**Table S1.** Review of studies on prenatal characteristics and Apgar scores in singletons

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| N | Authors | Sample (country) | Sample size n infants (n twin pairs) | Sample and Setting | Apgar score (min) | characteristics associated with low Apgar score (↓) | characteristics not associated with Apgar score |
| 1 | Camilleri, 1981 | Maltese Islands | 2791 | maltese women deliveries | not indicated | mother's short stature  |   |
| 2 | Marbury et al, 1984 | USA | 11173 | working and not-working during pregnancy women | 1, 5 min |  | working during pregnancy |
| 3 | Ladehoff et al, 1986 | Denmark | 641 | small birth weight cohort (2500g) | 5 min | low birthweight | mode of delivery, presentation at birth |
| 4 | Hemminki et al, 1990 | Finland | 2912 | population-based | 1 min | lower social class (occupation and years of education)  |  |
| 5 | Krebs et al, 1999 | Denmark | 19476 | case-control, infants delivered in breech presentation | 5 min | planned vaginal delivery in breech presentation  | maternal age, parity |
| 6 | Stevenson et al, 2000 | USA | 6738 | very low birth weight infants | 1 min, 5 min | sex (males ↓ vs females) |  |
| 7 | Kallen, 2001 | Sweden | 1,413,811 | population based cohort | 5 min | maternal smoking |  |
| 8 | Krebs et al., 2001 | Denmark | 344 | neurological disorders, case-control | 5 min | breech infants with vaginal delivery  |  |
| 9 | Milsom et al, 2002 | Sweden | 42,203 | term neonates with group of birth asphyxia (Apgare <7) | 5 min | single civil status, intrauterine meconium release, operative delivery, breech delivery, oxytocin augmentation, cord complication, external compression to assist delivery, low cardiotocography score | maternal age, smoking and illnesses, time of delivery, previous caesarean section |
| 10 | Rode et al, 2005 | Denmark | 8092 | cohort with different mother's BMI | 5 min | delivery via vacuum extraction or emergency cesarean delivery  | mother's obesity, parity |
| 11 | Kiran et al, 2005 | Wales | 60,167 | population-based, primigravid, uncomplicated, term delivery | 5 min |  | mother's obesity |
| 12 | Kalland et al, 2006 | Finland | 1668 | children from foster families | 1 min | adverse social circumstances  |  |
| 13 | Chen et al., 2007 | USA | 3.9 mln | nulliparous pregnancies | 5 min | mother's age at birth younger 17  |  |
| 14 | Gilman S.E. et al., 2008 | Collaborative Perinatal Project, USA | 52919 | normal | 5 min |  | maternal smoking |
| 15 | Jahromi et al, 2008 | Taiwan | 400 | case control, deliveries mothers >40 years | 5 min | mother's age at birth > 40  |  |
| 16 | Odd et al, 2008 | Sweden | 183637 | population based cohort | 1 min, 5 min | maternal occupation: manual worker mothers, low maternal level of education with instrumental mode of delivery  | paternal socioeconomic factors |
| 17 | Chen et al, 2010 | USA | 58,089 | singleton infants delivered from white non-Hispanic women with different BMI | 5 min | maternal weight status: obesity, early gestational age, preeclampsia  | gestational diabetes mellitus |
| 18 | Straube S. et al. 2009 | Germany | 465,964 | population-based cohort | 5 min | biological maternal factors: age (older mothers), higher BMI, higher parity  | socio-economic factors (maternal occupation, working during pregnancy cigarette smoking), mothers age below 20 |
| 19 | Grunebaum et al, 2013 | USA | 13,9 mln | term singleton home and hospital deliveries  | 5 min | home delivery, births in free-standing birth centers  |  |
| 20 | Ramoglu et al, 2014 | Turkey | 125 | preterm infants ART and spontaneous | 1 min, 5 min | Apgar score 5 min: mode of conception (spontaneous pregnancy group ↓) | Apgar score 1 min: mode of conception  |
| 21 | Iliodromiti et al., 2014 | Scotland, UK | 1 mln | population-based cohort | 5 min | early gestational age  | birthweight |
| 22 | Van der Ven et al, 2014 | Netherlands | 52,397 | preterm births | 5 min | preterm home delivery  |  |
| 23 | Svenik et al, 2015 | Sweden | 21,126 | population-based cohort, one twin from multiple pregnancies | 5 min | preterm birth, post-term birth, multiple pregnancy, previous cesarean section, non normal CTG (cardiotocography) at admission, maternal height <158 cm, BMI >30 kg/m2, nulliparity, preeclampsia |  |
| 24 | Khandwala et al, 2018 | USA | 40,529,905 | population-based cohort | 5 min | higher paternal age >55  |   |

studies are indicated with \* in the reference list

**Table S2.**Multi-group model fitting results

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Model | N parameters | df | χ2 | Compare model | Δ df | χ2 | p | RMSEA |
| **1** | **Baseline model. SAT (different thresholds of Apgar scores and means of covariates for 1st and 2nd born, sex and zygosity groups)**  | 198 | 264 | 438.6 | - | - | - | - | 0.026 |
| 2 | SAT (equal thresholds of Apgar scores in males and females monozygotic twins) | 190 | 272 | 449.1 | Model 1 | 8 | 10 | 0.255 | 0.027 |
| 3 | SAT (equal thresholds of Apgar scores in males and females in monozygotic and dizygotic twins) | 182 | 280 | 461.4 | Model 1 | 16 | 23.87 | 0.092 | 0.027 |
| 4 | SAT (equal thresholds of Apgar scores for monozygotic and dizygotic)  | 174 | 288 | 480.7 | Model 3 | 8 | 23.38 | **0.003** | 0.028 |
| **5** | **Model of choice. SAT (with zygosity differences in thresholds of Apgar scores and without sex differences in regression coefficients of covariates)** | 166 | 296 | 462.2 | Model 3 | 16 | 8.882 | 0.918 | 0.026 |
| 6 | ACE (with sex differences) | 144 | 318 | 500.9 | Model 5 | 22 | 45.337 | **0.002** | 0.026 |
| 7 | CE (different covariates for sex groups) | 138 | 324 | 509.9 | Model 6 | 6 | 10.307 | 0.112 | 0.026 |
| **8** | **Model of choice. CE (without sex differences in C and E components)** | 134 | 328 | 513.2 | Model 7 | 4 | 3.958 | 0.412 | 0.026 |

df – degree of freedom

χ2 – chi squared

Δ df – difference in degree of freedom

RMSEA – root mean square error of approximation

SAT – saturated model

ACE – additive genetic, common environmental, and unique environmental model

CE – common environmental and unique environmental model

**Table S3.** Distribution of MZ and DZ twin pairs in three categories of 1- and 5-minute Apgar scores in MZ and DZ twins (%)

|  |  |  |
| --- | --- | --- |
|   | 1-minute Apgar 2nd born | 5-minute Apgar 2nd born |
| 0-6 |  7-9 | 10 | 0-6 |  7-9 | 10 |
| **Monozygotic pairs**  |  |  |  |  |  |  |
| 1-min Apgar 1st born |  0-6 | 3.8% | 4.7% | 0.2% | 1.5% | 5.3% | 2.2% |
|  7-9 | 10.6% | 65.6% | 3.4% | 2.7% | 33.7% | 44.6% |
| 10 | 0.5% | 4.0% | 7.1% | 0 | 1.3% | 8.6% |
| Total | 1694 pairs (100%) | 1612 pairs (100%) |
| 5-min Apgar 1st born |  0-6 | 1.8% | 1.9% | 0.1% | 1.2% | 1.8% | 0.7% |
|  7-9 | 7.2% | 23.8% | 1.1% | 1.9% | 23.1% | 6.9% |
| 10 | 6.3% | 49.8% | 8.0% | 1.0% | 15.2% | 48.0% |
| Total  | 1607 pairs (100%) | 1627 (100%) |
| **Dizygotic pairs**  |  |  |  |  |  |  |
| 1-min Apgar 1st born |  0-6 | 2.9% | 3.5% | 0.3% | 0.9% | 4.2% | 1.9% |
|  7-9 | 12.2% | 62.2% | 3.4% | 1.9% | 34.4% | 43.4% |
| 10 | 1.2% | 5.3% | 9.1% | 0.1% | 1.9% | 11.2% |
| Total  | 3253 pairs (100%) | 3058 pairs (100%) |
| 5-min Apgar 1st born |  0-6 | 0.9% | 1.4% | 0 | 0.5% | 1.2% | 0.6% |
|  7-9 | 6.8% | 20.3% | 0.9% | 1.2% | 20.6% | 6.2% |
| 10 | 9.1% | 50.7% | 10.1% | 1.3% | 18.6% | 49.8% |
| Total | 3052 pairs (100%) | 3097 pairs (100%) |
|  |  |  |

**Table S4.** Comparison of 1- and 5-minute Apgar score means between first- and second-born monozygotic and dizygotic twins (continuous variables) and between monozygotic and dizygotic first and second born (continuous and ordinal variables)

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|   |  | 1st born | 2nd born | Sig\* | t | df | 95% CI of the difference |
|   | N pairs | Mean | SD | SE | Mean | SD | SE |  |  |  | lower | Upper |
| **Continuous variables** |  |  |  |  |  |  |  |  |  |  |
| MZ |   |   |   |   |   |   |   |   |   |   |   |   |
| 1-min Apgar  | 1694 | 8,45 | 1,546 | 0,038 | 8,1 | 1,853 | 0,045 | 0,000000 | 7,513 | 1693 | 0,258 | 0,441 |
| 5-min Apgar  | 1627 | 9,36 | 1,192 | 0,03 | 9,22 | 1,238 | 0,031 | 0,000006 | 4,551 | 1626 | 0,083 | 0,209 |
| DZ |  |  |  |  |  |  |  |  |  |  |  |  |
| 1-min Apgar  | 3253 | 8,61 | 1,416 | 0,025 | 8,03 | 1,895 | 0,033 | 0,000000 | 16,714 | 3252 | 0,511 | 0,647 |
| 5-min Apgar  | 3097 | 9,51 | 0,991 | 0,018 | 9,26 | 1,128 | 0,02 | 0,000000 | 12,022 | 3096 | 0,215 | 0,299 |

*\*paired t-test*

|  |  |  |  |
| --- | --- | --- | --- |
|   | MZ | DZ |   |
|   | N | Mean | SD | N | Mean | SD | Sig\* |
| **Continuous variables** |  |  |  |  |  |
| 1st born |  |  |  |  |  |  |  |
| 1-min Apgar  | 1726 | 8,43 | 1,574 | 3324 | 8,6 | 1,436 | 0,000109 |
| 5-min Apgar  | 1652 | 9,36 | 1,194 | 3174 | 9,51 | 0,998 | 0,000006 |
| 2nd born |  |  |  |  |  |  |  |
| 1-min Apgar | 1712 | 8,08 | 1,875 | 3287 | 8,01 | 1,913 | 0,211302 |
| 5-min Apgar | 1650 | 9,21 | 1,248 | 3151 | 9,24 | 1,148 | 0,335751 |
| **Ordinal variables** |  |  |  |  |  |  |  |
| 1st born |  |  |  |  |  |  |  |
| 1-min Apgar  | 1726 | 2,0255 | 0,45229 | 3324 | 2,0872 | 0,46506 | 0,000006 |
| 5-min Apgar  | 1652 | 2,6035 | 0,56191 | 3174 | 2,6717 | 0,51633 | 0,000025 |
| 2nd born |  |  |  |  |  |  |  |
| 1-min Apgar  | 1712 | 1,9556 | 0,50747 | 3287 | 1,9605 | 0,53962 | 0,758669 |
| 5-min Apgar  | 1650 | 2,5109 | 0,58083 | 3151 | 2,5332 | 0,55728 | 0,195344 |

*\* ANOVA*

**Table S6.** Effects of prenatal characteristics on ordinal 1- and 5-minute Apgar score in first and second-born twin

|  |  |  |
| --- | --- | --- |
|   | 1-minute Apgar  | 5-minute Apgar |
|   | B | SE | 95% Wald Confidence Interval | df | Sig. | B | SE | 95% Wald Confidence Interval | df | Sig. |
|   | Lower | Upper | Lower | Upper |
| **1st born**  | n=4554 | n=4369 |
| **Gestational age (Z-scores)** | .355 | .063 | .231 | .479 | 1 | **.000** | .526 | .059 | .410 | .641 | 1 | **.000** |
| **Birthweight (z-scores)** | .211 | .062 | .088 | .333 | 1 | **.001** | .208 | .059 | .091 | .324 | 1 | **.000** |
| **Mode of delivery** *(vaginal, interventions)* | -.508 | .085 | -.674 | -.342 | 1 | **.000** | -.781 | .076 | -.931 | -.631 | 1 | **.000** |
| **Fetal presentation at birth** *(cephalic, non-cephalic)* | .229 | .101 | .030 | .428 | 1 | **.024** | .320 | .090 | .143 | .497 | 1 | **.000** |
| Intertwin delivery time (Z-scores) | -.054 | .036 | -.125 | .016 | 1 | .132 | -.057 | .032 | -.120 | .006 | 1 | .076 |
| **Sex (M, F)** | .176 | .074 | .031 | .320 | 1 | **.017** | .120 | .069 | -.014 | .255 | 1 | .080 |
| **Zygosity (MZ, DZ)** | .188 | .079 | .033 | .343 | 1 | **.017** | .038 | .072 | -.103 | .179 | 1 | .597 |
| Mother's age at birth (Z-scores) | .069 | .050 | -.029 | .167 | 1 | .169 | -.003 | .046 | -.094 | .087 | 1 | .945 |
| Father's age at birth (Z-scores) | -.087 | .049 | -.183 | .008 | 1 | .073 | .026 | .045 | -.062 | .115 | 1 | .560 |
| Mother's BMI at birth (Z-scores) | -.051 | .038 | -.126 | .025 | 1 | .189 | -.067 | .035 | -.135 | .001 | 1 | .055 |
| **2nd born** | n=4428 | n=4267 |
| **Gestational age (Z-scores)** | .388 | .055 | .280 | .496 | 1 | **.000** | .480 | .053 | .376 | .584 | 1 | **.000** |
| **Birthweight (z-scores)** | .148 | .054 | .041 | .254 | 1 | **.007** | .184 | .052 | .083 | .285 | 1 | **.000** |
| **Mode of delivery** *(vaginal, interventions)* | .026 | .069 | -.108 | .161 | 1 | .701 | -.153 | .065 | -.280 | -.026 | 1 | **.018** |
| **Fetal presentation at birth** *(cephalic, non-cephalic)* | -.495 | .069 | -.631 | -.360 | 1 | **.000** | -.362 | .064 | -.487 | -.236 | 1 | **.000** |
| **Intertwin delivery time (Z-scores)** | -.140 | .043 | -.224 | -.055 | 1 | **.001** | -.149 | .034 | -.216 | -.083 | 1 | **.000** |
| Sex (M, F) | .067 | .068 | -.066 | .200 | 1 | .321 | .053 | .064 | -.072 | .178 | 1 | .406 |
| Zygosity (MZ, DZ) | -.117 | .072 | -.258 | .024 | 1 | .104 | -.101 | .068 | -.234 | .032 | 1 | .137 |
| Mother's age at birth (Z-scores) | -.023 | .046 | -.113 | .067 | 1 | .617 | .032 | .043 | -.052 | .117 | 1 | .455 |
| Father's age at birth (Z-scores) | .014 | .044 | -.073 | .101 | 1 | .752 | .078 | .042 | -.005 | .161 | 1 | .065 |
| Mother's BMI at birth (Z-scores) | -.014 | .035 | -.084 | .055 | 1 | .681 | -.030 | .033 | -.095 | .035 | 1 | .368 |

SD=standard deviation, SE=standard error, Sig=significance, df=degree of freedom

M=male, F=female

**Table S7.** Effect size on ordinal 1- and 5-minute Apgar score of prenatal characteristics and chorionicity in first and second-born twin in subset of MZ twins

|  |  |  |
| --- | --- | --- |
|   | 1-minute Apgar  | 5-minute Apgar |
|   | B | SE | 95% Wald Confidence Interval | df | Sig. | B | SE | 95% Wald Confidence Interval | df | Sig. |
|   | Lower | Upper | Lower | Upper |
| 1st born |  |  | n=497 |  |  |  |  |  | n=481 |  |  |  |
| Gestational age (Z-scores) | .150 | .1930 | -.228 | .528 | 1 | .437 | .332 | .1533 | .032 | .633 | 1 | .030 |
| Birthweight (z-scores) | .174 | .1959 | -.210 | .558 | 1 | .374 | .318 | .1732 | -.021 | .657 | 1 | .066 |
| **Delivery (vaginal, instrumental)** | -.866 | .2605 | -1.377 | -.356 | 1 | **.001** | -.617 | .2274 | -1.063 | -.171 | 1 | **.007** |
| Fetal presentation at birth *(cephalic, non-cephalic)* | -.173 | .4033 | -.964 | .617 | 1 | .668 | -.154 | .2209 | -.587 | .279 | 1 | .486 |
| Sex (M, F) | -.075 | .2337 | -.533 | .383 | 1 | .748 | -.199 | .2097 | -.610 | .212 | 1 | .343 |
| Chorionicity (MC, DC) | -.182 | .2414 | -.655 | .291 | 1 | .450 | -.081 | .2385 | -.548 | .386 | 1 | .734 |
| 2nd born |  |  | n=488 |  |  |  |  |  | n=472 |  |  |  |
| Gestational age (Z-scores) | .425 | .1635 | .104 | .745 | 1 | .009 | .297 | .1557 | -.008 | .602 | 1 | .056 |
| Birthweight (z-scores) | .070 | .1778 | -.279 | .418 | 1 | .695 | .442 | .1716 | .106 | .778 | 1 | .010 |
| **Delivery (vaginal, instrumental)** | -1.041 | .2041 | -1.441 | -.641 | 1 | **.000** | -.479 | .1968 | -.864 | -.093 | 1 | **.015** |
| Fetal presentation at birth *(cephalic, non-cephalic)* | .109 | .2948 | -.469 | .687 | 1 | .712 | -.431 | .1986 | -.820 | -.041 | 1 | .030 |
| Sex (M, F) | .016 | .1981 | -.373 | .404 | 1 | .937 | -.130 | .1898 | -.502 | .242 | 1 | .492 |
| Chorionicity (MC, DC) | .250 | .2226 | -.186 | .686 | 1 | .262 | .043 | .2132 | -.375 | .461 | 1 | .839 |

SD=standard deviation, SE=standard error, Sig=significance, df=degree of freedom

M=male, F=female, MC=monochorionic, DC=dichorionic

**Table S8.** Polychoric correlation matrices of ordinal 1- and 5-minute Apgar scores in six zygosity-sex groups corrected for gestational age, birthweight, mode of delivery, and fetal presentation

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 1-min Apgar twin 1 | 5-min Apgar twin 1 | 1-min Apgar twin 2 | 5-min Apgar twin 2 |
| MZM |  |  |  |  |
| 1-min Apgar twin 1 | 1 |  |  |  |
| 5-min Apgar twin 1 | 0.651 (0.604 / 0.698) | 1 |  |  |
| 1-min Apgar twin 2 | *0.552 (0.508 / 0.595)* | 0.297 (0.223 / 0.371) | 1 |  |
| 5-min Apgar twin 2 | 0.394 (0.318 / 0.470) | *0.540 (0.496 / 0.584)* | 0.647 (0.609 / 0.684) | 1 |
| DZM |  |  |  |  |
| 1-min Apgar twin 1 | 1 |  |  |  |
| 5-min Apgar twin 1 | 0.785 (0.746/0.823) | 1 |  |  |
| 1-min Apgar twin 2 | *0.485 (0.437/0.533)*  | 0.310 (0.219 / 0.400) | 1 |  |
| 5-min Apgar twin 2 | 0.394 (0.323/0.466) | *0.518 (0.466 /0.569)*  | 0.700 (0.651/0.749)  | 1 |
| MZF |  |  |  |  |
| 1-min Apgar twin 1 | 1 |  |  |  |
| 5-min Apgar twin 1 | 0.714 (0.662 / 0.767) | 1 |  |  |
| 1-min Apgar twin 2 | *0.537 (0.494 / 0.580)* | 0.303 (0.226 / 0.381) | 1 |  |
| 5-min Apgar twin 2 | 0.357 (0.278 / 0.435)  | *0.507 (0.462 / 0.552)* | 0.704 (0.659 / 0.749) | 1 |
| DZF |  |  |  |  |
| 1-min Apgar twin 1 | 1 |  |  |  |
| 5-min Apgar twin 1 | 0.655 (0.603 / 0.708) | 1 |  |  |
| 1-min Apgar twin 2 | *0.551 (0.510 / 0.591)* | 0.297 (0.207 / 0.387) | 1 |  |
| 5-min Apgar twin 2 | 0.338 (0.260 / 0.416) | *0.508 (0.461 / 0.555)* | 0.682 (0.629 / 0.735) | 1 |
| DZMF |  |  |  |  |
| 1-min Apgar twin 1 | 1 |  |  |  |
| 5-min Apgar twin 1 | 0.739 (0.695 / 0.783) | 1 |  |  |
| 1-min Apgar twin 2 | *0.479 (0.435 / 0.523)* | 0.231 (0.140 / 0.323) | 1 |  |
| 5-min Apgar twin 2 | 0.318 (0.238 / 0.398) | *0.455 (0.401 / 0.509)* | 0.646 (0.594 / 0.697) | 1 |
| DZFM |  |  |  |  |
| 1-min Apgar twin 1 | 1 |  |  |  |
| 5-min Apgar twin 1 | 0.738 (0.688 / 0.787) | 1 |  |  |
| 1-min Apgar twin 2 | *0.536 (0.491 / 0.580)* | 0.208 (0.118 / 0.298) | 1 |  |
| 5-min Apgar twin 2 | 0.369 (0.292 / 0.447) | *0.435 (0.375 / 0.495)* | 0.691 (0.659 / 0.722) | 1 |

In Italic, the twin 1 - twin 2 correlation for the ordinal 1- and 5-minute Apgar scores (with 95% CI: lower/upper)

**Table S9.**Shared and non-shared environment effects and correlations for ordinal 1- and 5-minute Apgar score (95% CI)

|  |  |  |
| --- | --- | --- |
|   | 1-min Apgar  | 5-min Apgar  |
| SC | .526 [.497/.555]  | .502 [.468/ .535]  |
| SE | .474 [.445/ .503]  | .498 [.465/.532] |
| rc | .634 [.573/ .695] |
| re | .769 [.697/ .842]  |
| rph | .699 [.683/ .716] |
| covariance C | .326 [.285/ .366] |
| covariance E | .374 [.349/ .400] |

SC=standardized estimate of phenotypic correlation explained by shared (common) environmental factors

SE= standardized estimate of phenotypic correlation explained by non-shared environmental factors

rc=shared environmental correlation between both phenotypes

re=unshared environmental correlation between both phenotypes

rph= phenotypic correlation between both phenotypes

C=shared prenatal environment

E=unshared prenatal environment

**Figure S1.** Path diagram including parameter estimates (and 95% CI in parentheses) and thresholds for ordinal 1- and 5-minute Apgar scores in first and second-born MZ and DZ twins



*C – shared prenatal environment*

*E – unshared prenatal environment*

Note .526 and .502 are the C variances at 1st minute and 5th minute, respectively. The C contribution to the phenotypic correlation is .725\*.634\*.708 = .326. The contributions of E (unshared environment) to the phenotypic variances are .474 and .498, and the E contribution to the phenotypic correlation is .689\*.769\*.706 = .374. The expected phenotypic correlation is .70 (i.e., .326+.374).