



## Supplementary Information for

### **Triplets, birthweight, and handedness**

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Supplementary text  
Figs. S1 to S3  
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References for SI reference citations

### **Supplementary methods.**

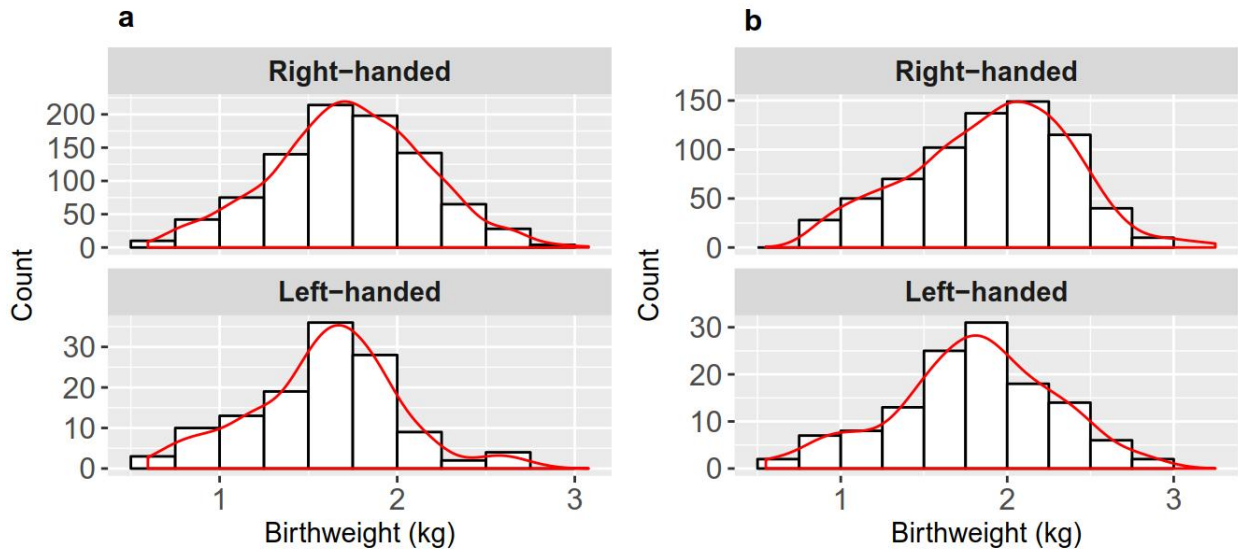
*Triplets from Japan.* For Japanese births, the triplets' birthweight, gestational age, sex, parity, within-triplet-birth-order and maternal age at delivery were obtained from records in the Maternal and Child Health Handbooks. This handbook was established by the Maternal and Child Health Law in Japan and is provided to the expecting mother by the authorities after a report of pregnancy. The purpose of this handbook is the maintenance of maternal and child health, and it includes information on health check-ups during pregnancy, the condition of the newborn, the progress of the infant, and periodic medical check-ups and vaccinations recorded by obstetricians or pediatricians. In addition, information on infertility treatment was obtained.

In Japan, the health check-up system after birth differs with life stage. Until six years of age, children participate in health check-ups administered by the Ministry of Health, Labor and Welfare based on age, which is counted as actual weeks, months, or years after birth. After six years of age, health check-ups are administered by the Ministry of Education, Culture, Sports, Science and Technology under the School Health Law. The data from these school-based health check-ups are routinely recorded in the school records and are made available to the family. Mothers have access to the school records from every grade and can check them directly. Mothers participating in this study were advised to refer to the named records when completing the questionnaire.

In case of several handedness classifications available, the most recent information was used. Handedness measurement age varied from 1 to 36 years with a mean of 12 years (90% were  $\geq 5$  years).

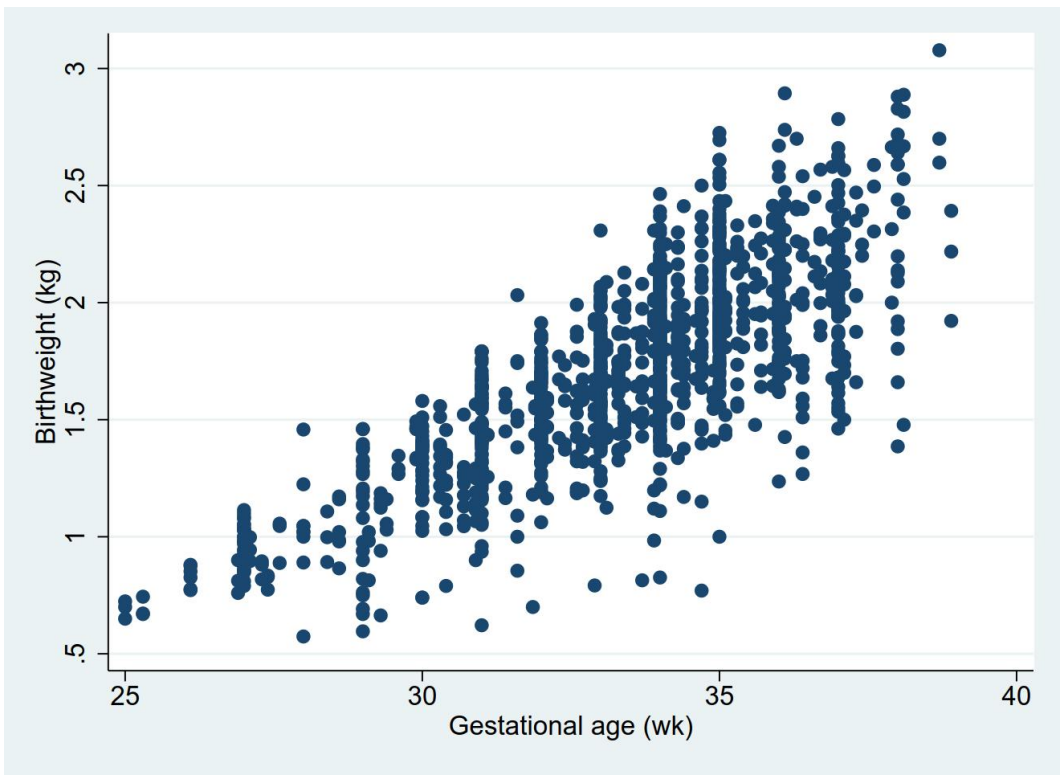
***Triplets from the Netherlands.*** The NTR sample included 947 triplet children from 332 births (96% were complete trios). The NTR sample consisted of triplets whose parents registered their offspring with the NTR (1-3), except for a few triplets born before 1986 who registered as young adults. Information on pregnancy, delivery, birthweight and handedness were obtained by various surveys. A first survey (Survey 1) was completed by the mother of the triplets shortly after registration and included items regarding maternal age and sex, birth order, gestational age and birthweight of the triplets. A second survey (Survey 2), including information on handedness of the triplets and their parents, was sent to the mothers of triplets around the triplets' second birthday. Regarding birthweight and gestational age, the NTR triplets were representative of the Dutch triplet population that is still alive after 28 days (2, 4). The triplets were born between 1970 and 2006, with the largest proportion born after 1986 (95.6%).

***Motor milestone measures.*** The counts of non-missing motor development items in the five sub-items varied from 434 to 544 in the Japanese sample and from 74 to 98 in the Dutch sample. The first un-rotated principal component of five motor development items accounted for 61% and 67% of the variance in Japanese (N=386) and Dutch (N=65) triplets, respectively.

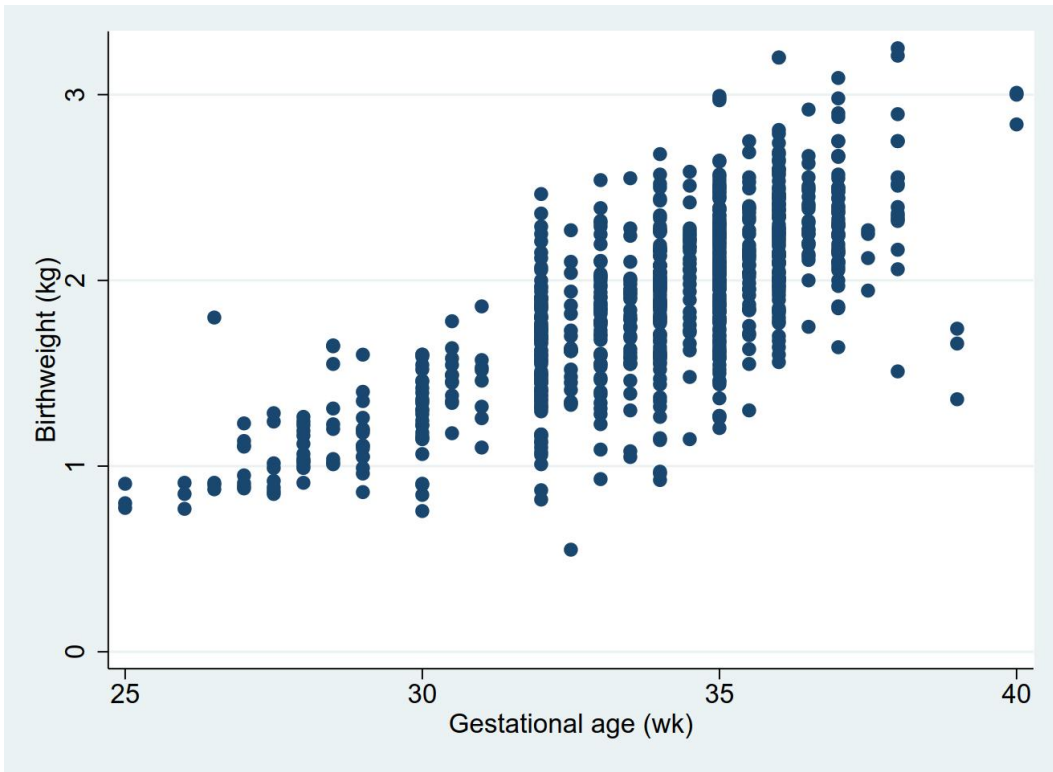


NOTE: Class of ambidextrous excluded

**Fig. S1.** Histogram of birthweight by handedness; in Japanese (a) and Dutch (b) triplets



**Fig. S2.** Scatter plot of birthweight by gestational age in Japanese triplets



**Fig. S3.** Scatter plot of birthweight by gestational age in Dutch triplets

**Table S1.** Handedness and mean age by country and reporter

	Handedness	Maternal report, N	%	Mean Age	Self-report, N	%	Mean Age
Japan	Right	924	70.8%	8.8	-	-	-
	Left	125	9.6%	8.6	-	-	-
	Ambidextrous	42	3.2%	7.5	-	-	-
	Missing	214	16.4%	-	-	-	-
Total		1305	100%	8.3	-	-	-
The Netherlands	Right	502	72.9%	11.5	217	84.1%	15.7
	Left	93	13.5%	12.1	39	15.1%	15.3
	Ambidextrous	33	4.8%	7.1	2	0.8%	15.5
	Do not know	28	4.1%	3.2	-	0%	-
	Missing	33	4.8%	-	-	0%	-
Total		689	100%	11.0	258	100%	15.6

**Table S2.** Logistic regression models reporting odds ratios (ORs) with 95% confidence intervals (CI) for left-handedness (left- versus right-handed) in the combined dataset including triplets from Japan and The Netherlands (N=1876)

Model	Variable	OR (95% CIs)	<i>P</i>
Model 1	Birthweight (kg)	0.60 (0.45 - 0.81)	<0.01
Model 2	First Born	(reference)	
	Second Born	0.87 (0.63 - 1.19)	0.39
	Third Born	0.98 (0.72 - 1.35)	0.92
Model 3	Sex (Boys)	(reference)	
	Sex (Girls)	0.91 (0.71 - 1.17)	0.47
Model 4	Birthweight (kg)	0.61 (0.40 - 0.93)	0.02
	Sex (Boys)	(reference)	
	Sex (Girls)	0.89 (0.31 - 2.50)	0.82
	Birthweight*Sex	0.98 (0.55 - 1.75)	0.94
Model 5	Birthweight (kg)	0.57 (0.42 - 0.79)	<0.01
	First Born	(reference)	
	Second Born	0.84 (0.60 - 1.18)	0.33
	Third Born	0.92 (0.66 - 1.28)	0.62
	Sex (Boys)	(reference)	
	Sex (Girls)	0.89 (0.68 - 1.17)	0.41
	Maternal age (years)	0.99 (0.95 - 1.04)	0.69
	Infertility Treatment	0.76 (0.55 - 1.06)	0.11



**Table S3.** Logistic regression models reporting odds ratios (OR) with 95% confidence intervals (CI) for left-handedness (left- versus right-handed) in Japanese (N=1005) and Dutch (N=739) triplets

Model	Variable	Japanese triplets		Dutch triplets	
		OR (95% CIs)	<i>P</i>	OR (95% CIs)	<i>P</i>
Gestational age model 1	Gestational age (wk)	0.93 (0.86 - 1.00)	0.06	0.91 (0.86 - 0.97)	0.01
Gestational age model 2	Gestational age (wk)	0.88 (0.80 - 0.98)	0.02	0.91 (0.82 - 1.01)	0.08
	Sex (Boys)	(reference)		(reference)	
	Sex (Girls)	0.05 (0.00 - 5.44)	0.21	0.97 (0.01 - 99.3)	0.99
	Gestational age*Sex	1.11 (0.96 - 1.28)	0.17	0.99 (0.86 - 1.13)	0.84
Zygoty model 1	Monozygotic	(reference)		(reference)	
	Dizygotic	0.95 (0.34 - 2.67)	0.92	1.09 (0.54 - 2.18)	0.82
	Trizygotic	1.02 (0.44 - 2.40)	0.96	0.84 (0.43 - 1.64)	0.62
Birthweight model 1	Birthweight (kg)	0.33 (0.15 - 0.70)	<0.01	0.64 (0.34 - 1.18)	0.15
	Gestational age (wk)	1.07 (0.94 - 1.20)	0.30	0.98 (0.89 - 1.09)	0.74
Birthweight model 2	Birthweight (kg)	0.36 (0.16 - 0.81)	0.01	0.55 (0.30 - 1.03)	0.06
	Gestational Age (wk)	1.06 (0.93 - 1.20)	0.39	0.99 (0.90 - 1.10)	0.88
	Sex (Boys)	(reference)		(reference)	
	Sex (Girls)	1.23 (0.83 - 1.84)	0.31	0.57 (0.38 - 0.86)	0.01
Birthweight model 3	Birthweight (kg)	0.55 (0.31 - 0.97)	0.04	0.59 (0.41 - 0.85)	0.01
	Monozygotic	(reference)		(reference)	
	Dizygotic	0.97 (0.34 - 2.80)	0.96	1.37 (0.62 - 3.01)	0.44
	Trizygotic	1.06 (0.44 - 2.55)	0.89	1.07 (0.50 - 2.28)	0.86

**Table S4.** Within-triplet analyses on left- versus right-handedness by birthweight and sex (N = 206)

Dataset	Variable	OR (95% CIs)	<i>P</i>
Japan (N = 102)	Birthweight (kg)	0.67 (0.22 - 1.99)	0.47
	Sex (Boys)	(reference)	
	Sex (Girls)	1.43 (0.79-2.58)	0.23
The Netherlands (N=104)	Birthweight (kg)	0.51 (0.21-1.23)	0.13
	Sex (Boys)	(reference)	
	Sex (Girls)	0.49 (0.31-0.79)	<0.01
Combined data (N=206)	Birthweight (kg)	0.63 (0.32-1.24)	0.18
	Sex (Boys)	(reference)	
	Sex (Girls)	0.82 (0.56-1.18)	0.28

Note. In this method, triplets with the same handedness value are excluded when discordant triplet-member-pairs are explained by the respective differences in covariates (sex and birthweight). Because of a maximum of two pairs per triplet, the standard errors were adjusted for non-independence. N = Number of discordant triplet pairs; OR = Odds Ratio; CI = 95% Confidence Interval

**Table S5.** Regression analysis of motor development milestone items by handedness and birthweight

Sample/Measure	Left-handers		Right-handers		Model 1		Model 2		Model 3			
	M (SD)	N	M (SD)	N	Handedness		Birthweight		Handedness		Birthweight	
					Beta	<i>P</i>	Beta	<i>P</i>	Beta	adjusted <i>P</i>	Beta	adjusted <i>P</i>
<b>Japan</b>												
Turning	6.59 (1.40)	63	6.13 (1.41)	411	0.45	0.04	-1.46	<0.001	0.14	0.398	-1.44	<0.001
Sitting	8.36 (1.67)	55	7.75 (1.33)	379	0.64	0.02	-1.58	<0.001	0.32	0.117	-1.54	<0.001
Crawling	9.09 (1.53)	53	8.69 (1.44)	413	0.39	0.12	-1.48	<0.001	0.11	0.569	-1.50	<0.001
Standing alone	10.39 (1.91)	59	9.99 (1.99)	421	0.39	0.16	-1.52	<0.001	0.10	0.716	-1.58	<0.001
Walking alone	14.21 (2.27)	66	13.46 (2.27)	478	0.77	0.01	-1.85	<0.001	0.45	0.129	-1.82	<0.001
<b>The Netherlands</b>												
Turning	7.45 (1.85)	10	6.80 (1.61)	75	0.70	0.29	-1.30	0.001	0.47	0.409	-1.44	<0.001
Sitting	8.95 (3.90)	10	9.72 (2.93)	64	-0.53	0.64	-1.37	0.079	-0.72	0.446	-1.38	0.138
Crawling	10.65 (3.04)	10	10.36 (2.65)	73	0.50	0.64	-0.72	0.226	0.38	0.697	-0.72	0.271
Standing alone	13.77 (2.97)	11	12.81 (3.05)	80	1.11	0.22	-1.95	0.035	0.74	0.342	-1.41	0.135
Walking alone	16.82 (2.50)	11	16.04 (2.80)	87	0.76	0.41	-1.89	0.004	0.31	0.691	-1.77	0.010

Each of the five motor development milestones were modeled separately. Higher values indicate more delayed motor development. All models included sex as a covariate. Models 1 and 2 had only one covariate of importance, handedness and birthweight, respectively. Model 3 included both covariates.

## References

1. Boomsma DI, *et al* (2002) Netherlands twin register: A focus on longitudinal research. *Twin Res* 5(5): 401-406.
2. van Beijsterveldt CE, *et al* (2013) The young netherlands twin register (YNTR): Longitudinal twin and family studies in over 70,000 children. *Twin Res Hum Genet* 16(1): 252-267.
3. Glasner TJ, van Beijsterveldt CE, Willemsen G & Boomsma DI (2013) Multiple births in the netherlands. *Ned Tijdschr Geneeskd* 157(30): A5962.
4. Lamb DJ, *et al* (2011) Birth weight in a large series of triplets. *BMC Pediatr* 11: 24.