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# *SMN1* duplications contribute to sporadic amyotrophic lateral sclerosis susceptibility: Evidence from a meta-analysis

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ABSTRACT

*Objective:* To investigate the association between *SMN1* and *SMN2* copy number variations (CNVs) and sporadic amyotrophic lateral sclerosis (SALS) by a meta-analysis.
*Methods:* Through searching PubMed and EMBASE database (or manual searching) up to November 2013 using the following keywords: "survival motor neuron gene", "*SMN*", and "amyotrophic lateral sclerosis", "ALS" or "motor neuron disease". Nine studies were identified as eligible for this meta-analysis. The association between *SMN* genes and the SALS risk was investigated based on *SMN1* and *SMN2* CNVs. The heterogeneity across the studies was tested, as was publication bias.
*Results:* The analysis showed significant association for *SMN1* duplications in SALS risk: the risk estimates were OR = 1.76, 95%CI = 1.33–2.32, $p < 0.0001$ (still significant when the p value was Bonferroni adjusted to 0.01). However, there was no significant association between *SMN1* deletions and SALS risk after Bonferroni correction (OR = 1.78, 95%CI = 1.02–3.11, $p = 0.04$). In addition, *SMN2* copy number statuses were not associated with SALS in our pooled study. No evidence of publication bias was observed.
*Conclusion:* Our meta-analysis suggested that *SMN1* duplications are a genetic risk factor in SALS, while there was no modulator effect of the *SMN2* gene. In addition, it was possible that *SMN1* deletions in predisposition to SALS vary across different countries. More studies were required to warrant the findings of this study.


## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that results in the loss of motor neurons, and a progressive paralysis and invariably death [1]. Although some ALS cases are familial, about 90% are sporadic [2]. Sporadic amyotrophic lateral sclerosis (SALS) is considered to be a multifactorial disease with environmental genetic risk factors [3,4]. Among candidate genes proposed as SALS susceptibility factors [5], recent attention has focused on the survival motor neuron (*SMN*) gene. It is present in 2 copies: *SMN1* and a centromeric copy *SMN2* which produces only approximated 20% of full-length transcripts coding for *SMN* protein [6,7]. Homozygous deletions of *SMN1* cause spinal muscular atrophy (SMA) [8,9], and *SMN2* gene copy number modulates the phenotype of SMA [10].

Phenotypic similarities between SMA and SALS result in that various studies focused on the possible involvement of *SMN* genes in SALS. A series of studies reported the association between SALS risk and abnormal copy numbers of *SMN* genes, mainly focusing on *SMN1* heterozygous deletions [11,12], *SMN1* duplications [13] and *SMN2* homozygous deletions [11–22]. Despite strong evidences for the relevance of several studies, the results for the association were controversial. Considering a single study may lack the power to provide a reliable conclusion, we hypothesized that a meta-analysis combining all available studies of *SMN* genes and SALS would increase the power to detect a true association. This is the largest genetic meta-analysis to discuss this association so far.

## 2. Materials and methods

### 2.1. Literature search methods

This meta-analysis focuses on the association between *SMN1* and *SMN2* CNVs and SALS. We conducted a literature search in the PubMed (from January 1965 to November 2013) and EMBASE (from January 1974 to November 2013) database using the following keywords: "survival motor neuron gene", "*SMN*" and "amyotrophic lateral sclerosis", "ALS" or "motor neuron disease". Additional studies were identified by manual search from the references of original studies or

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**Table 1**
Characteristics of epidemiological studies of the association between *SMN* genes copy number variations and SALS risk.

| First author | Year | Country | Cases/controls | Method of copy number calculation | Confounding variables | Associated factor | NOS[a] |
|---|---|---|---|---|---|---|---|
| Corcia [15] | 2012 | Sweden | 502/502 | Quantitative multiplex PCR | Gender, site and age of onset, duration of the disease | Homozygous *SMN2* deletions (protective factor) | 9 |
| Blauw [13] | 2012 | Holland | 847/984 | MLPA | Gender, site and age of onset | *SMN1* duplications (risk factor) | 8 |
| Lee [17] | 2012 | Korea | 25/100 | MLPA | Onset age, initial motor grade | Homozygous *SMN2* deletions (risk factor) | 6 |
| Corcia [11] | 2006 | France | 433/454 | Quantitative multiplex PCR | Gender, site and age of onset, survival duration | Abnormal *SMN1* copy number (risk factor) | 9 |
| Veldink [21] | 2005 | Holland | 242/175 | Quantitative multiplex PCR | Gender, site and age of onset | 1 *SMN1* copy,1 *SMN2* copy and 2 *SMN2* copies (risk factor) | 8 |
| Corcia [12] | 2002 | France | 167/167 | Quantitative multiplex PCR | Gender, site and age of onset, duration of the disease | Abnormal *SMN1* copy number (risk factor) | 8 |
| Gamez [16] | 2002 | Spain | 124/200 | PCR-RFLP | Survival duration, time taken for FVC | Homozygous *SMN2* deletions (irrelevant factor) | 7 |
| Veldink [22] | 2001 | Holland | 110/100 | Quantitative multiplex PCR | Gender, onset type, El Escorial classification | Homozygous *SMN2* deletions (prognostic factor) | 7 |
| Parboosingh [20] | 1999 | Canada | 194/262 | PCR-SSCP | None | Homozygous *SMN2* deletions (irrelevant factor) | 7 |

[a] NOS: Newcastle-Ottawa Quality Assessment Scale.

review articles on this topic. Full texts or abstracts of all related reports were then reviewed. The literature retrieval was performed by two independent reviewers (Ning-hua Cui, Jia-jia Gao).

### 2.2. Selection criteria

The selected studies were required to meet all the following criteria: (1) each included study must be an unrelated case–control study; (2) the studies should refer to the association between *SMN1* and *SMN2* CNVs and SALS; (3) case–control studies had sufficient published data to estimate an odds ratio (OR) with 95% confidence intervals (CI) or provide raw data that allowed us to calculate them; (4) the following were excluded: animal studies, review articles, abstracts, reports with incomplete data, studies based on pedigree data, studies about lower motor neuron disease (LMND) or progressive muscular atrophy (PMA) etc.

### 2.3. Quality assessment of primary studies

Quality assessment was performed in each of the acceptable studies in duplicate by independent reviewers (Xue-bin Wang, Jia-Jia Gao) using the Newcastle-Ottawa Quality Assessment Scale [23] for all case–control studies. Any discrepancies were resolved by a third reviewer (Ning-hua Cui).

### 2.4. Data extraction

Two reviewers (Xue-bin Wang and Xue-ping Qiu) independently extracted the required information from all primary studies. The following information was extracted from the selected studies: first author, year of publication, available number of participants in case and control groups, country of origin, method of copy number calculation, confounding variables, associated factors and quality assessment of studies.

**Table 2**
Frequency of *SMN1* and *SMN2* copy numbers in pooled SALS and control groups.

| | SALS [11–13,15,17,21] | Control [11–13,15,17,21] |
|---|---|---|
| *SMN1* copies | | |
| 1 | 84 | 50 |
| 2 | 1992 | 2248 |
| 3 | 140 | 84 |
| *SMN2* copies | | |
| 0 | 175 | 186 |
| 1 | 900 | 905 |
| 2 | 1073 | 1199 |
| 3 or more | 68 | 92 |
| Total | 2216 | 2382 |

Chi-square test: *SMN1* (SALS vs control): $p = 0.001$ *SMN2* (SALS vs control): $p = 0.175$.

In such cases, when the same patient population was included in more than one publication, only the most recent or complete study was selected for the meta-analysis.

In this meta-analysis, the method of copy number calculation was classified by quantitative analysis or non-quantitative analysis (PCR-RFLP, PCR-SSCP). If *SMN* copy numbers were calculated by non-quantitative analysis, only the cases of the homozygous *SMN* genes and healthy control were extracted for final analyses.

The criteria for SALS diagnosis followed the International Classification of Disease (ICD-8 348, ICD-9 335.2 and ICD-10G12.2) and the World Federation of Neurology El Escorial.

### 2.5. Meta-analysis

First, we analyzed frequencies of *SMN1* and *SMN2* copy number categories between pooled cases and controls using a $\chi^2$-test. The statistically significant level was 0.05.

Second, we performed a meta-analysis for each abnormal *SMN1* (i.e., 1 and 3) and *SMN2* (i.e., 0, 1, 3 and more) copy number using the summary data from all studied population. The ORs and 95% CIs were calculated to assess the association between *SMN* genes CNVs and SALS. The significance of the pooled ORs were determined by the Z-test; and the p values were adjusted using Bonferroni correction [24] by the number of compared CNVs ($p = 0.05/5 = 0.01$). Subgroup analyses for different countries (Holland or other countries) were conducted for *SMN1* heterozygous deletions (i.e., 1 copy) and *SMN2* homozygous deletions (i.e., 0 copy). However, for other *SMN* gene CNVs, participants and the number of the included studies were relative small, thus subgroup analyses were not performed.

Results were combined using either a fixed effects (Mantel–Haenszel) model or random effects model depending on the heterogeneity between studies. Heterogeneity between studies was assessed by $\chi^2$-based Q-tests and $I^2$ tests, where $I^2$ (%) > 50% or $P < 0.10$ was considered significantly heterogeneous [25]. Sensitivity analysis was conducted to evaluate the stability of the combined results after sequential removal of each study. Publication bias was assessed graphically by funnel plots and formally by both Begg's test [26] and Egger's test [27]. All statistical analyses were conducted using RevMan 5.0 (The Nordic Cochrane Centre, The Cochrane Collaboration) & STATA 12.0 (Stata, College, TX, USA).

## 3. Results

### 3.1. Eligible studies

A total of twenty one relevant studies were identified, of which nine were included (Table 1). Six studies were excluded because of animal

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