, Alaee M, Dadgar S and Houshmand M. Usage of mitochondrial D-loop variation to predict risk for Huntington disease. Mitochondrial DNA 2015; 26: 579-82.