**Supplementary Material**

**Supplementary Data 1: Phenotype description NTR**

In this study we analysed information from multiple NTR surveys. Information was obtained from a 2005 survey of the NTR aimed at all mothers of young and adult twins (N = 19,357), referred to as Twinning2005 (Hoekstra et al., 2008). Two other large sources of information were the first survey that mothers of young twins receive when joining the NTR, referred to as YNTR1 (Van Beijsterveldt et al., 2013) and the first survey adult twins received since 2015 upon registration, which we shall refer to as ANTR0 (Willemsen et al., 2013).

*Zygosity*

The zygosity of the twins was determined based on opposite-sex status, multiple survey questions or on DNA/blood group testing. The zygosity assessment of the NTR twins is described in more detail in Ligthart et al. (2019) showing 97.2% and 95.9% concordance in children and adults respectively between the assessment by survey and DNA-based methods (Ligthart et al., 2019). Data were excluded for mothers who gave birth to both MZ and DZ twins (N=64) or if the zygosity of the twins was unknown (N=378), this resulted in complete zygosity data for 33,648 participants.

*Maternal age*

Maternal age at the birth of the twins was calculated based on the dates of birth of mothers and twins and survey questions such as: “How old were you when you had your first child/children?”. A distribution of the year of birth of the twin mothers is shown is Figure 1. We were able to determine the maternal age for 33,625 participants.

*Age at first birth*

We determined the age at birth of the first offspring for 28,200 participants using, dates of birth of twins, mothers and siblings, or by the answers of questions such as: “How old were you when you had your first child/children”.

*Height*

Height was available for 32,341 mothers. Data were checked for consistency across longitudinal surveys and measured height from experimental studies in the NTR participants. More details can be found in (Ligthart et al., 2019)

*BMI before twin pregnancy*

Mothers were asked to indicate their weight before the twin pregnancy in the TWINNING2005 and YNTR1 surveys. BMI before pregnancy was calculated as weight(kg)/(height(m)2, using the height variable described above*.* We calculated the BMI before the pregnancy for 28,129 participants.

*Number of children before the twin pregnancy*

In the ANTR surveys, mothers were asked to provide a date of birth for all their children, including the twins. In YNTR1, mothers reported on the number of pregnancies before the twins (excluding miscarriages). In TWINNING2005, mothers were asked to report the total N pregnancies (including miscarriages), the N miscarriages, total N pregnancies before the first multiple pregnancy, and the number of singletons. Based on this information, the number of older siblings could be derived for come, but not all cases (Supplement data 1). The information from ANTR, YNTR1 and TWINNING2005 was checked for consistency and combined into a single variable. In total, we determined the number of older siblings for 22,010 participants.

*Primigravida*

Primigravida, has the mother been pregnant before the twin pregnancy (yes/no), information was available for 28,136 mothers. Information was checked on consistency and mothers with inconsistent information were excluded from the sample.

*Number of miscarriages*

Number of miscarriages was assessed by the questions: “How many miscarriages did you have?” and “How often did you have a miscarriage?”. We were able to establish the number of miscarriages for 22,678 participants.

*Smoking*

We created a dichotomous variable for smoking before and/or during the pregnancy (yes/no) based on the question: “Did you smoke before or during the twin pregnancy?”. We assume that mothers who smoked during the pregnancy were also smoking before their pregnancy. Smoking was defined as yes if the question was answered with “yes, before and during the pregnancy”, “yes, but only before the pregnancy” or “yes, but only during the pregnancy”. If the question was answered with ”no”, smoking was defined as no. In total we had information about smoking before the pregnancy for 30,836 mothers.

**Supplementary Data 2: Phenotype description MoBa**

MoBa is based on data from 111,668 pregnancies, of which 1,968 pregnancies where twin births to 1,965 mothers. One mother of twins was removed because her twins were born 10 weeks apart. Among the remaining 1,964 twin births, zygosity was unknown for 283 pairs, 1,277 pairs were classified as DZ, and 404 pairs were classified as MZ, resulting in a sample of 1,660 mothers of twins with known zygosity.

*Zygosity*

Zygosity was determined from either DNA analyses for pairs in which both twins were genotyped or maternal questionnaire, all sex-discordant pairs were classified as DZ regardless of genotype status. If inconstancy between maternal report and genotype, the genotype inferred zygosity was used. The remaining pairs had undetermined zygosity and were not included.

*Maternal age*

Maternal age was recorded at time of birth by the Medical Birth Registry of Norway (MBR), and was treated as a continuous variable, but censored at 15 and 45 years of age.

*Height*

Height was self-reported from the questionnaire completed at recruitment to the study during pregnancy.

*BMI*

Weight at the onset of the pregnancy was self-reported, and BMI was calculated from height and weight using the standard formula: weight(kg)/(height(m))2.

*Number of children before the twin pregnancy*

The MBRN registers if the mothers have given birth previously. This is treated as a factor, ranging from 0 to 4+. The vital status of the siblings is not provided, and it is assumed that all are alive when the twin birth is registered, and that they are singletons. Since previous pregnancies where not recorded by twin status, we do not know how many of the older siblings are twins.

*Primiparous*

The MBR does not record primigravida, however, it does record parity, this was used as a proxy variable, and all mothers recorded as primiparous were assumed to be primigravida.

*Smoking*

Smoking status during pregnancy was based on self-report from the first three questionnaires completed at weeks 15, 22, and 30 during pregnancy. Smoking was coded as: never smoker, previous smoker, stopped early in pregnancy, and smoked throughout pregnancy.

**Supplementary Table 1**

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| --- | --- | --- | --- |
| Group | N participants NTR | N total relatives NTR | N participants MoBa |
| Mother of naturally conceived DZ twins | 14,694 | 235 | 881 |
| Mother of MAR DZ twins | 8,255 | 8 | 396 |
| Mother of naturally conceived MZ twins | 10,699 | 45 | 383 |
| Total | 33,648 | 526 (288)\* | 1660 |

The number of mothers of twins in NTR and MoBa. Relatives can be sister-sister or mother-daughters pairs. MAR = medically assisted reproduction; DZ = dizygotic; MZ = monozygotic. Note: relatedness information is not readily available for the participants of MoBa

\*The number of complete related pairs/trios within the four groups (e.g. one pair counts as 2)