Transgenerational transmission of small for gestational age

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Running Head: Transgenerational effect of SGA.

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Abstract

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Objective: To evaluate the transgenerational transmission of small for gestational age.

Methods: Cohort study including a random sample of 2,043 offspring of deliveries occurring from 1975 to 1993. Of 623 offspring –now adults- that agreed to participate, 152 adults (72 born small-for-gestational age (SGA) and 80 with appropriate intrauterine growth) reported to have at least one child. Multiple regression analysis was used to determine the presence of SGA (defined as a birthweight < 10^{th} percentile) or placental mediated disease (defined as the presence of SGA, preeclampsia or gestational hypertension) in the following generation.

Results: Descendants from SGA adults presented lower birthweight percentile (median 26 [interquartile range 7-52] vs. 43 [19-75]; p<0.001) and higher prevalence of SGA (40.3% vs. 16.3%; p=0.001) and placental mediated disease (43.1% vs. 17.5%; p=0.001). After adjustment for confounder variables, parental SGA background was associated with an almost three-fold increased risk of subsequent SGA or any placental mediated disease in the following generation. This association was stronger in SGA mothers as compared to fathers.

Conclusions: Our data provides evidence suggesting a transgenerational transmission of SGA highlighting the importance of public health strategies for preventing intrauterine growth impairment.

Introduction

Fetal growth restriction is defined as a failure to reach the growth potential of an individual and is considered as a placental-mediated disease that commonly associates maternal hypertension/preeclampsia.[1] It is usually diagnosed as small-for-gestational age (SGA) by birth weight below the 10th centile for gestational age.[2] It affects about 5 – 10 % of all pregnancies, and is one of the main causes of perinatal morbidity and mortality worldwide.[3] Apart from perinatal complications, individuals born small present long-term consequences with increased risk of adult cardiovascular and metabolic disease. [4–8]

Experimental and human studies also suggest not only effects on the born-small individual but also transmission on the subsequent generation,[9–12] mainly recurrence of low birth weight and preeclampsia in offspring of SGA individuals.[13,14] A transgenerational transmission is defined when the latter generations -not exposed to an adverse intrauterine condition- develop the same phenotype of their ancestors without a genetic inheritance. Interestingly, this transmission is supposed to be mediated by epigenetic changes, mainly DNA-methylation, that modulate DNA expression in order to better adapt to environment.[15] Although most of studies have been focused on transgenerational effect of low birth weight on the first generation of offspring, there is increasing evidence from animal research suggesting that this programmed phenomena can be also present in later generations.[15] Most studies in humans are based on north European or American populations born before the 80s and use birth weight –not centile- as a surrogate of intrauterine growth.[14,16,17]

In the present study, we aimed to assess the transgenerational transmission of SGA –defined by birth weight centile- in a contemporary Mediterranean population with relatively lower prevalence of placental mediated diseases. We used a well-phenotyped modern population to assess the transmission of SGA and placental

mediated diseases into first/subsequent offspring taking into account also paternal gender.

Methods

Study design and patient´s selection

A cohort study including a random sample of offspring (Generation 1, G1) from 2,043 deliveries occurring from 1975 to 1993 at Hospital Sant Joan de Déu in Barcelona (Figure 1). Delivery books and maternal medical files were reviewed to obtain maternal characteristics of generation 0 (G0) and pregnancy outcomes including the diagnosis of SGA in the offspring's (G1) defined as birth weight below the 10th centile for gestational age and appropriate growth for gestational age (AGA) was defined as birth weight centile above 10th centile, according to both a contemporary Spanish reference normograms[18] and a current local customized curve calculator.[19]. Only newborns with concordance of classification (AGA/SGA) by both birth weight curves were included. Patient´s selection was performed randomly in a 1:1 ratio of SGA and AGA of G1. Exclusion criteria were multiple pregnancies, aneuploidy or genetic syndrome, major birth defects, major mental diseases and macrosomia (birthweight percentile above 95th).

Current contact data could be retrieved from 1,165 offspring's (G1) that fulfilled inclusion criteria. A total of 623 G1 individuals -now adults- agreed to participate in the study after an invitation letter and a phone call. From those, 152 individuals reported to have at least one offspring (G2) and an oral questionnaire was applied to obtain information about pregnancy characteristics of their offspring.

The study was approved by the Ethical Committees of Hospital Sant Joan de Deu (Registry N° PIC-101-14) and Hospital Clinic (Registry N° HCB/2014/0598). All patients included signed a written informed consent.

Definitions and variables

For current study, the following definitions were used: The term generation 0 (G0) was applied to the mother of the index patient. Generation 1 (G1) was applied to the index patient, born between 1975 and 1993, and contacted as an adult. Generation 2 (G2) was applied to the offspring of G1, born between 2005 and 2015 (Figure 1).

The main exposure variable was defined as the presence of SGA in G1. The primary outcome was considered as the presence of SGA in G2. The presence of any placental disease (SGA, preeclampsia, gestational hypertension and/or placental abruption) was considered as a secondary outcome. SGA was defined as birth weight below 10th percentile according to local reference curves,[18,19] for G1, and current customized curves for G2.[19] Preeclampsia was defined as the presence of hypertension and proteinuria after 20 weeks of pregnancy in a previously normotensive patient. Hypertension was considered as at least two measurements with systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, with 6 hours apart. Proteinuria was considered with a 24-hours sample > 300mg or a spot urine protein/creatinine ≥ 30mg/mmol.[20]

Sample size estimation

For sample-size calculation, the freely available software OpenEpi, with corrected Fleiss method, was used. Based on a hypothetical 30% incidence of SGA in exposed G2 offspring and a 10% incidence of SGA in unexposed group, with a 5% two-sided alpha-error and with a power of 80%, a total of 143 patients were estimated to be needed.

Statistical analysis

Study groups were described and compared using mean ± standard deviation, median (interquartile range) or relative frequencies with corresponding t-test, Mann-

Whitney U, X^2 tests or Fisher´s exact test when appropriated. Multiple logistic regressions were applied to adjust for potential confounders including gender, perinatal characteristics, sociodemographic data, cardiovascular risk factors (life style and chronic status) and anthropometry of G1. The analysis was performed by combining both first and second offspring (G2-any offspring) and stratified by offspring position. When both pregnancies were analysed, robust standard errors were obtained to account for non-independency (pregnancies are clustered within mothers). Additionally, an exploratory analysis was performed to evaluate the effect of parental gender on placental mediated disease. For all statistical analyses, STATA 14.2 (Statacorp, Texas USA) was used. A p-value less than 0.05 was considered as significant.

Results

Maternal and pregnancy characteristics of G0

Pregnancy and perinatal characteristics of G0 pregnancies when delivering G1 are described in Table 1. G0-pregnant women that delivered a SGA offspring were characterized by a lower maternal height and body surface area, with no difference in body mass index. In addition, these patients showed a higher rate of smoking during pregnancy, compared to patients that delivered a normal birth weight offspring. As expected, G1-SGA showed lower birth weight, birth weight percentile and placental weight than G1-AGA. There was a non-significant trend to higher rate of preeclampsia and cesarean section in G0-SGA as compared to G0-AGA. Gestational age at delivery was similar among groups.

Current adult demographic and medical characteristics of G1

G1-SGA as adults were characterized by a lower educational level, lower salary and higher tobacco use than G1-AGA (Table 2). Current anthropometric analyses showed that G1-SGA adults were shorter and lighter than G1-AGA, with no differences in gender, age, personal or familiar history of cardiovascular diseases.

Perinatal outcomes of G2

Regarding G2 population, birth weight and birth weight percentile was lower when a parental background of SGA was present (Figure 2), with also a higher rate of preeclampsia and placental-mediated disease (Table 3). Although these differences were observed both in first and second offspring, changes were more evident in the first offspring (Table 3). The adjusted odds ratio for SGA and placental-mediated disease in the subsequent pregnancy was 2.79 and 3.15 respectively (Table 4).

A stratified analysis according to G1 gender demonstrated a stronger transgenerational effect among G1-females: G2 birth weight was lower (Figure 3) and rate of placental-mediated disease higher (Figure 4) in G1-SGA females as compared to G1-SGA males. Among G1-females, the incidence of SGA in any G2 offspring was 21.9% and 48.7% for G1-AGA and G1-SGA, respectively (p=0.02). This trend was similar for both, first and second pregnancy. Among G1-male parents, the incidence of SGA in any G2 offspring was 12.5% and 30.3% for G1-AGA and G1-SGA, respectively (p=0.048).

Discussion

Our study supports the transgenerational transmission of SGA and placental mediated diseases in a contemporary population. This transmission occurs both in the first and second offspring and seems to be stronger through the maternal lineage.

Our data provide evidence suggesting a transgenerational transmission of SGA in the subsequent generation using a contemporary Mediterranean population. SGA background increased the risk of having a SGA offspring in almost three times, even after adjustment for confounders. Interestingly, the risk of developing any placental-mediated disease (including SGA or maternal pregnancy-induced hypertension) in the following generation was also increased more than three times. These results are in line with most recent studies describing the transmission of low birth weight and SGA into the following generation.[14,21–23] Castrillio *et al* demonstrated an adjusted risk for SGA transmission of 2.1 (2.0 – 2.2) and 1.5 (1.5 – 1.6) for white and African-American women, respectively.[21] Wikström *et al* reported that SGA mothers have also an increased risk of developing early preeclampsia (aOR=1.87; 95% CI= 1.38 – 2.35) and placental abruption (aOR=1.60; 95% CI= 1.23 – 2.09), with a non-significant impact on spontaneous preterm delivery or stillbirth.[23] Selling *et al*, also reported a two-fold increased risk of delivering a SGA offspring if the mother was SGA at birth (OR= 2.68; 95% CI= 2.11 – 3.41). However, a background of spontaneous preterm delivery did not increase the risk of SGA in the following generation.[21,22] Indeed, our data also suggest no effect of gestational age at delivery on SGA transmission. Overall, most recent data suggest a 2 to 3-fold increase of SGA or placental related diseases in the subsequent population. However, data from the Dutch Famine cohort failed to demonstrate a transgenerational effect on birth weight or cardiovascular diseases.[24] This discordance could be explained by relevant differences in the populations regarding main factors

contributing to fetal smallness -mainly related to low maternal intake of nutrients- and timing -Dutch Famine occurring in the 40s-.

Interestingly, our data further suggest a differential impact of parental gender on the transmission of SGA in offspring. Female SGA parents are associated with almost two-times higher rates of SGA as compared with male SGA parents. These results are in agreement with previous publications also suggesting a stronger maternal linkage effect. Coutinho *et al* demonstrated a greater impact of maternal birth weight rather than paternal birth weight, in both white and African-American population.[14] In the same line, Skjærven *et al* showed that daughters of preeclamptic mothers demonstrated a two-fold higher risk of developing preeclampsia compared to son´s partners pregnancies.[13] These findings are in line with experimental data suggesting that placental mediated diseases (mainly SGA and PE) are associated with genetic or epigenetic modifications that can be transmitted to the following generations via maternal germ-cell line, an altered in-utero environment or by maternal mitochondrial DNA.[10,15,25]

In addition, to our knowledge, our study is the first to report a transmission of the transgenerational effect of SGA in the first and subsequent offspring of the same G2-generation, with a stronger association in the former. Regarding preeclampsia, again both first and subsequent offspring presented a higher risk, although first child accumulated a greater risk as compared to the second one. However, the rate of preeclampsia decreased from the first offspring to the second. This reduction in rate of PE in the second G2-offspring could be explained by the immunologic tolerance theory based on the observed higher risk of preeclampsia in a first pregnancy or after change of the male partner.[28] It has been postulated that long-term exposure to paternal seminal plasma tolerizes the woman to male alloantigens, reducing the risk of an altered immunologic tolerance during the placentation process, allowing an adequate

trophoblast implantation, and therefore, reducing the risk of PE-in the current pregnancy.

The present study has some strengths and limitations that merit comment. The main strength is the use of a well-phenotyped contemporary population with detailed maternal and pregnancy data from G0 and G1. Our database was constructed by an extensive review of medical charts and delivery registries in a single institution. This strategy allowed us to obtain very accurate data regarding perinatal outcomes. We acknowledge several limitations. Firstly, the diagnosis of SGA was based on birth weight centile as data on feto-placental ultrasound was not available on G1. Secondly, first trimester ultrasound in G0 pregnancy was not available to estimate gestational age, then patients with a doubtful last menstrual period at first obstetric control were excluded to minimize the inaccuracy in dating. In addition, maternal height and weight could not be used to customize the size of fetuses and newborns. Cases and controls were randomly selected and not paired by demographical characteristics such as educational level or smoking habits. However, logistic regressions were applied to adjust for potential confounders such as gender, perinatal characteristics, sociodemographic data, cardiovascular risk factors and anthropometry of G1. Finally, the non-significant trend of lower birthweight in G2-offspring of male parents could be related to an underpowered sample size for sub-analysis of parental gender.

The clinical relevance of the present study resides in the impact of growth restriction not only on the affected individual but also on the future generations, offering a unique window of opportunity to improve health and prevent long-term consequences. From a public health perspective, the transgenerational evidence reinforce the importance and high value of strategies preventing or treating intrauterine growth restriction for improving the health of present and future generations.

Disclosure of interests

All authors declare no conflict of interests. Full disclosure of interests is available online as supporting information.

Contribution to authorship

AS-M co-designed the study and wrote the manuscript. MR-L co-designed the study, carried out the statistical analysis and co-wrote the manuscript. FP and GC assisted with data collection and database design. EG co-designed the study, applied for funding and ethical approval, and assisted with edition of the manuscript. FC co-designed the study, applied for funding, helped with interpretation of analyses and edited the manuscript.

Details of ethics approval

Project approval was obtained from Ethics Committees of Hospital Sant Joan de Deu (Registry n° PIC-101-14) and Hospital Clínic (Registry n° HCB/2014/0598). All patients included signed a written informed consent before enrollment.

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Tables

Table 1. Maternal and perinatal characteristics of Generation 0 (G0) according to SGA status of Generation 1 (G1).

Characteristics	G1-AGA (n= 80)	G1-SGA (n= 72)	p-value*
G0 Maternal pre-pregnancy characteristics			
Age, *years*	27 (24 – 30)	25 (22 – 28)	0.01
Low socio-economic status	3 (3.8)	8 (11.1)	0.08
University education	8 (10.3)	6 (8.3)	0.7
Height, *m*	1.59 ± 0.05	1.55 ± 0.06	0.0001
Weight, *kg*	56 (53 – 62)	54 (50 – 59.5)	0.1
Body mass index, kg/m^2	22.8 (20.8 – 24.4)	22.2 (20.4 – 24.3)	0.7
Smoking habit	2 (2.5)	9 (12.5)	0.02
Chronic hypertension	2 (2.5)	2 (2.8)	0.9
Diabetes Mellitus	0	1 (1.4)	0.3
G0 Pregnancy characteristics			
Nulliparity	31 (40.8)	51 (70.8)	<0.0001
Gestational diabetes	0	0	NA
Preeclampsia	1 (1.3)	2 (2.8)	0.5
Gestational hypertension	0	2 (2.8)	0.1
G0 Perinatal characteristics			
Induction of labor	3 (4.0)	6 (8.3)	0.5
Labor – delivery interval, *min*	260 (190 – 355)	320 (230 – 420)	0.2
Gestational age at birth, *weeks*	40.0 (39.0 – 40.9)	40.2 (39.3 – 41.1)	0.2
Cesarean section	6 (8.2)	11 (15.3)	0.19
Placental weight, *g*	600 (530 – 660)	470 (430 – 512)	<0.0001
Birthweight of G1, *g*	3,400 (3,190 – 3,525)	2,625 (2,445 – 2,710)	<0.0001
Birthweight percentile of G1	52.9 ± 18.9	3.4 ± 2.4	<0.0001
Male gender of G1	48 (60.0)	33 (45.8)	0.08

AGA= adequate for gestational age; SGA= small for gestational age.

Continuous variables expressed as mean ± standard deviation or median (interquartile range). Categorical variables expressed as n (%).

* p-value from chi2 or Fisher´s exact test was used to compare proportions, t-student to compare means and Mann-Whitney U to compare medians

Table 2. Current adult demographic and medical characteristics of Generation 1 (G1) according to SGA condition at birth.

Characteristics	G1-AGA (n=80)	G1-SGA (n=72)	p-value**
Male gender, *%*	46 (57.5)	32 (44.4)	0.11
Age, *years*	35.5 (33.0 – 37.4)	34.7 (32.2 – 37.4)	0.39
Low socio-economic status*, *%*	4 (5.6)	10 (15.6)	0.09
University education, *%*	47 (58.8)	18 (25)	<0.001
Height, *m*	1.71 ± 0.08	1.63 ± 0.09	<0.001
Weight, *kg*	73.6 (62.2 – 84.5)	65.9 (56.9 – 79.7)	0.047
Body mass index, kg/m^2	24.9 (22.1 – 26.9)	24.6 (21.7 – 28.7)	0.78
Smoking habit, *%*	20 (25)	29 (40.3)	0.04
Sedentarism, *%*	9 (12)	16 (24.2)	0.07

AGA= appropriate growth for gestational age; SGA= small for gestational age.
*Monthly income ≤ 750 euros.
Categorical data expressed as n (%). Continuous data expressed as mean ± standard deviation or median (interquartile range).
** p-value from chi2 or Fisher´s exact test was used to compare proportions, t-student to compare means and Mann-Whitney U to compare medians

Table 3. Perinatal characteristics of Generation 2 (G2), according to SGA status in Generation 1 (G1).

Characteristics	G1-AGA (n=80)	G1-SGA (n=72)	p-value**
Any offspring			
Gestational age at birth, *weeks*	39.5 (38 – 40)	40 (38 – 40)	0.001
Birthweight, *g*	3200 (2900 – 3500)	2950 (2680 – 3275)	0.51
Birthweight percentile	43 (19 – 75)	26 (7 – 52)	<0.001
Small for gestational age, *%*	13 (16.3)	29 (40.3)	0.001
Preeclampsia or GH, *%*	1 (1.25)	7 (9.72)	0.03
Placental mediated disease*, *%*	14 (17.5)	31 (43.1)	0.001
First offspring			
Gestational age at birth, *weeks*	39.5 (38 – 40)	40 (38 – 40)	0.5
Birthweight, *g*	3200 (2830 – 3465)	2950 (2570 – 3288)	0.009
Birthweight percentile	38 (19 – 65)	18 (5 – 50)	0.004
Small for gestational age, *%*	10 (13.0)	24 (34.8)	0.02
Preeclampsia or GH, *%*	1 (1.25)	6 (8.3)	0.05
Placental mediated disease*, *%*	11 (12.6)	25 (33.3)	0.002
Second offspring			
Gestational age at birth, *weeks*	39.5 (38 – 40)	40 (38 – 40)	0.89
Birthweight, *g*	3291 ± 87.1	3048 ± 88.2	0.05
Birthweight percentile	70 (22 – 90)	27.5 (7 – 55)	0.02
Small for gestational age, *%*	3 (10)	9 (28.1)	0.07
Preeclampsia or GH, *%*	0	1 (1.03)	0.33
Placental mediated disease*, *%*	3 (10)	10 (31.3)	0.04

AGA= appropriate growth for gestational age; SGA= small for gestational age; GH= gestational hypertension.
*Placental mediated disease defined as the presence of SGA, preeclampsia or placental abruption
Categorical data expressed as n (%). Continuous data expressed as mean ± standard deviation or median (interquartile range).
** p-value from chi2 or Fisher´s exact test was used to compare proportions, t-student to compare means and Mann-Whitney U to compare medians

Table 4. Odds ratios (95% confidence interval) for small for gestational age and placental mediated diseases in a subsequent pregnancy.

	Any offspring	First offspring	Second offspring
Small for gestational age			
Odds Ratio	3.47 (1.62 – 7.43)	3.57 (1.56 – 8.18)	3.52 (0.85 – 14.57)
Adjusted Odds Ratio*	2.79 (1.11 – 6.97)	2.90 (1.06 – 7.91)	2.80 (0.37 – 21.12)
Placental-mediated diseases			
Odds Ratio	3.56 (1.69 – 7.50)	3.33 (1.49 – 7.47)	4.13 (1.09 – 15.69)
Adjusted Odds Ratio*	3.15 (1.26 – 7.91)	3.19 (1.14 – 8.94)	2.79 (0.60 – 12.95)

*Adjusted Odds Ratio by G1 gender, salary, educational level, body surface area and smoking status, obtained by univariate and multiple logistic regression.

Figure legends

Figure 1. Flowchart of recruitment.

AGA= adequate for gestational age; SGA= small for gestational age; G0= generation 0; G1= generation 1; G2= generation 2.

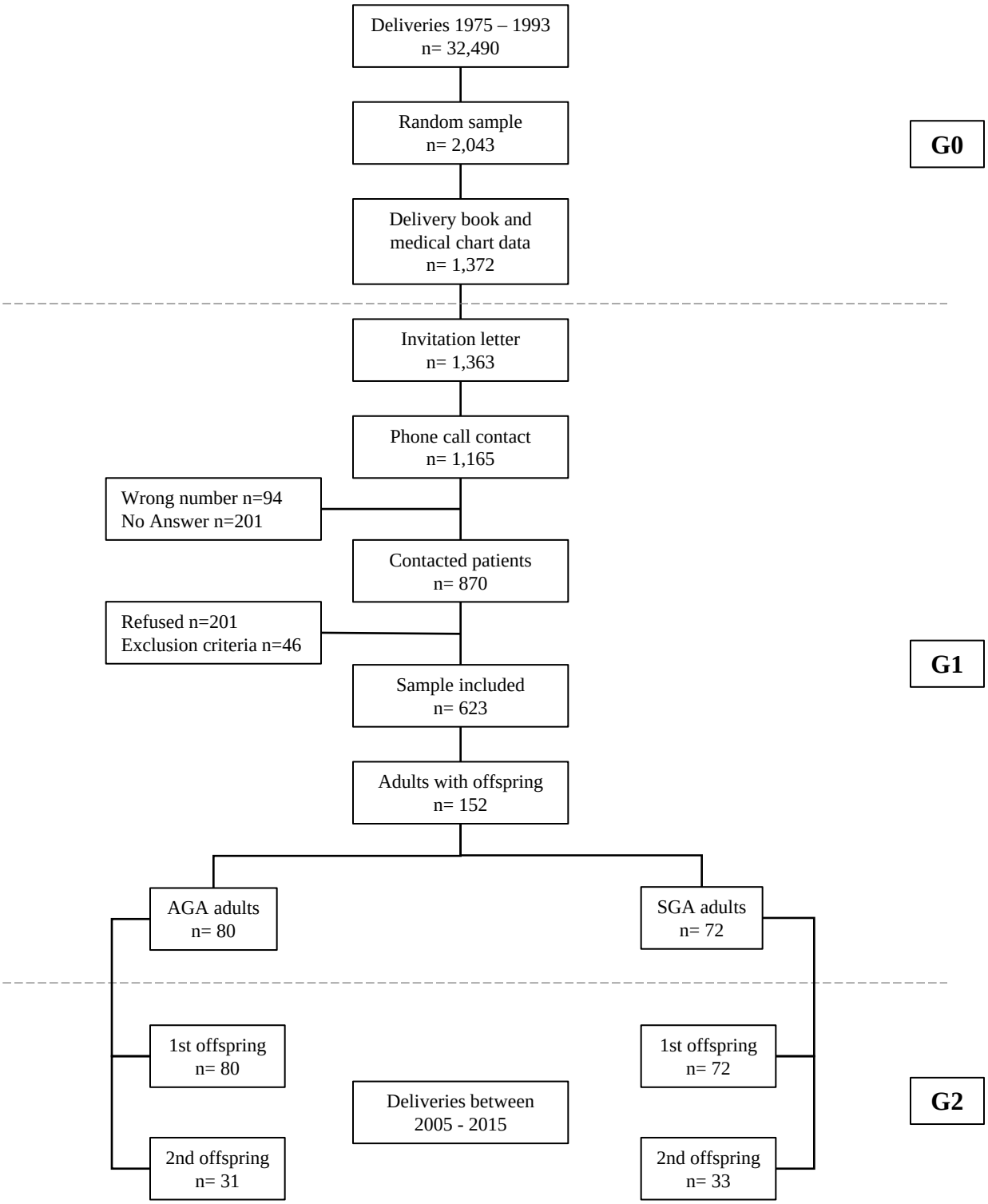
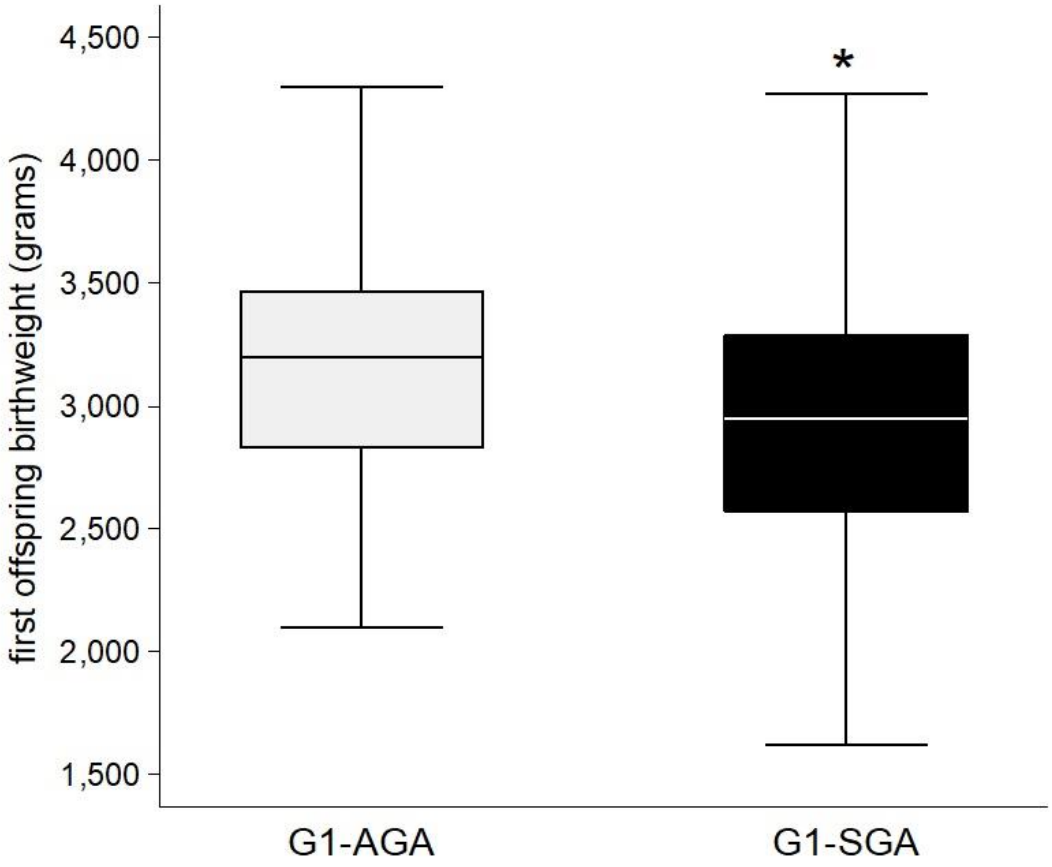


Figure 2. First offspring´s birth weight from AGA and SGA parents.

*p<0.01



AGA= adequate for gestational age; SGA= small for gestational age.

Figure 3. First offspring´s birth weight from AGA and SGA parents, according to parental gender.

*p<0.005.

AGA= adequate for gestational age; SGA= small for gestational age.

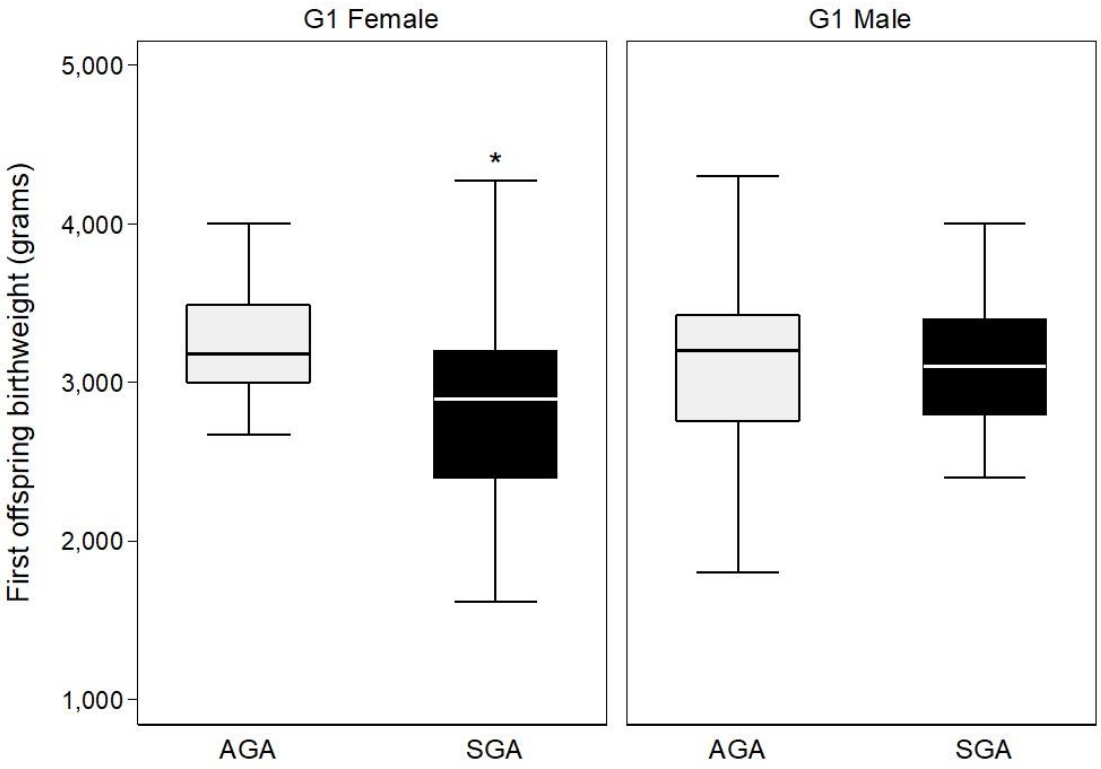


Figure 4. Rate of placental mediated diseases based on parental gender and SGA status of G1.

AGA= adequate for gestational age; SGA= small for gestational age.

*p=0.01; **p=0.05