Birth order and the genetics of amyotrophic lateral sclerosis

**Background**

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease predominantly affecting motor neurons, resulting in progressive weakness and death usually within a few years [1]. In about 10% of cases, a family history of ALS is obtained, and for about 20% of these cases, mutation is found in the SOD1 gene [2, 3]. Such mutations are also found in 2% or more of sporadic cases, suggesting incomplete penetrance, spontaneous mutation or both [4–6]. Evidence for reduced penetrance comes from studies of the D90A and I113T mutations of SOD1, and the observation that in general, females in autosomal dominant pedigrees are slightly less likely to develop ALS. In at least one case, spontaneous mutation of SOD1 has been observed in an affected individual with confirmed paternity [7]. The cause of ALS remains largely unknown for the 90% with no known family history, but spontaneous mutation to risk alleles of as yet unidentified genes is possible. It has long been recognized that genetic diseases may be more likely to occur in the last born children of a sibship because increased paternal age is associated with an increased spontaneous point mutation rate in sperm. To test the hypothesis that such a mechanism is responsible for sporadic ALS, we have performed a retrospective analysis of birth order position. We have analyzed sibships of size greater than four using a binomial test for birth position. The 478 pedigrees studied show no birth order effect, suggesting that any genetic contributions to sporadic ALS are more likely to be through deletion in large genes or interactions of common polymorphisms, rather than frequent spontaneous point mutation. This is encouraging for the prospect of finding sporadic ALS susceptibility genes using genome-wide association mapping.

**Abstract** The cause of ALS remains largely unknown for the 90% with no known family history, but spontaneous mutation to risk alleles of as yet unidentified genes is possible. It has long been recognized that genetic diseases may be more likely to occur in the last born children of a sibship because increased paternal age is associated with an increased spontaneous point mutation rate in sperm. To test the hypothesis that such a mechanism is responsible for sporadic ALS, we have performed a retrospective analysis of birth order position. We have analyzed sibships of size greater than four using a binomial test for birth position. The 478 pedigrees studied show no birth order effect, suggesting that any genetic contributions to sporadic ALS are more likely to be through deletion in large genes or interactions of common polymorphisms, rather than frequent spontaneous point mutation. This is encouraging for the prospect of finding sporadic ALS susceptibility genes using genome-wide association mapping.

**Key words** genetic · spermatogenesis · sibship · paternal age effect · birth order
There are no other cell divisions and therefore no other chromosomal replication steps. In contrast, for males, germ cells undergo about 30 mitotic divisions to puberty, after which there is a stem cell division every 16 days to make a further stem cell and a gonial cell. The gonial cells then undergo a further four mitotic and two meiotic divisions. Because of the stem cell division every 16 days, by the age of 30, there will have been about 400 chromosomal replications, and more than 800 by the age of 50. Each replication carries with it the risk of DNA replication error and spontaneous mutation. This is thought to be the explanation for the paternal age effect.

As a result, diseases which occur more commonly in the last born of a sibship are likely to be genetically determined, dominant (or X-linked), and caused by point mutation [10, 11].

We therefore hypothesized that if there was a significant genetic contribution from such spontaneous point mutations to sporadic ALS, affected individuals would be more likely to be born later in a sibship.

Methods

Clinical methods

Sibships examined were those of individuals attending a tertiary referral centre for ALS in London (UK) and Boston (MA, USA). The diagnosis of ALS was made by a specialist in neurology after exclusion of other conditions. All participants gave their informed consent prior to inclusion in the study in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Sibships

Birth order was established by review of medical notes from the specialist ALS clinics. Because about 10% of those attending ALS clinics have a family history of ALS, both tertiary referral centres collect detailed pedigree information as part of the normal clinical assessment.

In the case of second relationships, only the full sibs of the affected individual were included in the analysis when the mother was the common parent, but all sibs when the father was. Non-paternity and miscarriages were not formally identified. Because small sibships could be the result of young parents that had not yet had all their children, or older parents who were either therefore less fertile, or had decided they were now too old for more children, we only included sibships of size four or greater. Because we were looking for spontaneous germ line mutation, we excluded those with a family history of ALS in previous generations. For the same reason, identical twins were counted as a single individual, and non-identical twins given equal ranking.

Statistical methods

For each individual, the sibship size (S) and birth position (X) were recorded, and a scaled birth position rank (R) assigned using the formula:

\[ R = \frac{X - 1}{S - 1}. \]

R therefore ranged from 0 (first born) to 1 (last born) with the midpoint defined by \( R = 0.5 \).

The distribution associated with the resulting statistic under the null hypothesis is \( R \sim U(0,1) \). When individuals with \( R = 0.5 \) are excluded, we can also say that \( P(R > 0.5) \sim B(n,0.5) \), (and therefore \( P(R < 0.5) \sim B(n,0.5) \)) with \( n \) the number of sibships tested. We therefore tested the hypotheses that our observed data were consistent with these distributions; in other words that ALS and birth order are unrelated. Calculations were performed with SPSS v11.0 (SPSS Inc).

Power calculations assuming a binomial test and \( p = 0.5 \) for the null hypothesis, show 80% power to detect a binomial \( p > 0.6 \) or \( < 0.4 \) at \( \alpha = 0.05 \) requires \( n = 194 \), and 99% power requires \( n = 449 \).

Results

In the London set, there were 350 sibships that met the inclusion criteria. There were 179 with \( R < 0.5 \), 152 with \( R > 0.5 \), and 19 with \( R = 0.5 \). A binomial test gave \( p = 0.153 \) (two-tailed) for accepting the null hypothesis that the distribution of ALS cases was equally likely in either half of the sibship.

In the Boston set, there were 128 sibships that met the inclusion criteria. There were 58 with \( R < 0.5 \), 62 with \( R > 0.5 \) and 8 with \( R = 0.5 \). A binomial test gave \( p = 0.784 \) (two-tailed).

When both data sets were considered together, there were 478 sibships that met the inclusion criteria, 237 with \( R < 0.5 \), 214 with \( R > 0.5 \), and 27 with \( R = 0.5 \). A binomial test gave \( p = 0.300 \) (two-tailed).

Plotting the ranked values of R from 0 to 1 showed a good approximation to a uniform distribution (Fig. 1).

Discussion

For the London set, the Boston set, or both considered together, those with ALS were not more likely to have been born later in the sibship. Thus our hypothesis that