Does the survival motor neuron copy number variation play a role in the onset and severity of sporadic amyotrophic lateral sclerosis in Malians?

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A B S T R A C T

Introduction: Spinal muscular atrophy (SMA) and sporadic amyotrophic lateral sclerosis (SALS) are both motor neuron disorders. SMA results from the deletion of the survival motor neuron (SMN1) gene. High or low SMN1 copy number and the absence of SMN2 have been reported as risk factors for the development or severity of SALS.

Objective: To investigate the role of SMN gene copy number in the onset and severity of SALS in Malians.

Material and Methods: We determined the SMN1 and SMN2 copy number in genomic DNA samples from 391 Malian adult volunteers, 120 Yoruba from Nigeria, 120 Luyha from Kenya and 74 U.S. Caucasians using a Taqman quantitative PCR assay. We evaluated the SALS risk based on the estimated SMA protein level using the Veldink formula (SMN1 copy number + 0.2∗SMN2 copy number). We also characterized the disease natural history in 15 ALS patients at the teaching hospital of Point G, Bamako, Mali.

Results: We found that 131 of 391 (33.5%) had an estimated SMN protein expression of ≤2.2; 60 out of 391 (15.3%) had an estimated SMN protein expression ≤2 and would be at risk of ALS and the disease onset was as early as 16 years old. All 15 patients were male and some were physically handicapped within 1–2 years in the disease course.

Conclusion: Because of the short survival time of our patients, family histories and sample DNA for testing were not done. However, our results show that sporadic ALS is of earlier onset and shorter survival time as compared to patients elsewhere. We plan to establish a network of neurologists and researchers for early screening of ALS.

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1. Introduction

Copy number variants are described in other neurodegenerative diseases such as hereditary sensory motor neuropathy (CMT1A), Alzheimer's disease (with Down syndrome), and Parkinson's disease [14,9,4]. Spinal muscular atrophy (SMA) and sporadic amyotrophic lateral sclerosis (SALS) are both motor neuron diseases. The former, a lower motor neuron disease is due to a reduced survival motor neuron (SMA) protein resulting from deletion of the SMN1 gene and the inability of a highly similar gene, SMN2 to compensate for the loss of SMN1. Abnormal SMN1 copy number distribution in SALS provides additional evidence that gene copy number variants may also contribute to...
neurodegeneration in the disease [5]. The estimated incidence of SMA is 1/6000 to 1/10,000 live births and its carrier frequency is 1/30 to 1/50 in populations of European and Asian origin [17,15,18]. The rarity of SMA and unexpectedly high rate of alleles with three or more SMN1 copies in individuals with black ancestry [8] have been reported [10,20]. Despite a consanguinity rate of 17% and a number of patients diagnosed with other autosomal recessive neurological diseases, SMA is rare in Mali [11,12]. SALS is an upper and lower motor neuron disease with an average incidence of 1.9 per 100,000/year and average prevalence of 5.2 per 100,000 in Western countries. The mean age of onset is 55–60 years and the mean duration is 4–5 years and riluzole is the only drug that has been shown to extend the survival [1,22]. In the last decade, few cases of SALS have been suspected or diagnosed in Mali. In recent years, high SMN1 copy number (SMN1 gene duplications) has been proposed as a risk factor for the development and/or severity of SALS [21]. We hypothesize that Malians may be at a higher risk as compared with others.

2. Materials and methods

We used the socio-demographic data from 420 of 632 Malian study participants who were sampled for our SMN copy number distribution study in which we determined the SMN1 and SMN2 copy number in genomic DNA samples from 391 Malian adult volunteers, 120 Yoruba from Nigeria, 120 Luyha from Kenya and 74 U.S. Caucasians using a Taqman quantitative PCR assay. We used SMN copy number data from 391 (with known SMN1 and SMN2 copy number) of 420 who consented for future use of their specimens and data. Our study participants were 69% male, 99% aged 18 to 29 years old and 97% single (Table 1). We obtained information from a register to compile on ALS inpatients (Patient#1 to Patient#10) in the Neurology Department of the Teaching Hospital of Point G. We reviewed the physicians’ notes to obtain information for ALS outpatients (Patient#11 to Patient#15). We also used data generated from de-identified 120 Nigerian and 120 Kenyan samples from Coriell (Camden, NJ) as well as 74 anonymous U.S. Caucasians for control purpose in SMA related studies [12]. We calculated the cumulative copy number of SMN1 and SMN2 and estimated the SMN protein expression using the Veldink formula (SMN1 copy number + 0.2 × SMN2 copy number). We then calculated the relative risk (RR) for SALS using a 2 × 2 table.

3. Results

3.1. Description of the study population

For the SMN copy number and sporadic ALS study in Malians, we used the stored data from 420 out of 632 study participants [12] who consented for the use of their specimens and data for future SMA and related studies. We found that our study participants were male in 69% of the cases, aged 18 to 29 years old in 99.3% of the cases and single in 97.3% of the cases (Table 1).

3.2. Total SMN (SMN1 + SMN2) copy number in Malians

To check the distribution of the total SMN copy number, we calculated the cumulative copy number of SMN1 and SMN2 for each individual and the average SMN1 or SMN2 copy number in our study. We found that up to 15% (57/391) of Malians had 2 as a total SMN copy number and only 5% (20/391) had 6 or 7 total SMN copies (Table 2). Fifty four percent (210/391) of the individuals had 4 or 5 total SMN copies. The average copy number of SMN1 was 2.7 and the average copy number of SMN2 was 1.1.

3.3. SMN protein expression estimation based on Veldink formula

To evaluate the risk for amyotrophic lateral sclerosis (ALS) in our study population, we used a 2 × 2 table from the Veldink et al. 2005 paper to determine the SALS odds ratio (Table 2) and estimated the SMN protein expression level using the Veldink formula. The cut off being 2.2, we found that 131 of 391 (33.5%) had an estimated SMN protein expression of ≤2.2; 60 out of 391 (15.3%) had an estimated SMN protein expression <2.2 and would be at risk of ALS according to Veldink et al. 2005 (Table 3).

3.4. Early onset and severe disease course in Malians with sporadic ALS

To characterize the disease natural history in Mali, we identified 15 ALS patients.

All patients were male, the disease onset was as early as 16 years old, and some patients were physically handicapped within 1–2 years in the disease course (Table 4).

4. Discussion

Despite growing interest in recent years, the role of SMN copy number in SALS is still controversial. On the one hand, increased copies of SMN1 have been reported to be associated with increased risk of SALS. Homozygous SMN2 deletion is not a risk factor for ALS, and SMN2 copy numbers have no effect on the disease [3,5,6,16]. On the other hand, decreased SMN copy number has also been reported as a risk factor for SALS and low SMN protein level may play a role in the disease [21].

SMN protein levels can be estimated through the following formula: SMN protein = SMN1 copy number + 0.2 × SMN2 copy number [21]. We have two concerns with the Veldink formula: (i) the calculation is based only on SMN copy number instead of an accurate determination of SMN expression level. A new exonic splicing enhancer element in SMN2, c.859G>C in exon 7 of the patients was identified and found to increase the amount of full-length SMN transcripts, thus resulting in less severe phenotypes [19] (ii) SMN hybrid genes (from SMN1 to SMN2 and vice versa) have been reported [12] with no information on how their SMN expression level. Therefore, it is not known whether all SMN copies in a given person are similar in structure and equally functional or not.

Nevertheless, one copy of SMN1 was associated with an increased risk of developing ALS (odds ratio: 4.1, 95% CI: 1.2 to 14.2, p = 0.02). Sixty-one percent of 242 clinically well-defined SALS had an estimated SMN protein level of 2.2 or less as compared to only 36% healthy controls, suggesting that an estimated SMN protein of 2.2 or less was associated with a higher risk for SALS (odds ratio: 1.3, 95% CI: 1.1 to 1.6, p = 0.03) [21]. Using a 2 × 2 table, we estimated the relative risk (RR) to be

Table 1
Socio-demographic description of our adult volunteer study participants.

| Socio-demographic data     | Frequency (n) | Percentage (%) |
|----------------------------|---------------|----------------|
| Sex                        |               |                |
| Male                       | 290           | 69             |
| Female                     | 130           | 31             |
| Total                      | 420           | 100            |
| Age group (in years)       |               |                |
| 18–29                      | 417           | 99.3           |
| 30–35                      | 2             | 0.5            |
| >35                        | 1             | 0.2            |
| Total                      | 420           | 100            |
| Marital status             |               |                |
| Single                     | 409           | 97.4           |
| Married                    | 11            | 2.6            |
| Total                      | 420           | 100            |

Table 2
A 2 × 2 table to determine the SALS odds ratio.

| Estimated SMN protein level | SALS patients | Healthy controls |
|-----------------------------|---------------|------------------|
| ≤2.2                        | 147           | 63               |
| >2                          | 242           | 175              |

The table above shows the counts of patients with SMN protein expression ≤2.2 and >2, respectively.
All patients were male. Hospital discharge.

Parental consanguinity history of smoking and alcoholism i.e., the disease did not worsen clinically from the first to the most recent outpatient visit or from the hospitalization to the hospital discharge. All patients were male.

Table 3

| Estimated SMN protein | Sub-Saharan Africa | *U.S. Caucasians (n = 74) |
|-----------------------|-------------------|--------------------------|
| Mali                  | Nigeria           | Kenya                     |
| ≥2                    | 60 (15.3%)        | 15 (12.5%)               |
| 2.1–2.3               | 71 (18.2%)        | 38 (31.7%)               |
| 2.4–4.6               | 258 (66%)         | 64 (53.3%)               |
| >4.6                  | 2 (0.5%)          | 3 (2.5%)                 |

CEPH DNA + NIH BB samples. We used the Veldink formula (SMN1 copy number + 0.2·SMN2 copy number) to estimate the SMN protein expression in 391 out of 420 study participants.

1.7 (147 × 175/242 × 63) (Table 2). In other words, by extrapolation, 36 of 100 healthy controls and 61 of 100 SALS patients would have a low estimated SMN protein level. Based on Veldink’s formula and this estimated RR, 60 out of our 391 study participants would be at a low risk of developing SALS. The onset of SALS is 40–60 years old in Europeans [2]. Our 60 healthy study participants at risk are only 18 to 26 years old (Table 1) and the onset of the disease had a strikingly wide range from 18 to 66 years old in our SALS patients (Table 4). Three patients, including two in their early 20s reported parental consanguinity, but the family history was negative. A thorough genetic study may be needed to exclude anticipation as the genetic risk factor for the early onset of the disease. Currently, it will be difficult for us to verify the predictability of the Veldink formula reliably due to the small number of our current SALS patients and adult volunteers estimated to be at risk of SALS. To determine the genetic risk factor, we plan to perform SMN1 and SMN2 copy number determination, SOD1, C9orf72, FUS and TDP mutation screening in these patients. A long term follow-up of a larger Malian adult population at risk of SALS would answer this and other questions related to genetic risk. Regarding other risk and disease severity factors, only one case with a history of head trauma and smoking was found. We did not look for either a high ratio LDL/HDL resulting in a 12-month longer survival in ALS or hypercholesterolemia, which has been reported to result in 25% reduced the risk for ALS (Schmitt et al. 2014). Alternatively, with the increased number of adult neurologists in the country, which allow the compilation of our SALS patients (Table 4), a good collaboration between neurologists in the teaching hospitals and researchers at the faculty of medicine will allow a careful screening for SALS among neurology outpatients and a subsequent SMN copy number quantification for SMN protein estimation in such patients.

5. Conclusion

Due to the limited survival of our patients and our inability to establish family history and obtain DNA samples for a comprehensive genetic testing, our results are preliminary and inconclusive. In the future we plan to establish a network of neurologists and researchers for early ALS screening and genotype–phenotype correlation.

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Table 4

| Sporadic ALS Age at onset (years) | Reason for consultation | Disease severity | Bulbar symptoms | Co-morbidity | Treatment Received | Disease course/ outcome |
|----------------------------------|------------------------|------------------|----------------|--------------|--------------------|------------------------|
| Patient 1 16                     | Bilateral upper limb weakness and soreness | Wasting of hands and arms at age 21 | Dysphagia (both liquid and solid) and dyspnea at age 21 | High blood pressure | Cortico-steroids | Spastic paraparesis at age 45 |
| Patient 2 48                     | Tetraplegia            | Tetraplegia within 5 months after the onset of the disease | Hypernasality | None | Muscle relaxant | Stationary disease evolution**** |
| Patient 3 25                     | Tetraparesia           | Tetraparesia predominant in the distality at age 28 | Dyspnea (liquid only) and Hypernasality | Bilateral inguinal hernia | Multi-vitamin | Worsening dysphagia Patient alive |
| Patient 4* 38                    | Left upper limb weakness | Tetraplegia within 2 years after a head trauma | Dysphagia (liquid only) | Insomnia and lung infection | Large spectrum antibiotics | Stationary disease evolution Patient alive |
| Patient 5 39                     | Bilateral lower limb weakness | Spastic paraparesis within one year of the onset of the disease | Intermittent dysphagia, atrophic tongue and slurred speech | None | Supportive | Stationary disease evolution Patient alive |
| Patient 6** 27                   | Right upper limb weakness | Tetraparesia within 11 months after the onset of the disease | Lingual fasciculation | None | Muscle relaxant | Stationary disease evolution Patient alive |
| Patient 7 30                     | Facial paresthesia and anorexia | Face atrophy and wasting of hands and arms within 2 years | Slight dysphagia (solid only) Fasciculation and atrophy of the tongue | Lung infection | Riluzole 50 mg 1 tablet twice a day | Stationary disease evolution Patient alive |
| Patient 8* 24                    | Wasting and muscle cramps of hands and arms | Walking difficulty within a year after the onset of the disease | Dysphagia | None | Supportive | Stationary disease evolution Patient alive |
| Patient 9** 55                   | Right upper limb weakness | Tetraplegia within 5 months of the onset | Absent | None | Tricyclic anti-depressant | Stationary disease evolution Patient alive |
| Patient 10 38                    | Slurred speech         | Walking difficulty within 6 months after the onset of the disease | Dysphagia (liquid only) within 6 months after the onset of the disease | Lung infection | None | Stationary disease evolution Patient alive |
| Patient 11 36                    | Tetraparesia           | –                | –              | –            | –                  | Patient died at age 37 |
| Patient 12 48                    | –                      | Tetraparesia 6 months ago | –              | –            | –                  | Patient alive |
| Patient 13 46                    | –                      | –                | –              | –            | –                  | Patient alive |
| Patient 14 59                    | –                      | –                | –              | –            | –                  | Patient alive |
| Patient 15 66                    | –                      | –                | –              | –            | –                  | Patient alive |

*Parental consanguinity **history of smoking and alcoholism ***i.e., the disease did not worsen clinically from the first to the most recent outpatient visit or from the hospitalization to the hospital discharge.
Conflict of interest

Authors declared no conflict of interest.

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