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Phenotypic variance in monozygotic twins with SCA3

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Abstract

Background: Spinocerebellar ataxia type 3 (SCA3) is a hereditary neurodegenerative disorder with high clinical heterogeneity. Twin study is valuable to estimate the contributions of gene and/or environment to phenotypic variance. However, SCA3 twins were extremely sparse and rarely reported.

Methods: A pair of monozygotic twins with SCA3 was assessed using well-acknowledged scales. Genetic modifiers and methylation levels were determined by Sanger sequencing and pyrosequencing.

Results: Sharing identical CAG repeat lengths, the twins presented with similar symptoms, whereas, the younger sister had an earlier age at onset of two years. The occurrence time and severity of constipation, blepharospasm and fasciculation were markedly different between the twins. Notable methylation level differences of several CpG sites existed between the twins.

Conclusions: It is the first time to report SCA3 monozygotic twin worldwide. The role of epigenetic factors in the phenotype variance deserved more attention. The DNA methylation may influence the phenotypic variance by altering the occurrence time and severity of symptoms, indicating its potential in alleviating the disease.

KEYWORDS
DNA methylation, monozygotic twin, phenotypic variance, spinocerebellar ataxia type 3

1 | INTRODUCTION

Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease, represents the most common type of spinocerebellar ataxias (SCAs) worldwide (Sun, Lu, & Wu, 2016). The predominant clinical feature of SCA3 is progressive ataxia due to primary involvement of cerebellum. Besides, SCA3 manifests as a variety of neurologic symptoms such as dysphagia, dysarthria, pyramidal and extrapyramidal signs, progressive external ophthalmoplegia, autonomic dysfunction (Yeh et al., 2005), and sleep disturbances. Mutation in *ATXN3* gene (MIM: 109150) reflecting as an expanded polyglutamine-encoding CAG repeat was discovered as the cause (Kawaguchi et al., 1994). Although the pathogenic gene is confirmed, SCA3 is significantly heterogeneous in phenotype. Age at onset (AAO) is the most common and objective feature of phenotype. In general, larger size of CAG repeat is associated with earlier AAO. The expanded repeat, however, accounted for 44.3% to 74.9% of AAO variance (Warrenburg et al., 2005), suggesting that other genetic or environmental factors contribute a lot.

For inherited neurological disorders, twin study is particularly valuable to estimate the contributions of gene and/

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or environment to phenotypic variance (Boomsma, Busjahn, & Peltonen, 2002). Nevertheless, twin study on SCAs was sparse. A pair of monozygotic (MZ) twins with SCA2 was reported (Anderson et al., 2002). Sharing identical repeat lengths, the twins presented with similar symptoms, yet markedly different AAOs, saccadic velocities and amplitudes, and postural stabilities. In addition, a pair of MZ twins with SCA17 was reported to have the same size of CAG expansion within pathogenic alleles but different size within normal alleles, and consequently presented with discordant symptoms and AAOs (Nethisinghe et al., 2018).

Considering the great weight attached to twin study, we described a pair of MZ twins with SCA3 for the first time worldwide. Further we assessed environmental and genetic factors, and epigenetic modifiers to investigate their contributions to phenotypic variance.

2 | MATERIALS AND METHODS

2.1 | Subjects and assessment scales

The MZ twins were originally from the Central China. The clinical characteristics were obtained, and brain MRI was performed to assess their affected regions. The Scale for the Assessment and Rating of Ataxia (SARA; Schmitz-Hubsch et al., 2006) and the International Cooperative Ataxia Rating Scale (ICARS; Trouillas et al., 1997) were used to assess the severity of ataxia. The SARA score ranges from 0 to 40 with 0 suggesting no sign of ataxia and 40 the most severe degree of ataxia. The 100-point ICARS consists of 19 items with 0 indicating absence of ataxia and 100 the most severe degree of ataxia. Nonataxia signs were assessed by means of the Inventory of Non-Ataxia Symptoms (INAS; Jacobi et al., 2013). INAS consists of 30 items and is appropriate for clinical description. Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used to assess cognitive level. Taking the emotional states into consideration, the Hamilton Depression Rating Scale (HAMD), Hamilton anxiety scale (HAMA), and the Patient Health Questionnaire-9 (PHQ-9) were used. The assessments above were performed independently by an experienced investigator to avoid interobserver variability. This study was approved by the Ethics Committee of Second Affiliated Hospital of Zhejiang University School of Medicine. Written informed consent was obtained from each participant.

2.2 | Molecular analysis

Genomic DNA of the MZ twins was extracted from peripheral blood with QIAamp DNA Blood Minikit (QIAGEN, Hilden, Germany). The genetic relationship of the MZ twins was confirmed by short tandem repeat loci detection. Amplification of the CAG repeat expansion was performed by polymerase chain reaction using MJD52/MJD25 primers (Gan et al., 2010). The number of CAG repeats was further identified by Sanger sequencing as previously reported (Gan et al., 2010). To evaluate the genetic background, we selected single-nucleotide polymorphisms (SNPs) and (CAG)n loci that have been reported to be modifiers of AAO in SCA3 patients. The reported SNPs included rs709930/rs910369 in *ATXN3*, *APOE* ε2 allele, rs7969300 in *ATXN2* (Ding et al., 2016; Long et al., 2015; Peng et al., 2014). The (CAG)n loci included normal CAG repeats within *ATXN3*, *CACNA1A*, *ATXN1*, *ATXN7*, *ATXN2*, *RAI1*, *ATN1*, *HTT*, and *KCNN3* (Chen et al., 2016). The modifiers were amplified with reported primers and identified by Sanger sequencing.

2.3 | Pyrosequencing

To explore the influence of epigenetic factor, we detected the DNA methylation levels of the twins and one of their unaffected siblings by pyrosequencing. The genomic DNA was treated with bisulfite. The primers targeting the *ATXN3* promotor were designed by PyroMark Assay Design 2.0 that harbored two main CpG islands including the first island 289 bp in length from ATG −779 to −491 containing 15 CpG sites, and the second island 254 bp in length from ATG −258 to −5 containing 23 CpG sites. After purification, the treated DNA was amplified and then performed pyrosequencing reaction (PyroMark Q96 ID, QIAGEN). Pyro Q-CpG was used to analyze DNA methylation level of each site. The DNA methylation level was determined as the ratio of methylated CpG to total CpG. And the methylation level at each CpG site and the mean methylation levels of the two islands were obtained.

3 | RESULTS

3.1 | Clinical features

Twin 1 (Figure1a; III-6), the younger sister, initially presented with progressive gait disturbance and dizziness at age 28. She suffered from fasciculation of facial muscles, especially periocular muscles at age 30. Blepharospasm appeared at age 35 and was exacerbated by nervousness. After the age of 38, a series of clinical manifestations including dysarthria, dysphagia, diplopia, and sleep disturbance gradually emerged. Moreover, the patient turned to need assistance while walking. The patient had no notable past medical history. And interestingly, she mentioned a visible improvement in walking after Christianization at 36.

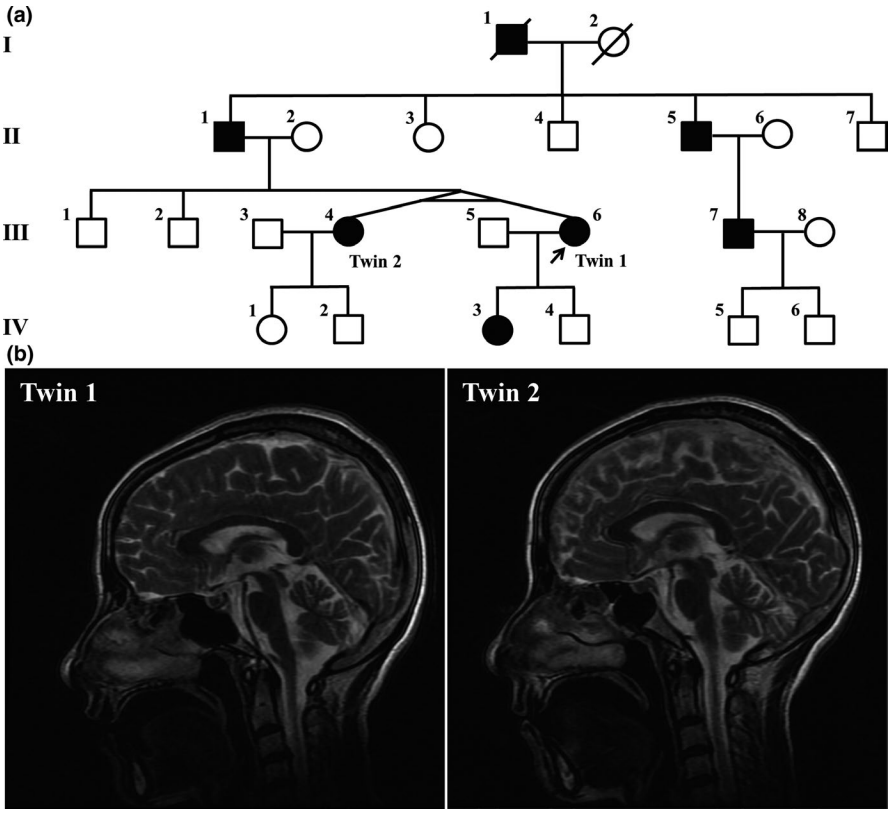

FIGURE 1 (a) Pedigree of the family (arrow, proband; square, male; circle, female; diagonal line, decreased members; black, patients); (b) Brain MRI of the monozygotic twins showed cerebellar and brainstem atrophy

TABLE 1 Landmarks of the twins' medical histories and life

	Twin 1	Twin 2
Education (age)	Finished junior high school (15)	Finished junior high school (15)
Employment	3 years as factory worker	1 years as factory worker
Marriage age	21	21
The first delivery (age)	1999 (22)	1999 (22)
The second delivery (age)	2003 (26)	2002 (25)
Gait ataxia onset (age)	2005 (28)	2007 (30)
Time of diagnosis (age)	2010 (33)	2011 (34)
Chronic gastritis (age)	Absent	2015 (38)
Fasciculation onset (age)	2007 (30)	2016 (39)
Blepharospasm onset (age)	2012 (35)	2016 (39)
Walk with assistance (age)	2015 (38)	2016 (39)
Christianization (age)	2013 (36)	2018 (41)

Twin 2 (III-4) presented with similar initial symptoms to her younger sister at age 30 (Table 1). While fasciculation and blepharospasm occurred when she was 39 years old, which was different from twin 1. Concomitant with worsening gait ataxia, a range of symptoms including dysarthria, dysphagia, and diplopia came out from age 39. She had noticeable leg cramp in sleep for one year and constipation for 15 years while her younger sister did not. She was Christianized at 41, and insisted on producing a considerable improvement in gait ataxia and constipation for a few months. Unlike her younger sister, she suffered from chronic gastritis for 4 years. The brain MRI result was consistent with the clinical symptoms, as cerebellar atrophy of twin 1 was more severe (Figure 1b).

The results of scales were shown in Table 2. Twin 1 presented with more severe ataxia in accordance with higher SARA score and ICARS score. The SARA score of twin 1 was 19, whereas that of twin 2 was 16. Consistently, the ICARS score of twin 1 was 47, whereas that of twin 2 was 41. Among the items, the kinetic functions, especially knee-tibia test and pronation-supination alternating movements, leaded to the major difference. Neurological examinations revealed that the twins exhibited similar nonataxia signs including hyperreflexia, positive extensor plantar reflex, spasticity, muscle atrophy, fasciculations, myoclonus, gaze-evoked nystagmus, and ophthalmoparesis, and twin 2 was affected to a lesser extent. With regard to cognitive assessment, they both had no cognitive impairment.

TABLE 2 Results of the twins' scale assessments

	Twin 1	Twin 2
SARA	19/40	16/40
ICARS	47/100	41/100
INAS	Hyperreflexia, positive extensor plantar reflex, spasticity, paresis, muscle atrophy, fasciculations, myoclonus	Hyperreflexia, positive extensor plantar reflex, spasticity, paresis, muscle atrophy, fasciculations, myoclonus
MMSE	28/30	27/30
MoCA	19/30	18/30
HAMA	11/56	17/56
HAMD	25/77	25/77
PHQ–9	10/27	14/27

TABLE 3 Different DNA methylation levels of nine CpG sites in the subjects

	Twin 1 (%)	Twin 2 (%)	Unaffected brother (%)
Pos.1	16.0	26.7	17.8
Pos.2	38.4	57.3	49.5
Pos.3	42.8	0	0
Pos.4	31.6	12.0	11.5
Pos.5	22.4	11.9	10.2
Pos.6	14.1	4.8	5.4
Pos.7	35.4	15.0	20.3
Pos.8	19.0	5.0	6.7
Pos.9	36.4	17.0	18.6

TABLE 4 SNP modifiers and repeat length of (CAG)n loci in the twins

	Twin 1	Twin 2
ATN1	14	14
ATXN1	28	28
ATXN2	19	19
ATXN3	14/77	14/77
ATXN7	10	10
CACNA1A	12	12
HTT	20	20
KCNN3	19	19
RAI1	12	12
APOE	ε3/ε3	ε3/ε3
rs709930	NM_004993.6:c.*151G>A	NM_004993.6:c.*151G>A
rs910369	NM_004993.6:c.*382G>T	NM_004993.6:c.*382G>T
rs7969300	NM_002973.3:c.743G>A	NM_00297 3.3:c.743G>A

3.2 | Genetic and epigenetic features

The twins had the same CAG repeat numbers of 14 and 77, respectively, within the normal and expanded alleles. We detected the methylation levels of two CpG islands among the twins and their unaffected brother (III-2). For the twin 1, the mean methylation levels of the two islands were 68.5% and 19.8%, respectively, whereas those of the twin 2 were 69.4% and 16.1%. And those of the normal brother were 65.8% and 14.5%. Although no significant difference was observed in the mean methylation levels of two islands between the twins, the methylation levels of nine CpG sites particularly in the second island markedly differed (Table 3). In addition to the methylation levels, we also detected the SNPs and (CAG)n loci that were reported to be modifiers of SCA3, but found no difference (Table 4).

3.3 | Environmental features

When exploring the difference between the MZ twins, we found they had strikingly similar milestones including growth and development, education, career, time of marriage, religion, the age of first delivery (Table 1). The financial and spiritual support of the two families was similar. As regards emotional state, though manifesting worse symptoms, twin 1 was less anxious than twin 2. The HAMA and PHQ-9 scores of twin 1 were 11 and 10, respectively, whereas those of twin 2 were 17 and 14.

4 | DISCUSSION

As MZ twin is precisely matching for genotype and environment background, it is preferentially used to understand gene–environment interactions in genetic diseases. However, SCAs twin was rare, especially SCA3. In this study, we reported a pair of MZ twins with SCA3 for the first time worldwide, and explored their differences from genetic and clinical aspects. The pair of twins shared identical CAG repeat lengths. However, the elder sister had a two years earlier onset, validating that CAG repeat lengths could not completely explain the AAO variance. With earlier onset, twin 1

presented with more severe ataxia in accordance with more severe degree of cerebellar atrophy showed in brain MRI.

Besides the onset age of disease, the twins presented with different occurrence time and severities of some symptoms. Constipation, blepharospasm, and fasciculation of facial muscles deserved attention. Autonomic dysfunction is not uncommon and possibly underestimated in SCA3 patients (Yeh et al., 2005), and the discordance of constipation between the MZ twins might be attributed to the SCA3 disease. Dystonia was one other predominately different feature between the twins. Marked involuntary twisting and cramping of the hands were found in twin 1 but not in twin 2. The pair of MZ twins both presented with blepharospasm and fasciculation, yet with the disparity in occurrence time of 4 years and 9 years, respectively. Previous studies (Gan, Zhao, Wu, Wang, & Murong, 2009) classified SCA3 into five different subtypes. Accordingly, the twins presented as subtype 1, characterizing by an early onset (20-30 years) with prominent pyramidal and extrapyramidal signs in addition to cerebellar ataxia. Dystonia is often found in SCA3 patients with subtype 1. And blepharospasm and fasciculation are two of the most frequent presentations of dystonia in SCA3 (Nunes et al., 2015). These manifestations usually occur in the advanced progression, which was consistent with the twins.

Though sharing identical CAG repeat lengths, the pair of MZ twins differed in many details. As the twins had a similar environmental background, the environmental influence on the phenotype of the twins may be scarce. Therefore, we focused more on genetic and epigenetic factors between the twins. The mechanism of how genetic modifiers influence phenotype remains obscure. It was postulated that they might impact by altering the structure, function or expression of the proteins associating with neurodegeneration (Chen, Sequeiros, Tang, & Jiang, 2018). We detected reported modifiers and not surprisingly found no difference, indicating that epigenetic modification may play a subtle but important role.

It is widely acknowledged that DNA methylation is one of the crucial epigenetic modifications participating in gene expression and function. Hypomethylation or hypermethylation pathologically contributes to neurodegenerative disease, such as SCA1 (Dion, Lin, Hubert, Waterland, & Wilson, 2008), SCA2 (Laffita-Mesa et al., 2012), SCA7 (Libby et al., 2008), Alzheimer's disease (De Jager et al., 2014), and Parkinson's disease (Jowaed, Schmitt, Kaut, & Wullner, 2010). The CpG methylation in the promotor region was reported to involve in phenotypic variance through modifying the disease progression and/or influencing the stability of CAG repeats (Liu, Tang, & Guo, 2018). Higher DNA methylation levels in the *ATXN3* promotor were detected in SCA3 patients compared with controls (Wang et al., 2017). In this study, mean DNA methylation level of two CpG islands was detected no difference between the twins. The limited number of CpG sites detected by pyrosequencing and exiguous subjects should confine the findings. Nevertheless, we observed notable difference of several sites between the twins, which might subtly account for the phenotype variance.

Regarding the twin study in the polyglutamine disease, previous studies (Anderson et al., 2002; Nethisinghe et al., 2018) attributed the phenotypic difference to nongermline or external factors, that is, epigenetic or environmental factors. In Huntington's disease, the majority of MZ twin pairs present concordant phenotype. For MZ twins with discordant phenotype, the possible involvement of environment and epigenetic mechanism was emphasized (Friedman, Trieschmann, Myers, & Fernandez, 2005). In this study, no apparent genetic and environmental differences were observed in the twins. Thus, the phenotypic discordance of the twins was probably due to the intervention of epigenetic mechanism. Our findings revealed that DNA methylation might participate by altering the occurrence time and severity of symptoms, and aided to better understand the function of epigenetic modifications in the pathogenesis of SCA3.

More recent neuropathological and pathoanatomical studies revealed that the brain damage in SCA3 extended beyond the brainstem and cerebellum. Dentate nucleus, basal ganglia, substantia nigra, thalamus, midbrain, and pons were widely affected as well (Klockgether, Mariotti, & Paulson, 2019). The pathogenic mechanism was triggered by CAG repeat expansion in the *ATXN3* gene. Somatic mosaicism referred to the phenomenon that CAG repeat instability occurred in different cells from the same tissue. While in SCA3, it may occur in the brain (LopesCendes et al., 1996). Thus, the existence of somatic mosaicism in the widespread affected regions could not be excluded, which might contribute to the phenotypic variance in the MZ twins.

This is the first study to report a pair of MZ twins with SCA3 worldwide. Sharing identical CAG repeat lengths and reported genetic modifiers, the twins presented with the similar symptoms, indicating genetic factors played a major role in its etiology. However, the occurrence time and severity of symptoms were markedly different. Notable methylation level difference of several CpG sites was observed between the twins. As environmental and genetic background in the twins was almost equal, the role of epigenetic factor in the phenotype variance requires more attention. The DNA methylation may influence the phenotypic variance by altering the occurrence time and severity of symptoms, indicating its potential in alleviating the disease.

5 | ETHICS APPROVAL

The study was approved by the Ethics Committee for clinical medical research. Informed consents were obtained from each participant.

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CONFLICT OF INTEREST

All authors reported no biomedical financial interests or potential conflicts of interest.

AUTHOR CONTRIBUTIONS

All authors gave approval for the final version of manuscript. Zhi-Ying Wu conceptualized the study. Hua Zhao and Lu Yang performed physical examination and sequencing. Hua Zhao, Lu Yang, and Yi Dong contributed to data acquisition and interpretation. Hua Zhao and Lu Yang drafted the manuscript and Zhi-Ying Wu critically revised the manuscript.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

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