

## Letter to the Editor

# Do Mothers of Dizygotic Twins Have Earlier Menopause? A Role for Fragile X?

### To the Editor:

Dizygotic (DZ) twinning appears to run in families and the underlying trait, multiple ovulation, appears to be genetically influenced [Lewis et al., 1996; Meulemans et al., 1996]. Multiple ovulation is considerably more frequent in mothers of DZ twins than in controls [Martin et al., 1991b] although its hormonal control is far from understood [Martin et al., 1991a]. Whatever the cause, if the supply of ovarian follicles is finite, then it might be expected that a woman predisposed to multiple ovulation would more quickly exhaust her supply. If this in turn is a precipitator of menopause [te Velde, 1993], one might expect mothers of DZ twins to have earlier menopause than controls.

One predisposing factor to DZ twinning may be heterozygosity for expansion of the CGG repeat number in the fragile X (FRAAX) locus into the premutation range (50–200 repeats) [Fu et al., 1991]. Previously we have shown that such women have a significantly higher DZ twinning rate than control women and have speculated that they should have earlier menopause [Turner et al., 1994]. Now Conway et al. [1995] have found 2 out of 9 women with premature ovarian failure with FRAAXA premutations. This has prompted us to investigate: (i) the frequency of early menopause in mothers of DZ twins (MODZTs), and (ii) whether FRAAXA premutations contribute to any tendency to earlier menopause among MODZTs.

Health and Lifestyle questionnaires were sent to mothers of 3,220 adult twin pairs enrolled on the Australian NHMRC Twin Registry; 65% responded (ages 38–96, mean 58) and 59% answered “Have you reached menopause (‘natural change of life’)?” and “How old were you when your periods stopped?” (Table I). They also answered questions about hysterectomy and its temporal relation to natural menopause. While it is difficult to evaluate the validity of self-reported age at menopause, the consistency of this reporting over time

appears to be good; for 357 postmenopausal twins who reported age at natural menopause in 1980 and again in 1988, the 8-year test-retest correlation was  $0.80 \pm 0.03$ , and in an overlapping sample of 85 women, the 2-year consistency was  $0.92 \pm 0.04$ .

From Kaplan-Meier survival analysis, the mean age at menopause was  $49.9 \pm .2$  in MODZTs and  $50.9 \pm .2$  in MOMZTs ( $P = 0.0013$ ). Smoking (pack-years;  $P < .0001$ ), education ( $P = 0.034$ ) and body mass index ( $P = 0.031$ ) were significant covariates, as found by other [Willett et al., 1983; Luoto et al., 1994], but adjusting for these only marginally influenced the significance of the zygosity effect ( $P = 0.0031$ ). Within MODZTs, there was no effect on menopausal age of having twins early (<32) rather than later in reproductive life, nor on having a positive vs. negative family history of DZ twinning.

Examination of the survival plots shows that the disparity between mothers of MZ and DZ twins arises before age 40 (Fig. 1) and that thereafter they parallel each other. Treating menopause before 40 as a disease (premature ovarian failure, POF) and using logistic regression with censoring, the zygosity effect is significant ( $P = 0.003$ ); entering smoking (pack-years) as a covariate alters the significance to  $P = 0.007$ . The odds ratio for premature ovarian failure in MODZT compared with MOMZT is 3.21 (95% CI 1.55–7.18).

Menopause <40 in MOMZTs may still be more frequent than in mothers of singleton controls since there are hints of etiological connections between DZ and MZ

TABLE I. Responses to a Questionnaire Item on Menopausal Age Answered by 1,906 Mothers of Twins

	Mothers of DZ twins	Mothers of MZ twins
	n %	n %
Responded	1,012	894
Not reached	266 (26)	281 (32)
menopause (%)		
Hysterectomy before	52 (5)	56 (6)
menopause (%)		
Natural meno-	694 (69)	556 (62)
pause (%)		
Menopause	32 [3.2]	9 [1.0]
before 40 (%)		
Mean age at	$49.9 \pm .2$	$50.9 \pm .2$
menopause		

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\*Correspondence to: N.G. Martin, Ph.D., Queensland Institute of Medical Research, 300 Herston Road, Brisbane 4029, Australia.

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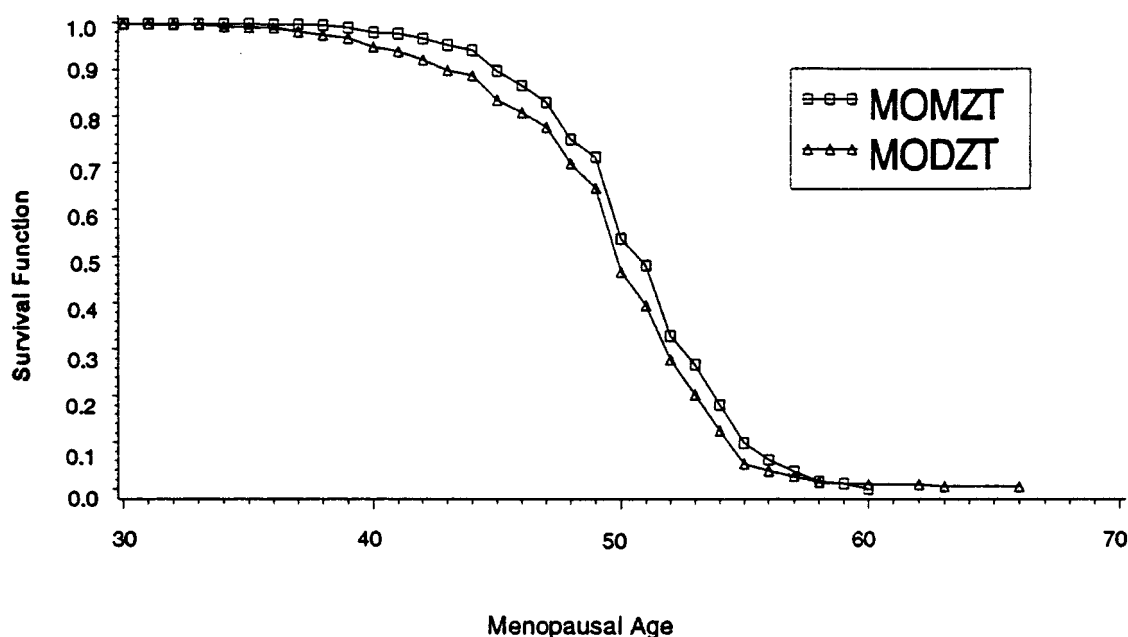


Fig. 1. Kaplan-Meier survival curves for menopausal age in mothers of DZ vs. MZ twins.

twinning [Parisi et al., 1983]. Rates may also be altered differentially by premenopausal hysterectomy, although there is no difference in the frequency of this. The question posed by the finding of Conway et al. [1995] is whether FRAXA premutations play a role in the high rate of premature ovarian failure in our sample of mothers of twins.

We therefore attempted (following a full informed consent procedure) to obtain DNA samples from the 41 mothers in our sample who had reported menopause <40 years in the questionnaire (Table II). We were unable to contact 9 (at least 3 had died) and of the 32 successfully interviewed 6 reported complicating pathology accounting for early menopause and 2 others failed to confirm menopause <40 years. Two women refused FRAXA testing and the DNA of one was degraded. No FRAXA premutations were detected by combined polymerase chain reaction (PCR) and Southern analysis [Fu et al., 1991]. The 42 alleles tested ranged in copy number from 19 to 40 (mean 28.1), within the usual range (6–54).

This negative result is despite 5 of the women with POF in our sample (3 MODZT, 2 MOMZT), reporting at

least one female first- or second-degree relative also having menopause <40. It was among 9 such familial cases of POF that Conway et al. [1995] found 2 FRAXA premutations. Nevertheless, it must be recognised that the 0/16 in our sample is not significantly different ( $P = 0.12$ ) from the 2/9 observed by (Conway et al. [1995] and that samples at least twice as large would be needed for such a disproportionality to be significant at the 5% level 95% of the time.

Our data show no differential misreporting by questionnaire of premature menopause between mothers of DZ vs. MZ twins. In those confirming menopause  $\leq 40$  at interview, the odds ratio for premature ovarian failure in MODZT compared with MOMZT remains significant at 3.40 (95% CI 1.36–10.29).

We conclude that being a mother of DZ twins is a significant risk factor for premature ovarian failure, but that FRAXA premutations can play no more than a minor role in this increased risk.

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TABLE II. Confirmation of Early Menopause and FRAXA premutation Status in 41 Mothers of Twins Who Reported Menopause Before 40 Years

	Mothers of DZ twins	Mothers of MZ twins
Reported menopause <40 in questionnaire	32	9
Interviewed	24	8
Confirmed menopause <40	19	5
Tested for FRAXA premutation	16	5
FRAXA premutation	0	0

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**N.G. Martin\***

**S.C. Healey**

**T.S. Pangan**

Queensland Institute of Medical Research  
Brisbane, Australia

**A.C. Heath**

Department of Psychiatry  
Washington University  
St. Louis, Missouri

**G. Turner**

Newcastle Medical Genetics Unit  
Newcastle, Australia