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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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ABSTRACT

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 (*SMN*) 1 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.

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 (SMN) 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

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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).

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

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 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