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EXTENDED REPORTS

Rheumatoid arthritis, HLA identity, and age at menarche

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Abstract

***Objective*—To determine whether women with rheumatoid arthritis (RA) had differences in obstetric and gynaecological histories when compared with sisters without RA (controls)**

***Methods*—Ninety eight RA discordant sister pairs, 36 of whom were identical for histocompatibility locus antigen (HLA-A, HLA-B, and HLA-Cw) types, were asked to recall their age of menarche, duration of use of contraceptive pill, pregnancy history, and age of menopause.**

***Results*—The 98 siblings with RA had an older mean age of menarche (13·90 (95% confidence interval (95% CI) 13·56 to 14·24) years) than their sisters (13·49 (95% CI 13·22 to 13·76) years; mean difference within pairs 0·41, 95% CI 0·09 to 0·73 years, paired *t* test $t=2·54$, $p=0·013$). When the pairs were divided into identical HLA and non-identical HLA groups, the first showed no significant difference (mean difference 0·17 (95% CI −0·40 to 0·73) years), whereas the second did (mean difference 0·55 (95% CI 0·16 to 0·94) years, $t=2·80$, $p=0·007$). A multiple regression analysis to predict differences in menarche in the non-identical HLA sibling pairs failed to show any demographic or reproductive confounding variables. In 19 RA concordant sibling pairs, the seven HLA identical pairs had similar ages of menarche, whereas the 12 non-identical HLA pairs had interpair differences that narrowly missed significance ($p=0·054$). All other obstetric and gynaecological variables were not significantly different within the pairs.**

***Conclusions*—The interpretations of these results are that either delayed menarche may predispose to or act as a marker of RA, or HLA linked genes are important in determining the age of menarche irrespective of disease state. This study fails to support a significant role for other obstetric and gynaecological variables in RA.**

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The finding that women are three times more likely to develop rheumatoid arthritis (RA) than men[1] raises the possibility that sex hormones may be important in the aetiology of this disease. The greatest difference in this sex ratio is seen in the 40 to 59 age group,[2] when most women are perimenopausal with sex hormones undergoing dramatic changes. Women with RA are reported to be more likely to be nulliparous before onset of symptoms,[3] and the contraceptive pill seems to confer mild protection from susceptibility to RA.[4] These findings suggest that pregnancies and the contraceptive pill may confer life long protection from RA. Alternatively, their absence may increase susceptibility to development of RA some time after the years when women usually have families or require contraception.

An idea that has received little attention is that the age of menarche may exert an influence over the predisposition to RA. There is evidence that both genetic and environmental variables determine the onset of the menarche,[5 6] and there is a documented association between early onset of menses and a greater risk of developing breast cancer[7] and Hashimoto's thyroiditis.[8] Conversely, the risk of thyroid cancer may be greater in women with a delayed menarche.[9] These findings raise the possibility that the mechanisms controlling menarche may be of importance in pathological states that do not declare themselves for many years; alternatively the total number of years of fluctuating adult hormone concentrations in the menstrual cycle could be important in susceptibility to disease.

Recent studies that have mentioned age of menarche and RA have given conflicting results, with two studies suggesting an older age of menarche in older patients compared with unrelated controls,[10 11] one the opposite,[12] and two no difference.[13 14] We have studied the inter-relationships between RA, age of menarche, and HLA identity in RA discordant pairs of sisters. We have also considered other obstetric and gynaecological variables within the pairs, to determine whether this potentially highly informative population could help to clarify previous work in this area.

Methods

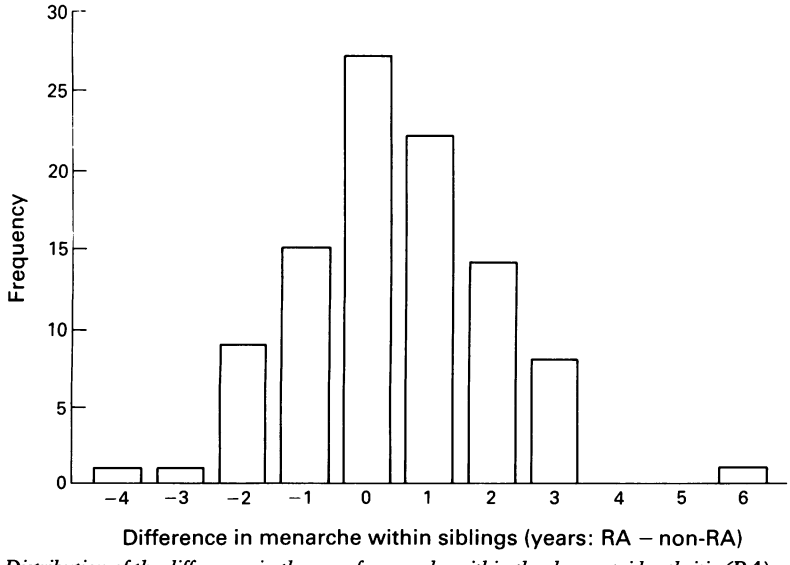
The population for this study has been described in detail previously.[15] Female patients with classical and definite RA according to the 1958 American Rheumatism Association criteria[16] were identified from rheumatology outpatients. Entry criteria for the original study of the genetic epidemiology

of RA included having a sister who was geographically accessible and willing to be examined for the presence of RA, and to have blood taken for histocompatibility locus antigen (HLA) class I typing by standard serological techniques.[15] Female sibships were subsequently approached for epidemiological studies. The second approach was biased towards the pairs who were identical for their HLA-A, HLA-B, and HLA-Cw types, as these were considered to be of interest for a wide range of genetic, serological, and epidemiological studies. Details of the RA disease in the probands were already available. A record was made of the age when each sibling recalled having started her periods. Age, duration of use of the contraceptive pill, pregnancy history, and age of the menopause (where appropriate) were also recorded.

The means and 95% confidence intervals (95% CIs) were calculated for the age of menarche and other demographic and reproductive details for the probands with RA and their sisters without RA. The significance of any differences within the pairs was tested by paired t test, χ^2 test, or Fisher's exact test where appropriate. The statistics were calculated for the whole population of sibling pairs, and then repeated for the identical HLA, and non-identical HLA (sharing 1 or 0 HLA haplotypes) subsets. Multiple regression analysis was performed to determine whether any significant differences in age of menarche could be predicted by any of the other demographic or reproductive variables, suggesting a potential confounding effect.

Table 1 Mean ages of menarche in the probands with RA and their unaffected sisters

Sibling	*Mean age (years) (95% CI)*	*Mean difference (years) (95% CI)*	*Paired t test*
RA	13·90 (13·56 to 14·24)		
(n=98 pairs)		0·41 (0·09 to 0·73)	t=2·54, p=0·013
Non-RA	13·49 (13·22 to 13·76)		



Distribution of the differences in the age of menarche within the rheumatoid arthritis (RA) discordant sibling pairs (age of siblings without RA subtracted from age of siblings with RA).

Results

Ninety eight RA discordant pairs of sisters were identified. The probands with RA had a mean age of 57·7 (standard deviation (SD) 11·6, range 30–82) years and duration of disease of 11·8 (SD 9·8, range 1–44) years. A rheumatoid factor test was seropositive for 69·8% (RAHA titre >1/40, Fugizoki, Tokyo, Japan), and 82·7% had erosive disease on hand radiographs. None of the probands had developed RA before the menarche, with a mean age of onset of 46·2 (SD 11·8, range 23–74) years.

Table 1 and the figure show the mean ages of menarche, mean differences within pairs, and 95% CIs. The difference was significant by paired t test, with members of the sibling pairs with RA having an older age of menarche than those without RA (p=0·013). When the population was divided into 36 identical HLA and 62 non-identical HLA pairs (table 2), it became clear that difference within the pairs resided in the second (p=0·007), and not the first group (p=0·55).

Multiple regression analysis was performed in the non-identical HLA pairs to determine whether other demographic and reproductive variables were significantly predictive of interpair differences in age of menarche and raising the possibility that such variables might be confounding factors in determining differences between disease discordant pairs. With the dependent variable difference in age of menarche the interpair differences considered as independent variables were age, years taking the contraceptive pill, number of pregnancies, number of failed pregnancies (miscarriages, stillbirths, and abortions), age of menopause (where appropriate), and total number of menstruating years (in those who were postmenopausal). No variables emerged as significant predictors in the interpair differences in age of menarche.

Because of the similar ages of menarche in the identical but not the non-identical HLA RA discordant sibling pairs, differences in age of menarche in 19 RA concordant sibling pairs were considered (table 3). The seven identical HLA pairs had similar ages of menarche, with differences within the non-identical HLA pairs narrowly missing conventional significance (p=0·054). Comparison of absolute differences in ages of menarche within pairs between the non-identical HLA and identical HLA groups also narrowly missed significance (means 1·8 *v* 1·0 respectively, unpaired t test p=0·08).

The 19 RA concordant pairs were combined with the 98 RA discordant pairs in a multiple regression equation with interpair differences in the age of menarche as the dependent variable and disease state of the pair and HLA haplotype sharing within the pair as independent variables. Neither emerged as significant predictors.

Table 4 shows demographic and reproductive details and interpair differences. There were no significant differences within sibling pairs for these variables. This also applied when the pairs were divided into identical and non-identical HLA groups (data

Table 2 Mean ages of menarche in probands and their unaffected sisters divided into HLA identical and HLA non-identical groups

Sibling	Mean age (y) (95% CI)	Mean difference (y) (95% CI)	Paired t test
HLA identical			
RA	13·81 (13·30 to 14·32)		
(n=36 pairs)		0·17 (−0·40 to 0·73)	t=0·60, p=0·55
Non-RA	13·64 (13·19 to 14·09)		
HLA non-identical			
RA	13·95 (13·50 to 14·41)		
(n=62 pairs)		0·55 (0·16 to 0·94)	t=2·80, p=0·007
Non-RA	13·40 (13·06 to 13·75)		

Table 3 Mean ages of menarche in the rheumatoid arthritis (RA) concordant sibling pairs, divided into HLA identical and HLA non-identical groups

Sibling	Mean age (y) (95% CI)	Mean difference (y) (95% CI)	Paired t test
HLA identical			
RA	14·14 (13·80 to 14·48)		
(n=7 pairs)		0·14 (−1·10 to 1·39)	t=0·28, p=0·79
Non-RA	14·29 (13·01 to 15·56)		
HLA non-identical			
RA	13·42 (12·54 to 14·29)		
(n=12 pairs)		1·08 (−0·01 to 2·18)	t=2·17, p=0·053
Non-RA	13·40 (11·51 to 13·16)		

Table 4 Demographic and reproductive variable differences within the rheumatoid arthritis discordant sibling pairs

	No of pairs	Proband (mean)	Sibling (mean)	Mean difference (95% CI)	Paired t test (p value)
Age (years)	98	57·7	57·1	0·60 (−1·01 to 2·20)	0·46
Years taking pill	98	1·9	2·3	0·42 (−0·58 to 1·41)	0·41
Number of pregnancies	98	2·0	2·0	0·04 (−0·29 to 0·37)	0·81
Number of failed pregnancies*	98	0·3	0·3	0·07 (−0·09 to 0·23)	0·38
Age of menopause†	65	47·0	47·2	0·22 (−1·37 to 1·81)	0·79
Number of years menstruating‡	65	33·2	33·6	0·43 (−1·24 to 2·50)	0·61

*Miscarriages, stillbirths and abortions.
†Natural menopause, oophorectomies excluded. For hysterectomies, age of start of menstrual symptoms.
‡Difference between age of menarche and menopause.

Table 5 Prevalence of ever having taken the contraceptive pill in rheumatoid arthritis (RA) discordant female sibling pairs

Pair	No	Neither sibling taken pill No (%)	RA sibling only taken pill No (%)	Non-RA sibling only taken pill No (%)	Both siblings taken pill No (%)
HLA non-identical	62	51 (82·3)	2 (3·2)	7 (11·4)	2 (3·2)
HLA identical	36	30 (83·3)	1 (2·8)	3 (8·3)	2 (5·5)
Combined	98	81 (82·7)	3 (3·1)	10 (10·2)	4 (4·1)

No significant difference in distributions between groups by χ^2 or Fisher's exact test as appropriate.

Table 6 Prevalence of nulliparity in rheumatoid arthritis (RA) discordant female sibling pairs

Pair	No	Neither sibling nulliparous No (%)	RA sibling only nulliparous No (%)	Non-RA sibling only nulliparous No (%)	Both siblings nulliparous No (%)
HLA non-identical	62	3 (4·8)	6 (9·7)	8 (12·9)	45 (72·6)
HLA identical	36	4 (11·1)	4 (11·1)	5 (13·9)	23 (63·9)
Combined	98	7 (7·1)	10 (10·2)	13 (13·3)	68 (69·4)

No significant difference in distributions between groups by χ^2 or Fisher's exact test as appropriate.

not shown). Table 5 shows the percentages of siblings ever having taken the pill. For this data, only 17 pairs of sisters had one or both members with a history of ever having taken the pill. Although it was more common for the sibling without RA rather than the sibling with RA to have taken the pill in both the HLA non-identical and identical sibling pairs, these differences were not significant. Table 6 shows the percentages of siblings who were nulliparous. There were no significant differences between the groups. Differences in the mean ages of first pregnancy within the pairs were not significant (siblings with RA *v* siblings without RA 23·2 (SD 3·1) years *v* 24·4 (SD 3·9) years). This finding applied to both the identical and non-identical HLA pairs.

Discussion

The sister pairs have provided a potentially informative population to investigate further previously described epidemiological associations with RA. The opportunity to investigate HLA identity also enabled us to analyse possible interactions between HLA linked factors and reproductive variables. By making comparisons within sibships, it is also possible to ensure a greater similarity of other social and environmental variables than is the case in unrelated control groups.

Our population was not ideal for considering the role of the contraceptive pill, as most of our sibling pairs had never taken the pill. This presumably reflects the age of the population and unavailability of the pill during their reproductive years. For those pairs in whom at least one member had ever taken the pill, there was a tendency in both the identical HLA and non-identical HLA pairs for the sibling without RA to have been ever exposed more often than the sibling with RA. These data are not incompatible with the possibility that the contraceptive pill confers a mild protective effect on the predisposition to RA.[4] Another study considering the use of the pill in pairs of sisters came to similar conclusions.[17]

Our study does not support the protective effect of pregnancy on RA in that we found similar frequencies of nulliparity in the siblings with and without RA. Although recent studies on RA and unrelated controls have suggested a predisposing effect for nulliparity[3 10] and one study suggested a protective effect from an early first pregnancy,[18] these results have not been reproduced in all studies.[12] Doubt has to remain, therefore, on the role of pregnancy in influencing the pathogenesis of RA. In agreement with most recent studies,[3 18 19] we failed to support a role for adverse pregnancy outcome in RA. The principal contribution of the present study has been in highlighting a possible role for interactions between delayed menarche, RA, and HLA linked factors.

The main problem in determining the age of menarche retrospectively is that of accurate recall and the discrepancies between subjects as to when the periods are defined as starting. A recent study suggested that 84% of women could recall the age of menarche to within one

year of the event.[20] Moreover, we can think of no reason why the biases introduced by inaccurate recall should be any greater in siblings who go on to develop RA than in their sisters without RA.

The finding that a delayed menarche is associated with development of RA needs to be confirmed in other populations. Spector and colleagues found an older age of menarche in patients with RA compared with normal controls and patients with osteoarthritis, although they did not comment on this.[10] Linos *et al* reported an older age of menarche in patients with RA and unrelated controls, although no details were given.[11] By contrast, an American study of a large cohort of nurses suggested that when compared with women who experienced menarche earlier than 13 years of age, the age adjusted relative risk of RA among women with an early menarche was 1·9 (95% CI 0·9–2·4).[12] Hazes *et al* and Del Junco *et al* found no difference in age of menarche between patients with RA and unrelated controls.[13 14] The explanation for these discrepancies may reside in the fact that if there is a true predisposition to RA due to a delay in the age of menarche, the biological effect shown in our potentially highly informative sibling pairs was not great, as the mean difference within the sibling pairs was only a few months and was confined to the HLA non-identical pairs. This effect could not be explained, however, by some of the other potential confounding variables that have previously been associated with RA, such as the contraceptive pill, parity, and failed pregnancies.[3 4]

If other studies confirm our finding of an older age of menarche in the siblings with RA, this raises the possibility that as the menarche predated the onset of symptomatic RA in all our probands, this may predispose to, or act as a marker of, susceptibility to disease. During childhood the ovary is active but does not produce significant quantities of sex steroids.[21] Women with an older age of menarche will therefore be relatively deficient in sex hormones for a longer period. As suggested by the protective effect of the contraceptive pill[4] and the increased susceptibility of nulliparous women,[3] these hormones may exert a prolonged protective effect against the development of RA so that hormone deficiency at any stage may have longstanding consequences. The total number of menstruating years did not seem to be different, however, within the RA discordant pairs.

It is of interest that the finding of delayed menarche was confined to the HLA non-identical sisters and that within the few RA concordant pairs similar observations were made. Many factors determine the timing of the menarche, such as body size and composition, socioeconomic conditions, energy expenditure, and dietary patterns.[5] There is evidence that genetic factors are important. The correlation for the onset of menarche in monozygotic twins is 0·65 compared with 0·18 in dizygotic pairs,[6] and a positive association between age of menarche in mothers and daughters has been described.[5] Which genes may be important in controlling the menarche is not known. We know of no evidence to implicate HLA linked genes in the timing of the menarche. There is evidence that HLA linked genes may be important in determining testosterone concentrations in men, in that identical HLA brothers have more similar values than non-identical HLA brothers.[22] HLA-B15 is associated with low concentrations of testosterone in men,[23] and HLA-B5 and HLA-B12, but not HLA-B8, with high concentrations in women.[24] It has been noted that the major histocompatibility complex in mice may exert an influence over gonadal and other organ size as well as testosterone concentrations.[25] These findings raise the possibility that genes within the major histocompatibility complex may be important in determining phenotypic variants that could exert a profound effect over characteristics such as the age of the menarche. This needs to be studied in healthy sibling populations. Such a study would have to be of a reasonable size, as in 117 sibships we found that HLA haplotype sharing was not a significant predictor of interpair differences in age of menarche. This is probably a reflection of the many environmental and possibly other genetic factors that determine this event.

In summary, this study raises some interesting possibilities. The first is that delayed menarche predisposes to, or is a marker of, RA. The second is that irrespective of eventual disease state, HLA factors are a determinant of age of menarche. We have previously shown that identical HLA siblings have about double the concordance rates for RA compared with siblings sharing 1 or 0 HLA haplotypes.[15] The HLA predisposition to RA and other autoimmune disease may not only be a manifestation of the gene products that are important in antigen presentation, but also a reflection of linked genes within the major histocompatibility complex that influences sex hormones and reproductive variables such as the age of the menarche. If confirmed in other populations, these findings have profound implications for all diseases in which there is a role for sex hormones, reproductive variables, and major histocompatibility complex genes.

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