Is the High Frequency of Machado-Joseph Disease in China Due to New Mutational Origins?

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Machado-Joseph disease (MJD, also known as spinocerebellar ataxia 3 or SCA3) is the most common dominant ataxia worldwide, with an overall average prevalence of 1–5/100,000. To this date, two major ancestral lineages have been found throughout the world. In China, the relative frequency of MJD among the SCAs reaches as high as 63%, however, little is known about its mutational origin in this country. We analyzed 50 families with MJD patients in two or more generations to study the hypothesis that new mutational events have occurred in this population. Haplotypes based on 20 SNPs have shown new genetic backgrounds segregating with MJD mutations in our cohort from China. We found the “Joseph-derived” lineage (Joseph lineage with a G variant in rs56268847) to be very common among Chinese MJD patients. Moreover, we estimated the time for the origin of this MJD SNP background based on STR diversity flanking the (CAG)n of ATXN3. It was surprising to find that the Chinese MJD population originated from 8,000 to 17,000 years ago, far earlier than the previous literature reports, which will be an important evidence to explain the origin, spread and founder effects of MJD.

Keywords: spinocerebellar ataxia type 3, SCA3, Machado-Joseph disease, founder effect, haplotype, mutational origins

INTRODUCTION

Machado-Joseph disease (MJD, OMIM#109150), also known as spinocerebellar ataxia type 3 (SCA3), is one of the polyQ diseases. It is a rare autosomal dominantly inherited neurodegenerative disease that causes progressive cerebellar ataxia, resulting in lack of muscle control and coordination of the upper and lower extremities, with symptoms including dysarthria, dysphagia, pyramidal signs, progressive external ophthalmoplegia, and dystonia (Bettencourt and Lima, 2011; Costa Mdo and Paulson, 2012; Wang et al., 2015; Paulson et al., 2017). This autosomal dominant disease is caused by an elongated polyglutamine stretch encoded by more than 51 CAG
participants agreed with our request for collection of
including 109 patients, and 105 healthy individuals. All
We analyzed 50 families with MJD, from Southern China,
Asian population.
however, little information on MJD haplotypes is available
the purpose of haplotypes identification (Martins et al., 2012),
determined using a total of 20 SNPs and 4 microsatellites for
MJD lineages in different populations, genetic distances were
Japan (Martins et al., 2007). To determine the occurrence
Portuguese-Azorean ancestry, in 1972 (Nakano et al., 1972),
and was believed to be very frequent in Portugal due to founder
effects. Also, Japan, Brazil and France all had founder effects of
MJD (Marie-Françoise Chesselet, 2001). Furthermore, a
linkage disequilibrium analysis was carried out with three
single nucleotide polymorphisms (SNPs, A669-TG/G669-TG,
C987-GG/G987-GG, and TAA1118-TAC1118) and five short tandem
repeats (STRs, D14S1015, D14S995, D14S973, D14S1016, and
D14S977), in 249 families of various ethnic backgrounds. Four
different SNP haplotypes were identified segregating with MJD
expansions: A-C-A, A-G-A, G-G-A, and G-G-C (Gaspar et al.,
2001). Following this discovery, a worldwide study of extended
haplotypes was performed in 264 MJD families, and two major
ancestral lineages were confirmed: the GTGGCA background or
the Machado lineage, probably originated in Portugal; and the
TTACAC or Joseph lineage, observed in 19 countries, including
Japan (Martins et al., 2007). To determine the occurrence
of new mutation events and clarify the spread of ancestral
MJD lineages in different populations, genetic distances were
determined using a total of 20 SNPs and 4 microsatellites for
the purpose of haplotypes identification (Martins et al., 2012),
however, little information on MJD haplotypes is available
in China, although the frequency of MJD is very high in this
Asian population.
In this study, to determine the origin and estimate the
age of MJD mutational event(s) in China, we performed a
haplotype analysis of 20 SNPs and 7 microsatellites in 50 Chinese
MJD families.

MATERIALS AND METHODS

Patients and Controls
We analyzed 50 families with MJD, from Southern China,
including 109 patients, and 105 healthy individuals. All
participants agreed with our request for collection of
peripheral blood samples and signed an informed consent
form. The study was approved by Ethics Committee of
Xiangya Hospital of Central South University in China.
Genomic DNA was isolated using a standard protocol. Each
family has at least two generations and has at least four
individuals. Healthy spouses served as the control group.
In one of the families studied, a patient homozygous for an
MJD expansion was analyzed together with both parents
and his sister.

Genotyping and Haplotype Analysis
We analyzed 20 SNPs located upstream and downstream the
(CAG)n, including the previously analyzed six core SNPs and
variants within a 4 kb area flanking the repeat (Figure 1;
Martins et al., 2006; Costa et al., 2019). PCR amplification
reactions were done with TSE 101 Golden Star T6 Super
PCR Mix (1.1×). The location of all loci and Primers
for amplification are listed in the Supplementary Table S1.
Genotyping of SNPs was performed through Sanger sequencing.
We inferred allelic phases by segregation for most of analyzed
families; some haplotypes were reconstructed by PHASE software
version 2.1.1 (only haplotypes with probabilities greater than
0.6 were used for subsequent analyses). We compared the
distribution of SNPs in the Chinese MJD population with
the two ancestral lineages described previously, and selected
four different SNPs among these to test for Hardy-Weinberg
equilibrium and Chi-square test (χ²). In this test, 49 healthy
spouses of probands served as controls. We first distinguished
the haplotypes of the six core SNPs and conducted a Fisher's
exact test. Basically, the formula δ = (Fd-Fc)/(1-Fc) is used
to calculate the approximate risk of population attribution and
provide evidence of LD. Then, we extended the haplotype
to 20 SNPs for further analysis (Devlin and Risch, 1995;
Gaspar et al., 2001).

STR-Based Haplotype Analysis and Age
Estimation
Capillary electrophoresis was used to detect the seven
STRs (TAT_223)n, (GT_199)n, (ATA_194)n, (AC_21)n,
(AAAC)n, (GT)n, and (AC_190)n (Figure 1), and haplotypes
reconstructed by segregation and with the PHASE software
version 2.1.1 (only haplotypes with probabilities greater
than 0.6 were used for subsequent analyses). We determine

1http://stephenslab.uchicago.edu/phase/download.html

FIGURE 1 | Microsatellite loci flanking the (CAG)n of ATXN3, analyzed in this study. Distances from the (CAG)n, included in the STR name, are expressed in kilobases.
## Table 1: SNP-based haplotypes of MJD Chinese families in comparison to Joseph MJD lineage.

| SNP       | refSNP ID       | Joseph lineage | Haplotypes of MJD Chinese families<sup>abc</sup> |
|-----------|-----------------|----------------|-----------------------------------------------|
| IVS6-300G > T | rs12590497     | T              | A (n = 10), B (n = 12), C (n = 1), D (n = 8), E (n = 1), F (n = 1), G (n = 11), H (n = 1), I (n = 1), J (n = 1), K (n = 2), L (n = 1), M (n = 1) |
| GT<sup>128</sup>/GTG<sup>527</sup> | rs16999141     | T              | T |
| IVS8-86T > G  | rs10146519      | G              | G |
| A<sup>665</sup>TG<sup>665</sup>TG | rs1046755       | A              | A, A, A, A, G, A, A, A, A, A, A, G |
| C/T:124     | rs12586535      | T              | T, T, T, T, C, T, T, T, T, T, T |
| T/C:248     | rs12586471      | C              | C, C, C, C, C, T, C, C, C, C, C, C, C, C, C |
| A/G:485     | rs56268847      | A              | A, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G |
| G/A:866     | rs10467658      | A              | A, A, A, A, A, A, A, A, A, A, A, A, A, A, A, A, A |
| C/G:910     | rs10467657      | G              | G, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G |
| T/C:921     | rs10467656      | C              | C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C |
| (CA<sup>187</sup>G)<sup>187</sup>G<sup>665</sup>TG | rs12895357     | C              | C, C, C, C, G, G, G, G, G, G, G, G, G, G, G, G, G |
| TAA<sup>1118</sup>/TAC<sup>1118</sup> | rs7158733      | A              | A, A, A, A, A, A, A, A, C, A, A, A, A, A, A, A, A |
| C<sup>178</sup>/A<sup>178</sup> | rs3092822      | C              | C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C |
| C/T         | rs70086230      | C              | C, C, C, T, C, C, C, C, C, C, C, C, C, C, C, C, C |
| C/T         | rs79316375      | C              | C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C |
| G/A:3738    | rs8004149       | A              | A, A, A, A, A, A, A, A, A, A, A, A, A, A, A, A, A |
| G/A:3770    | rs11773934      | G              | G, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G |
| C/T3874     | rs181752420     | C              | C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C |
| A/G:3912    | rs142326        | G              | G, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G, G |
| C/T:3980    | rs74071847      | C              | C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C, C |

- **SNP**: single-nucleotide polymorphism.
- **n**: the number of families. Haplotype A: families 1, 5, 8, 10, 11, 24, 29, 34, 44, 47; Haplotype B: families 4, 16, 23, 25, 30, 35, 37, 40, 41, 45, 48, 49; Haplotype C: family 15; Haplotype D: families 1, 3, 7, 9#, 19, 26, 38#, 50; Haplotype E: family 31; Haplotype F: family 14; Haplotype G: families 6, 13, 17, 22, 27, 32, 33#, 36, 42, 43, 46; Haplotype H: family 18; Haplotype I: family 12; Haplotype J: family 2; Haplotype K: families 20, 39#; Haplotype L: family 21; Haplotype M: family 28# (# indicate families with some alleles inferred by PHASE).
- **n**: the number of families. Haplotype A: families 1, 5, 8, 10, 11, 24, 29, 34, 44, 47; Haplotype B: families 4, 16, 23, 25, 30, 35, 37, 40, 41, 45, 48, 49; Haplotype C: family 15; Haplotype D: families 1, 3, 7, 9#, 19, 26, 38#, 50; Haplotype E: family 31; Haplotype F: family 14; Haplotype G: families 6, 13, 17, 22, 27, 32, 33#, 36, 42, 43, 46; Haplotype H: family 18; Haplotype I: family 12; Haplotype J: family 2; Haplotype K: families 20, 39#; Haplotype L: family 21; Haplotype M: family 28# (# indicate families with some alleles inferred by PHASE).
- **bold** indicates different SNP alleles when compared to the Joseph lineage.
- **#** indicates families with some alleles inferred by PHASE.
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ancestral haplotypes and steps of mutation by STR-based haplotype from the most common haplotype based on 20 SNPs and assess the age of mutation events. This formula $\varepsilon = 1 - [(1 - c) (1 - \mu)]$ is used to calculate the probability of change for each generation, where $c$ represents the recombination rate and $\mu$ represents the mutation rate. The multiplication of $\varepsilon$ and $t$ yields the average of mutations and reorganizations ($\lambda$), where $t$ is the number of generations (Martins et al., 2007).

RESULTS

SNP Haplotyping of Chinese MJD Families

We identified 13 disease-associated haplotypes in our cohort of Chinese MJD families. We compared these haplotypes with the Joseph lineage and indicated their differences (Table 1). It is worthy of mentioning that, for four SNPs, we found new alleles segregating with expanded alleles, not previously associated to Machado or Joseph ancestral MJD lineages (Table 2). The G allele occurs in the rs56268847 of the pathogenic chromosomes in the 28 families, exceeding 50% of the total fifty families involved in the study. Regarding SNPs rs16999141, rs10467857 and rs77086230, alleles never found in phase with expanded MJD alleles were here observed in families 21, 31 and 15, respectively.

Hardy-Weinberg Equilibrium and Chi-Square Test for the 4 of SNPs

Hardy-Weinberg equilibrium and chi-square test were carried out to examine the SNP variations. The control group consisting of 49 spouses confirmed by the Hardy-Weinberg equilibrium that satisfy the genetic equilibrium in the four heterogeneous SNPs. In rs16999141, no genotype CC was found in MJD patients, 57 of 109 patients shared TT, and the rest were TC. For SNP rs56268847, significant variation from AA, AG to GG was detected with the $p$-value of the Hardy-Weinberg equilibrium at this position is 0.007. More specifically, 46 out of 109 SCA3/MJD patients had a genotype of AA, while 60 patients had AG, and by contrast, only three patients had GG. Different distribution for SNP rs10467857 was detected. Of the 109 identical patients, 56 are genotyped as GG and 53 as GC ($p$-value of the Hardy-Weinberg equilibrium 0.004), respectively. Similar situation was found in the SNP rs77086230 in a way that Only CC and CT were significant by chi-square test, and all the $p$-values were less than 0.001 (Table 2).

Analysis of Haplotype Based on Six SNPs

Through linkage analysis of six SNPs such as IVS6-30G > T, GTT/527/GTC/527, A669>TG/G669>TG, C987GG/G987GG, TAA1118/TAC1118, and C1178/A1178, we found that Chinese MJD patients only shared 7 haplotypes, while healthy people shared 21 haplotypes (Table 3). Among them, T-T-A-G-C-C only appears in the MJD patients. The remaining 6 haplotypes were confirmed statistically significant by chi-square test, and all the $p$-values were less than 0.001.

Age Estimation

We performed STR analysis and age estimation on four major haplotype A, B, D, and G based on 20 SNPs, and inferred the Phylogenetic networks of the four haplotypes (Figure 2).

| refSNP ID | Group | N | Genotype | P-value | Allele | P-value |
|-----------|-------|---|----------|---------|--------|---------|
| rs16999141 | MJD | 109 | TT | 0.005 | T | 166 (0.76) | 52 (0.24) | <0.001 |
| | Control | 49 | TC | 0.407 | C | 53 (0.54) | 45 (0.46) | 
| rs56268847 | MJD | 109 | AG | 0.007 | G | 152 (0.70) | 66 (0.30) | <0.001 |
| | Control | 49 | GG | 0.271 | A | 91 (0.93) | 7 (0.07) | 
| rs10467857 | MJD | 109 | CC | 0.004 | G | 165 (0.76) | 53 (0.24) | <0.001 |
| | Control | 49 | GG | 0.901 | C | 52 (0.53) | 46 (0.47) | 
| rs77086230 | MJD | 109 | TT | 0.957 | T | 212 (0.97) | 6 (0.03) | <0.001 |
| | Control | 49 | CT | 0.932 | C | 93 (0.95) | 5 (0.05) | 

MJD, Machado-Joseph disease; SNP, single-nucleotide polymorphism.

- The number of each group.
- Value of the Hardy-Weinberg equilibrium for each comparison (MJD vs. controls).
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- Value of the Hardy-Weinberg equilibrium for each comparison (MJD vs. controls).
- The $p$-value of chi-square test.
- 49 healthy spouses of probands of 50 Chinese families with MJD served as controls.
TABLE 3 | Overall linkage disequilibrium analysis of Chinese families for intragenic haplotypes based on 6 SNPs.

| Haplotype<sup>a</sup> | Frequency in Control subjects | Frequency in Subjects with MJD | $\chi^2$<sup>b</sup> | P-value |
|----------------------|-----------------------------|-------------------------------|----------------|---------|
| T-T-A-C-A-C          | 0.283                       | 0.482                         | 0.278          | <0.001  |
| T-T-A-C-C-A          | 0.013                       | 0                             | ...            | ...     |
| T-T-A-G-A-C          | 0.119                       | 0.382                         | 0.298          | <0.001  |
| T-T-A-G-C-C          | 0.000                       | 0.045                         | 0.045          | ...     |
| T-T-A-G-C-A          | 0.003                       | 0                             | ...            | ...     |
| T-T-G-A-A-C          | 0.013                       | 0.009                         | 0.003          | <0.001  |
| T-T-G-C-C-A          | 0.013                       | 0                             | ...            | ...     |
| T-T-G-C-A-C          | 0.006                       | 0.018                         | 0.012          | <0.001  |
| T-C-G-G-C-A          | 0.019                       | 0                             | ...            | ...     |
| G-C-G-G-C-A          | 0.321                       | 0                             | ...            | ...     |
| G-C-G-G-A-C          | 0.006                       | 0                             | ...            | ...     |
| G-C-G-C-C-A          | 0.035                       | 0                             | ...            | ...     |
| G-C-G-C-A-C          | 0.013                       | 0                             | ...            | ...     |
| G-C-A-G-C-A          | 0.028                       | 0                             | ...            | ...     |
| G-C-A-G-A-C          | 0.009                       | 0.009                         | 0              | <0.001  |
| G-T-A-G-A-C          | 0.016                       | 0.055                         | 0.040          | <0.001  |
| G-T-A-G-C-A          | 0.003                       | 0                             | ...            | ...     |
| G-T-A-C-C-A          | 0.009                       | 0                             | ...            | ...     |
| G-T-A-C-A-C          | 0.009                       | 0                             | ...            | ...     |
| G-T-G-G-C-A          | 0.066                       | 0                             | ...            | ...     |
| G-T-G-C-C-A          | 0.013                       | 0                             | ...            | ...     |

<sup>a</sup>Haplotype based on IVS6-30G > T, GTT<sup>527</sup>/GTC<sup>527</sup>, A<sup>669</sup>TG/G<sup>669</sup>TG, C<sup>987</sup>GG/G<sup>987</sup>GG, TAA<sup>1118</sup>/TAC<sup>1118</sup>, C<sup>1178</sup>/A<sup>1178</sup>.

<sup>b</sup>The approximate risk of population attribution.

FIGURE 2 | Phylogenetic networks of the four major haplotypes based on 7 microsatellite loci. (A) Phylogenetic network of haplotype A. (B) Phylogenetic network of haplotype B. (C) Phylogenetic network of haplotype D. (D) Phylogenetic network of haplotype G.

Surprisingly, MJD haplotypes A and D seemed to be present in the Chinese population as remote as 16,335 ± 1,966 and 11,837 ± 1,871 years, respectively; whereas introduction of haplotypes B and G probably occurred simultaneously, a few million years later, 9,272 ± 1,352 and 9,254 ± 1,411 years ago, respectively (Table 4).
TABLE 4 | Haplotypes and age estimation with 7 STR flanking the (CAG)n at the ATXN3, from families sharing the four most common SNP-based haplotypes.

| SNP-based haplotype | Family number | (TAT_223)n | (GT_199)n | (ATA_194)n | (AC_21)n | (AAAC)n | (GT)n | (AC_190)n | Agea |
|---------------------|---------------|------------|-----------|------------|----------|---------|------|-----------|------|
| A:TTGATCAAGC-(CAG)_exp-GACCCAGGC | Fam 10 | 10 | 20 | 10 | 13 | 7 | 16 | 15 | 16,335 ± 1,966 |
|                      | Fam 44 | 10 | 20 | 10 | 13 | 7 | 16 | 15 | |
|                      | Fam 34 | 11 | 20 | 10 | 13 | 7 | 15 | 15 | |
|                      | Fam 1  | 10 | 20 | 10 | 13 | 7 | 20 | 18 | |
|                      | Fam 8  | 10 | 22 | 10 | 13 | 7 | 16 | 15 | |
|                      | Fam 5  | 10 | 26 | 10 | 13 | 7 | 16 | 15 | |
|                      | Fam 11 | 10 | 24 | 10 | 12 | 7 | 14 | 18 | |
|                      | Fam 24 | 14 | 22 | 10 | 12 | 8 | 20 | 15 | |
|                      | Fam 29 | 16 | 22 | 10 | 13 | 7 | 20 | 15 | |
|                      | Fam 47 | 16 | 24 | 10 | 14 | 5 | 20 | 16 | |
|                      | Fam 41 | 10 | 22 | 10 | 12 | 7 | 20 | 16 | 9,272 ± 1,352 |
| B:TTGATCGAGC-(CAG)_exp-GACCCAGGC | Fam 23 | 10 | 22 | 10 | 13 | 7 | 20 | 16 | |
|                      | Fam 16 | 10 | 21 | 11 | 13 | 7 | 20 | 16 | |
|                      | Fam 4  | 11 | 24 | 10 | 12 | 7 | 20 | 15 | |
|                      | Fam 49 | 10 | 22 | 10 | 12 | 7 | 16 | 16 | |
|                      | Fam 25 | 10 | 22 | 10 | 14 | 7 | 16 | 16 | |
|                      | Fam 40 | 10 | 22 | 9  | 12 | 7 | 17 | 19 | |
|                      | Fam 30 | 10 | 22 | 9  | 12 | 7 | 20 | 16 | |
|                      | Fam 37 | 10 | 22 | 9  | 12 | 8 | 20 | 16 | |
|                      | Fam 45 | 10 | 20 | 9  | 12 | 7 | 20 | 16 | |
|                      | Fam 35 | 15 | 22 | 8  | 12 | 7 | 20 | 16 | |
|                      | Fam 48 | 16 | 22 | 9  | 12 | 6 | 20 | 15 | |
|                      | Fam 1  | 10 | 20 | 10 | 13 | 7 | 16 | 15 | 11,837 ± 1,871 |
| D:TTGATCAAGC-(CAG)_exp-GACCCAGGC | Fam 7   | 10 | 20 | 10 | 13 | 7 | 16 | 15 | |
|                      | Fam 38 | 10 | 20 | 10 | 13 | 7 | 16 | 15 | |
|                      | Fam 50 | 11 | 19 | 12 | 13 | 7 | 15 | 15 | |
|                      | Fam 19 | 13 | 22 | 9  | 13 | 7 | 16 | 15 | |
|                      | Fam 3  | 16 | 23 | 10 | 13 | 7 | 16 | 15 | |
|                      | Fam 9  | 17 | 21 | 10 | 13 | 7 | 17 | 15 | |
|                      | Fam 26 | 16 | 21 | 10 | 13 | 7 | 20 | 15 | |
|                      | Fam 13 | 10 | 22 | 9  | 12 | 7 | 20 | 16 | 9,254 ± 1,411 |
| G:TTGATCGAGC-(CAG)_exp-GACCCAGGC | Fam 43 | 10 | 22 | 8  | 12 | 7 | 20 | 16 | |
|                      | Fam 22 | 10 | 22 | 9  | 12 | 7 | 20 | 15 | |
|                      | Fam 33 | 10 | 22 | 9  | 15 | 7 (5) | 19 | 16 | |
|                      | Fam 17 | 10 | 22 | 9  | 14 | 8 | 16 | 16 | |
|                      | Fam 6  | 12 | 22 | 10 | 12 | 7 | 20 | 16 | |
|                      | Fam 32 | 16 | 22 | 9  | 12 | 7 | 20 | 16 | |
|                      | Fam 46 | 16 | 21 | 10 | 12 | 7 | 20 | 15 | |
|                      | Fam 27 | 10 | 22 | 9  | 12 | 5 | 20 | 16 | |
|                      | Fam 36 | 10 | 22 | 10 | 12 | 5 | 20 | 15 | |
|                      | Fam 42 | 11 | 22 | 11 | 12 | 5 | 20 | 20 | |

aAge is the estimated age of occurrence for each haplotype based on 20 SNP.

DISCUSSION

For the selection of participants, we require that the proband must have more than one children or proband's family must bear more than two generations. The stringency of the selection criteria for the participants made only 5 of the families inferred as haplotypes by software, suggesting the vast
majority of families inferred haplotypes through the pedigree structure to make our results more accurate and unquestionable.

The rs56268847 found in Asian SCA3/MJD patients was not statistically significant in the previous report (Martins et al., 2012), most probably due to the small sample size and different national backgrounds. Pathogenic chromosomes of 28 probands in 50 families carry G allele with a frequency of 0.56. The p-value of the Hardy-Weinberg equilibrium is <0.05 in the SCA3/MJD group, indicating that this allele does not reach a genetic equilibrium. However, the difference between the control group confirmed to achieve the genetic balance after the same test and Chi-square test, and the SCA3/MJD group is statistically significant. Thus, it is reasonable to speculate that the difference might be primarily caused by the disease. Furthermore, since the G allele at rs56268847 has been observed to segregate with the expanded allele in more than half of our Chinese MJD families, this suggests that a point mutation at this SNP must have occurred early in the introduction of MJD in China or, even, in other Asian countries.

Additionally, for the first time we detected base variations (new base addition) in three SNPs rs16999141, rs10467857, and rs77086230 in the Chinese SCA3/MJD patients compared to the previously reported two ancestral lineages. Both ancestral lineages are reported as T on rs16999141, and we detected T/C. While G is reported in the ancestral lineage SNP rs10467857, we detected G/C instead of G only. Similarly, C/T is found in the Chinese SCA3/MJD patients other than C only as reported in the ancestral lineage SNP rs77086230. As for the significance in our new discovery of the new base variation in 3 sites, although so far only one family were examined for each SNP, two test methods have confirmed the significant difference. To be more conclusive to say that the new base variations occurred relatively recently, tests of more families are required.

Previous study showed that the A-G-A haplotype for the SNPs rs1048755, rs12895357, and rs7158733 in the 249 of families, exist in MJD patient (Gaspar et al., 2001), while they were not statistically significant most probably due to the different backgrounds. More convincingly, the p-value obtained by Fisher's exact test is less than 0.001. Thus, we believe the significant difference in the frequency of A-G-A haplotype between the SCA3/MJD and the control group.

The first studies on the epidemiology of MJD were based on families described before the gene ATXN3 was known (Sequeiros and Coutinho, 1993). Later, two major MJD haplotypes have been identified (Gaspar et al., 2001; Martins et al., 2007); here, in addition to the worldwide spread Joseph lineage, we found three other major haplotypes that differ from Joseph at (1) rs56268847 (lineage B, 12 families), (2) rs12895357 (lineage D, 8 families), and (3) both rs56268847 and rs12895357 (lineage G, 11 families). Interestingly, lineage B has been previously described among Australian aborigines, probably introduced in this population via Asia (Martins et al., 2012). Independent mutational origins do not necessarily underlie these four MJD SNP-haplotypes since recurrent mutations on the 2 SNPs may have occurred: backgrounds B and D may have evolved from the Joseph lineage by recurrence at rs56268847 and rs12895357, respectively; recombination could be a possible explanation for the origin of SNP backgrounds G, although the short distance between the two SNPs and the deleterious (CAG)n makes it unlikely. Other MJD backgrounds, phylogenetically more distant from the Joseph lineage, were found in single MJD families; de novo expansions may be on the origin of some of them, but a larger cohort should confirm that their low frequency is explained by a recent event or genetic drift.

This discovery is of importance to clarify the prevalence of SCA3/MJD and could become indispensable evidence that supports the founder effect in this disease. More importantly, this finding could help decipher the genetic basis of the SCA3/MJD by further study on the haplotypes.

INFORMED CONSENT
Informed consent was obtained from all individual participants included in the study.

AUTHOR CONTRIBUTIONS
TL completed the collection of samples, analysis of data, and writing of the manuscript. SM, JS, ZH, KK, BT, and HJ completed the design of the experiments. YP, PW, XH, ZC, CW, and ZT assisted in the collection of samples. RQ and CC contributed to the analysis of the data.

FUNDING
This work was supported by the National Key Research and Development Program of China (Nos. 2016YFC0905100 and 2016YFC0901504 to HJ), the National Natural Science Foundation of China (Nos. 81771231 and 81471156 to HJ), the Clinical Research Funds of Xiangya Hospital (2014L03 to HJ), the Clinical and rehabilitation fund of Peking University Weiming Biotech Group (No. xywm2015I10 to HJ), Youth Foundation of Xiangya Hospital (No. 2017Q03 to ZC), and Independent Exploration and Innovation Project of Graduate Students of Central South University (No. 1053320170177 to TL). Moreover, SM is funded by the project IF/00930/2013 from FCT. This article is a result of the project NORTE-01-0145-FEDER-000008, supported by Norte Portugal Regional Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, and through the European Regional Development Fund (ERDF).
ACKNOWLEDGMENTS

We sincerely appreciate all patients and their families that participated in the study voluntarily and supported this study. Moreover, we hope to thank all those who gave this study help.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2018.00740/full#supplementary-material

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