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RESEARCH ARTICLE

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Second generation of the 1993 birth cohort, Pelotas (Brazil): Aims, design, preliminary results

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Abstract

Background and Aims: Longitudinal cohort studies examining different generations can explain how health problems can be transmitted through genetic and environmental mechanisms and their effects on the health of offspring. This study aimed to present the design and to describe the characteristics of the baseline sample of a second generation cohort.

Methods: The 93Cohort-II is a dynamic prospective cohort composed of a second generation from the original 1993 Pelotas Birth Cohort (offspring), whose parents had their last follow-up at 22 years old. Biological parents were asked to answer questions addressing the type of birth, general health status, family composition, dietary habits, breastfeeding habits, and child-caregiver(s), among others, and the children's anthropometric measurements were evaluated.

Results: Of 1650 children identified, 1212 were evaluated (response rate, 73.4%), and 21 died before the baseline assessment. The age of the offspring ranged from 0 to 10 years (mean [±SD], 2.9 ± 2.1 years); most children (65.6%) lived with both parents and were born to young mothers and poor families. One-third of the children were breastfed until 6 months of age, one-half were born by cesarean section, 63.9% had used medication in the previous 15 days, 26.4% experienced hospitalization at least once since birth, and 14% had no updated vaccination; asthma/bronchitis (20.4%) and bronchiolitis (13.4%) were the most frequently reported diseases. More than 60% consumed ultra-processed foods, and the prevalence of overweight among those <5 and ≥6 years of age was 10.2% and 18.9%, respectively. The mean total Child Behavior Checklist score was 44.1 ± 23.61 (≥16 months), and the mean intellectual quotient score in children ≥6 years of age was 97.9 ± 15.4.

Conclusion: Despite the difficulties in conducting intergenerational cohort studies, the results of the present investigation provide evidence supporting the feasibility of performing these types of studies in middle-income countries.

KEYWORDS

birth, child, cohort, design, intergenerational study

1 | INTRODUCTION

Birth cohort studies have advantages over other study designs because they enable the assessment of the consequences of lifetime exposures and parallel events.[1] Thus, critical events and experiences early in life can be identified as cumulative effects of specific exposures, and their associations with future health can be explored over time.[1] Several birth cohort studies have been performed worldwide, including the Avon Longitudinal Study of Parents and Children (ALSPAC) in the United Kingdom, Birth to Twenty in South Africa, and the Dunedin Multidisciplinary Health and Development Study in New Zealand.[2-4] The next generation of the original cohort has been followed in some of these cohorts, including ALSPAC,[5] the Dunedin Multidisciplinary Health and Development,[6] and The 1970 British Cohort Study, which followed individuals born in England, Scotland, and Wales.[7] Thus, the data collected allow the exploration of the intergenerational nature of health.[8,9]

In Brazil, a middle-income country, some birth cohorts (eg, Ribeirão Preto and São Luís) have been systematically monitored over time.[10] Among the four birth cohorts from the city of Pelotas (birth years, 1982, 1993, 1994, and 2015), only the 1993 cohort study evaluated the children of the original cohort (named in the manuscript as "the second generation"). The original 1993 birth cohort study included all children born in Pelotas and had the initial objective of assessing maternal and child health indicators and to compare them with the results of a previous cohort (the 1982 cohort) in the same city. Subsequently, it has investigated associations between variables of birth and early years of life and later results, with a particular emphasis on the detection of critical windows and on the social determinants of health, since there were children from every social stratum.[11]

The purpose of this article is to present the second generation of the 1993 Pelotas Birth Cohort (93Cohort-II), in which data from biological parents and children were collected. Some studies performed in high-income countries have shown, among other results, that the lifestyle and health of children are largely influenced by maternal and paternal habits.[12] For example, babies born to overweight/obese mothers exhibit a higher percentage of fat mass than do the children of mothers with normal body mass index (BMI).[13]

Information from this baseline and the new expected follow-ups for the 93Cohort-II will enable us to assess, in the future, associations in the intergenerational process throughout life and the possible interaction of human and socioeconomic capital and cultural factors with later outcomes including health conditions and behaviors in a middle-income country, whose health inequities persist.[14]

2 | COHORT DESCRIPTION

The 93Cohort-II is a study linked to the 1993 Birth Cohort. From January 1 to December 31, 1993, all maternity wards in Pelotas (Rio Grande do Sul, Brazil) were visited, and mothers living in urban areas of the city were eligible to participate in the cohort study. A total of 5265 women were identified and 16 refused to participate; thus, the 1993 Birth Cohort (referred to as "original cohort" in this manuscript) comprised 5249 individuals (Figure 1); all women from the original cohort were surveyed about their own health and that of their children, and anthropometric measurement data were collected by trained interviewers (Victora et al 2008).[15,16] Over the years, follow-up with a subsample was conducted at 1, 3, and 6 months, and at 1, 4, 6, 9, and 12-13 years. When cohort participants reached the age of 11, 15, 18, and 22 years, they were invited to participate in this study. The follow-up rates at these ages were 87.5%, 85.7%, 81.4%, and 76.3%, respectively. The last follow-up was conducted when they were 22 years old.[16] Additional methodological information is available elsewhere (Victora et al 2006).[15,16]

During the 22-year follow-up of the 1993 Birth Cohort, a new study was started in January 2016 and was named 93Cohort-II, which included the children of the 1993 Birth Cohort members that give rise to this new cohort study, which this article will describe. A summary of the main follow-ups of the original cohort, including the 93Cohort-II, is shown in Figure 1. This longitudinal study can be characterized as a dynamic, prospective cohort, in which all the children (offspring) of the original cohort members will be invited to participate, including new children born after the last visit, when a new follow-up from the original cohort will be conducted.

In order for the first follow-up to begin, all the members of the original 1993 Birth Cohort were contacted to update their registration data. They were queried as follows: if they already had a child, the number of children, the age of the children, whether any pregnancies were in progress, if the father or mother of the born child lived with the children, and their name, telephone number, and address. Finally, the participants were also asked who would be best suited to answer questions about the health of the child or children. Further, the participants were informed that their children would be invited to participate in the study within a few months.

After most interviews were conducted with members of the original cohort, fieldwork for the 93Cohort-II was initiated (January 2016). When scheduling each interview, the biological parents of children who did not belong to the cohort (born at another location and/or year; nonmember parents of the original cohort) were also invited to accompany their partner, as they are an important source of information for the triad data.

A total of 1650 children of the participants of the main cohort were identified. Some cohort participants had more than one child, and the aforementioned questions were asked. Of the 1650 children identified, 21 died before 93Cohort-II. Of the remaining 1629 children, 1212 were assessed, corresponding to a response rate of 73.4% (including deaths). Some children (N = 44) were unable to attend the clinic. Thus, trained interviewers visited their homes to administer the questionnaires to their mother and/or father and to perform anthropometric measurements (Table 1). The main reasons for not evaluating 373 children are as follows: father no longer in contact with the child, thus making contact with the team infeasible; no longer residing in the city; parents did not visit the study site or did not respond to invitations via telephone (nonverbal refusal). Interviews with parents and

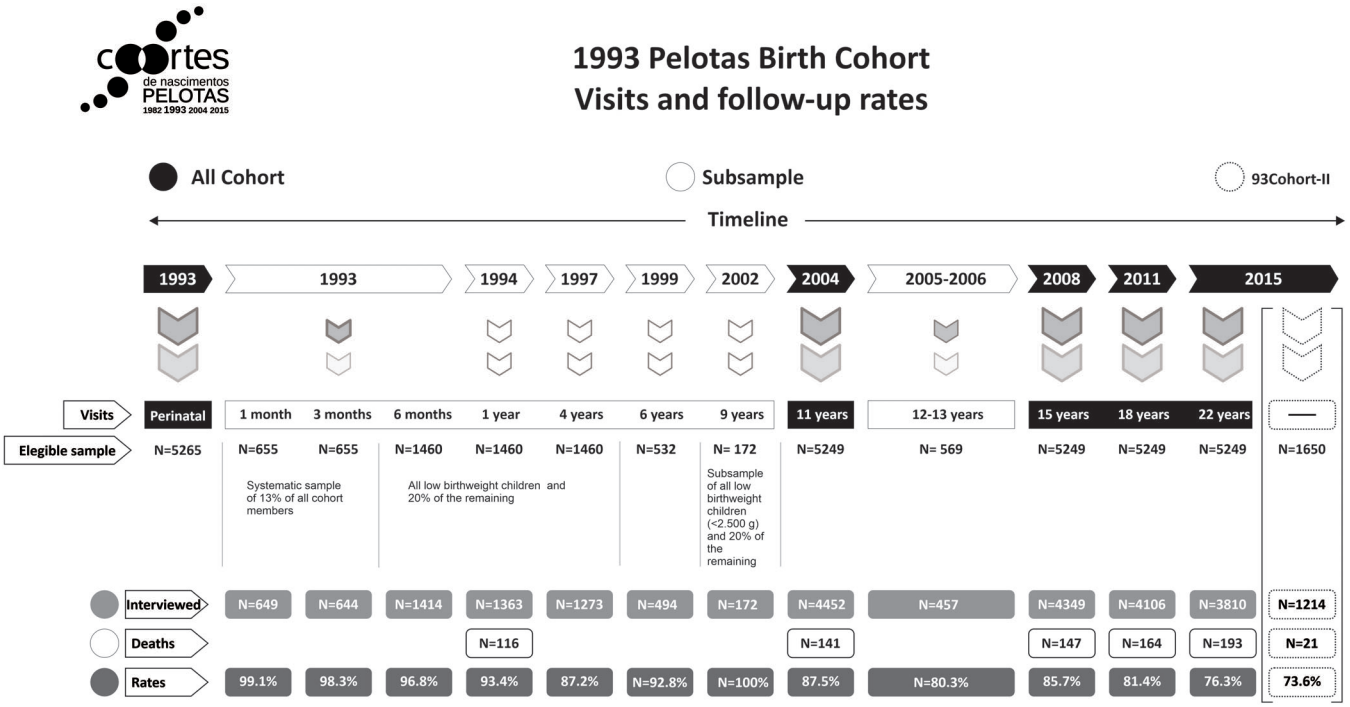

FIGURE 1 The main follow-ups of the 1993 birth cohort study and 93Cohort-II study

anthropometric measurements of the children were recorded at the local cohort study clinic. Prior to data collection, proof of maternity and/or paternity was verified through a child's birth certificate and the identification of the father and/or mother in the 1993 Birth Cohort data. Anthropometric measurements and questionnaires were administered by personnel trained by the researchers responsible for the study. The questionnaire was tested for correcting possible errors or misunderstandings in the questions by a sample that did not include eligible children. Parental questionnaires were also tested on adults who were not part of the study. Thus, at baseline, the following areas were investigated: family composition; parental schooling; substance use before and during pregnancy (for father and mother); childcare; and children's health.

Ethical approval was obtained from the Medical School Ethics Committee of the Federal University of Pelotas, and written informed consent was obtained from the cohort and nonmembers of the original cohort (biologial father or mother) interviewed for 93Cohort-II (N 1.250.366). The baseline study of the 93Cohort-II was funded by the Science and Technology Department, Brazilian Ministry of Health, with resources transferred through the Brazilian National Council for Scientific and Technological Development (CNPq).

2.1 | Data collection

This section contains information on the content of the questionnaires (93Cohort-II questionnaire for mothers/fathers and the children's questionnaire, applied to noncohort mothers and original cohort mothers), measures, tests applied, and biological sample collected at the baseline with the biological parents (members or nonmembers of the original cohort) and their offspring.

All instruments were developed by the research team and previously tested with individuals with age ranging from 21 to 25 years. The specific content of the questionnaires and the data of the interviewed individuals are summarized in Table 1.

2.2 | Interviews (parents)

Parents from the original cohort answered questions about their children during the 22-year follow-up. In the 93Cohort-II baseline, other questions on the following aspects were included: day care and school education, health problems and disease diagnosis, medication use, and hospitalization.

Nonmember parents (only biological) of the original cohort were invited to the study clinic and an instrument was applied on family composition, skin color, parent's schooling, number of children, tobacco, and alcohol (consumption before during pregnancy).

In order to be able to compare the data for children at the baseline, nonmember biological mothers of the original cohort also answered the questions answered by mothers who are members. Mothers were identified as those who spent the most time with children during the telephone scheduling process; therefore, their instrument included data on the following: child feeding and vaccination, breastfeeding, food consumption, and eating habits.

TABLE 1 Themes investigated and measures taken at the first follow-up 93Cohort-II (second generation)

Instruments (questionnaires) and measures (physical exams)	Parents			
	Father		Mother	
	Biologic not member of the 1993 birth cohort	Member of the 1993 birth cohort	Biological or social not member of the 1993 birth cohort	Member of the 1993 birth cohort
Mother/Father questionnaire—Cohort 93-II				
Family composition Schooling of parents Tobacco and alcohol (consumption before during pregnancy) Vaccination Child health (problems of health, use medicine, hospitalization) Child eating habits Child behavior checklist (CBCL)	X		X	
22 years questionnaire (1993 birth cohort)				
School achievement; employment and salary; family composition; family income; bone fractures; self-perceived health; self-perception of oral health; body image; injuries; nicotine consumption including cigarette smoking; alcohol intake; physical activity; morbidity history and hospitalizations; use of health services and of medicines; offspring (date of birth, birthweight, breastfeeding duration, delivery, number of children); marital status; assessment of relationship with partner; head injury; restless legs syndrome; sleep problems and drowsiness; parental morbidity and mortality; body pain; headaches; use of social media; wheezing and asthma; health insurance; common mental disorders (Self-Reporting Questionnaire, SRQ-20) Confidential questionnaire: Stressful life events; violence (suffered and perpetrated); illicit drug use; use of contraceptive methods; history of abortions; number of sexual partners Self-reported food frequency; facial expression recognition task (Brazilian version) Psychological interview (test) Mini International Neuropsychiatric Interview (M.I.N.I.): major depressive episode, suicide attempt, bipolar, social phobia, generalized anxiety disorder, attention-deficit/hyperactivity disorder, post-traumatic stress, antisocial personality; Digit Span (subtest WAIS-III); DSM-5 Self-Rated Level 1 Cross-Cutting Symptom; Snaith-Hamilton Pleasure Scale; Center for Epidemiologic Studies Depression Scale (CESD-R); Well-being (Warwick-Edinburgh Mental Well-being Scale, WEMBWS) Physical examinations: Weight, height, BMI, sitting height; waist circumference; whole-body three-dimensional photonic scanning (3D photonic scanner); blood pressure; blood samples; aortic pulse wave velocity, dynamometry, accelerometry; spirometry; diffusing capacity of the lungs for carbon monoxide; dual-energy X-ray absorptiometry (DXA Lunar Prodigy): fat mass, free fat mass, bone density and content; air-displacement plethysmography (BodPod): fat mass, lean mass		X		X
Child questionnaire—Cohort 93-II				
Residents of the house and age people who take care of the child; who are they and the care time; attend day care or school and the shift self-reported health problem; health problem diagnosed by doctor; drug use in the last 15 years; hospitalization; motive in the last year if you were vaccinated; breast feeding; usual food			X	X

For biological fathers who were nonmembers of the original cohort, we asked questions on age, education, work, if they had/did not have contact with the child, skin color, health problems, and alcohol and tobacco consumption before and during pregnancy. In 17 cases, the father or adult responsible answered questions about the child.

The children's mental health assessment was performed by applying the CBCL questionnaire (pre-school and school versions) for mothers of children ≥16 months. The test identifies behavioral problems. The two versions used were validated in Brazil and translated into Portuguese.[17-19]

2.3 | Interviews (children)

Only the cube and vocabulary subtests of the Wechsler Child Intelligence Scale, fourth Edition (WISC-IV) were applied when children were ≥6 years old.[20] This scale assesses cognitive development according to IQ, measuring intellectual capacity and problem-solving abilities. Trained professional psychologists applied the WISC-IV test.[21]

2.4 | Physical examinations (children)

Saliva samples were collected, and anthropometric measurements were recorded during the children's visit to the clinic (Figure 2). To assess BMI-for-age in the children, weight and height measurements were recorded; children ≤5 years of age underwent measurement of head circumference (cephalic perimeter [occipital-frontal]). Head circumference of younger children was measured due to a Zika virus outbreak in Brazil.

For children >5 years of age, a more accurate examination of body composition was performed using air-displacement plethysmography (BodPod Gold Standard, COSMED, Rome, Italy). In this technique, the volume of a body is measured indirectly (volume of air it displaces inside an enclosed chamber—plethysmograph).[22] In children <25 months of age, weight and length were measured by professionals trained according to the techniques described by Lohman et al[23] and standardized by Habicht,[24] according to the World Health Organization. Weight was measured using a balance scale (Tanita, Arlington Heights, Illinois) with the mother being weighed alone initially and then with the child; the weight of the child was calculated as the difference between the two weights. Length (height) was measured using an infantometer to an accuracy of 0.1 cm.

BodPod assessment of body composition was performed in children ≥61 months only; this method is not recommended in younger children because they move during the examination, which can lead to errors in body density measurement.[25] Weight and height measured according to the techniques described by Lohman and standardized by Habicht. Height was measured using a horizontal aluminum infantometer, which was designed especially for this study, with an accuracy of 0.1 cm.

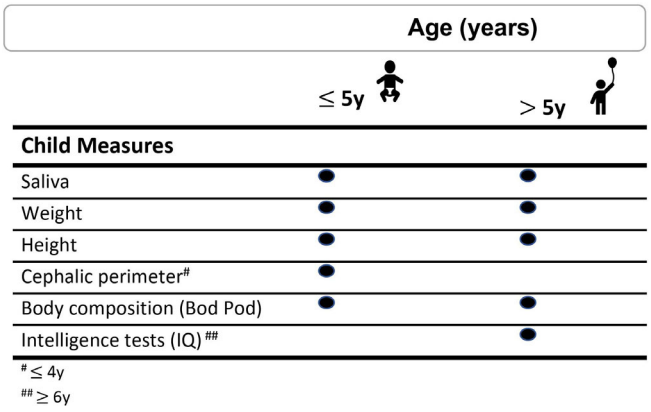

FIGURE 2 Measures taken at the first follow-up 93Cohort-II (second generation)

2.5 | DNA (parents and children)

For cohort member parents, DNA was isolated from blood samples collected by venipuncture using vacutainer tubes (Vacutainer, BD, Franklin Lakes, New Jersey), as done during the 18-year follow-up.[15] DNA extraction was performed using the salting-out procedure according to a modified protocol described by Miler et al.[26] The DNA samples will be used for the analysis of polymorphisms for some morbidities and will be stored for future analysis. The samples may also be used for epigenetic analyses. Saliva samples were only collected from participants who had a phobia of needles or the sight of blood.

For biological nonmember parents of the original cohort and children ≥6 years of age, DNA was isolated from saliva samples that were collected using a commercially available saliva collection kit (Oragene, OG-500, DNA Genotek Inc, Kanata, Ontario). In the case of children <5 years of age or those who were unable to provide saliva samples, the same collection device was used, and the swab technique was employed to improve the quantity of buccal cell collection. DNA extraction was performed according to manufacturer's instructions.

DNA samples were eluted in TE buffer and quantified using spectrophotometry. A total of 1162 DNA samples from the children and 754 from biological parents not belonging to the original cohort were collected.

2.6 | Statistical analysis

Descriptive statistics were used to describe some characteristics of the 93Cohort-II children at baseline. Variables had a normal distribution; continuous variables were expressed as mean ± SD, and categorical variables were expressed as absolute and relative frequencies. Statistical analysis was performed using Stata version 13.2 (StataCorp, College Station, Texas).

3 | PRELIMINARY RESULTS

Children's baseline characteristics of the 93Cohort-II are summarized in Table 2. It is important to note that the results showed here

TABLE 2 Child characteristics in the first follow-up of the 93Cohort-II (second generation), Pelotas, 2016 (*N* = 1212)

Variable	*N* (%)
Sex	
Male	648 (53.5)
Female	564 (46.5)
Delivery	
Vaginal birth	564 (46.6)
Cesarean	646 (53.4)
Multiple birth	
Single birth	1204 (99.3)
Twin birth	8 (0.7)
Maternal age at birth	
<18	310 (26.0)
18-24.9	856 (71.7)
25-29.0	22 (1.8)
≥30	6 (0.5)
Birthweight (grams)	
<2500	133 (12.1)
2500-2999	231 (21.0)
3000-3499	394 (35.7)
≥3500	344 (31.2)
Feeding	
Never breastfed	143 (11.8)
Exclusive breast feeding in the first 6 months[a]	334 (29.0)
Age (years)	
<1	258 (21.3)
≥1 to 2.9	418 (34.5)
≥3 to 4.9	317 (26.1)
≥5	219 (18.1)
Family composition	
Lives with both parents	795 (65.6)
Lives only with mother	376 (31.0)
Lives only with father	17 (1.4)
Shared custody	11 (0.9)
Family asset index (quintiles)[b]	
First (poorest)	469 (38.7)
Second	291 (24.0)
Third	245 (20.2)
Fourth	146 (12.1)
Fifth (richest)	61 (5.0)
Nutritional status (*z*-scores BMI-for-age)	
<5 years	
Healthy weight (−2 to +2 SD)	838 (89.8)
Overweight (>+2 SD)	95 (10.2)
≥5 years	
Eutrophic (−2 to +1 SD)	112 (62.2)
Overweight (>+1 to ≤+2 SD)	34 (18.9)
Obesity (>+2 SD)	34 (18.9)
%Fat mass (N—mean)	180 (16.8)
5-5.9 years	82 (15.2)
≥6 years	98 (18.2)
Ultra-processed foods consumption[c]	
Soft drinks	699 (63.3)
Snacks (*chips*)	706 (74.6)
Nuggets, hamburgers, or sausages	685 (72.3)
Sweet cookies	788 (83.2)
Health characteristics	
Medicine use in the last 15 days	434 (36.1)
Any hospitalization after birth	318 (26.4)
Complete vaccination scheme	1035 (85.9)
Asthma or bronchitis[d]	247 (20.4)
Bronchiolitis[d]	162 (13.4)
CBCL total score (≥1 year and 4 months; *N*—mean)	747 (44.1)
Intelligence quotient (≥6 years; *N*—mean)	106 (97.9)

[a]Excluding 55 actual exclusive breast feeding children.
[b]Quintiles generated based on the original cohort sample at 22 years.[27,28]
[c]Habitual consumption, daily (≥1 year-old children).
[d]Health problems in any moment of life.

represent a description of the data collected at the baseline for this study. It is noted that the children and their parents are still very young, a fact that will change in subsequent follow-ups. The age of the children ranged from 0 to 10 years, with a mean (±SD) of 2.9 ± 2.1 years. Regarding family composition, the majority of children (65.6%) lived with both parents. The percentage of cesarean deliveries was 53.4%. Approximately 29% of the children were exclusively breastfed until 6 months of age, and 12% were never breastfed. Regarding the current consumption of ultra-processed foods, >60% regularly consumed soft drinks, snacks, nuggets, hamburgers or sausages, and sweet cookies. The prevalence of overweight in children <5 years (>+2SD) and in children ≥5 years (>+1 to ≤+2SD) was 10.2% and 18.9%, respectively. The mean percentage fat mass was 16.8% (95% confidence interval [CI] 15.5-18.1). No children were underweight. Thirty-six percent of the children had used some type of medication in the previous 15 days, 26.4% had been hospitalized at least once since birth (hospitalized <12 months of age, 24.6%; hospitalized ≥12 months of age, 26.5%; with the vast majority with respiratory diseases, such as asthma and bronchitis), and 14% did not have their vaccination schedule up to date. The most frequent diseases in children as reported by the parents, at any time during their life, were asthma or bronchitis (20.4%) and bronchiolitis (13.4%). Regarding behavioral problems (CBCL), for children aged ≥1 year and 4 months to 5 years (*N* = 747), the mean of the total score was 44.1 (normal; SD = 23.5). Intelligence quotient (IQ,

≥6 years) was evaluated in 106 children, who presented a mean score of 97.9 (average IQ; SD = 15.4). The score showed a normal distribution.

Future follow-up of 93Cohort-II will enable the analyses of intergenerational transmission of health problems.[29,30] It is important to integrate life-course information as possible precursors to complex chronic diseases and other outcomes among participants and their parents, whose genetic data are available for the biological triad. In addition, more recent sociocultural and family changes in Brazil, including the reduced number of offspring and an increase in schooling, raise new research questions about how these variables influence the health of recent offspring.[31-33]

In its first assessment, the 93Cohort-II study obtained a high response rate (73.4%). It should also be noted that because we did not evaluate these children at birth, some of the information may be subject to recall bias.

As a dynamic, prospective cohort, new participants were eligible for inclusion during the entire study period. This will improve statistical power due to an increase in sample size and is a strength of this study. The high response rates achieved for years in the original cohort, due to a great effort to search for its members, may allow for the success of new follow-ups and the quality of data for 93 Cohort-II.

4 | DISCUSSION AND FUTURE PLANS

The most significant contribution of the present study is the collection of data regarding physical and mental health in more than one generation of the same families. Future monitoring of the second generation will enable us to analyze the intergenerational transmission of health problems.[29,30] We will be able, with the data of the future follow-ups, to understand the consequences for the child's health, comparing the baseline data (mothers, fathers, and children), and the follow-up that will come. Having data collected to analyze the genetic information of the biological father-mother-child triad is important for understanding the genetics and epigenetics of health problems (eg, diseases and/or symptoms) and their consequences, allowing access to information from parents and children, which is rare in middle-income countries such as Brazil. Changes in the country's economy and others, such as the increase in schooling, raise research questions, for example, how variables like these influence the life of the father-mother-child and, especially, the health since the children's childhood.[31,33]

We hope to perform a future follow-up of the original cohort at the age of 30 years, reevaluate their children (93Cohort-II) during this follow-up. At each new follow-up, children not found in the baseline or born thereafter will be eligible for inclusion in the study, and this will enable new analyses at different age stages. The fact that all the children will be of different ages is a challenge in this study; it means that data collection will require the use of appropriate development tools, and additional analytical work is required to standardize data according to age groups and, if possible, compare them.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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All authors have read and approved the final version of the manuscript.

Helen Gonçalves had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

The authors affirm that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

DATA AVAILABILITY STATEMENT

The authors confirm that the data are available on request from the authors, that is, the findings of this study are available upon reasonable request.

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