## Handedness and 23 early life characteristics in 37,495 Dutch twins

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## SUPPLEMENTARY INFORMATION

Supplementary Table 1. Overview of the association studies of handedness and early life characteristics ..... 2
Supplementary Table 2. Early life characteristics included in the analysis of association with handedness ..... 6
Supplementary Table 3. Cross-tabulation of handedness defined by drawing on paper and other items on handedness from the NTR survey at 5 years old ..... 8
Supplementary Table 4. Prevalence of right-handedness, left-handedness and mixed- handedness in twins by parental handedness ..... 9
Appendix 1. Neurodevelopmental delay ..... 9
Supplementary Figure 1. Frequencies in categorical early life characteristics in left- handed (LH), mixed-handed (MH) and right-handed (RH) children ..... 11
Supplementary Figure 2. Boxplots of continuous early life characteristics in left-handed (LH), mixed-handed ( MH ) and right-handed ( RH ) children ..... 12
Supplementary Figure 3. Correlations between 23 early life characteristics ..... 13
Appendix 2. Multiple testing correction ..... 14
Supplementary Table 5. Results of univariate regression analysis ..... 15
Supplementary Table 6. Prevalence of handedness in term and preterm twin births ..... 16
Supplementary Table 7. Prevalence of left-handedness, mixed-handedness and non- right-handedness in same-sex and opposite-sex twins ..... 16

Supplementary Table 1. Overview of the association studies of handedness and early life characteristics

| First author, year | Sample <br> size | Age of handedness measurement | Handedness measurement | Handedness phenotype | Characteristics associated with handedness | Characteristics not associated with handedness |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Mixed twin-singleton population studies |  |  |  |  |  |  |
| De Kovel, 2019 | 421,776 | 40-69 years | Self-report | RH/LH | Being multiple, year of birth, sex, season of birth, birthweight, being breastfed | Maternal smoking |
| Vuoksimaa, 2009 | 30,161 | 18-69 years | Self-report on childhood and | LH in RH/LH/MH | Being multiple, sex | Zygosity, birth order |
|  |  |  | current hand preference | MH in RH/LH/MH | Sex; higher MH in triplets vs singletons and twins | Zygosity, birth order |
|  |  |  |  | RH/NRH | Birthorder |  |
| Dragovic, 2013 | 1,031 | Mean 16.2 | Self-report | RH/NRH | Maternal smoking, low Apgar scores | Being multiple, birth order, parental age, mode of delivery |
| Singleton studies |  |  |  |  |  |  |
| Hujoel, 2019 | 62,129 | Children | Different measurements in metaanalysis | RH/NRH | Being breastfed | None |
| Searleman, 1989 | 46,699 | Children-adults (meta-analysis) | Different measurements in metaanalysis | RH/NRH | Mode of delivery, gestational age, sex, birthweight, fetal presentation, maternal smoking, birth order position in family | Mother's age at birth, being multiple |
| Zhu, 2009 | 35,206 | 7 years | Report on "Which hand does your child use most?" or "Is your child right-handed or left-handed?" (3 response categories: RH, LH, both) and Annett Hand Preference Questionnaire | RH/MH | Gestational age, parental handedness, mode of conception, maternal smoking, contraception during 1st trimester | None |
| Denny, 2012 | 21,847 | 7 years | Mother's survey | $\begin{aligned} & \text { RH/NRH (LH+ } \\ & \mathrm{MH}) \end{aligned}$ | Being breastfed |  |
| Van der Feen, 2020 | 20,539 | Mean 41.3 | Hand skill assessed by alternating key press task ("tapping task") | Continuous | Aggression score | None |


| First author, year | Sample size | Age of handedness measurement | Handedness measurement | Handedness phenotype | Characteristics associated with handedness | Characteristics not associated with handedness |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Johnston, 2013 | 12,686 | Mean 7.4 | Question on writing hand | RH/LH | Being breastfed, sex, parental age at birth, birthweight | Mode of delivery, gestational age, maternal smoking, socioeconomic status |
| Domellof, 2011 | 10,117 | 3-19 years | Different measurements in metaanalysis | RH/NRH | Gestational age, neurological and neuropsychological outcomes | None |
| BaileyMcKeever, 2004 | 2,151 | Undergraduate students | Writing hand | RH/LH | Mother's age at birth | List of 25 factors, including mode of delivery, gestational age, birthweight, fetal presentation |
| Van der Hoorn, 2010 | 2,096 | Mean 13.6 | Report on "What is the hand you are writing with?" (3 response categories: RH, LH, alternating) | RH/NRH | Thought problems, social problems, being withdrawn and depressed (psychotic items) | Obstetric factors (gestational age, birthweight, caesarean section, vacuum or forceps assisted birth), treated with oxygen or incubator as neonate, externalizing problems |
| Sutcliffe, 2005 | 1,525 | 5 years | McCarthy Scale of children's abilities (motor scale), parental report: child handedness for drawing and writing. | Continuous | None | Parental handedness, mode of conception |
| Fagard, 2021 | 1,129 | 5 years | Handedness index based on 8-item hand preference test (from LH to RH) and absolute handedness index (from non-lateralized to nonlateralized). Binomial variable based on handedness index: RH vs LH. | Continuous and RH/LH | Father's handedness, season of birth, gestational age, fetal presentation (breech), being breastfed | Sex, mother's handedness |
| Karev,2008 | 870 | Mean 16.6 | Drawing hand (Chapman and Chapman's inventory) | RH/MH/LH | None | Parental age at birth, season of birth |
| Obel, 2003 | 824 | 3 years | Mixed-handedness based on maternal report at 3 years old (use of hand in 5 activities) | RH/MH | Prenatal stress in 3d trimester | None |
| Logue, 2015 | 692 | 4-18 years (clinical setting, predominantly | Writing hand | RH/LH | Psychiatric diagnosis including oppositional defiant disorder | None |


| First author, year | Sample <br> size | Age of handedness measurement | Handedness measurement | Handedness phenotype | Characteristics associated with handedness | Characteristics not associated with handedness |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | AfricanAmerican ancestry) |  |  |  |  |
| Dinsdale, 2011 | 395 | Mean 19.2 | Edinburgh Inventory of Handedness | Continuous | Aggression score | Sex |
| van der Elst, 2021 | 294 | $5.67-15.08$ <br> years | Lateral preference (hand, foot, eye, ear preference) | $\mathrm{RH} / \mathrm{LH}(\mathrm{LH}+\mathrm{MH})$ | None | Sex, fetal presentation, mode of delivery |
| Marlow, 2007 | 241 | 6 years | Neuropsychological battery (fingertip tapping) | Continuous | Gestational age | None |
| Van Heerwaarde, 2020 | 179 | 5 years | Movement Assessment Battery for Children second edition, Dutch version (MABC-2-NL): "the hand used to write or draw with" at school age in preterm clinical group (<28 weeks GA) | RH/NRH (MH and LH combined) | Parental handedness, gestational age | Being multiple, sex, season of birth, birthweight, Apgar scores, parental education level |
| Gutteling, 2007 | 110 | 6 years | Hand preference based on 8 activities assessed by independent observers | RH/MH | Gestational age, mother's handedness, prenatal stress | None |
| Twin studies |  |  |  |  |  |  |
| Sicotte, 1999 | 19,938 | Children-adults (meta-analysis) | Different measurements in metaanalysis | RH/LH | Being multiple | Zygosity |
| van Beijsterveldt, 2016 | 1,8222 | 5 years | Parental report about which hand is used for drawing at the survey of age 5 | RH/LH/MH | None | Chorionicity |
| Medland, 2003 | 14,838 | Children-adults (meta-analysis) | Self-report on "Which hand would you use to write a letter?" and "Which hand would you use to throw a ball to hit a target" | RH/LH | None | Birth order, being multiple, zygosity |
| Vuoksimaa, 2010 | 4,736 | 14 years | Self-report | RH/LH/MH | Sex | Birth order |
| Ooki, 2006 | 4,164 | 1-15 years | Parental report on "Which hand would your twin children | RH/LH | None | Being multiple |


| First author, year | Sample <br> size | Age of handedness measurement | Handedness measurement | Handedness phenotype | Characteristics associated with handedness | Characteristics not associated with handedness |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  |  | predominantly use, if possible, to write a letter?" |  |  |  |
| Orlebeke, 1996 | 3,400 | Mean 17.8 | Report on "Do you consider yourself predominantly righthanded or predominantly lefthanded?" | RH/LH | Birth order, being multiple, zygosity | None |
| Heikkila_2015 | 2,252 | Mean 12 [1-36 years] | Parental report and self-report | RH/LH | Birthweight (in triplets) | None |
| Derom, 1996 | 1,616 | 6-28 years | Parental report | RH/LH | Being multiple | Sex, birth order, chorionicity, zygosity |
| Elkadi, 1999 | 1,476 | Mean 23.5 | Survey on hand use for 3 activities | Continuous | None | Birth order |
| James\&Orlebeke, 2002 | 606 | Mean 17.8 | Report on "Do you consider yourself predominantly righthanded or predominantly lefthanded?" | RH/LH | Birth order | None |

 groups: mixed twin-singleton population studies, singleton population studies, and twin studies.

|  | Characteristic | Type | Codification in our study | Expected association with handedness (non-right-handedness) | Reference* (First author, year) |
| :---: | :---: | :---: | :---: | :---: | :---: |
| General characteristics |  |  |  |  |  |
| 1 | Sex (being male) | Categorical | Female $=0$, Male $=1$ | Effect of being male | de Kovel, 2019 (RH/LH) |
| 2 | Year of birth | Continuous | Scaled | Strong effect | de Kovel, 2019 (RH/LH) |
| 3 | Mother's handedness | Categorical | LH: RH $=0, \mathrm{LH}=1$ <br> $\mathrm{MH}: \mathrm{RH}=0, \mathrm{MH}=1$ <br> NRH: RH $=0, \mathrm{NRH}=1$ | Strong effect | Johnston, 2010 (RH/LH), Zhu, 2010 (RH/MH) |
| 4 | Father's handedness | Categorical | LH: RH $=0, \mathrm{LH}=1$ <br> $\mathrm{MH}: \mathrm{RH}=0, \mathrm{MH}=1$ <br> NRH: RH = 0, NRH = 1 | Strong effect | Zhu, 2010 (RH/MH), Fagard, 2021 (Handedness score) |
| Prenatal characteristics |  |  |  |  |  |
| 5 | Mother's age at birth | Continuous | Scaled | Effect of older mother's age | Bailey\&McKeever, 2004 <br> (RH/LH); Johnston, 2010 <br> (RH/LH) |
| 6 | Father's age at birth | Continuous | Scaled | No effect | Karev, 2008 (RH/LH, RH/MH) |
| 7 | Mode of conception | Categorical | Spontaneous $=0$, Artificial (with hormones/ IVF/ICSI/IUI) $=1$ | Effect of being conceived with ART | Zhu, 2010 (RH/MH) |
| 8 | Prenatal maternal smoking | Categorical | No = 0, yes $=1$ | No effect | de Kovel, 2019 (RH/LH) |
| 9 | Maternal stress during pregnancy | Categorical | No =0, yes $=1$ | Effect | Searleman, 1989 (RH/NRH); Obel, 2003 (RH/MH); Gutteling, 2007 (RH+LH/MH) |
| Perinatal characteristics |  |  |  |  |  |
| 10 | Season of birth (being born in summer) | Categorical | Summer months = 1, other months $=0$ | Being born in the summer | de Kovel, 2019 (RH/LH) |
| 11 | Fetal presentation at birth | Categorical | Cephalic $=0$, non-cephalic (breech and horizontal) $=1$ | Effect of noncephalic presentation (breech presentation) | Sealerman, 1989 (RH/NRH); Fagard, 2021 (Handedness score) |
| 12 | Mode of delivery | Categorical | Vaginal spontaneous=0, intervention (caesarean section, vacuum/forceps extraction) $=1$ | Effect of instrumental delivery or no effect | Sealerman, 1989 (RH/NRH) No effect: Bailey\&McKeever 2004 (RH/LH); Van der Elst, 2011 RH/LH (LH+MH); Johnston, 2010 (RH/LH) |
| 13 | Gestational age | Continuous Categorical | Scaled <37 weeks, $\geq 37$ weeks | Effect of preterm birth or no effect | Zhu, 2010 (RH/MH); Domellof, 2011 (RH/LH, RH/NRH); van Heerwaarde, 2020 (RH/LH, RH/NRH) <br> No effect: Bailey\&McKeever, 2004 (RH/LH); Johnston, 2010 (RH/LH) |
| 14 | Birthweight | Continuous Categorical | $\begin{aligned} & \text { Scaled } \\ & <2500 \mathrm{~g},>2500 \mathrm{~g} \end{aligned}$ | Effect of low birthweight | de Kovell, 2019 (RH/LH) |
| 15 | Apgar score | Continuous Categorical | 1-10 points, scaled $<7$ points, $\geq 7$ points | Effect of low Apgar score or no effect | Dragovic, 2013 (RH/NRH). <br> No effect: van Heerwaarde I, <br> 20202020 (RH/LH, RH/NRH) |
| Postnatal characteristics |  |  |  |  |  |
| 16 | Breastfeeding | Categorical | $\begin{aligned} & \mathrm{No}=0, \\ & \text { yes }=1 \end{aligned}$ | Effect of being nonbreastfed | de Kovel, 2019 (RH/LH); Hujoel, 2019 (RH/NRH), Denny, |

2012 (RH/LH, RH/NRH), Johnston, 2010 (RH/LH)

| 17 | Neurodevelopme ntal delay at 5 years old | Categorical | $\begin{aligned} & \text { No delay = } 0, \\ & \text { delay }=1 \end{aligned}$ | More NRH in children with neurodevelopment al delay |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| 18 | Aggression score at 7 years old | Continuous <br> Categorical | Scaled $<5$ points, $\geq 5$ points | More NRH in children with higher aggression score | van der Feen, 2020; Dinsdale, 2011 <br> No effect: van der Hoorn, 2010 |
| Twin-specific characteristics |  |  |  |  |  |
| 19 | Birthorder | Categorical | $\begin{aligned} & 1^{\text {st }} \text { born }=0, \\ & 2^{\text {nd }} \text { born }=1 \end{aligned}$ | More NRH in $1^{\text {st }}$ born | Derom, 1996 (RH/LH); <br> Orlebeke, 1997 (RH/LH) |
| 20 | Zygosity | Categorical | $\begin{aligned} & \mathrm{DZ}=0, \\ & \mathrm{MZ}=1 \end{aligned}$ | More NRH in MZ compared to DZ | Orlebeke, 1997 (RH/LH) |
| 21 | Chorionicity | Categorical | $\begin{aligned} & D C=0 \\ & M C=1 \end{aligned}$ | No effect | Derom, 1996 (RH/LH); Carlier, 1996 (Handedness scores) |
| 22 | Amnionicity | Categorical | $\begin{aligned} & \mathrm{DA}=0, \\ & \mathrm{MA}=1 \end{aligned}$ | No effect or more NRH in MA compared to DA |  |
| 23 | Time interval between the birth of the $1^{\text {st }}$ and $2^{\text {nd }}$ twin | Continuous Categorical | Scaled $\leq 30$ minutes, >30 minutes | Effect of longer time interval between birth of twins |  |

RH, right-handed; LH, left-handed; MH, mixed-handed; NRH, non-right-handed; DZ, dizygotic; MZ, monozygotic; DC, dichorionic; MC, monochorionic; DA, diamniotic; MA, monoamniotic

* for characteristics not available in study of de Kovel et al, 2019, references on largest studies are reported.

Supplementary Table 3. Cross-tabulation of handedness defined by drawing on paper and other items on handedness from the NTR survey at 5 years old

|  |  | Which hand do the children usually use to: draw on paper? |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | left-handed | righthanded | no hand preference | "I don't know" | Total |
| Which hand do the children usually use to: drink from a cup? | left-handed | 4695 | 223 | 50 | 3 | 4971 |
|  |  | 85.90\% | 0.70\% | 10.20\% | 5.80\% | 13.40\% |
|  | right-handed | 374 | 29929 | 198 | 11 | 30512 |
|  |  | 6.80\% | 95.90\% | 40.40\% | 21.20\% | 82.00\% |
|  | no hand | 329 | 897 | 237 | 4 | 1467 |
|  | preference | 6.00\% | 2.90\% | 48.40\% | 7.70\% | 3.90\% |
|  | "I don't know" | 65 | 166 | 5 | 34 | 270 |
|  |  | 1.20\% | 0.50\% | 1.00\% | 65.40\% | 0.70\% |
|  | Total | 5463 | 31215 | 490 | 52 | 37220 |
| Which hand do the children usually use to: eat? | left-handed | 5035 | 223 | 56 | 5 | 5319 |
|  |  | 91.50\% | 0.70\% | 11.30\% | 9.60\% | 14.30\% |
|  | right-handed | 290 | 30364 | 208 | 8 | 30870 |
|  |  | 5.30\% | 97.10\% | 41.90\% | 15.40\% | 82.70\% |
|  | no hand | 155 | 628 | 232 | 0 | 1015 |
|  | preference | 2.80\% | 2.00\% | 46.80\% | 0.00\% | 2.70\% |
|  | "I don't know" | 21 | 43 | 0 | 39 | 103 |
|  |  | 0.40\% | 0.10\% | 0.00\% | 75.00\% | 0.30\% |
|  | Total | 5501 | 31258 | 496 | 52 | 37307 |
| Which hand do the children usually use to: throw a ball? | left-handed | 4592 | 230 | 49 | 2 | 4873 |
|  |  | 84.30\% | 0.70\% | 10.00\% | 3.80\% | 13.10\% |
|  | right-handed | 373 | 29466 | 151 | 4 | 29994 |
|  |  | 6.90\% | 94.70\% | 30.80\% | 7.70\% | 80.80\% |
|  | no hand | 328 | 984 | 277 | 6 | 1595 |
|  | preference | 6.00\% | 3.20\% | 56.50\% | 11.50\% | 4.30\% |
|  | "I don't know" | 151 | 451 | 13 | 40 | 655 |
|  |  | 2.80\% | 1.40\% | 2.70\% | 76.90\% | 1.80\% |
|  | Total | 5444 | 31131 | 490 | 52 | 37117 |
| Which hand do the children usually use to: pick up a coin? | left-handed | 4543 | 264 | 45 | 2 | 4854 |
|  |  | 83.70\% | 0.80\% | 9.10\% | 3.90\% | 13.10\% |
|  | right-handed | 312 | 28847 | 137 | 3 | 29299 |
|  |  | 5.80\% | 92.70\% | 27.70\% | 5.90\% | 79.00\% |
|  | no hand | 415 | 1408 | 289 | 3 | 2115 |
|  | preference | 7.60\% | 4.50\% | 58.40\% | 5.90\% | 5.70\% |
|  | "I don't know" | 156 | 591 | 24 | 43 | 814 |
|  |  | 2.90\% | 1.90\% | 4.80\% | 84.30\% | 2.20\% |
|  | Total | 5426 | 31110 | 495 | 51 | 37082 |
| Which hand do the children usually use to: comb hair? | left-handed | 4346 | 204 | 40 | 4 | 4594 |
|  |  | 79.90\% | 0.70\% | 8.10\% | 7.70\% | 12.40\% |
|  | right-handed | 319 | 27958 | 138 | 4 | 28419 |
|  |  | 5.90\% | 89.90\% | 28.00\% | 7.70\% | 76.60\% |
|  | no hand | 315 | 1102 | 233 | 1 | 1651 |
|  | preference | 5.80\% | 3.50\% | 47.40\% | 1.90\% | 4.50\% |
|  | "I don't know" | 459 | 1832 | 81 | 43 | 2415 |
|  |  | 8.40\% | 5.90\% | 16.50\% | 82.70\% | 6.50\% |
|  | Total | 5439 | 31096 | 492 | 52 | 37079 |
| Which hand do the children usually use to: thumb suction during sleep? | left-handed | 1389 | 2207 | 47 | 0 | 3643 |
|  |  | 26.50\% | 7.40\% | 10.10\% | 0.00\% | 10.30\% |
|  | right-handed | 449 | 8851 | 68 | 4 | 9372 |
|  |  | 8.60\% | 29.70\% | 14.60\% | 7.70\% | 26.40\% |
|  | no hand | 198 | 1219 | 61 | 4 | 1482 |
|  | preference | 3.80\% | 4.10\% | 13.10\% | 7.70\% | 4.20\% |
|  | "I don't know" | 3206 | 17478 | 290 | 44 | 21018 |
|  |  | 61.20\% | 58.70\% | 62.20\% | 84.60\% | 59.20\% |
|  | Total | 5242 | 29755 | 466 | 52 | 35515 |

Supplementary Table 4. Prevalence of right-handedness, left-handedness and mixed-handedness in twins by parental handedness

|  | RH both parents <br> $\left(R H \_R H\right)$ | NRH one parent <br> $\left(N R H \_R H\right)$ | $P$ NRH_RH vs | NRH both parents | $P$ NRH_NRH |
| :--- | :---: | :---: | :---: | :---: | :---: |
|  | RH_RH | $($ NRH_NRH $)$ | vs RH_RH |  |  |
| Left-handedness | $13.8 \%(n=3120)$ | $18 \%(n=1409)$ | $1.79 E-19$ | $24.04 \%(n=176)$ | $6.48 \mathrm{E}-15$ |
| Mixed-handedness | $1.2 \%(n=276)$ | $1.41 \%(n=110)$ | 0.226 | $1.1 \%(n=8)$ | 0.892 |
| Non-right-handedness | $15.0 \%(n=3396)$ | $19.41 \%(n=1519)$ | $7.85 \mathrm{E}-20$ | $25.14 \%(n=184)$ | $1.02 \mathrm{E}-13$ |

## Appendix 1. Neurodevelopmental delay

The variable "Neurodevelopmental delay" was created based on two variables - delay in bowel control toilet skill and delay in bladder control toilet skill at 5 years based on questions "How often do the children poop in their pants?" and "How often do the children pee in their pants during the day?". Answers for both questions were "Never", "<1 per month", "1 per month", " 1 per week", and "Every day". Coding of both variables was $1=$ delay (answer "Every day"), $0=$ no delay (other answers).

## Crosstab of bladder and bowel skill delay

|  | Bowel control toilet skill delay |  |  |  |
| :--- | :--- | :---: | :---: | :---: |
|  | No delay | Delay | NA |  |
| Bladder control toilet skill | No delay | 36073 | 145 | 47 |
| delay | Delay | 524 | 174 | 6 |
|  | NA | 132 | 5 | 389 |

Neurodevelopmental delay variable:
Coded as '0': bladder and/or bowel control toilet skill delay = "No delay" (blue in the table);
Coded as ' 1 ': bladder and/or bowel control toilet skill delay = "Delay" (red in the table)
$N_{\text {cases }}=37106$, of them $N_{\text {no delay }}=36252, N_{\text {delay }}=854$.
$N_{\text {missing }}=389$.




Supplementary Figure 1. Frequencies in categorical early life characteristics in right-handed (RH), mixedhanded (MH) and left-handed (LH) children


Father's age at birth


Apgar score at 1 min


Time interval between birth of twins


Aggression score at 7 years


Supplementary Figure 2. Boxplots of continuous early life characteristics in right-handed (RH), mixedhanded (MH) and left-handed (LH) children


## Supplementary Figure 3. Correlations between 23 early life characteristics

Pearson correlations between continuous variables, polychoric correlations between ordinal variables, and point biserial correlations between continuous and ordinal variables. Handedness-associated are the characteristics that were associated with handedness in one of three definitions in regression analysis at $p<0.0011$. Handedness-unassociated are the characteristics that were not associated with handedness in regression analysis. For coding of variables see Supplementary Table 1. $\mathrm{N}=37,495$.

## Appendix 2. Multiple testing correction

The correction suggested by Nyholt (2004) involves calculating the effective number of tests given the currelations among the predictors. The effective number of tests ( $M_{\text {eff }}$ ) taking in account the proportional reduction in the number of variables in a set that is the ratio of observed eigenvalue variance to its maximum is calculated as:

$$
M_{\text {eff }}=1+(M-1)\left(1-\left(\operatorname{Var}\left(\lambda_{\mathrm{obs}}\right) / M\right),\right.
$$

where $M$ is the total number of variables included in the correlation matrix, $\operatorname{Var}\left(\lambda_{\text {obs }}\right)$ is the observed eigenvalue variance derived from correlation matrix (Nyholt, 2004).

There were 23 early life characteristics (predictors) in the current study. Number of calculations 23*24/2-23=253 correlations (mean 0.14, sd 0.14, range [-0.62:0.98]). Distribution of correlation coefficients between 23 early life characteristics is presented on histogram:

$M_{\text {eff }}=1+(23-1)(1-(0.472507 / 23)$. The effective number of tests is 22.5 . Given two definitions of handedness (left-handedness and mixed-handedness), alpha-per-test equalled $\alpha=0.05 /\left(22.5^{*} 2\right)=0.001108744$.

Supplementary Table 5. Results of univariate regression analysis

|  | RH vs LH |  |  |  |  | RH vs MH |  |  |  |  | RH vs NRH |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | N | $\beta$ | SE | z | P | N | $\beta$ | SE | z | $P$ | N | $\beta$ | SE | z | $P$ |
| General characteristics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Sex (F/M) | 36,997 | 0.183 | 0.030 | 6.199 | 6.68E-10 | 31,923 | 0.811 | 0.100 | 8.113 | 1.39E-16 | 37,495 | 0.232 | 0.029 | 8.120 | $4.66 \mathrm{E}-16$ |
| Year of birth (scaled) | 36,997 | -0.010 | 0.015 | -0.668 | 0.526 | 31,923 | 0.028 | 0.044 | 0.638 | 0.621 | 37,495 | -0.007 | 0.015 | -0.485 | 0.628 |
| Mother's handedness* | 30,349 | 0.470 | 0.047 | 9.976 | 1.14E-23 | 26,179 | -0.109 | 0.197 | -0.556 | 0.034 | 31,869 | 0.387 | 0.041 | 9.335 | $6.74 \mathrm{E}-21$ |
| Father's handedness* | 30,088 | 0.236 | 0.047 | 5.036 | 3.05E-07 | 25,922 | 0.078 | 0.162 | 0.482 | 0.002 | 31,643 | 0.202 | 0.041 | 4.950 | 4.75E-07 |
| Prenatal characteristics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Mother's age at birth (scaled) | 35,885 | -0.019 | 0.015 | -1.269 | 0.199 | 30,955 | 0.001 | 0.054 | 0.016 | 0.912 | 36,364 | -0.018 | 0.015 | -1.240 | 0.204 |
| Father's age at birth (scaled) | 35,520 | -0.012 | 0.015 | -0.749 | 0.454 | 30,621 | 0.043 | 0.054 | 0.797 | 0.453 | 35,990 | -0.007 | 0.015 | -0.477 | 0.626 |
| Mode of conception (Natural/Assisted) | 34,058 | -0.056 | 0.036 | -1.558 | 0.133 | 29,380 | 0.027 | 0.111 | 0.243 | 0.764 | 34,526 | -0.049 | 0.034 | -1.424 | 0.173 |
| Maternal smoking (no/yes) | 35,878 | 0.081 | 0.039 | 2.076 | 0.039 | 30,945 | 0.280 | 0.121 | 2.302 | 0.015 | 36,360 | 0.099 | 0.038 | 2.607 | 0.009 |
| Prenatal stress (no/yes) | 7,117 | 0.107 | 0.071 | 1.505 | 0.135 | 6,134 | 0.103 | 0.226 | 0.456 | 0.642 | 7,202 | 0.105 | 0.069 | 1.522 | 0.126 |
| Perinatal characteristics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Gestational age (scaled) | 35,795 | -0.055 | 0.015 | -3.764 | 0.00016 | 30,883 | -0.175 | 0.046 | -3.833 | 4.92E-05 | 36,277 | -0.066 | 0.014 | -4.640 | $2.55 \mathrm{E}-06$ |
|  | 36,993 | 0.053 | 0.034 | 1.558 | 0.102 | 31,919 | 0.071 | 0.111 | 0.639 | 0.473 | 37,491 | 0.054 | 0.033 | 1.650 | 0.083 |
| Fetal presentation (cephalic/noncephalic) | 7,182 | -0.044 | 0.070 | -0.630 | 0.479 | 6,189 | 0.399 | 0.220 | 1.809 | 0.077 | 7,268 | -0.009 | 0.067 | -0.134 | 0.826 |
| Mode of delivery (vaginal/instrumental) | 31,373 | -0.026 | 0.033 | -0.791 | 0.426 | 27,090 | 0.018 | 0.102 | 0.180 | 0.799 | 31,818 | -0.021 | 0.032 | -0.656 | 0.510 |
| Birth weight (scaled) | 35,568 | -0.023 | 0.015 | -1.538 | 0.125 | 30,677 | -0.147 | 0.051 | -2.895 | 0.002 | 36,045 | -0.034 | 0.015 | -2.318 | 0.019 |
| Apgar score 1 min (scaled) | 5,217 | 0.016 | 0.023 | 0.684 | 0.506 | 4,515 | -0.133 | 0.059 | -2.247 | 0.025 | 5,281 | 0.001 | 0.022 | 0.054 | 0.971 |
| Postnatal characteristics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Breastfeeding (no/yes) | 31,624 | -0.107 | 0.033 | -3.289 | 0.00099 | 27,261 | -0.001 | 0.109 | -0.006 | 0.953 | 32,038 | -0.100 | 0.032 | -3.184 | 0.0014 |
| Neurodevelopmental delay (no/yes) | 36,615 | 0.019 | 0.098 | 0.197 | 0.844 | 31,599 | 0.920 | 0.218 | 4.225 | 2.38E-05 | 37,106 | 0.134 | 0.091 | 1.466 | 0.143 |
| Aggression score at 7 y (scaled) | 20,327 | 0.022 | 0.019 | 1.157 | 0.244 | 17,516 | 0.325 | 0.048 | 6.846 | $1.46 \mathrm{E}-12$ | 20,595 | 0.053 | 0.018 | 2.892 | 0.004 |
| Twin-specific characteristics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Birthorder (1st/2nd) | 36,968 | -0.059 | 0.028 | -2.077 | 0.042 | 31,896 | 0.164 | 0.084 | 1.948 | 0.077 | 37,465 | -0.041 | 0.027 | -1.503 | 0.138 |
| Zygosity (DZ/MZ) | 36,827 | -0.056 | 0.031 | -1.790 | 0.076 | 31,777 | 0.119 | 0.106 | 1.122 | 0.261 | 37,321 | -0.042 | 0.030 | -1.386 | 0.169 |
| Chorionicity (DC/MC) | 11,043 | 0.055 | 0.059 | 0.923 | 0.347 | 9,544 | -0.209 | 0.201 | -1.040 | 0.304 | 11,201 | 0.032 | 0.057 | 0.557 | 0.567 |
| Amnionicity (DA/MA) | 11,000 | 0.190 | 0.167 | 1.143 | 0.260 | 9,506 | -1.396 | 1.004 | -1.391 | 0.153 | 11,155 | 0.119 | 0.164 | 0.723 | 0.479 |
| Time between birth of $1^{\text {st }}$ and $2^{\text {nd }}$ twin (scaled) | 30,312 | 0.022 | 0.014 | 1.517 | 0.113 | 26,167 | -0.059 | 0.067 | -0.880 | 0.368 | 30,746 | 0.017 | 0.015 | 1.246 | 0.213 |

 monochorionic; DC, dichorionic; MA, monoamniotic; DA, diamniotic. ${ }^{*}$ Mother's and father's handedness are included in the same definition as the offspring handedness.

Supplementary Table 6. Prevalence of handedness in term and preterm twin births

|  | Full-term $>37$ weeks | Preterm $<37$ weeks | $P$ |
| :--- | :---: | :---: | :---: |
| Left-handedness | $14.39 \%(n=3102)$ | $15.58 \%(n=2292)$ | 0.00018 |
| Mixed-handedness | $1.21 \%(n=261)$ | $1.5 \%(n=221)$ | 0.019 |
| Non-righthandedness | $15.60 \%(n=3363)$ | $17.08 \%(n=2513)$ | 0.00017 |

$\mathrm{N}_{\text {full-term }}=21563$. $\mathrm{N}_{\text {preterm }}=14714$. $P, p$-value in 2-sample test for equality of proportions.

Supplementary Table 7. Prevalence of left-handedness, mixed-handedness and non-right-handedness in same-sex and opposite-sex twins

|  | Females from same- | Females from opposite- | PSSF vs | Males from same- | Males from opposite- | PSSM vs |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: |
|  | sex pairs (SSF) | sex pairs (OSF) | OSF | sex pairs (SSM) | sex pairs (OSM) | OSM |
| Left-handedness | $14.22 \%(\mathrm{n}=1818)$ | $12.91 \%(\mathrm{n}=776)$ | 0.017 | $15.71 \%(1970)$ | $16.38 \%(980)$ |  |
| Mixed-handedness | $0.8 \%(\mathrm{n}=102)$ | $0.93 \%(\mathrm{n}=56)$ | 0.393 | $1.72 \%(216)$ | $2.01 \%(120)$ |  |
| Non-right-handedness | $15.01 \%(\mathrm{n}=1920)$ | $13.85 \%(\mathrm{n}=823)$ | 0.037 | $17.43 \%(2186)$ | $18.38 \%(1100)$ |  |

$N_{\text {SSF }}=12788, N_{\text {OSF }}=6009, N_{\text {SSM }}=12540, N_{\text {OSM }}=5984$. $P, p$-value in 2-sample test for equality of proportions.

