Type 2 diabetes gene TCF7L2 polymorphism is not associated with fetal and postnatal growth in two birth cohort studies.

**TCF7L2 and early growth**

Dennis O Mook-Kanamori, MD\(^1, 2, 3^*\) d.mook@erasusmc.nl
Sandra WK de Kort, MD\(^3^*\) s.dekort@erasusmc.nl
Cornelia M van Duijn, PhD\(^2\) c.vanduijn@erasusmc.nl
Andre G Uitterlinden, PhD\(^2, 4\) a.g.witterlinden@erasusmc.nl
Albert Hofman, MD, PhD\(^2\) a.hofman@erasusmc.nl
Henriëtte A Moll, MD, PhD\(^3\) h.a.moll@erasusmc.nl
Eric AP Steegers, MD, PhD\(^5\) e.a.p.steegers@erasusmc.nl
Anita CS Hokken-Koelega, MD, PhD\(^3\) a.hokken@erasusmc.nl
Vincent WV Jaddoe, MD, PhD\(^1, 2, 3\) v.jaddoe@erasusmc.nl

\(^1\)The Generation R Study Group, 
\(^2\)Department of Epidemiology, 
\(^3\)Department of Pediatrics, 
\(^4\)Department of Internal Medicine, 
\(^5\)Department of Obstetrics & Gynaecology, 
Erasmus Medical Center, Rotterdam, The Netherlands

* Both authors contributed equally to the paper

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**Corresponding author:**
Vincent. W.V. Jaddoe, MD, PhD, The Generation R Study Group (AE-006),
Erasmus Medical Center, PO Box 2040, 3000 CA Rotterdam, The Netherlands
Phone: +31 (0) 10 7043405, Fax: +31 (0) 10 7044645
E-mail: v.jaddoe@erasusmc.nl
ABSTRACT

Background:
An inverse association between birth weight and the risk to develop type 2 diabetes (T2D) in adulthood has been reported. This association may be explained by common genetic variants related to insulin secretion and resistance, since insulin is the most important growth factor in fetal life. Thus, the objective of this study was to examine whether T2D gene polymorphism TCF7L2 rs7903146 is associated with growth patterns from fetal life until infancy.

Design and Methods:
This study was performed in two independent birth cohort studies, one prospective population-based (Generation R cohort), and one of subjects born small-for-gestational-age (SGA cohort). Fetal growth was assessed by ultrasounds in second and third trimesters of pregnancy in Generation R. Growth in infancy was assessed in both cohorts at birth and at 6, 12 and 24 months postnatally. TCF7L2 rs7903146 genotype was determined in 3,419 subjects in Generation R and in 566 subjects in the SGA cohort.

Results:
Minor allele frequency did not differ significantly (p=0.47) between Generation R (T-allele: 28.7%) and the SGA cohort (T-allele: 29.8%). No differences at birth were found in gestational age or size (head circumference, length, weight) between the genotypes in either cohort. TCF7L2 genotype was also not associated with any pre- or postnatal growth characteristic in either Generation R or the SGA cohort.

Discussion:
We found no evidence for an association between TCF7L2 genotype and early growth. TCF7L2 would therefore not appear to be involved in the previously demonstrated associations of low birth weight with T2D.
INTRODUCTION

Several epidemiological studies have shown inverse associations between birth weight and metabolic diseases, including type 2 diabetes (T2D) in adulthood [1, 2]. These associations may be explained by common genetic variants [2]. Insulin is the most important fetal growth factor and insulin-mediated fetal growth might be affected by fetal genetic factors that regulate fetal insulin secretion or insulin sensitivity [2]. Therefore, gene variants associated with T2D have been suggested as candidate genes for influencing early growth [2].

Genome-wide association (GWA) studies have consistently shown that the C>T substitution in TCF7L2 gene (rs7903146) increases the risk of T2D approximately 2-fold when two risk allele copies (TT) are present [3-5]. It has been suggested that the T-allele of this TCF7L2 polymorphisms reduces proinsulin to insulin conversion [6], though the exact mechanism has not been elucidated yet. Other single nucleotide polymorphisms (SNPs) of the TCF7L2 gene have been shown to be associated with type 2 diabetes, although less strongly [7]. The T-allele of rs7903146, which has an estimated allele frequency amongst Caucasians of about 25%, has been shown to be associated with reduced insulin response and secretion in both diabetic and non-diabetic individuals [8-10], though results in non-diabetics are not consistent [11]. This polymorphism may also lead to an increased risk of gestational diabetes [12]. These findings make TCF7L2 one of the most important candidate genes for explaining the associations between low birth weight and T2D.

Freathy et al. were the first to investigate the association between TCF7L2 genotype and birth weight, and found an association with maternal TCF7L2 genotype [13]. Each maternal copy of the risk allele was associated with a 30 grams increase in offspring birth weight, probably as a result of higher maternal glucose levels stimulating fetal insulin production [13]. After adjustment for maternal genotype, fetal TCF7L2 genotype did not influence fetal birth weight [13]. In another study, no association was found between fetal TCF7L2 genotype and the risk of small size for gestational age [14]. Birth weight might be
an inappropriate measure of the individual growth potential since different fetal growth rates may lead to the same birth weight [15]. Furthermore, early postnatal growth has also been shown to be associated with metabolic phenotype in adulthood, independent of birth weight [16].

Therefore we hypothesized that longitudinally measured pre- and postnatal growth are better parameters than one specific growth characteristic such as birth weight, to investigate the possible effect of TCF7L2 genotype on growth. We first assessed the associations of TCF7L2 rs7903146 with fetal and postnatal growth characteristics in a population-based prospective cohort study among 3,419 subjects followed from early fetal life onwards. Second, for replication, we assessed associations of this genotype with postnatal growth in 566 small-for-gestational-age (SGA) children participating in an ongoing cohort study.
RESULTS

Subject characteristics of Generation R and SGA cohort are presented in Table 1. The minor allele frequency distributions did not differ significantly (p=0.47) between Generation R (T-allele: 28.7%) and the SGA cohort (T-allele: 29.8%) (Table 2).

Fetal growth characteristics in Generation R were analyzed with dominant, recessive and additive models and no significant differences between genotype groups were observed (Table 3). No differences in birth characteristics (head circumference, length and weight), between genotype groups were observed in either cohort (Table 4). Postnatal growth characteristics for both cohorts are shown in Table 5. No significant differences were found in either cohort for head circumference, weight or height at any age, except that the T-allele was associated with a lower weight at the age of 2 years in the SGA cohort using an additive model after adjusting for age and gender (p=0.04).

Finally, no differences were found in weight growth rate (SDS/week) from birth until the age of 2 years in either Generation R or the SGA cohort. Compared to the CC genotype, differences were -0.04 (95% confidence interval (CI): -0.11 to 0.03) SDS/week and -0.01 (95% CI: -0.13 to 0.10) SDS/week, for the CT and TT genotype, respectively, in Generation R. In the SGA cohort, differences were -0.08 (95% CI: -0.36 to 0.20) SDS/week and 0.03 (95% CI: -0.47 to 0.53) SDS/week, for the CT and TT genotype, respectively, using the CC genotype as a reference. Similarly, no differences were found in height growth rate from birth to 2 years in either cohort (data not shown).
DISCUSSION

In the current study, we showed that T2D gene polymorphism *TCF7L2* rs7903146 is not associated with growth in early life in the general population and in a selected cohort of subjects born SGA. Furthermore, we demonstrated that this polymorphism is not related to size at birth and that minor allele frequency in an SGA cohort did not differ from that in a cohort representing the general Caucasian population.

To our knowledge, this study is the first to examine the association of *TCF7L2* rs7903146 with longitudinally measured growth patterns in fetal and early postnatal life in two independent birth cohorts. In the Generation R Study, DNA for genotyping was available in 59% of all subjects and was isolated from cord-blood. Missing cord-blood was mainly caused by logistical restraints at delivery. Children who were not genotyped had a shorter gestational age (p < 0.001) and were lighter at birth (p < 0.001) than subjects who were genotyped. Of all genotyped eligible subjects at baseline, 22% did not participate in follow-up measurements. In the SGA cohort, genotyping was successful in 91% of the subjects and longitudinally growth data were available in 48% of the cohort. Our effect estimates could be biased if the associations between genotypes and growth characteristics differ between those with and without postnatal growth data available. In the Generation R cohort, no differences were observed between children with and without postnatal growth measurements. In the SGA cohort the T-allele was slightly more frequent in subjects with postnatal growth measurements than in subjects without these measurements (p < 0.05). This may bias our effect estimates, though this seems unlikely.

Low birth weight and postnatal catch-up growth are associated with an increased risk of developing T2D in adulthood [2, 17, 18]. Insulin is the most important fetal growth factor and insulin-mediated fetal growth might be affected by fetal genetic factors that regulate fetal insulin secretion or insulin sensitivity [2]. These fetal genetic factors could also explain the association between low birth weight and T2D risk [2]. Support for this hypothesis comes
from monogenic forms of diabetes and described associations between paternal T2D and reduced offspring birth weight [19]. Several studies investigated the effect of common genetic variants related to insulin action on early growth and found no or inconsistent associations [15, 20, 21], possibly because they investigated gene polymorphisms that appear to be less strongly associated with T2D than TCF7L2 rs7903146. The association between TCF7L2 rs7903146 and T2D has been replicated in various studies and it is to date the strongest genetic association with T2D [3-5]. Therefore TCF7L2 rs7903146 is a very important candidate gene for explaining the association between low birth weight and T2D risk.

Our study is the first to investigate the effect of TCF7L2 rs7903146 on longitudinal growth in early life. Longitudinal assessment of growth provides more information than just measurements at birth as we have demonstrated earlier that different fetal growth patterns may result in a similar birth weight [15]. Furthermore, most SGA born children have catch-up growth during the first months of life but 15% remains small [22]. Thus, to investigate whether TCF7L2 rs7903146 influences fetal and postnatal growth, longitudinal growth data provide more information than birth weight alone.

Freathy et al. studied the effect of TCF7L2 rs7903146 on birth weight in over 24,000 individuals and found that each fetal copy of the risk allele was associated with an increase of 18 grams in weight at birth and each maternal risk allele with an increase of 30 grams in birth weight [13]. However, the association of fetal genotype with birth weight did not remain after adjustment for maternal genotype. The authors concluded that the most likely mechanism was that maternal genotype was associated with a reduction of maternal insulin secretion, leading to an increased glucose level and increased birth weight, rather than a direct effect of the fetal genotype on birth weight. In our study, we did not find any effect of fetal genotype on birth weight in the general population and also not in a specific population of children with insufficient fetal growth resulting in small size for gestational age at birth.
Our findings would therefore be in line with the conclusions of Freathy et al. Furthermore, we found no effect of fetal genotype on weight before birth or during infancy, indicating that there is no evidence for any association between this fetal genotype and weight or change in weight during early life. The effect of this polymorphism on the metabolic phenotype found in adults would therefore appear to develop after early childhood.

Regarding intra-uterine growth retardation, one previous study examined the effect of TCF7L2 rs7903146 genotype on SGA. In this study, Cauchi et al. found no association between this genotype and SGA, using family-based association analyses in over 3,000 subjects of which 627 subjects were SGA [14]. In their analyses, the SGA group was slightly larger than in our current study and included parents, but postnatal growth data were not analyzed longitudinally. In the current study, we did not find a difference in minor allele frequency between the general population (Generation R) and the SGA cohort. With two independent negative studies, one may conclude that there is no association between this genetic polymorphism and risk of SGA.

In summary, our results suggest that TCF7L2 rs7903146 does not influence growth from early fetal life to infancy. Furthermore, minor allele frequency was not different in SGA subjects than in non-SGA subjects from which we can conclude that there was no association between genotype and risk of being born SGA. Systematic searches for common genetic variants by means of genome-wide association studies will enable us to obtain a more complete understanding of what genes are involved in growth in fetal life and infancy.
MATERIALS AND METHODS

Cohort Descriptions

The Generation R Study

The Generation R Study is a population based prospective cohort study from early fetal life onwards. This study is designed to identify early environmental and genetic determinants of growth, development and health from fetal life until young adulthood and has been described previously in detail [23, 24]. Fetal and postnatal growth and their main determinants were repeatedly measured by physical examinations, fetal ultrasounds, biological samples and questionnaires. We have previously shown that of all eligible children born in the study area 61% participated in the study [24]. The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. Written informed consent was obtained from all parents or their parents.

Fetal growth and birth characteristics

Fetal ultrasound examinations were carried out at the visits in one of the research centers. These fetal ultrasounds were used for establishing gestational age in the first trimester (conception to 12 weeks of gestational age) and for assessing fetal growth characteristics in second (17-25 weeks of gestational age) and third trimester (>25 weeks of gestational age) of pregnancy [25]. Fetal growth measurements used for the present study included head circumference (HC), abdominal circumference (AC) and femur length (FL) measured in second and third trimesters to the nearest mm using standardized ultrasound procedures [26]. Estimated fetal weight (EFW) was calculated using the formula by Hadlock using head circumference, abdominal circumference and femur length (log$_{10}$ EFW = 1.5662 – 0.0108 (HC) + 0.0468 (AC) + 0.171 (FL) + 0.00034 (HC)$^2$ – 0.003685 (AC * FL)) [27]. First trimester ultrasound measures were not included as growth characteristics since these ultrasound examinations were primarily performed to establish gestational age.
**Birth and postnatal growth**

Birth weight, date of birth and gender were obtained from community midwife and hospital registries. Information on head circumference or length at birth was not available but many children were measured during the first two months of life. Well-trained staff in community health centers obtained postnatal growth characteristics using standardized procedures. Based on the routine health care program, visits for these growth characteristics were grouped into three age periods: 6 (range 5 to 8.99) months; 12 (range 9 to 12.99) months; and 24 (range 23 to 34.99 months). Postnatally, head circumference was not measured at the age of 24 months.

**Population for analysis**

Analysis were restricted to singletons of whom DNA was available for *TCF7L2* genotyping and with Dutch or other Caucasian ethnicity as defined by having both parents born in the Netherlands or another European country (n = 3,419)(Figure 1). Fetal growth measurements were available in 3,320 and 3,384 children in second and third trimester, respectively. Of these children, those living outside the study area postnatally (10%) were not followed up in infancy and a further 12% were lost in postnatal follow-up, leaving 2,675 subjects eligible for the postnatal analyses (Figure 1).

**The SGA Cohort**

The SGA cohort is designed to assess growth and development of subjects born SGA. Subjects were included at childhood age (n = 367) or at young adult age (n = 252). Children were included in the SGA cohort when they were SGA at birth, had short stature (height standard deviation score (SDS) for age and gender of below – 2 [28]), did not show catch-up growth in height, and had no growth failure caused by any other identified disorder. These inclusion criteria have previously been described [29]. Young adults included in the SGA cohort were randomly selected from hospitals in The Netherlands, where they had been
registered because of being SGA. Only those young adults born at 36 weeks or more of gestation, being singleton and Caucasian and not suffering from conditions or receiving treatment known to interfere with growth, were invited to participate. SGA was defined as a birth length and/or birth weight SDS of below –2.0 for gestational age [30]. The Medical Ethics Committees of Erasmus Medical Center, Rotterdam, and of the participating centers approved all studies and written informed consent was obtained from all participants or their parents.

**Birth and postnatal growth**

Birth characteristics of the SGA cohort were collected from hospital registries. The gestational age of the subjects was determined by ultrasound in the first trimester, if available, and otherwise calculated from the date of the last menstruation. Growth data (head circumference, height and weight) measured during the first two years of life was collected from records of hospitals, community health services and general practitioners. In 272 participants of the SGA cohort longitudinal growth data were available (Figure 1).

**Genotyping**

DNA was collected from cord blood samples in the Generation R cohort and from peripheral venous blood samples in the SGA cohort. Cord blood for DNA isolation was available in 59% of all participating children of the Generation R cohort. Missing cord blood samples were mainly due to logistical constraints at the delivery. Venous blood samples were available in the complete SGA cohort. Genotyping of the C>T substitution in *TCF7L2* (rs7903146) gene was performed using Taqman allelic discrimination assay (Applied Biosystems, Foster City, CA) and Abgene QPCR ROX mix (Abgene, Hamburg Germany). The genotyping reaction was amplified using the GeneAmp® PCR system 9600 (95°C (15 minutes), with 40 cycles of 94°C (15 seconds) and 60°C (1 minute)). The fluorescence was detected on the 7900HT Fast Real-Time PCR System (Applied Biosystems) and individual genotypes were
determined using SDS software (version 2.3, Applied Biosystems). Genotyping was successful in 98% and 91% of the samples in the Generation R and SGA cohort, respectively. To confirm the accuracy of the genotyping results, 276 randomly selected samples from the Generation R Study were genotyped for a second time with the same method. The error rate was less than 1%. The frequency distribution in Generation R did not deviate from the Hardy-Weinberg equilibrium in subjects with Dutch ethnicity or in the SGA cohort.

**Data analysis**

With a sample size in the Generation R Study of 3,419 subjects and assuming a statistical power level (1 – β) of 0.80, a level of significance (α) of 0.05 and a variance of 1.0, we were able to detect differences in growth characteristics of about 0.05 SDS. First, differences in allele distribution between children born SGA and the general population (Generation R) were assessed. Differences were calculated using the Chi-square test. Second, we examined the differences in birth characteristics between genotype groups with independent sample t-tests, Mann-Whitney U-tests and linear regression analyses using three models: additive (change of each additional risk allele), dominant (TT/CT vs. CC), and recessive (CC/CT vs. TT). Weight, length and head circumference at birth and at the different ages were analyzed using gender and age adjusted standard deviation scores (SDS) [30, 31]. For Generation R, we used the first length SDS and head circumference SDS that was measured after birth and before the second month of life, since these measurements were not available at birth. Third, we compared fetal (only Generation R) and postnatal characteristics between the genotypes with independent sample t-test or Mann-Whitney U test. Finally, to assess longitudinally measured weight and length patterns from fetal life to infancy, we performed repeated measures regression analysis in both cohorts with weight and length from birth to 24 months as outcome variable. This regression technique takes the
correlation of multiple measurements within one subject into account, assesses both the time-independent and time-dependent effect of TCF7L2 genotype on growth, and allows for incomplete outcome data [32]. In these models, genotype was included as both intercept and interaction with age. To account for (gestational) age at each specific measurement, these analyses were conducted with age-adjusted standard deviation scores. The models can be written as:

$$\text{Height (SDS) or weight (SDS) = } \beta_0 + \beta_1 \cdot \text{age} + \beta_2 \cdot \text{TCF7L2 genotype} + \beta_3 \cdot \text{TCF7L2 genotype} \cdot \text{age}. $$

In this model, the term including ‘$\beta_0$’ reflects the intercept and the term including ‘$\beta_1$’ reflects the slope of growth (weight or length) per week for the reference group (CC genotype). The terms including ‘$\beta_2$’ and ‘$\beta_3$’ reflect the age independent growth differences in weight (and length) between the different categories of the TCF7L2 genotype, respectively [33]. All models were unadjusted (all growth characteristics are age and gender adjusted SD scores) since population genotype distribution is assumed to be unrelated to covariates and the effect estimates were not materially affected by adjusting for maternal age, pre-pregnancy body mass index or parity [34]. The occurrence of gestational diabetes in the entire cohort was 0.6% and did not affect the effect estimates. Therefore, occurrence of gestational diabetes was not included in the analyses.

All effect estimates are presented with their 95% confidence interval (95% CI). Statistical analyses were performed using the Statistical Analysis System version 9.1.3 (SAS, Stata corporation, College station, TX, USA), including the PROC MIXED module for unbalanced repeated measurements and the Statistical Package of Social Sciences version 15.0 for Windows (SPSS Inc, Chicago, IL, USA).
ACKNOWLEDGEMENTS
The Generation R Study is conducted by the Erasmus Medical Center in close collaboration with the School of Law and Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, Rotterdam, the Rotterdam Homecare Foundation, Rotterdam and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam. We gratefully acknowledge the contribution of general practitioners, hospitals, midwives and pharmacies in Rotterdam. The first phase of the Generation R Study is made possible by financial support from the Erasmus Medical Center, Rotterdam, the Erasmus University Rotterdam and the Netherlands Organization for Health Research and Development (ZonMw). Also, we would like to thank all participants and their parents of the SGA cohort. We greatly acknowledge R.W. Leunissen, W.A. Ester, C. Bruinings-Vroombout, M. Huibregtse-Schouten, J. van Houten, J. van Nieuwkastelee, J. Dunk and E. Lems, for their assistance with data collection of the SGA subjects.

CONFLICT OF INTEREST STATEMENT
All authors of this manuscript confirm there they have no conflict of interest to declare.

AUTHOR’S CONTRIBUTION
DOMK, SWKdK, ACSHK and VWJV made substantial contributions to conception and design of the manuscript and the analysis and interpretation of data. CMvD and AGU were involved in the genetic analysis of the data. AH and HAM were involved in the design of the cohort. EAPS was responsible for the prenatal growth data collection. All authors were involved in drafting and revising the manuscript and have given final approval of the version to be published.
REFERENCES


FIGURE LEGENDS

Figure 1. Flow diagram indicating number of subjects in the two cohorts

* All live-born, Caucasian, singleton subjects within Generation R
Table 1. Subject characteristics by cohort.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Generation R</th>
<th>The SGA cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% boys)</td>
<td>50.8%</td>
<td>47.2%</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>40.1 (36.7 – 42.4)</td>
<td>38.0 (29.9 – 41.0)</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>3513 (511)</td>
<td>1819 (716)</td>
</tr>
<tr>
<td>Premature (gestational age &lt; 37 weeks) (%)</td>
<td>2.9%</td>
<td>44.9%</td>
</tr>
<tr>
<td>Birth weight &lt; 2500 grams (%)</td>
<td>2.5%</td>
<td>82.7%</td>
</tr>
<tr>
<td>Small for gestational age (weight &lt; -2 SDS) (%)</td>
<td>1.6%</td>
<td>100%</td>
</tr>
<tr>
<td>Gestational diabetes (%)</td>
<td>0.6%</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values are means (SD), medians (95% range) or percentages. N/A = not available
Table 2. Distribution of TCF7L2 rs7903146 minor allele frequency according to cohort.

<table>
<thead>
<tr>
<th></th>
<th>C-Allele</th>
<th>T-Allele</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population (Generation R) (%)</td>
<td>4875 (71.3)</td>
<td>1963 (28.7)</td>
<td></td>
</tr>
<tr>
<td>SGA (SGA cohort) (%)</td>
<td>795 (70.2)</td>
<td>337 (29.8)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

SGA = birth weight SDS and/or birth length SDS < -2

p-value express differences in distribution between SGA and General population tested with Chi-square test.
Table 3. Fetal characteristics according to fetal TCF7L2 rs7903146 genotype in the Generation R study.

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fetal characteristics second trimester</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference (SDS)</td>
<td>0.04 (1.0)</td>
<td>0.02 (1.0)</td>
<td>0.05 (0.9)</td>
<td>0.88</td>
</tr>
<tr>
<td>Femur length (SDS)</td>
<td>-0.01 (1.0)</td>
<td>-0.01 (1.0)</td>
<td>0.05 (0.9)</td>
<td>0.64</td>
</tr>
<tr>
<td>Estimated fetal weight (SDS)</td>
<td>-0.06 (1.0)</td>
<td>-0.07 (1.0)</td>
<td>0.00 (1.0)</td>
<td>0.57</td>
</tr>
<tr>
<td><strong>Fetal characteristics third trimester</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference (SDS)</td>
<td>0.11 (1.0)</td>
<td>0.13 (1.0)</td>
<td>0.15 (0.9)</td>
<td>0.45</td>
</tr>
<tr>
<td>Femur length (SDS)</td>
<td>0.01 (1.0)</td>
<td>-0.02 (1.0)</td>
<td>-0.04 (1.0)</td>
<td>0.34</td>
</tr>
<tr>
<td>Estimated fetal weight (SDS)</td>
<td>0.12 (1.0)</td>
<td>0.14 (1.0)</td>
<td>0.11 (0.9)</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Values are means (SD). SDS = standard deviation score for age and gender. Differences were tested using linear regression analyses or independent sample t-test.

*p-values for additive models. Dominant models (TT/CT vs. CC) and recessive models (CC/CT vs. TT) did not result in significant differences.
Table 4. Birth characteristics in both cohorts according to TCF7L2 rs7903146 genotype of child.

<table>
<thead>
<tr>
<th>Generation R</th>
<th>CC (n = 1762)</th>
<th>CT (n = 1351)</th>
<th>TT (n = 306)</th>
<th>p-value*#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>40.3 (36.7 – 42.3)</td>
<td>40.3 (36.6 – 42.4)</td>
<td>40.1 (37.1 – 42.6)</td>
<td>0.83</td>
</tr>
<tr>
<td>Birth head circumference (SDS)*</td>
<td>0.22 (0.9)</td>
<td>0.24 (0.9)</td>
<td>0.26 (0.9)</td>
<td>0.55</td>
</tr>
<tr>
<td>Birth length (SDS)*</td>
<td>-0.07 (1.0)</td>
<td>-0.08 (1.0)</td>
<td>0.00 (1.1)</td>
<td>0.66</td>
</tr>
<tr>
<td>Birth weight (SDS)</td>
<td>0.21 (1.0)</td>
<td>0.22 (1.0)</td>
<td>0.20 (1.0)</td>
<td>0.97</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SGA Cohort</th>
<th>CC (n = 270)</th>
<th>CT (n = 255)</th>
<th>TT (n = 41)</th>
<th>p-value*#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (weeks)</td>
<td>38.0 (28.6 – 42)</td>
<td>38.0 (28.6 – 41.0)</td>
<td>38.0 (29.0 – 42.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>Birth head circumference (SDS)</td>
<td>-1.51 (1.4)</td>
<td>-1.20 (1.6)</td>
<td>-1.31 (1.4)</td>
<td>0.32</td>
</tr>
<tr>
<td>Birth length (SDS)</td>
<td>-3.11 (1.4)</td>
<td>-3.27 (1.5)</td>
<td>-3.02 (1.5)</td>
<td>0.41</td>
</tr>
<tr>
<td>Birth weight (SDS)</td>
<td>-2.40 (1.0)</td>
<td>-2.46 (0.9)</td>
<td>-2.32 (0.9)</td>
<td>0.58</td>
</tr>
</tbody>
</table>

* Length and head circumference were measured in the first two months of after birth. 
Values are means (SD) or medians (95% range). SDS = standard deviation score for age and gender. 
Differences were tested using linear regression analyses, independent sample t-test or Mann-Whitney U-test. 
* p-values for additive models. Dominant models (TT/CT vs. CC) and recessive models (CC/CT vs. TT) did not result in significant differences. 
Of the total group, data at birth were missing in The Generation R Study for length (n=1,460), head circumference (n=1,105), SGA cohort length (n=491) and head circumference (n=203).
Table 5. Postnatal characteristics at 6, 12, and 24 months according to TCF7L2 rs7903146 genotype.

<table>
<thead>
<tr>
<th>Generation R</th>
<th>CC (n = 1375)</th>
<th>CT (n = 1063)</th>
<th>TT (n = 237)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference (SDS)</td>
<td>-0.02 (0.93)</td>
<td>-0.03 (0.89)</td>
<td>-0.06 (0.91)</td>
<td>0.83</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>0.03 (0.91)</td>
<td>0.03 (0.90)</td>
<td>0.07 (0.93)</td>
<td>0.81</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>0.41 (0.96)</td>
<td>0.44 (0.95)</td>
<td>0.54 (0.99)</td>
<td>0.14</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference (SDS)</td>
<td>0.00 (0.89)</td>
<td>-0.04 (0.94)</td>
<td>-0.03 (1.12)</td>
<td>0.54</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-0.01 (0.90)</td>
<td>-0.05 (0.90)</td>
<td>-0.01 (0.90)</td>
<td>0.70</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>0.18 (0.98)</td>
<td>0.18 (0.99)</td>
<td>0.24 (1.00)</td>
<td>0.63</td>
</tr>
<tr>
<td><strong>24 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-0.19 (0.93)</td>
<td>-0.21 (0.89)</td>
<td>-0.18 (0.87)</td>
<td>0.82</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>-0.11 (0.99)</td>
<td>-0.13 (1.00)</td>
<td>-0.09 (0.96)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SGA cohort</th>
<th>CC (n = 143)</th>
<th>CT (n = 107)</th>
<th>TT (n = 22)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>6 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference (SDS)</td>
<td>-1.38 (0.92)</td>
<td>-1.36 (0.90)</td>
<td>-1.74 (1.04)</td>
<td>0.41</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-2.39 (1.37)</td>
<td>-2.43 (1.26)</td>
<td>-2.51 (1.55)</td>
<td>0.93</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>-2.18 (1.40)</td>
<td>-2.22 (1.26)</td>
<td>-2.37 (2.05)</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>12 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference (SDS)</td>
<td>-1.21 (0.83)</td>
<td>-1.24 (0.88)</td>
<td>-1.72 (1.06)</td>
<td>0.16</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-2.25 (1.25)</td>
<td>-2.30 (1.06)</td>
<td>-2.30 (1.47)</td>
<td>0.94</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>-2.14 (1.41)</td>
<td>-2.25 (1.15)</td>
<td>-2.17 (1.89)</td>
<td>0.82</td>
</tr>
<tr>
<td><strong>24 months</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Head circumference (SDS)</td>
<td>-1.10 (0.82)</td>
<td>-1.13 (0.87)</td>
<td>-1.60 (1.06)</td>
<td>0.20</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-2.39 (1.24)</td>
<td>-2.47 (1.05)</td>
<td>-2.94 (1.04)</td>
<td>0.20</td>
</tr>
<tr>
<td>Weight (SDS)</td>
<td>-2.19 (1.33)</td>
<td>-2.31 (1.21)</td>
<td>-3.06 (1.65)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Values expressed as mean (SD). SDS = standard deviation score for age and gender.

* p-values for additive models. Dominant models (TT/CT vs. CC) and recessive models (CC/CT vs. TT) did not result in significant differences.

Differences were tested using linear regression for additive models and using independent samples t-test for dominant and recessive models.

In the postnatal follow-up of Generation R (n=2675), data were missing at 12 months (n=116) and at 24 months (n=310). In the postnatal follow-up of the SGA cohort (n=272), data were missing at 12 months (n=4) and at 24 months (n=28).
Figure 1. Flow diagram indicating number of subjects in the two cohorts

* All live-born, Caucasian, singleton subjects within Generation R