ORIGINAL ARTICLE

Mitochondrial NADH Dehydrogenase Subunit 3 Polymorphism Associated with an Earlier Age at Onset in Male Machado–Joseph disease Patients

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Keywords

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SUMMARY

Aims: To investigate the potential effect of six previously reported candidate single nucleotide polymorphisms on age at onset (AAO) among Chinese patients with Machado–Joseph disease (MJD). **Methods:** Three hundred and twenty-four unrelated molecular-confirmed MJD patients were recruited between January 2006 and December 2014. The screening of candidate polymorphisms was first performed in 173 subjects using the SNaPshot® Multiplex System. The mitochondrial NADH dehydrogenase subunit 3 (*MT-ND3*) polymorphism 10398A>G (rs2853826) was further verified with Sanger sequencing in additional 151 patients. **Results:** An inverse correlation was found between expanded CAG repeat length and AAO. The expanded CAG repeat length can explain 63% of AAO variance. The 10398A polymorphism was significantly associated with a 3-year earlier AAO in male patients with MJD ($P = 0.001$). Stepwise multiple regressions revealed that the 10398A polymorphism could account for nearly 2% of AAO variance in male patients. **Conclusion:** Six candidate SNPs have been screened in Chinese patients with MJD. A remarkable earlier AAO was noted in male Chinese MJD patients with *MT-ND3* gene 10398A polymorphism.

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Introduction

Machado–Joseph disease (MJD), also known as spinocerebellar ataxia type 3 (SCA3), is a progressive and degenerative spinocerebellar disorder associated with a variety of clinical manifestations – including progressive ataxia, pyramidal, and extrapyramidal signs – ophthalmoplegia, and facial myokymia [1]. It is caused by a pathogenic CAG expansion in exon 10 of the *ATXN3* gene [2]. The length of pathogenic CAG expansion can approximately explain 45–87% of the total age at onset (AAO) variance in MJD [3]. Additionally, it has been demonstrated that the *APOE* ϵ 2 allele is associated with an earlier AAO among patients with MJD [4,5]. However, our previous study with larger population casted doubt on the role of *APOE* in MJD [3]. This has led to a search for additional genetic factors that modulate the AAO variance.

Even though few single nucleotide polymorphisms (SNPs) associated with AAO have been reported in MJD, they have been reported frequently in another common type of polyQ disease, Huntington disease (HD). More than fifty latest researches were carefully reviewed and six candidate SNPs were selected, which from genes is considered to have potential impact on the pathogenesis process of polyQ disease. The c.977C>G (p.Ser326Cys) polymorphism (rs1052133) of 8-oxoguanine DNA glycosylase gene (*OGG1*) has been reported to have susceptibility to oxidative pathology in patients with HD [6]. The c.587G>A (p.Arg196Lys) polymorphism (rs12738235) in human caspase-activated DNase gene (*hCAD*) was suspected to have an impact on DNA degradation during apoptosis [7]. A study of 276 French patients with HD showed the c.53C>A (p.Ser18Tyr) polymorphism (rs5030732) in ubiquitin carboxyl-terminal esterase L1 gene (*UCHL1*) associated with AAO variance in patients with HD [8]. The c.220G>A

(p.Val74Met) polymorphism (rs6265) of brain-derived neurotrophic factor gene (*BDNF*) may also be a potential modulator for the AAO of patients with HD [9]. Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha gene (*PPARGC1A*) has been implicated in the pathogenesis of several neurodegenerative disorders, such as Parkinson's disease (PD) [10] and Alzheimer's disease (AD) [11], besides HD [12]. The c.235-19637A>G polymorphism (rs7665116) in *PPARGC1A* has been reported to be a significant modifier of AAO in three European HD cohorts [13]. Several reports suggest that mitochondrial dysfunction may be involved in the pathogenic process of neurodegenerative disorders [14,15]. And the mitochondrial polymorphism 10398A>G (rs2853826) located on mitochondrial DNA NADH dehydrogenase 3 (*MT-ND3*) which encodes a component of the complex I may significantly reduce the risk of PD [16]. However, in a study of SCA2, this variant is associated with an earlier AAO in Cuban patients [17]. All association studies mentioned above have never been conducted in patients with MJD.

Considering a fact that MJD is a monogenic disorder, we supposed that the introduction of a control group may result in an unexplainable situation that the modifying genes were detected as causative gene. Therefore, we did not include a control group. Referring to the previous studies [4,5], we randomly selected a study population of 173 patients in the SNaPshot® screening. Another 151 patients with MJD were recruited in the further verifying of potential SNPs, which made the total number of our study population attain 324.

Methods

MJD Patients

A total of 324 unrelated molecular-confirmed patients with MJD were recruited between January 2006 and December 2014: 173 of them (90 males and 83 females) were randomly selected in the SNaPshot® screening. The study was approved by the ethics committee of Huashan Hospital and First Affiliated Hospital of Fujian Medical University. Written informed consents were signed by patients or their statutory guardians. AAO was defined as the age that the first symptom of ataxia occurred.

Molecular Analysis

Genomic DNA was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following standard procedures. Polymerase chain reaction (PCR) amplification of the CAG repeat expansion was performed with MJD52/MJD25 primers [2]. The number of CAG repeats was further verified by Sanger sequencing as previously reported [3,18]. The screening of candidate polymorphisms was first performed for 173 patients with the SNaPshot® Multiplex System (Life Technologies, Foster city, CA, USA). Then, SNaPshot® genotyping was following the "ABI PRISM SNaPshot Multiplex Kit" Protocol (genotyping primers are listed in Table S1). Furthermore, to avoid suspects for the accuracy of the SNaPshot® screening, the *MT-ND3* polymorphism (rs2853826) was amplified with PCR primers (mt25F: AACACCTCCTAGCCTTAC, mt25R: AGGATATGAGGTGTGAGCG) and identified in another 151 patients using Sanger sequencing.

Statistical Analysis

Statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Quantitative measures were summarized with descriptive statistics. Linear regression model was used to assess the relationship between CAG repeat length and AAO. Correlation between candidate SNPs and pathogenic CAG repeat length was assessed by Spearman's correlation coefficients. Differences in AAO caused by the effect of expanded *ATXN3* allele were adjusted by analysis of covariance (ANCOVA) and Bonferroni correction was also used. Stepwise multiple linear regressions were used to calculate the effect of several factors on the AAO: the number of CAG repeats in *ATXN3* alleles, their interaction, the candidate SNP genotypes, and the patient gender. Moreover, to reduce potential deviation when defining the AAO, differences in the AAO according to the SNP genotypes in 184 MJD patients with a shorter duration of disease (<5 years) were examined to avoid recall bias using ANCOVA after adjusting for the expanded *ATXN3* allele. *P*-values were two-tailed, and a significant level of 0.05 was used.

Results

The clinical and genetic backgrounds of the first-stage 173 unrelated and 324 unrelated patients with MJD are summarized in Table 1. In our study population, the length of expanded CAG repeats can explain 63% of AAO variance in total 324 patients (Figure 1A) and 73% in 167 male patients (Figure 1B). Stepwise multiple linear regressions indicated that different genders or normal *ATXN3* allele length did not have remarkable influence on AAO variance ($P = 0.803$ and $P = 0.567$).

After screening the candidate genes, we found *MT-ND3* polymorphism 10398A was significantly associated with an earlier AAO in male patients with MJD when adjusting for the effect of expanded *ATXN3* allele ($P = 0.002$, Table 2 & Figure 2), however, no significant differences in female patients ($P = 0.311$). To further study our findings, we increased the number of patients up to 324. It is still revealed that male MJD patients with 10398A polymorphism appeared to experience a 3-year earlier AAO when adjusting for the effect of expanded *ATXN3* allele ($P = 0.001$, Table 2 & Figure 2), while still no significant difference in female patients ($P = 0.791$). Stepwise multiple regressions revealed that the 10398A polymorphism can account for nearly 2% of AAO variance in male patients and 0.7% in total, in agreement with the result of the ANCOVA. Meanwhile, no interaction was found between 10398A polymorphism and the causative CAG repeat length in male patients with MJD ($P = 0.677$, Table S2). To avoid potential deviation of AAO, AAO differences between *MT-ND3* A10398G polymorphism in 184 MJD patients with duration of disease <5 years were further evaluated. Male patients with 10398A polymorphism still appeared to have earlier AAO ($P = 0.011$, Table 2 & Figure 2).

In the screening of the SNPs in *OGG1*, *hCAD*, *UCLH-1*, *BDNF* and *PPARGC1A*, no significant differences in AAO were found even when adjusting for the effect of expanded *ATXN3* allele (Table S3). The evaluation of patients with a shorter duration of disease revealed that no significant difference were found in AAO according to the other SNP genotypes (Table S3). Furthermore,

Table 1 Demographics of two stages of patients with MJD

	Average AAO (mean $\pm$ SD)	Average number of CAG repeats	
		Normal (mean $\pm$ SD)	Expanded (mean $\pm$ SD)
173 unrelated patients with MJD			
Male(90)	35.5 $\pm$ 11.4 (15–59)	19.7 $\pm$ 6.6 (14–34)	76.0 $\pm$ 3.6 (67–84)
Female(83)	37.8 $\pm$ 9.7 (16–60)	21.2 $\pm$ 7.7 (14–36)	75.2 $\pm$ 3.6 (60–84)
Total(173)	36.6 $\pm$ 10.7 (15–60)	20.4 $\pm$ 6.9 (14–36)	75.6 $\pm$ 3.6 (60–84)
324 unrelated patients with MJD			
Male(167)	35.7 $\pm$ 11.0 (14–72)	19.8 $\pm$ 6.9 (14–38)	76.1 $\pm$ 3.3 (67–84)
Female(157)	36.5 $\pm$ 10.9 (10–60)	20.1 $\pm$ 7.1 (14–37)	75.7 $\pm$ 4.2 (53–87)
Total(324)	36.1 $\pm$ 11.0 (10–72)	20.0 $\pm$ 7.0 (14–38)	75.9 $\pm$ 3.8 (53–87)
184 unrelated MJD patients with shorter duration of disease			
Male(104)	35.4 $\pm$ 11.9 (14–72)	20.1 $\pm$ 7.0 (14–35)	76.2 $\pm$ 3.6 (67–84)
Female(80)	36.2 $\pm$ 12.3 (10–60)	20.2 $\pm$ 7.1 (14–37)	75.6 $\pm$ 5.1 (53–87)
Total(184)	35.7 $\pm$ 12.0 (10–72)	20.1 $\pm$ 7.0 (14–37)	76.0 $\pm$ 4.3 (53–87)

Figure 1 (A) The correlation between the number of expanded CAG repeats and the age at onset (AAO) in a total 324 patients. The expanded CAG repeats can explain 63% of AAO. (B) The correlation between the number of expanded CAG repeats and the AAO in 167 male patients. The expanded CAG repeats can explain 73% of AAO.

sex-specific effects failed to show significant difference in AAO among the other candidate SNPs (Table S3), and no interaction were found between candidate SNPs and the causative length of CAG repeats (Table S2).

Discussion

MJD is caused by toxic accumulation of pathogenic polyQ tracts encoded by expanded CAG repeats in exon 10 of *ATXN3*. However, the variation of causative CAG expansion cannot explain all the AAO variance. In the current study, we identified a negative correlation between AAO and expanded CAG repeats, the latter can explain 63% to 73% AAO variance in patients with MJD, similar to previous reports [5,19,20]. More importantly, we investigated 6 previously reported candidate polymorphisms in Chinese patients with MJD. Interestingly, we found a remarkable association between earlier AAO of male patients with MJD and *MT-ND3* 10398A polymorphism. Stepwise multiple regressions revealed that the 10398A polymorphism can account for nearly 2% of AAO variance in male patients and 0.7% in total.

Mitochondrion is essential for the survival of neuron, as it provides usable energy in the form of ATP. Mitochondrial dysfunction has been frequently linked to neurodegenerative disorders [21]. Actually, the presence of mtDNA dysfunction has been reported several times in MJD, even the precise mechanism remains unclear [22,23]. It has also been revealed in transgenic mice models of MJD that the mtDNA 3876-bp deletion accumulated with age and phenotype development [24]. The 10398 polymorphism is the most common variation in the mitochondrial complex I gene. Normally, complex I produce reactive oxygen species (ROS) during cellular activity. It is conceivable that 10398A allele, compared to 10398G, may inhibit the performance of complex I in the brain or cerebellar [16]. Once it has been compromised, generation of ROS enhanced, leading to oxidative stress [25]. Therefore, the toxic effects of oxidative injury may strengthen the toxic effects of misfolded pathogenic polyglutamine tracts, which may cause an earlier onset of MJD.

Unexpectedly, it has also been suggested in our study that the effect of 10398A polymorphism in *MT-ND3* is sex specific. Only male patients with MJD showed a significant association with an

Table 2 Descriptive data for the mitochondrial A10398G polymorphism (rs2853826)

					Average number of CAG repeats		
SNPs	Gender	Genotypes	Number of patients (%)	Adjusted mean AAO $\pm$ SE*	Normal mean $\pm$ SD	Expanded mean $\pm$ SD	P value
rs2853826 (Part1)	Male	A	38 (42.2%)	33.5 $\pm$ 0.8	19.8 $\pm$ 6.7	76.3 $\pm$ 3.6	0.002
		G	52 (57.8%)	36.9 $\pm$ 0.7	19.6 $\pm$ 6.6	75.8 $\pm$ 3.6	
	Female	A	40 (48.2%)	38.6 $\pm$ 1.2	22.1 $\pm$ 7.2	74.9 $\pm$ 4.5	0.311
		G	43 (51.8%)	37.0 $\pm$ 1.1	20.3 $\pm$ 7.0	75.4 $\pm$ 2.6	
	Total	A	78 (45.1%)	35.9 $\pm$ 0.7	21.0 $\pm$.0	75.6 $\pm$ 4.1	0.234
		G	95 (54.9%)	37.1 $\pm$ 0.7	19.9 $\pm$ 6.7	75.6 $\pm$ 3.2	
rs2853826 (Total)	Male	A	68 (40.7%)	33.9 $\pm$ 0.7	19.5 $\pm$ 6.7	76.2 $\pm$ 3.6	0.001
		G	99 (59.3%)	36.9 $\pm$ 0.6	20.0 $\pm$ 7.0	76.0 $\pm$ 3.2	
	Female	A	69 (43.9%)	36.3 $\pm$ 0.9	20.5 $\pm$ 7.4	75.4 $\pm$ 5.1	0.791
		G	88 (56.1%)	36.6 $\pm$ 0.8	19.8 $\pm$ 6.9	75.9 $\pm$ 3.3	
	Total	A	137 (42.3%)	35.0 $\pm$ 0.6	20.0 $\pm$ 7.1	75.8 $\pm$ 4.4	0.016
		G	187 (57.7%)	36.8 $\pm$ 0.5	19.9 $\pm$ 7.0	76.0 $\pm$ 3.2	
rs2853826 (Short duration of disease)	Male	A	43 (41.3%)	33.5 $\pm$ 1.0	19.7 $\pm$ 7.0	76.1 $\pm$ 4.2	0.011
		G	61 (58.7%)	36.8 $\pm$ 0.8	20.4 $\pm$ 7.0	76.3 $\pm$ 3.1	
	Female	A	37 (46.3%)	36.5 $\pm$ 1.4	21.6 $\pm$ 7.4	74.3 $\pm$ 6.3	0.787
		G	43 (56.7%)	35.9 $\pm$ 1.3	19.0 $\pm$ 6.6	76.7 $\pm$ 3.6	
	Total	A	80 (43.5%)	34.6 $\pm$ 0.8	20.6 $\pm$ 7.2	75.2 $\pm$ 5.3	0.077
		G	104 (56.5%)	36.6 $\pm$ 0.7	19.8 $\pm$ 6.9	76.5 $\pm$ 3.3	

*Adjusted for the average expanded CAG repeat length in the studied series of patients with MJD. Bold values indicate statistically significant values $P < 0.05$.

Figure 2 Mean AAO of patients with MJD. Mean ($\pm$ SE) AAO in different stages. * $P < 0.05$. M: male, F: female.

earlier AAO. Actually, sex specificity in mitochondria function has been observed in various studies. A previous study had discovered sex-specific effects on life span and aging in mice with mild impairment of mitochondrial electron transport [26]. In this study, male *Risp*^{+/P224S} mice showed shortened life span, and aged *Risp*^{+/P224S} males tended to suffer a more rapidly deteriorating health. Their results suggested that even a small perturbation in electron transport of the mitochondrion can result in dramatic physiological changes in mammals. Meanwhile, sexual dimorphism of mtDNA polymorphism has been reported in the association study of neurodegenerative disorders as well. It has been reported that European mtDNA haplogroups J and K [27], both have 10398G in common, are associated with more significantly reduced risk of PD in women than in men [16]. Together with our study, it is reasonable to believe that the mtDNA 10398 polymor-

phism tended to show sex specificity in neurodegenerative disorders. Furthermore, 10398A polymorphism is more likely to display vulnerabilities among male patients with MJD.

It has been revealed that *OGG1* is remarkably related to age-dependent pathogenic CAG expansion in mouse HD model, and two haplotypes were found to be associated with younger AAO in patients with HD [28,29]. Similarly, younger AAO was also found to be associated with a polymorphism in *hCAD*, which might be involved in ultimate execution of apoptotic cell death [30]. *BDNF* polymorphism has been linked to the AAO variance in polyQ diseases [9]. But follow-up studies made this finding controversial [31]. *PPARGC1A*, also termed as *PGC-1 α* , has been reported to act as a key transcriptional coregulator in process of mitochondrial respiration, oxidative stress defense and adaptive thermogenesis [32]. Inhibition of *PPARGC1A* function by mutant Htt supports a link to mitochondrial dysfunction in HD [12]. However, neither significant differences in AAO nor interaction with causative CAG repeat length was found among these candidate SNPs. In spite of the fact that small samples may be one of the reasons that lead to research variation, we consider different study populations may contribute more. Our study was the first time to replicate these non-MJD but polyQ-related association studies of Caucasian population in Chinese patients with MJD. We believe that these candidate SNPs founded in association studies of Caucasian population might not be perfectly applied in Chinese patients with MJD, while we cannot rule out the possibilities of other SNPs in the same candidate gene.

In summary, using SNaPshot[®] Multiplex System, it was the first time that the 6 candidate SNPs had been screened in patients with

MJD, and we found the *MT-ND3* polymorphism 10398A>G (rs2853826) was significantly associated with a 3-year earlier AAO in male patients with MJD. Our findings provide a new AAO prediction factor of MJD. Furthermore, the findings involving mitochondrial dysfunction can be a promising target for disease modifying therapy in the future. However, we still need prospective studies with larger study population to better understand the pathogenic process and possible modulators of MJD.

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Conflict Of Interest

The authors declare no conflict of interest.

Author Contribution

Dr. Chen involved in data acquisition, analysis and interpretation of the data, statistical analysis and drafting the manuscript. Dr. Gan helps in funding and involved in data acquisition, and analysis and interpretation of the data. Dr. Cai, Dr. Ni, Dr. Zhou, Dr. Dong, and Prof. Wang involved in data acquisition. Prof. Wu helps in funding and involved in study design and conceptualization, data acquisition, analysis and interpretation of the data, technical and material support, and critical revision of the manuscript.

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Supporting Information

The following supplementary material is available for this article:

Table S1. Primers for 6 candidate polymorphisms.

Table S2. Interaction between candidate SNPs and the causative length of CAG repeats.

Table S3. Descriptive data for other 5 candidate SNPs.