American Journal of Medical Genetics Part A 146A:3147–3156 (2008)

Familial Twinning and Fertility in Dutch Mothers of Twins

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Received 7 December 2007; Accepted 30 September 2008

We studied twinning and fertility indices in mothers with spontaneous monozygotic (MZ) and dizygotic (DZ) twins and in mothers who conceived their twins after the use of assisted reproduction techniques (ART). Participants in this study consisted of 8,222 and 5,505 women with spontaneous DZ and MZ offspring and 4,164 and 250 women with ART DZ and MZ twin pairs, respectively. Women were compared with respect to the number of sibs and offspring, the presence of other relatives with twins and the time it took to conceive the twins. We also compared familial twinning between a younger and an older age group. Women with spontaneous DZ twins more often reported female relatives with twins than those with spontaneous MZ twins. The proportion of DZ versus MZ twin offspring in relatives was also larger in women with spontaneous DZ offspring than in women with MZ offspring. The first group of women reported a shorter time to conceive. Women with ART twins had fewer sibs and offspring and less often reported relatives with twins. We did not observe that DZ twinning was more familial in women who had their twins before age 36 years compared to older women. Familial DZ twinning is clearly present in mothers of spontaneous DZ twins. The mechanisms underlying spontaneous and non-spontaneous DZ twinning are different and fertility treatment should be taken into account in any study of twinning. Twinning is not more familial in women who have their twins at a younger age.

Key words: spontaneous DZ twinning; iatrogenic twins; familial twinning; fertility

How to cite this article: Hoekstra C, Willemsen G, van Beijsterveldt TCEM, Montgomery GW, Boomsma DI. 2008. Familial twinning and fertility in Dutch mothers of twins. Am J Med Genet Part A 146A:3147–3156.

INTRODUCTION

It has long been known that dizygotic (DZ) twinning runs in families [e.g., Hoekstra et al., 2008a]. In 1901, Weinberg described familial clustering of DZ twin pregnancies. He observed that mothers, sisters, and daughters of women who have given birth to multiples, had an increased risk of conceiving twins or multiples by 39%, 95%, and 30%, respectively. This increased risk was not found in relatives on the paternal side of the family. Some 60 years later, Wyshak and White came to the same conclusion when analyzing the inheritance of twinning using data obtained from the archives of the Genealogical Society of the Mormon Church in Salt Lake City [White and Wyshak, 1964; Wyshak and White, 1965]. Further support was obtained by Bulmer [1970] who found that the risk of conceiving twins was 1.7 times higher in female relatives than in male relatives of index mothers. Examining the DZ twinning rate among female relatives in greater detail, Bulmer showed that the twinning rate in women who had a sister with DZ twins was 2.5 times higher than the twinning rate in the general population. The risk of having twins for mothers and daughters of a woman with DZ twins was about

Grant sponsor: National Institute of Child Health and Human Development; Grant number: HD042157; Grant sponsor: Center for Neurogenomics and Cognition Research (CNCR) of the VU University Amsterdam; Grant sponsor: Twin-Family Database for Behavior Genetics and Genomics Studies; Grant number: NWO-MagW 480-04-004; Grant sponsor: Spinozapremie; Grant number: NWO/SPI 56-464-14192.

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Published online 14 November 2008 in Wiley InterScience (www.interscience.wiley.com)
DOI 10.1002/ajmg.a.32585

twice as high as the risk in the general population [Bulmer, 1970].

Additional studies support evidence for familial DZ twinning. Lewis et al. [1996] investigated familial twinning in 6,596 twin pairs from the Australian Twin Registry and found a relative risk of 1.7 for sisters and mothers of DZ twins and of 2.5 for the offspring of female DZ twins. Meulemans et al. [1996] investigated the inheritance of DZ twinning in 1,422 Dutch and Flemish families by formal segregation analysis. The phenotype of "having DZ twins" was consistent with an autosomal dominant monogenic model with incomplete penetrance. Thus, the genetic contribution to DZ twinning is observed in women, but the trait may be inherited from either parent [Greulich, 1934; Parisi et al., 1983].

Additional factors influencing DZ twinning, which may or may not interact with genotype, are maternal age, parity and the use of fertility treatments [Bulmer, 1970; Fauser et al., 2005]. The decline in the DZ twinning rate in the early 20th century reflected a decrease in mean maternal age and a lower number of maternities. The increase in twinning rate reported in the late 1970s has been mainly associated with an older age at childbearing [Derom et al., 1995; Lambalk et al., 2004]. The use of fertility treatments such as ovulation induction (OI), in vitro fertilization (IVF), intro cytoplasmatic sperm injection (ICSI), and intra-uterine insemination (IUI) has further added to the rising incidence in twin births [Fauser et al., 2005; Martin et al., 2005].

Monozygotic (MZ) twinning has a very different etiology than DZ twinning [Hall, 2003]. MZ twinning does not seem to be influenced by genetic factors, maternal age and parity, though families with a history of MZ twinning have been reported [Bulmer, 1970; Hamamy et al., 2004]. Recently, an increase in MZ twin births has been reported after IVF and OI [Steinman, 2003; Derom et al., 2006]. If MZ twinning is the result of a random event while DZ twinning is influenced by a genetic predisposition, the comparison of mothers who gave birth to DZ twins with mothers who gave birth to MZ twins may provide valuable clues concerning the processes involved in fertility and subsequently infertility. Such a comparison was undertaken by Lewis et al. [1996], who obtained information on familial twinning through the twins themselves. The prevalence of additional DZ twins in the family of DZ twins was much higher than the prevalence of additional MZ twins in the family of MZ twins, confirming a stronger familial component for DZ twinning.

We collected data on familial twinning from mothers of twins. Familial twinning was examined in mothers with spontaneous MZ and DZ twin offspring and in mothers of MZ and DZ twins who conceived their offspring after assisted reproduction techniques (ART). We compared these groups on the following familial and fertility related variables (1) the number of brothers and sisters of the index mother and the number of offspring of the index mother (2) the presence of other family members with twin offspring (3) the ratio of DZ and MZ familial twinning and (4) the time it took to become pregnant with the twins.

It is still unclear if maternal and iatrogenic factors affect twinning independently or interact with genetic factors causing DZ twinning. It is possible that women who conceived DZ twins after ART also had a genetic susceptibility for DZ twinning, which may be expressed as providing the embryos with an optimal uterine milieu or having embryos of high quality [Lambers et al., 2007]. If mothers of ART DZ twins have a genetic predisposition, they should have similar numbers of additional family members with twins as mothers of spontaneous DZ twins. We therefore compared women with spontaneous and with ART DZ twins on the proportion of other family members with twins. We also compared mothers of ART MZ twins with mothers of ART DZ twins. If having DZ twins after fertility treatment is influenced by genetic factors, while having MZ twins after ART is not, we would expect fewer mothers of ART MZ pairs to report a family history of twinning than ART DZ.

Women with multiple sets of spontaneous DZ twin pairs may be considered as an even more genetically predisposed subgroup of DZ twin mothers. We compared women with multiple sets of spontaneous DZ twins to those with one set of DZ twins on familial twinning and the time it took to become pregnant.

DZ twinning rates increase with maternal age; the chance of having DZ twins increases approximately fourfold up to the age of 36 years [Bulmer, 1970]. This means that any genetic predisposition to DZ twinning may be more apparent in younger women, so we examined whether women who had DZ pairs at a younger age (<36 years) reported more familial twinning than women who had DZ pairs at an older age.

Finally, in women who are twins themselves we examined whether their cotwin was also a mother of twins and if so, whether they were concordant for twin offspring zygosity.

MATERIALS AND METHODS

Participants

The Netherlands Twin Register (NTR) collects longitudinal data on twins and their family members in two samples: (1) in mothers of newborn or young twins (YNTR) who are registered at birth by their parents and (2) in adolescent and adult twins (ANTR) and their family members. Over 90% of the participants are born in the Netherlands [Boomsma et al., 2002, 2006; Bartels et al., 2007].

In 2005, a survey was mailed to all mothers of twins and multiples (N = 33,528) registered with the NTR

(referred to as index mothers). Index mothers were asked to complete a series of questions on maternal characteristics prior to the birth of the twins, familial twinning and mode of conception. From the mothers who participated in NTR studies before (N = 25,620), we received 17,683 completed questionnaires, and 1,674 completed questionnaires were received from mothers who had never previously participated. In 2006, the survey was also sent to newly registered mothers. In total, data from 20,150 surveys were available; 260 surveys were returned by mothers of triplets. We previously reported on the absence of response bias by comparing data from women who returned this survey with data from mothers who did not return the survey, but who had taken part in earlier NTR studies [Hoekstra et al., 2008b; Van Beijsterveldt et al., 2008].

Data were excluded when the index mother was not the biological mother of the twins or when the relation with the twins was unknown (N = 94); when data on fertility treatment were lacking or when the twins were conceived by other methods than IVF, ICSI, IUI, and OI (N = 294), such as egg donation; when data on familial twinning were missing (N = 406); or when the response to the familial twinning questions was unclear (N = 585). There were 134 women, who completed a similar questionnaire designed for mothers with multiple sets of twins. If the zygosity and/or mode of conception was different for the two pairs, for example, one twin pair was spontaneous and one twin pair was artificially conceived, data were excluded (N = 53). Data of 577 twin mothers were excluded due to missing information on zygosity of the twin offspring (in 388 cases the index mother indicated that she did not know the zygosity of her twins and in 189 cases the index mother did not answer the question or her answer was unclear). For these twin pairs, no other information on zygosity (e.g., from earlier NTR surveys) was available.

Zygosity data were available on 18,141 twin pairs. For same-sex pairs, zygosity was based on DNA polymorphisms obtained in participants in previous NTR studies (N = 1,656) or from previous survey questions (N = 7,403). When DNA and previous survey data were not available, zygosity was based on the answers of the mother in the current survey (N = 2,983). Previous questions regarding offspring zygosity asked whether the twins were alike in eye-, hair- or face color and in face form and whether the twins were often mistaken for each other by their parents, other relatives and by strangers. Based on the answers to these questions, zygosity was determined in same-sex twin pairs. The association between DNA and questionnaire zygosity is 93% in the YNTR sample [Rietveld et al., 2000] and 97% in the ANTR sample [Willemsen et al., 2005]. A comparison of the zygosity of same-sex twin pairs based on information from the current survey and the zygosity obtained from previous questionnaires also showed a high degree of agreement (90.7%). The sample included 5,943 (32.8%) index mothers with opposite-sex twin offspring which is comparable with the general Dutch population (34.4%) [Statistics Netherlands, 2007]. The sample included 78 mothers of sponteneous di- and tri-zygotic triplets, who were included in the mothers of DZ twins group and 24/6 mothers of spontaneous/ART MZ triplets.

The final sample consisted of 5,505 women with spontaneous MZ twin offspring (including 8 mothers of 2 sets of twin pairs), 8,222 women with spontaneous DZ twin offspring (including 61 mothers of multiple sets of twin pairs), 4,164 women with ART DZ twins (including 12 mothers of multiple sets of twins) and 250 women with ART MZ twins.

Family Size

Mother of twins were asked how many sibs they had with the same biological mother and father. They could indicate 0–8 or more brothers and 0–8 or more sisters.

Number of Children

The number of own offspring is obtained by summing the number of twins, number of triplets and the number of single children.

Relatives With Twin Offspring

The survey contained a series of items regarding familial twinning. Women were asked "Which of your own biological family members are also parents of twins/multiples?" Familial relationships and the number of index mothers who reported a relative with twin offspring are described in Table I. There were 5,520 index mothers who reported 1 additional type of relative with twins/multiples and 2,058 index mothers who reported more than 1 type of relative with multiples. The categories listed in Table I are used to describe the relationship of the index mother with her relatives in all analyses. When family members with twins were indicated, the index mother could report if the twins of these family members were girls (FF), boys (MM) or a girl and boy (FM). The index mother was also asked to report whether the twins of her relatives were monozygotic (MZ) or dizygotic (DZ). She could also report that she did not know the zygosity of the twins of her relatives.

Familial twinning was defined as follows: if an index mother indicated that she had a female relative with twins, regardless of zygosity and the number of family members that were reported, then the index mother was given a yes for familial twinning. Similarly, if an index mother reported a female relative with DZ twins she received a yes for DZ

TABLE I. Number of Index Mothers With Family Members Who Have Twin/Multiple Offspring (by Family Relation)

Relationship	Category	N of probands with 1 type of relative who has multiple offspring	N of probands with >1 type of relative who has multiple offspring
My daughter(s) has/have multiples	Daughter	15	29
My son(s) has/have multiples	Son	9	11
My sister(s) has/have multiples	Sister	415	382
My brother(s) has/have multiples	Brother	265	257
My mother has multiples	Mother	594	532
Sister(s) of my mother has/have multiples	Aunt (M)	686	671
Brother(s) of my mother has/have multiples	Uncle (M)	511	456
The parents of my mother has/have multiples	Grandma (M)	951	661
The sister(s) of my father has/have multiples	Aunt (P)	604	573
The brother(s) of my father has/have multiples	Uncle (P)	489	427
The parents of my father has/have multiples	Grandma (P)	981	642
Number of families with twins/multiples		5,520	2,058

P, paternal; M, maternal.

familial twinning. If an index mother had female relatives with MZ twins, she received a yes for MZ familial twinning. If both female relatives with MZ offspring and female relatives with DZ offspring were present (e.g., an aunt with DZ twins and a sister with MZ twins), she received a yes for both DZ and MZ familial twinning.

Use of Assisted Reproduction Techniques (ART) and Time to Become Pregnant

Whether the twins were conceived spontaneously or not and the time it took to become pregnant with twins were asked in a combined question. The index mother was asked "How did you become pregnant with your first twin pregnancy?" The answer categories were: (1) It was a spontaneous pregnancy, and I became pregnant in (a) 0–2 months (b) 3–5 months (c) 6–12 months (d) more than 12 months; (2) IVF; (3) ICSI; (4) IUI; (5) OI; and (6) other, specify. Based on the answers to these questions, we created a variable with 3-categories to indicate whether the birth was (1) spontaneous or the result of (2) IVF, ICSI, IUI or (3) OI. Mothers who ticked the category "other specify" in the original question were either classified as a mother of spontaneous twins or a mother of an ART twin pair depending on the answer. Data from women which did not fall within one of these classes of conception were excluded (N = 71). A 4-category variable was created to indicate for those who had a spontaneous pregnancy the time it took to conceive (0–2 months, 3–5 months, 6–12 months, more than 12 months).

Maternal Age

Maternal age at time of the twin birth was obtained by subtracting the birth date of twins from the birth date of the mother. Maternal age was recoded into two categories; younger than 36 years and 36 years or older at twin birth.

Maternal Twin Status

All index mothers were asked if they were twins themselves and, if yes, about their zygosity. If a woman was a twin herself and indicated that she had at least one sister who was a mother of twins, we tried to contact her by phone to obtain information on which of her sisters was the mother of twins.

Analysis

We tested whether the number of sibs and the number of own offspring differed between women with spontaneous DZ twins, women with spontaneous MZ twins and women with ART DZ and ART MZ twins using Chi-squared tests. Next, we compared the frequency of familial twinning (both MZ and DZ) in women with spontaneous DZ twins and women with spontaneous MZ twins. We also examined whether women with spontaneous DZ twins differed from women with spontaneous MZ twins in the proportion DZ versus MZ twin offspring in relatives. This was done for each familial relationship (e.g., sister, parents) as well as for familial twinning (having at least one female relative with twins). We repeated these analyses by using only relatives with DOS twins as zygosity is certain for DOS twins. There were 797 index mothers with a sister who also was a mother of twins. In some families (N = 249) both sisters were registered with the NTR and returned the questionnaire. As these sisters pairs were ascertained independently, all data were used in the analyses. However, repeating the analyses, with the data from one sister removed, did not change the results.

At the second step, women with spontaneous DZ twins were compared to women with ART DZ twins on the frequency of familial twinning and on the proportion of DZ versus MZ twin offspring in their relatives. The frequency of familial twinning was also compared between ART MZ and ART DZ group. At the third step, the frequency of familial twinning in

women who had multiple spontaneous DZ twin pairs was compared to the frequency of familial twinning in women who had one spontaneous DZ twin pair. We also examined whether women with spontaneous DZ twins differed in the time it took to conceive the twin pregnancy from women with spontaneous MZ twins. To investigate if genotype interacts with age, we examined whether women who had their DZ twins at an older age (36 or older) less often reported relatives with twins than women who had their DZ twins when they were younger. Finally, we looked at the women who were twins themselves and at the concordance with their twin sister for being a twin mother.

RESULTS

Table IIA shows the number of sibs of the index mothers. There was no difference in the number of sibs reported by index mothers with spontaneous DZ twins and spontaneous MZ twins ($\chi^2 = 3.710$, df = 8, $P = 0.882$). This was also the case when examining the number of brothers ($\chi^2 = 9.326$, df = 8, $P = 0.316$) and sisters ($\chi^2 = 6.760$, df = 8, $P = 0.563$) separately. However, women with spontaneous DZ twins reported significantly more sibs than women with ART DZ twins ($\chi^2 = 96.628$, df = 8, $P < 0.001$). This was also the case when looking at the number of brothers ($\chi^2 = 59.978$, df = 8, $P < 0.001$) and sisters ($\chi^2 = 49.961$, df = 8, $P < 0.001$) separately. We found no difference in number of sibs between women with spontaneous MZ twins and ART MZ twins ($\chi^2 = 6.472$, df = 8, $P = 0.595$). In Table IIB the number of own children is given for the women with spontaneous MZ and DZ twins, ART DZ and ART MZ twins. Women with spontaneous DZ twins had three or more children more often than women with ART DZ twins ($\chi^2 = 865{,}741$, df = 4, $P < 0.001$). Also, women with spontaneous MZ twins had three or more children more often than women with ART MZ twins ($\chi^2 = 44.322$, df = 4, $P < 0.001$). Women with spontaneous DZ twins had more families of four or more children than women with spontaneous MZ twins ($\chi^2 = 19.306$, df = 4, $P = 0.001$).

We first compared the frequency of familial twinning (at least one female relative with twins, irrespective of zygosity) between women with spontaneous DZ and MZ twins. Women with spontaneous DZ twin offspring had a female relative with twins significantly more often than women with spontaneous MZ twin offspring ($\chi^2 = 37.122$, df = 1, $P < 0.001$). Of all women with MZ twins, 21.9% (1,204 of 5,505) had female relatives with twins, while of all women with DZ twins, 26.4% (2,174 of 8,222) had female relatives with twins.

Table III shows the information on the proportion of DZ versus MZ twin offspring in relatives of women with spontaneous DZ and MZ twins. Because of the small numbers reported, we excluded the categories "my daughter has twins" (N = 44, 36 with known zygosity) and 'my son has twins' (N = 20, 16 with known zygosity) from the analyses. With respect to familial twinning, the proportion of DZ versus MZ twin offspring in relatives was greater in women with spontaneous DZ offspring than in those with spontaneous MZ offspring ($\chi^2 = 53.409$, df = 1, $P < 0.001$). Of the twin offspring in relatives of women with DZ twins 81.9% were DZ (1,873 of

TABLE II. Number and Percentage of Own Siblings (Part A) and Own Children (Part B) for Women With Spontaneous DZ Twins, Spontaneous MZ Twins, ART DZ Twins and ART MZ Twins

	Proband with spontaneous DZ twin offspring		Proband with spontaneous MZ twin offspring		Proband with ART DZ twin offspring		Proband with ART MZ twin offspring	
	n	%	n	%	n	%	n	%
A. Number of siblings								
0	373	4.6	256	4.7	218	5.3	10	4.0
1	2,485	30.9	1,687	31.3	1,518	37.2	82	33.2
2	2,154	26.7	1,437	26.6	1,098	26.9	68	27.5
3	1,217	15.1	845	15.7	564	13.8	43	17.4
4	696	8.6	441	8.2	300	7.4	22	8.9
5	392	4.9	254	4.7	146	3.6	9	3.6
6	281	3.5	166	3.1	80	2.0	5	2.0
7	174	2.2	115	2.1	63	1.5	1	0.4
8 or more	282	3.5	195	3.6	89	2.2	7	2.8
Total	7,990	100	5,396	100	4,068	100	263	100
B. Number of own children								
2	2,271	28.9	1,577	30.1	2,108	54.4	117	49.6
3	3,553	45.3	2,472	47.1	1,419	36.6	91	38.6
4	1,451	18.5	879	16.8	274	7.1	18	7.6
5	395	5	200	3.8	54	1.4	7	3.0
6 or more	177	2.3	119	2.3	18	.5	3	1.3
Total	7,847	100	5,247	100	3,873	100	236	100

ART, assisted reproduction technologies.

TABLE III. Number and Percentage of Relatives With MZ, DZ or DZ Opposite-Sex Twin Offspring in Index Mothers With Spontaneous DZ and MZ Twin Offspring

	Index mothers with DZ twin offspring (n = 8,222)						Index mothers with MZ twin offspring (n = 5,505)					
	Relative with MZ offspring		Relative with DZ offspring		Relative with DOS offspring		Relative with MZ offspring		Relative with DZ offspring		Relative with DOS offspring	
	n	%	n	%	n	%	n	%	n	%	n	%
Sister	57	15.9	301	84.1**	157	73.4**	51	27.0	138	73.0	54	51.4
Brother	63	31.2	139	68.8*	72	53.7	31	21.5	113	78.5	44	58.7
Mother	87	17.1	421	82.9**	201	69.8**	90	32.3	189	67.7	104	53.6
Aunt (M)	99	17.5	468	82.5**	250	71.6**	73	25.5	213	74.5	107	59.4
Uncle (M)	90	25.7	260	74.3	155	63.3	66	24.8	200	75.2	109	62.5
Grandmother (M)	90	15.3	497	84.7**	271	75.1**	96	29.1	234	70.9	134	58.3
Aunt (P)	87	18.0	396	82.0**	212	70.9**	91	32.9	186	67.1	98	51.9
Uncle (P)	75	24.8	227	75.2	112	59.9	79	31.1	175	68.9	105	57.1
Grandmother (P)	88	15.0	499	85.0*	267	75.2**	79	25.1	236	74.9	135	63.1
At least 1 female relative with twins	415	18.1	1,873	81.9**	1,110	72.8**	367	28.7	912	71.3	543	59.3

M, maternal; P, paternal; DZ offspring includes opposite sex twins (DOS) offspring.
Percentages in the 2nd and 4th columns (relative with MZ offspring/relative with DZ offspring) sum to 100% across rows.
Percentages in column 6 (relatives with DOS offspring) are calculated as percentages of relatives with MZ + DOS offspring.
*$P < .05$, **$P < .01$; significance levels are given for the comparison of the proportion of relatives with DZ versus MZ offspring in index mothers with spontaneous DZ and MZ twin offspring.

2,288), while this percentage was 71.3% (912 of 1279) in the relatives of women with MZ twins. The same direction of effect was found for all individual female relatives, but not for male relatives. One exception was the proportion of DZ offspring in brothers. The brothers of women with spontaneous MZ twins had significantly more DZ offspring (113 of 144) than the brothers of women with spontaneous DZ twins (139 of 202).

Since index mothers may overreport the zygosity of their relatives' twin based on their own twin offspring' zygosity (i.e., mothers of DZ twins may be more likely to report twins of relatives as dizygotic), we repeated the analyses including DOS twin offspring instead of all DZ twin offspring. We found similar results to the comparison with all DZ twins. The proportion of DZ offspring in female relatives was higher in women with DZ twins (72.8%) than in the female relatives of women with MZ offspring (59.3%).

Next, we examined differences in familial twinning between index mothers with spontaneous DZ and ART twins. We first tested whether DZ mothers who conceived their twins after OI (N = 1,103) and DZ mothers who conceived their twins after IVF, ICSI or IUI (N = 3,061) differed in familial twinning (MZ or DZ) and found no differences ($\chi^2 = .308$, df = 1, $P = 0.579$). We therefore treated these groups of mothers as one group.

Table IV shows the frequency of familial twinning in women with spontaneous DZ twins compared to women with ART DZ and ART MZ twins. Women with spontaneous DZ twins had a female family member with twins (MZ or DZ) significantly more often than women with DZ twins conceived after fertility treatment ($\chi^2 = 55.777$, df = 1, $P < 0.001$). Of the spontaneous DZ group, 26.4% (2,174 of 8,222) had at least one female relative with twins, compared to 20.3% (847 of 4,164) of the ART DZ index mothers. Differences in familial twinning between women with spontaneous DZ offspring and women with ART DZ offspring were found for all female relatives, but not in the male relatives with the exception of the uncle on mothers' side.

With respect to DZ familial twinning, women with spontaneous DZ twins had a female relative with DZ twin offspring more often than women with ART DZ twins (81.9% vs. 73.3%, $\chi^2 = 28.607$ df = 2, $P < 0.001$). The pattern of results regarding the ratio of DZ-MZ twinning was also found for both maternal and paternal aunts ($\chi^2 = 14.266$, df = 1, $P < 0.001$ and $\chi^2 = 8.209$, df = 1, $P = 0.004$, respectively) and both maternal and paternal grandmothers ($\chi^2 = 6.634$, df = 1, $P = 0.01$ and $\chi^2 = 9.688$, df = 1, $P = 0.002$, respectively). The proportion of brothers with DZ twins did not differ significantly between women with spontaneous DZ twins (139 of 202) compared to women with ART DZ twins (74 of 95).

The last columns in Table IV present the data for familial twinning for index mothers with ART MZ offspring. We only analyzed the data for familial twinning, as the numbers of women with ART MZ offspring were too small to compare the ratio for individual relationships. Women with ART DZ offspring did not differ from women with ART MZ offspring with respect to the presence of relatives with a twin ($\chi^2 = 0.876$, df = 1, $P = 0.349$). In addition, the groups did not differ in the proportion of DZ twins; 73.3% of the ART DZ mothers reported DZ familial twinning, compared to 77% of the ART MZ mothers ($\chi^2 = 0.404$, df = 1, $P = 0.525$).

TABLE IV. Number and Percentage of Relatives With Twin Offspring and Relatives With MZ/DZ Twin Offspring in Index Mothers With Spontaneous DZ Twin Offspring and Probands With ART DZ or ART MZ Twins

	Index mothers with DZ twin offspring (n = 8,222)						Index mothers with ART-DZ twin offspring (n = 4,164)						Index mothers with ART-MZ twin offspring (n = 250)					
	Relative with twins		Relative with MZ offspring		Relative with DZ offspring		Relative with twins		Relative with MZ Offspring		Relative with DZ offspring		Relative with twins		Relative with MZ offspring		Relative with DZ offspring	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Sister	353	4.3**	57	15.9	301	84.1*	120	2.9	30	24.6	92	75.4	11	4.4	3	25	9	75
Brother	202	2.5	63	31.2	139	68.8	95	2.3	21	22.1	74	77.9	9	3.6	2	22.2	7	77.8
Mother	507	6.2**	87	17.1	421	82.9**	150	3.6	43	28.5	108	71.5	6	2.4	1	16.7	5	83.3
Aunt (m)	555	6.8**	99	17.5	468	82.5**	218	5.2	66	29.6	157	70.4	14	5.6	2	14.3	12	85.7
Uncle (m)	346	4.2*	90	25.7	260	74.3	137	3.3	43	30.9	96	69.1	12	4.8	4	30.7	9	69.3
Grandmother (m)	580	7.1**	90	15.3	497	84.7*	235	5.6	55	22.8	186	77.2	22	8.8	6	27.3	16	72.7
Aunt (f)	473	5.8**	87	18.0	396	82.0**	154	3.7	45	28.7	112	71.3	14	5.6	5	35.7	9	64.3
Uncle (f)	300	3.6	75	24.8	227	75.2*	137	3.3	50	36.0	89	64.0	13	5.2	4	30.8	9	69.2
Grandmother (f)	582	7.1**	88	15.0	499	85.0**	241	5.8	58	24.1	183	75.9	15	6.0	2	13.3	13	86.7
At least 1 female relative with twins	2,174	26.4**	415	18.1	1,873	81.9**	847	20.3	237	26.7	652	73.3	57	22.8	14	23	47	77

The total number of female relatives with twins also includes families in which both MZ and DZ offspring occur (114 probands with DZ offspring had relatives with both MZ and DZ offspring); DZ offspring includes DOS offspring; percentages in the 4th and 6th columns (relative with MZ offspring/relative with DZ offspring) sum to 100% across rows.
*Significant ($P < 0.05$).
**Significant ($P < 0.01$).

Table V shows the frequency of familial twinning in women with one pair of spontaneous DZ twins and women with multiple sets of spontaneous DZ twins (N = 61). Women with MZ and ART DZ offspring were not included (N = 20). For the comparison of these two groups, we only examined familial twinning (having a female relative with twins) because there were too few observations to study each familial relationship separately. We found that women with multiple sets of spontaneous DZ twins had a female relative with twins (MZ or DZ) significantly more often than women with one set of spontaneous DZ twins ($\chi^2 = 10.081$, df = 1, $P = 0.001$). However, women with multiple sets of spontaneous DZ twins did not more often have a relative with DZ twin offspring (Fisher Exact test, $P = 0.458$).

The data for time to conceive for index mothers with spontaneous DZ and MZ twins are shown in Table VI. Women with spontaneous DZ twin offspring became pregnant more quickly with their twins than women with spontaneous MZ twin offspring ($\chi^2 = 31.873$, df = 3, $P < 0.001$). We did not find a difference in the time it took to become pregnant between women with one spontaneous DZ twin pair and women with multiple sets of spontaneous DZ twin pairs ($\chi^2 = 2.311$, df = 3, $P = 0.510$).

When comparing index mothers who gave birth to their twin at a younger (N = 7,353) versus a later age (N = 815), we found no differences in female familial twinning ($\chi^2 = 0.814$, df = 1, $P = 0.367$). Of the women who were younger than 36 years at the time of the twin birth, 26.3% had a female relative who had given birth to twins. Of the women of 36 years or older at the time of twin birth, 27.7% had a female relative who had given birth to twins. If only females relatives with DZ offspring were considered these percentages were 23.5% for women <36 years and 24.8% for the older group ($\chi^2 = 0.419$, df = 1, $P = 0.654$).

There were 482 index mothers who reported to be a twin themselves. Of these, 112 women indicated they were MZ, 323 were DZ and 47 mothers did not know their zygosity. In the group of 112 index mothers who were MZ twins themselves, there were 7 women with a sister who was also the mother of twins. Of these women, four women represent two twin pairs; one pair was concordant for having MZ twins, one pair was concordant for having DZ twins. In one family, the sister with twins was not the cotwin and in two families it was not clear if it was the co-twin or another sister; two of these families were discordant for twin offspring zygosity.

In the group of 323 index mothers with a DZ twin sister there were 23 women who had a sister with twins. In five cases the co-twin was also a mother of twins; two of them were concordant having DZ offspring and three were discordant for twin offspring zygosity. In nine cases the sister with twins

TABLE V. The Proportion of Relatives With Twins, Relatives With DZ Twin Offspring and MZ Twin Offspring and Relatives With Only DOS Offspring in Women With One Set of Spontaneous DZ Twins and Women With Multiple Sets of Spontaneous DZ Wins

	Index mothers with one DZ twin (n = 8,012)								Index mothers with multiple sets of spontaneous DZ twins (n = 61)							
	Relative with twins		Relative with MZ offspring		Relative with DZ offspring		Relative with DOS offspring		Relative with twins		Relative with MZ offspring		Relative with DZ offspring		Relative with DOS offspring	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
All relatives	2,105	26.3	403	18.2	1,813	81.8	1,071	72.7	27	44.3	3	10.7	25	89.3	12	80.0

DZ offspring includes DOS offspring; percentages in the 4th and 6th columns (relative with MZ offspring/relative with DZ offspring) sum to 100% across rows.

was not the cotwin (six were concordant for DZ twinning, one for MZ twinning and two were discordant) and in the other cases it was not clear whether the sister with a twin was the co-twin.

DISCUSSION

Family and fertility related characteristics were compared in women with spontaneous DZ and MZ twins and in women with ART MZ and DZ twins. For spontaneous twinning, we found that women with DZ twin offspring reported female relatives with DZ twins significantly more often than women with MZ twin offspring. This association predominantly applied to the female relatives, but equally to the female relatives on the fathers and on the mothers' side of the family. Thus, these results are consistent with the current understanding that DZ twinning is a trait which is passed on from both father and mother, which can only be expressed in women and for which men can be carriers [Meulemans et al., 1996]. Women with spontaneous MZ and DZ twins did not differ in the number of brothers and sisters, so this result is not biased because of a larger number of sibs in DZ twin mothers.

It is likely that the larger presence of DZ familial twinning (i.e., relatives with DZ twins) reported by women with DZ twins indicates a genetic predisposition to have DZ twins. A possible mechanism may be a higher rate of ovulation [Martin et al., 1991; James, 2007]. Martin et al. [1991] found that mothers of DZ twins had increased rates of ovulation compared to mothers of MZ twins. We found that women with spontaneous DZ twins conceived more quickly than women with spontaneous MZ twins, supporting the notion that mothers of spontaneous DZ twins can be viewed as being more fertile than mothers of spontaneous MZ twins.

A special group consists of women who have had multiple sets of spontaneous DZ twins. These women may have a stronger genetic predisposition for DZ twinning than women with a single set of DZ twins. If this is the case, we expect to see a higher frequency of familial DZ twinning and a shorter time to conceive in the women with multiple spontaneous DZ twins. Women with multiple sets of DZ twins indeed had significantly more familial twinning (DZ or MZ) than women with one set of DZ twins. Probably due to the small sample size, there was no significant difference with regard to DZ twinning only. There was a trend, however, for women with multiple sets of DZ twins to have more relatives with DZ twins than women with one set of DZ twins. Analysis of the time it took to conceive showed no significant difference between women with one set and more than 1 set of DZ twins. Again, the reason for not finding such a difference might be that the sample of women with multiple sets of DZ twins was small. Alternatively, these women have had access to contraceptives, and it therefore may be difficult to pick up differences in genetic susceptibility as women (and their husbands) may have decided after

TABLE VI. Time to Conceive in Mothers of Spontaneous Twins for Women With DZ Twins, MZ Twins and for Women With One Set and Multiple Sets of DZ Twins

Time it took to conceive twins	All DZ twin[a] offspring		MZ twin offspring		One set of DZ twins		Mothers with multiple sets of DZ twins	
	n	%	n	%	n	%	n	%
0–2 months	3,705	50.2	2,234	45.2	3,616	50.2	30	54.5
3–5 months	1,883	24.8	1,298	26.3	1,790	24.9	12	21.8
6–12 months	1,060	14.4	817	16.5	1,034	14.4	10	18.2
>12 months	784	10.6	593	12.0	760	10.6	3	5.5
Total	7,432	100	4,942	100	7,200	100	55	100

[a]All DZ offspring includes mothers with one set of DZ twin offspring and mothers with multiple sets of DZ twin offspring.

having had one set of twins that they would not further increase their number of offspring. A true comparison of familial twinning in mothers of one and of more spontaneous DZ twin pairs may only be obtained through designs such as that of Lummaa et al. [2007] who had access to extensive family data through church records in a time that contraceptives were not available or, alternatively, in present times by examining pedigrees in communities in which the use of contraceptives is restricted.

One of the earliest reported factors influencing DZ twinning is maternal age, with a fourfold increased risk of having DZ twins after up to the age of 36 years [Bulmer, 1970]. The etiology of having twins at an older age might be different from that of younger mothers [Lambalk et al., 1998]. Our findings do not support this idea; women who had spontaneous DZ twins at a young age (<36 years) reported relatives with DZ twins as often as women who had their twins at a later age.

Little is known about family size and familial twinning in families of twins conceived after fertility treatment. Iatrogenic factors might interact with genetic factors causing DZ twinning or these factors might affect twinning independently. In our study we found a significant difference in the proportion of female relatives with DZ twins between women with spontaneously conceived DZ twins and women with ART DZ twins. Women with ART DZ twins reported female relatives with DZ twins less often than women with spontaneous DZ twins. This confirms that the mechanisms underlying spontaneous and non-spontaneous DZ twinning are different. Some studies hypothesize that embryo quality plays an important role in the maintenance of a multiple pregnancy after IVF [Zegers-Hochschild et al., 2004]. This might be an inherited maternal characteristic in addition to a double ovulation in women with spontaneous DZ twins. If good embryo quality is also inherited in mothers of artificially conceived DZ twins one would expect to see an increased number of relatives with DZ twins in these mothers, compared to women with ART MZ twins. However, we did not find any differences for DZ familial twinning in mothers of ART DZ and ART MZ twins. This suggests that spontaneous DZ twinning is largely a function of double ovulation. In women with ART DZ twins, embryo's are selected from a better cohort of embryo's which increases the change of double implantation and continuation of the multiple pregnancy [Tummers et al., 2003; Lambers et al., 2007] and this process does not seem to be related to a genetic predisposition to have DZ twins.

Women with ART DZ twins came from smaller families. This should be kept in mind when comparing this group with spontaneous twin mothers with regard to familial twinning. Alternatively, the finding that mothers who had infertility treatment came from smaller families than mothers who had their twins spontaneously, may imply that mothers of ART DZ twins come from families that are less fertile, though we do not know whether the reason for fertility treatment was because of fertility problems in the mother or the father of the twins. Still, there is a well documented positive association in family size between parents and children [Axinn et al., 1994; Pouta et al., 2005] and while it is very likely there is a significant genetic component to this association, it is difficult to estimate the true contribution of genes to this association due to the large contribution of social or cultural circumstances [Pluzhnikov et al., 2007]. The lower number of sibs in women with ART DZ twins compared to women with spontaneous DZ twins seems in line with a genetic contribution to reproduction, at least with regard to decreased fertility.

The present study demonstrated that, at least with respect to the genetic susceptibility of having DZ twins, it is necessary to treat spontaneous and ART twins as two separate groups. This not only refers to the study of the mechanisms involved in DZ twinning, but may also apply to the study of twins in general as ART DZ twins come from smaller families, with fewer brothers and sisters.

A limitation of this study is that index mothers from "twinning" pedigrees may be more likely to report on familial twinning than others. This bias is likely to be small because the item about familial twinning was one among many others in the survey. We also have a relatively high proportion of mothers of spontaneous MZ (N = 5,505) compared to mothers of spontaneous DZ twins (N = 8,222) while population frequencies are closer to 2/3 [Bulmer, 1970].

A possible bias may have occurred in the form of an interaction between the zygosity of the offspring of the index mother and the zygosity of the offspring of her relatives. It is possible that an MZ twin mother is more likely to judge the twins of her relatives as MZ, while a DZ mother is more likely to judge them as DZ, thereby increasing the difference in the prevalence of DZ and MZ familial twinning between the two groups. However, the difference in the DOS twinning rate, which can be determined with certainty, between relatives of women with spontaneous MZ and DZ twins was 3.7%, while it was 6.2% when examining DZ twinning rate. Even when correcting for this additional difference due to reporter bias in MZ twinning rates, familial twinning is still increased in mothers of DZ twins.

In conclusion, familial DZ twinning in index mothers with spontaneous DZ twin offspring is clearly demonstrated in this study. With respect to the genetic predisposition to have twins, the mechanisms underlying spontaneous and non-spontaneous DZ twin births are very different and these two groups should be treated differently in studies of the genetics of DZ twinning.

ACKNOWLEDGMENTS

This work was supported by the National Institute of Child Health and Human Development (Grant nr HD042157), Center for Neurogenomics and Cognition Research (CNCR) of the VU University Amsterdam, Twin-Family Database for Behavior Genetics and Genomics Studies (NWO-MagW 480-04-004) and Spinozapremie (NWO/SPI 56-464-14192).

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