Ages of onset suggestive of genetic anticipation in rheumatoid arthritis multicase sibships can be explained by observational bias

C. Deighton, L. A. Criswell¹, R. F. Lum¹ and A. Silman²

Objectives. Previous work has suggested that features of genetic anticipation might be present in familial rheumatoid arthritis (RA), but bias is difficult to exclude when looking at disease in two consecutive generations. We used data from the North American Rheumatoid Arthritis Consortium (NARAC) and the Arthritis Research Campaign National repository for RA multicase pedigrees to determine whether differences in age of onset within multicase sibships were supportive of genetic anticipation.

Method. RA sibling pairs were identified from both data sets. The period of observation was defined as the time between the first sibling developing RA and the time that the sibship was ascertained for the study. A paired t-test for the difference in ages of RA onset within the pairs was calculated. Ages of conception of the parent were correlated with the age of RA onset.

Results. Information was available for 743 sibships in the NARAC data set and 396 sibships in the Arthritis Research Campaign (ARC) data set. In both data sets, the older siblings had an older age of onset than their younger siblings (39.3 vs 36.9 in the NARAC, and 43.8 vs 40.1 in the ARC data set, both \(P < 0.001\)). The two data sets were then stratified into tertiles by a period of observation. In both data sets, there was a progressive decline in the sibling age of onset differences. For the first tertile (shortest observation period), the older sibling had a significantly older age of onset than the younger. This difference decreased in the second tertile, and was not significant in the third tertile (longest observation period). There was no significant correlation between the age of RA onset and the maternal or paternal ages of conception in either data set.

Conclusion. Features compatible with genetic anticipation in RA multicase sibships are subject to observational bias. This does not support a role for genetic anticipation in familial RA.

Key words: Rheumatoid arthritis, Genetics, Epidemiology.

Introduction

Genetic anticipation describes the tendency in some diseases for successive generations to experience more severe and earlier onset disease. A well-documented example is myotonic dystrophy (MD) [1]. A molecular correlate has been identified for MD and other diseases such as Huntington’s disease and the fragile X syndrome [2]: tandemly repeated trinucleotide sequences close to or within the disease-associated gene expand, changing from the marginally expanded premutant alleles associated with normal or subclinical phenotype, to large increases in copy numbers, and the fully expressed disease. Thus, these diseases demonstrate genetic anticipation to some degree, with earlier age of disease onset and increasing disease severity over the generations correlating with a progressively expanding trinucleotide repeat sequence [2]. In both MD and Huntington’s disease, it has been demonstrated that paternal age at which the patient was conceived is negatively associated with the age of onset of the disease [3]. One potential explanation is that this reflects the germ cells continuing to divide mitotically (and hence be subject to continued expansion) in the post-embryonic state only in males [3]. Subsequently, genetic anticipation and premutation phenoma have been described in genetically complex diseases such as bipolar affective disorder [4], schizophrenia [5], Crohn’s disease [6] and psoriasis [7]. For schizophrenia, advancing paternal age has been found to be an important independent predictor of risk, consistent with premutation models [8].

In 1994, evidence of genetic anticipation in familial rheumatoid arthritis (RA) was reported [9]. In pedigrees where the mother had RA, the probands had a significantly younger age of onset than their mother’s [38 vs 54 (\(P=0.002\))]. There was also a negative correlation between the age of disease onset and the paternal age of conception (\(R=–0.60, \ P=0.005\)) [9]. In seven affected mother–proband pairs for which information was available, the probands had a tendency to more severe RA, despite shorter disease duration and younger age [9]. Some of these findings were replicated in two further studies [10, 11] with a significantly older age of onset in the affected parent (mother or father) of an affected proband.

There is a considerable potential for bias in such studies. Specifically, pedigrees in which the proband developed the disease at an older age, and the parent developed the disease much earlier, are much less likely to be ascertained in any cross-sectional study design [1]. Other treatments and secular trends might influence the age of onset and disease severity. The practical problem is that it is difficult to study diseases such as RA across generations with such a variable age of onset. An alternative and more robust approach would be to study multicase sibships where it would be predicted...
that within a sibship, younger siblings would be more likely to acquire RA at an earlier age. Paternally transmitted genes might expand during the lifetime of the father increasing the chances of each subsequent child inheriting alleles that might predispose to an earlier and a more severe disease. This may only have a small effect size in the overall predisposition to familial RA, but if large enough populations could be analysed, this observation might emerge significantly. Even here care needs to be taken with bias (as was shown in a study of Crohn’s disease [12]) as the longer the follow-up the greater the opportunity younger siblings have to develop the disease at an older age than their other siblings. An observation of a younger age of disease status in younger siblings restricted to sibships followed up over short-time periods only would not be supportive of genetic anticipation.

We used data from the North American Rheumatoid Arthritis Consortium (NARAC) and the Arthritis Research Campaign’s (ARC) national repository for RA multicase pedigrees to determine whether differences in age of onset within multicase sibships were supportive of genetic anticipation, and if so, if there was any evidence of observational bias to account for this. We also looked at parental ages at the time of conception of the siblings to identify any inverse correlation with the age of onset in the offspring in all index cases, and then in those where the mother had a documented diagnosis of RA.

Methods

The data for this analysis came from the data sets gathered as part of the NARAC and the UK ARC national repository for RA multicase pedigrees’ collections. Details of their case ascertainment are described elsewhere [13–16].

Sibling pairs identified from both data sets, where both members satisfied the ACR criteria for RA [17], were eligible for inclusion in this analysis. The gender and age of onset, defined as self-reported onset of joint swelling for the ARC data, the age of RA diagnosis for the NARAC data and the parental age of conception where available, were analysed for all affected siblings.

For each sibship a period of observation was calculated. This was defined as the time interval between the first sibling developing RA and the time that the sibship was ascertainment for the study. As the period of observation increases, then the at-risk period for the younger sibling to be ascertained as having RA increases. To test this as a source of bias, the period of observation was stratified into tertiles for the two data sets.

A paired t-test for the difference in ages of RA onset within the pairs was calculated. This was performed for the whole population, then stratified into female–female, female–male and male–male sibling pairs. These analyses were then repeated after stratification by tertiles of the distribution of the period of observation.

For sibships in whom more than two siblings were affected, the first affected sibling was compared in turn with the other affected, or younger siblings to identify any inverse correlation with the age of onset in the offspring.

Information was available for 743 sibships in the NARAC data set and 396 sibships in the ARC data set. The distribution of sibship sizes is shown in Table 1. In both data sets, the majority of the sibships consisted of two affected siblings. The age of onset differences for older and younger siblings in the two data sets is shown in Table 2. In both datasets, the older siblings had an older age of onset than their younger siblings.

Similar significant differences were seen in both data sets when the sibships were stratified according to female–female pairs or female–male pairs (irrespective of whether the sister was older or younger than the brother) (data not shown). In each case, the older sibling had a significantly older age of onset than their younger sibling, irrespective of their gender. Male–male pairs showed a similar trend in each data set, though numbers were small and the differences were not significant (data not shown).

The two data sets were then stratified by a period of observation as defined in the ‘Methods’ section. The age of onset differences were compared within each tertile of observation. The results for the NARAC data set are shown in Table 3 and for the ARC dataset in Table 4. The results for the two datasets were similar. For the first tertile, the older sibling has a significantly older age of onset than the younger. For the second tertile, this difference is diminished though still significant. For the third tertile, the difference was diminished further with the younger sibling still having a younger age of onset than the older sibling, but with the significance having been lost.

Pearson correlation coefficients for parental ages at conception and age of RA onset in the index cases were calculated for each data set. There was no significant correlation between the age of RA onset and the maternal or paternal ages at conception in either data set (Table 5). Reliable information had not been collected on the RA status of mothers in the NARAC data set. In the ARC data set, information was available on 20 RA individuals with a mother who also had a documented RA. The paternal age of conception in these mother–index case pairs showed no significant inverse correlation with age of symptomatic onset in the index case ($R = -0.10, P = 0.77$, data only available in 10 of the 20 families). The maternal age of conception almost reached significant levels ($R = -0.43, P = 0.06, n = 20$).

Conclusion

Previous work on familial RA in three separate studies had suggested that features compatible with genetic anticipation might be present, with second generations having a younger age of onset and possibly more severe disease than their parents [9–11]. Some studies, but not all, had suggested a correlation between parental age of conception and the age of RA onset of the proband, consistent with premutation models [9]. Unstable alleles would be an attractive possible explanation for some of the highly variable features seen in RA [18].

<table>
<thead>
<tr>
<th>Number of affected siblings</th>
<th>NARAC</th>
<th>ARC</th>
<th>Number of pairings per sibship</th>
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</tr>
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<tr>
<td>Total</td>
<td>743</td>
<td>396</td>
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</table>

Table 1. Distribution of multicase sibship sizes in the NARAC and ARC data sets, and number of pairings available per sibship.
A crude analysis, not allowing for observational bias, seemed to support genetic anticipation with older siblings having an older age of onset than their younger siblings. However, if this observation was a true phenomenon, it would be expected to persist irrespective of the period of observation of the sibship. Our analysis in fact showed that in the longest tertiles of observation in both the data sets the younger siblings had an older age of onset not statistically different from their older siblings. There was a trend for a gradually decreasing difference in the younger age of onset in younger siblings with increasing duration of observational tertile. This was true for both the data sets despite their different origins, and their overall difference in distribution of age at onset. These results are highly suggestive that there is a major bias of observation operating in this analysis. This is a type of right censorship bias based on truncating follow-up before ascertainment of all individuals in the cohort destined to develop RA. As a consequence it is those with the younger ages at onset who are selectively ascertained. This is consistent with work on potential bias in data on genetic anticipation in familial Crohn’s disease [12].

Previous work had suggested an inverse correlation of parental ages of conception with age of RA onset in those pedigrees where the mother had RA. In this analysis, we found no overall correlation with parental age of conception and index case age of RA onset. In the ARC data set where RA was documented carefully in parents, there was an inverse correlation with maternal, but not paternal, age of conception and the age of RA onset. Numbers however were small. Maternal transmissions of unstable alleles are more difficult to understand than paternal, as ova do not reproduce during the life-time of the female. However, there are examples of maternal transmission of genetic elements that demonstrate anticipation, such as congenital myotonic dystrophy being transmitted only by affected mothers [1], and the unstable trinucleotide repeat in the fragile X syndrome being restricted to maternal transmissions [19]. The underlying mechanisms are not understood.

There is still a possibility of unstable genetic effects in polygenic diseases such as RA, particularly as we are only beginning to understand the complexity of non-coding regions of the genome [20, 21]. However, if unstable alleles are operating in RA, they have failed to make themselves obvious in this analysis.

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The authors have declared no conflict of interest.

References