PROJECT TITLE: The Next Generation Cohort: Childhood Outcomes of Offspring of Parents with Early Onset Type 2 Diabetes

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SUMMARY:
The Next Generation Birth Cohort has recruited 127 offspring of parents with youth-onset type 2 diabetes (T2DM)since 2003. Despite the high rate of awareness of the risks of obesity and T2DM in the offspring, 66% are obese at the most recent assessment with an average BMI z-score of 1.9, and 34.4% of the offspring age 10 years and older have T2DM. All of the offspring with T2DM are overweight or obese, all were born to mothers with pre-pregnancy T2DM, and all have 1 or 2 copies of the private HNF-1α G319S polymorphism. The size of the cohort remains too small to make conclusions about the impact of the intrauterine environment and the S319 allele on the risk of T2DM in this highly selected population.

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Introduction

Compared with adults, youth with type 2 diabetes mellitus (T2DM) appear to have a more aggressive disease course with a higher risk of early-onset complications from T2DM.[1] People of indigenous origin are more likely to develop childhood-onset T2DM than children of other backgrounds.[1] Manitoba, a province in central Canada, has the highest incidence of youth-onset T2DM in Canada at 12.45 cases per 100 000.[2] These children in Manitoba are predominantly of Oji-Cree speaking heritage, a distinct First Nations language group residing in Northern remote communities. This population was among the first in the world to experience pediatric-onset T2DM in the 1980's.[3] In Canada, the highest reported prevalence of T2DM in First Nation youth age 4-19 years was 1.1% in an Oji-Cree speaking community in Northeastern Manitoba. [4] M. Mendelson *et. al.* described the Next Generation cohort (NextGen), a group of Oji-Cree speaking children at high risk of youth-onset T2DM because they were exposed to maternal T2DM throughout gestation. In the Next Generation cohort, it was noted that 43% of these children born to mothers with T2DM developed T2DM between the ages of 10 and 19 years.[3] This report will expand on the report by M. Mendelson *et. al.*, adding 3 more years of surveillance and new offspring in the cohort. In addition, this is the first time the outcomes of children of fathers have been compared to the outcomes of children of mothers in this cohort, in an attempt to understand the impact of the maternal intrauterine environment on the risk of obesity and T2DM in the children.

In addition to the high rates of obesity and lack of physical activity [3, 5-6], the increased susceptibility of the Oji-Cree speaking youth to T2DM is partly attributed to the private glycine to serine substitution at codon 319 of the hepatic nuclear transcription factor-1α gene (HNF-1α G319S).[7-8] The S319 allele increases the risk of developing diabetes because it causes an insulin secretory deficiency, thus preventing the ability of these children to mount a physiologic hyperinsulinemic response to overcome insulin resistance.[7] Accordingly, at diagnosis there is a dose-dependent decrease in age of T2DM onset, decrease in body mass index (BMI), and a reduced presence of acanthosis nigricans with each copy of the S allele.[7-8] The youth with T2DM with the S319 allele (either G/S or S/S genotype) also have lower insulin levels, and higher mean haemoglobin A1C than youth with T2DM but without the S319 allele (G/G genotype).[7]

There is evidence to suggest that exposure to an intrauterine environment complicated by maternal T2DM increases the risk for developing T2DM in the child, possibly due to influences on the early expression of genes involved in insulin secretion and beta cell development. Being born to a mother with pre-existing T2DM has life-long consequences including higher rates of obesity, higher insulin levels, greater pancreatic dysfunction, higher rates of glucose intolerance, higher rates of T2DM, and earlier age of T2DM onset.[9-13] In the Pima Indian community of Arizona, the rapid increase in prevalence of youth-onset T2DM over the past 30 years was mostly attributed to an increase in weight and an increasing frequency of exposure to T2DM *in utero.* In addition, offspring of mothers with diabetes were much more likely to develop T2DM (45%) than those born to non-diabetic mothers (1.4%) and pre-diabetic mothers (8.6%).[11, 14] Furthermore, siblings born after the mothers' diagnosis of T2DM in this Pima population had a 3.7-fold higher risk of developing T2DM than siblings born before T2DM diagnosis in the same mother.[15] In the Manitoba Oji-Cree speaking population, pre-existent T2DM in the mother was the strongest predictor of T2DM in the child (OR = 14.4), followed by gestational diabetes (OR = 4.4).[16] In the Next Generation cohort from 2003-2008, 25% of those children eligible

for blood glucose screening (> 7 years old) had T2DM and 43% of those >10 years old had T2DM.[3] Every child with diabetes in this cohort had at least 1 copy of the HNF-1α polymorphism, therefore it is difficult to know if this was a function of HNF-1α or gestational T2DM.[3] It is unknown how much of an impact exposure to T2DM *in utero* has on the children of this population as the outcomes of children born to mothers with pre-pregnancy T2DM have never been compared to the outcomes of children born to fathers with pre-pregnancy T2DM.

This report has four aims. Firstly, to describe the feasibility of conducting a longitudinal birth cohort surveillance program in a group of offspring of parents with T2DM before pregnancy, termed the Next Generation Cohort (NextGen), and to expand on the findings of Mendelson *et.al.* regarding the description of this unique cohort. Secondly, whether associations and altering factors; such as breastfeeding, smoking, birth weight, and congenital anomalies; demonstrated in other studies of T2DM have a similar effect in this cohort. Thirdly, to isolate the effect of the intrauterine environment on weight status and risk of developing T2DM in the children, by comparing the outcomes of children born to mothers with the outcomes of children born to fathers. Lastly, whether the HNF-1α G319S polymorphism has a similar effect on T2DM risk in this cohort who also possesses intrauterine T2DM exposure as a risk factor for T2DM. Results from this study will be used to inform intervention studies in the children born to mothers with pre-existing T2DM, and intervention studies in the pregnant mothers with T2DM themselves.

Methods

1. Population

The NextGen cohort includes children born to parents who had T2DM diagnosed in adolescence (age < 18 years) and reside in Manitoba. These parents received medical surveillance as children and adolescents at the Diabetes Education Resource for Children and Adolescents (DER-CA), a regional comprehensive and integrated multidisciplinary pediatric diabetes team located at the Winnipeg Children's Hospital in Winnipeg, Manitoba, Canada. Parents were identified either because they were expecting a child and still seen at the DER-CA, they were identified through community health care workers as being former DER-CA graduates who were expecting or had a child, or they were contacted through the Maestro Project and discovered to be expecting or had a child. The Maestro project is a transition service designed to help recent DER-CA graduates to maintain contact with adult T2DM services in the community. A database is kept on these graduates and annual follow up is performed by telephone to determine their needs for surveillance. Children in NextGen originate from First Nations communities throughout Manitoba, however the majority (59.8%) are from one Oji-Cree Tribal Council in Northeastern Manitoba. In 2003, only mothers were contacted to recruit their children into NextGen. In 2010 and 2011, fathers were contacted to identify their offspring and to determine eligibility of the offspring to be included in NextGen. In 2010, the number of male graduates of DER-CA was high enough to justify the intense search involved in seeking this group out to identify their children.

2. Clinical Assessments

The offspring of graduates from the DER-CA were recruited by telephone, written parental consent was obtained and clinical assessments were arranged. Parent-reported information was collected regarding birth weight, gestational age, breastfeeding history, smoking history during pregnancy, parental T2DM status, presence of congenital anomalies and family history of T2DM in first degree relatives. The parents were then contacted annually by telephone to arrange the offsprings' annual assessments. The assessments were arranged with the family's local family physician, with the DER-CA if the child lived in Winnipeg, or in the nursing station or health center of the First Nations community. The annual anthropometric assessments of the offspring included weight, height, waist circumference, blood pressure, and the presence of acanthosis nigricans for offspring aged 1-18 years. For the offspring 7 years and older, the assessments included a one-time blood draw for HNF-1α G319S genotyping followed by annual assessments of blood glucose (BG) preferably by venipuncture. Blood for genetic testing was collected either onsite at DER-CA or in the community and shipped to the Molecular Genetics Laboratory at the Health Sciences Centre in Winnipeg, Manitoba. Biochemical screening using fasting BG for T2DM is recommended by the Canadian Diabetes Association (CDA) for children above the age of 10 years old with 2 or more risk factors for T2DM. [17] Biochemical confirmation of T2DM was based on CDA criteria.[17] We chose to screen for T2DM as young as 7 years old because 11% of First Nations children in Manitoba present with T2DM before the age of 10 years.[2] Some children received biochemical assessment as young as 6.7 years old as it was decided that at this age waiting a full year before biochemical assessment could be done would be irresponsible in some of the highest risk children.

3. Ethics and Knowledge Translation

This study protocol has received annual approval since 2003 by the Research Ethics Board at the University of Manitoba in accordance with the Declaration of Helsinki. In planning the original protocol, broad consultation was achieved with the Northern Medical Unit, the Centre for Aboriginal Health Research at the University of Manitoba, and the Manitoba First Nations Diabetes Committee of the Assembly of Manitoba Chiefs. Annual reports were distributed to the partner organizations and to the communities using public media and written copies. Critical results were communicated directly to the parents, the pediatric endocrinologists at the DER-CA, to the community nursing stations and health centers, and to the community and family physicians. Biochemical confirmation of T2DM and medical follow-up was arranged at the DER-CA.

4. Data Analysis

BMI was converted to age and gender standardized BMI z-scores using CDC reference data.[18] Overweight was classified as a BMI between the 85^{th} and 94^{th} percentile, and obesity was defined as a BMI $\geq 95^{th}$ percentile. The mean annual change in BMI z-score (BMI z-score velocity) was calculated by determining the BMI z-score change per year of follow-up for each child who underwent >1 assessment (n=86). Large for gestational age was defined as all those children above the 90^{th} percentile of birth weight based on gestational age, and small for gestational age was defined as children that were below the 10^{th} percentile.[19] Linear data were

reported as mean with standard deviation if the data was parametric or median and range if the data was non-parametric. Categorical data was reported as absolute number with percentage. Comparisons between groups were performed using an unpaired students t-test for linear data, a paired students T-test for paired linear data (BMI z-score and haemoglobin A1C before and after diagnosis with T2DM), a Mann-Whitney-Wilcoxon test for non-parametric linear data, a Chi-squared test utilizing Yates correction to compare allele frequencies, and two-tailed Fischer exact test for the remaining categorical data. Statistical analyses were completed with SPSS Rel. 16. 2007 (SPSS Inc., Chicago, IL, USA).

Results

Demographics

Of the 336 graduates of the pediatric endocrinology clinic with T2DM and participants in Maestro, 180 were of First Nations heritage who live in Manitoba, whose children therefore are eligible to participate in NextGen. Including those participating in Maestro (52 parents), the mothers or expectant mothers still seen in the DER-CA (3 parents), and those identified by other health care workers as being former DER-CA graduates (2 parents), 57 parents were found to have 127 children, all of which agreed to participate in NextGen. Fathers contributed 7 (12.3%) of these parents and 50 (87.7%) were mothers, and they were found to have 15 (11.8%) children and 112 (88.2%) children, respectively. Of the father's offspring, the mean age was 5.2 (1.3-10.3) years and 5 (36%) were males. Of the mother's offspring, the mean age was 7.6 (0.3-22.1) years and 56 (50 %) were males. There was no difference in age ($p=0.08$) or gender (0.28) of offspring of mothers compared to those of fathers. The children of mothers were more likely to be from an urban community, 34/112 (30.4%), as 0 of the 15 children of males were from an urban area ($p=0.02$). The information regarding the recruitment and assessment of the children is represented in figure 1. Information illustrating the age distribution of the children in NextGen by gender of parent and by T2DM status is demonstrated in figure 2.

Recruitment

Recruitment into NextGen has been ongoing from July 2003 until July 2011. Since 2003, 18, 18, 13, 9, 7, 7, 7, 13, and 20 children were enrolled into NextGen in each study year. However, children of fathers have only been recruited since 2010 with enrolment of 10 in 2010 and 5 in 2011. The entire NextGen cohort demonstrates a mean age at recruitment of 3.4 (0.1-13.9) years, and for those children with two or more assessments an average duration of follow up of 4.5 (0.3-11.3) years was seen as well as a mean interval between consecutive assessments of 1.4 (0.0-7.1) years. Two children of one of the fathers had assessments in 2003 but were not followed again until 2010 and were never formally included in the project until 2010. The mean age at enrollment was 4.3 (0.2-9.2) years for children of fathers and 3.3 (0.1-13.9) years for children of mothers, representing no significant difference in age of recruitment between affected parent's gender ($p=0.26$).

Children of fathers had 28 assessments completed representing 14/15 (93.3%) of the eligible children ($\geq$ to 1 years old). One father was approached to recruit a child into NextGen but could not bring the child to be assessed. Of those that received assessments, 14 (93%) had anthropometric measures collected, 4/5 (80%) of those $\geq$ 7 years old had blood glucose screening

and none had HNF-1α G319S genotyping, 4 (27%) had a birth history collected, and 2 (13%) had breastfeeding information. Two or more assessments were performed on 9/14 (64.2%) of the children of fathers, with an average duration of follow up of 3.3 (0.7-10.3) years and a mean interval between consecutive assessments of 2.1 (0.2-7.1) years.

Eligible children of mothers had an assessment completion rate of 95/103 (92.3%), and 77/95 (81.1%) of these children had undergone multiple assessments. For those children of mothers who received multiple assessments, the average duration of follow up was 4.6 (0.3-11.3) years and the mean interval between consecutive assessments was 1.4 (0.0-6.4) years. There was no significant difference between the duration of follow-up between children of mothers and those of fathers (p=0.15), and when removing the two children of fathers that were assessed in 2003 and then left out of follow up until 2010, the mean interval between consecutive assessments is 1.3 (0.2-4.7) years, demonstrating no significant difference (p=0.78). Of the 95 children of mothers that had assessments, 95 (100%) had anthropometric measures collected, 52/60 (86.7%) of those ≥ 7 years old had blood glucose screening and 31/60 (51.7%) had HNF-1α G319S genotyping, 54 (48%) had a birth history collected, and 48 (43%) had breastfeeding information. When comparing male and female children and those from remote communites to those from urban communities, there was no difference between age, age at recruitment, duration of follow up, and mean interval between consecutive assessments. Table 1 summarizes the information on recruitment and assessments of children in NextGen.

Clinical Assessments

Birth History

Of the 127 children in NextGen, 64 (50.4%) had birth history collected, including 62 (96.9%) birthweights, and 64 (100%) gestational ages. In the cohort, 58 (46.5%) had both gestational age and birthweight recorded, therefore 1/59 (1.7%) were small for gestational age (SGA), 30/59 (50.9%) as appropriate for gestational age (AGA), and 27/59 (45.8%) as large for gestational age (LGA). There was no difference between children of mothers and fathers in regards to SGA (p=1.00), AGA (p=0.61), or LGA (p=0.62). Similarly, there was no difference between birthweight categories when comparing boys versus girls and comparing urban versus rural living. See table 1 for information on birthweights.

50/127 (39.4%) of the children had information on breastfeeding and 21/50 (42%) reported to have breastfed their children for any period of time. As reported by the mothers, 15/50 (30%) of these children were breastfed for <3 months, 3/50 (6%) were breastfed for 3-6 months, 3/50 (6%) were breastfed for >6 months. There was no difference between children of mothers and children of fathers (p=0.09), boys and girls (p=0.60), and urban or rural residence (p=0.60).

Smoking during pregnancy occurred in 14/25 (56%) of the children with information on the subject, and 13/28 (46.4%) of the children in NextGen are currently exposed to smoke in the household (either the children smoke themselves or share the home with an individual who smokes). There were differences between residence related to smoking history. Children residing in a remote area were more likely to be exposed to smoking *in utero*, 14/22 (66.3%), than children residing in an urban area, 0/3 (0%), (p=0.04). Also, children residing in a remote area were less likely to be exposed to smoking after birth, 10/25 (40.0%), than children residing in an urban area, 3/3 (100%), (p=0.05).

Lastly, 9 of the 87 children (10.4%) with information of the subject reported having some form of major congenital anomaly with no difference between parental gender, child's gender, or area of residence.

Anthropometric assessment

Anthropometric assessment was performed on 109 (92.4%) of the 118 eligible children (≥1 years old), including 14/15 (93.3%) children of fathers and 95/103 (92.2%) children of mothers. The mean BMI z-score at last assessment was 1.9 (1.0) for all NextGen children, with 13 (13.5%) classified as overweight and 63 (65.6%) classified as obese. Figure 3 demonstrates the trends in overweight and obesity at different ages for every assessment in NextGen since 2003. At the younger ages, the variation in weight status is high and tightens around a BMI z-score of 2.0 at around 6 years old. The difference in BMI z-scores between those children aged ≤6 years and those >6 years nearly reached significance (p=0.07). The mean BMI z-score is approximately 2.0 through the entire age spectrum. For children of fathers the mean BMI z-score at their last assessment was 2.0 (1.0), with 2 (15.4%) and 8 (61.5%) classified as overweight and obese. Children of mothers had a mean BMI z-score at their last assessment of 1.9 (1.0), with 11 (13.3%) and 55 (66.3%) classified as overweight and obese. There was no significant difference (p=0.74) between the BMI z-score of children of fathers and children of mothers. In addition, there was no difference in BMI z-score between boy and girls (1.9 vs. 1.8, p=0.50), nor between children living in a remote setting and children living in an urban setting (1.9 vs. 1.9, p=1.00). For those with ≥2 measures, the BMI z-score velocity in NextGen was 0.0 (-2.7-+0.8). There was no difference (p=0.18) in the mean BMI z-score velocity between children of fathers (-0.6 (-2.7-+0.6)) and children of mothers (0.0 (-1.0-+0.8)), or between boys and girls (-0.1vs. 0.0, p=0.60) or between remote and urban living (0.0 vs. 0.0, p=0.12).

Genetic Assessment

HNF-1α G319S genotyping was performed in 32 (51.6%) of the 62 eligible children (>/= 7 years old) in NextGen. Of the genotyped children, 5 (16.1%) possessed the G/G (wild type) allele, 21(67.7%) possessed the G/S allele (heterozygote), and 6 (18.8%) possessed the S/S allele (homozygote). Therefore, 83.9% of the genotyped children have at least one copy of the G319S allele. Of the 64 alleles sequenced, 33 were S alleles revealing an S allele frequency of 0.516. In the mothers, 33 S alleles were seen in 70 alleles sequenced, resulting in a similar S allele frequency of 0.472 (p=0.74). The 30 (48.4%) eligible children that had not received genotyping failed to receive the test due to limitations in access to the necessary facilities. None declined the test. The children with genotype G/G were compared to those with G/S, G/S was compared to S/S and G/G was compared to the children with at least one S allele (G/S and S/S). As can be seen in table 2, there was no significant differences between the three genotypes in BMI z-score at last assessment and BMI z-score velocity. Although statistically insignificant, there were noticeable trends suggesting that a child with the S/S genotype was more likely to be AGA than children with the G/S genotype (p=0.06), and the G/S genotype was more likely to be associated with LGA than the S/S genotype (p=0.06). Another trend suggested that the diagnosis of T2DM was more likely associated with possessing one S allele than with no S allele (p=0.07). Table 2 summarizes the differences between the 3 HNF-1α G319S genotypes.

Type 2 Diabetes Mellitus

There are currently 13 (10.2%) children with T2DM in NextGen. There were significantly more females (11/13) than males (2/13) (p=0.02). Children with T2DM were not more likely to be offspring of fathers (0/13) than children without T2DM (15/114 (13.2%)) (p=0.17), and accordingly the same can be said regarding children of mothers (13/13 vs. 99/114 (86.8%), p=0.17). There was no difference regarding area of residence. This information is summarized in table 4. The CDA recommends screening children with more than 2 risk factors for T2DM to be screened at 10 years old.[17] In the NextGen cohort 12/35 (34.4%) of those children over 10 years old have been diagnosed with T2DM. Every child diagnosed with T2DM had at least 1 copy of 3 HNF-1α G319S allele (G/S or S/S), while none had the wildtype G/G genotype (p=0.07). The mean A1C at diagnosis was 9.0 (1.7) and at last assessment was 10.3 (2.0), demonstrating no significant difference (p=0.06). The mean BMI z-score at last assessment among those with T2DM was 1.8 (0.6) and this was significantly different than those in the NextGen cohort without T2DM, 1.9 (1.1) (p=0.05). There was also a difference between BMI z-score velocity between these two groups (0.0 vs. -0.1, p=0.05). Interestingly, the difference between BMI z-score at diagnosis (2.1 (0.4)) and BMI z-score at last assessment (1.8 (0.6)) for those children diagnosed with T2DM was significantly (p=0.01). Table 4 summarizes the anthropometric measures for those children with or without T2DM. Acanthosis nigricans was more common in those children with T2DM (p=0.005) and exposure to tobacco smoke sometime after birth was trending to show a significantly higher association with T2DM (p=0.07). Lastly, strong trends were demonstrated in G319S allele status, as the possession of at least one G319S allele was nearly significantly higher (p=0.07) in those children with T2DM (13(100%)) than those without T2DM (14(73.7%)). This difference was also evident in G319S allele frequency as those with T2DM had an allele frequency of 0.654 and those without T2DM had a frequency of 0.421 (p=0.08). See table 4 for this data.

Of the 13 children diagnosed with T2DM, 8 were diagnosed by screening in NextGen (mean duration of follow-up = 5.3 (1.3) years), 1 was diagnosed on the first NextGen assessment, 2 were diagnosed in the community but were eligible for NextGen, and 2 were diagnosed before the NextGen cohort was created. There was no significant difference at diagnosis between those children with T2DM identified by screening in NextGen and those discovered through other ways for age, BMI z-score, and hemoglobin A1C.

Discussion

It has become evident to the researchers that maintaining a birth cohort in this population is feasible. No parent refused to participate, and there were no withdrawals from follow-up. Unfortunately, locating the parents in the Maestro Program was a significant challenge as contact information changes rapidly in these northern remote communities. However, with the expansion of the number of NextGen and Maestro personnel and widening of its contact strategies, recruitment has expanded from 11.3 children/year before 2010 to 24.0 children per year in 2010-2011. The contact strategies were expanded under the guidance of a First Nations cultural liason whose knowledge of key informants and contact media in the communities was indispensible for tracking the NextGen and Maestro participants. The mean age of recruitment was 3.4 years old for the cohort, suggesting that it takes the team a significant amount of time to track down a new child after birth. This time lapse has been reduced to 3.0

years following the NextGen and Maestro expansion in 2010 and 2011. The average interval between follow up for the cohort is 1.4 years, demonstrating that we are successful at contacting the existing NextGen children and arranging their assessments annually. With the aging of the cohort, it is now possible to make observations regarding longitudinal changes in the children as the mean duration of follow-up for the children is 4.5 years.

A number of protective factors, risk factors and associations have been demonstrated in other population in the context of T2DM in children including breastfeeding, smoking, and the presence of major congenital anomalies. Forty-two percent of the children in NextGen were breastfed, which is similar to the 41% rate presented by Mendelson *et.al.* in this cohort from 2003-2008.[3] This rate is lower than a random sample of Manitoban mothers who demonstrated a breastfeeding intiation rate of 91.2%.[20] These findings were particularly disturbing as it has been shown that breastfeeding for at least 2 months protects against developing T2DM in their first 40 years of life in the Pima Indians of Arizona.[21] Sellers *et al* demonstrated in a Manitoban population of Aboriginal mothers that breastfeeding longer than 12 months protected against developing pediatric-onset of T2DM (OR=0.24).[16] However, no protective effect was seen in this cohort (p=0.71). The study by Sellers *et al* demonstrating breastfeeding protection compared Aboriginal children with T2DM to Aboriginal controls and either group may or may not have been exposed to *in utero* T2DM. In this cohort, every child was exposed to *in utero* T2DM and it is therefore possible that this fetal exposure may somehow negate the protective effect of T2DM. Despite this finding, mothers of these children in NextGen, who are at high risk of developing T2DM in childhood, might be considered for information on the protective effects of breastfeeding.

As well, similar counselling regarding a tobacco smoke free environment is a necessity in this population. Exposure to smoke is a risk factor for the development of T2DM.[22] In the NextGen cohort, 56% were exposed to smoking *in utero* and 46% were exposed to smoke after birth. There is a trend in the NextGen cohort demonstrating that children developing T2DM were more likely (p=0.07) to be exposed to tobacco smoke after birth. Some unexpected associations were detected when examining smoking in detail. Children living in remote areas were more likely to be exposed to smoke *in utero* than those living in urban areas. Alternatively, children living in urban areas were more likely to be exposed to smoke after pregnancy than those living in remote areas. Explanations for these associations remain hidden, however due to the small number of children in the urban group this comparison is susceptible to chance and must be interpreted with caution.

Major congenital anomalies were reported at a rate of 10.4% in this report. This is similar to the rate of 12.3% seen in a random sample of Caucasian and Asian mothers with T2DM in England.[23] Yet, this rate is still greater than the 3% rate seen in the Canadian general population.[24] This data indicates the need for counselling of pregnant women with T2DM as it has been shown that modifiable factors such as the use of oral hypoglycaemic agents (OR = 1.8), BMI (OR=1.09), and folic acid supplementation (OR = 0.3) all have significant impact on the risk of fetal congenital malformations in pregnant women with T2DM.[23]

All baseline data, with the exception of location of residence, was statistically similar between children of fathers and children of mothers, allowing comparison between these groups. There were no differences between boys and girls in NextGen with the exception of the fact that girls were more likely to be diagnosed with T2DM. Despite our prediction, there was no significant differences between children of mothers and children of fathers in respect to weight

status and longitudinal weight change. This is in contrast to reports on the Pima Indians demonstrating that children born to mothers with pre-pregnancy T2DM had a greater body weight when compared to children born to mothers without T2DM and when compared to children born to fathers.[9, 13] This observation may represent a true finding in the NextGen population or a small sample size of the offspring of fathers.

When examining the entire cohort, 65.6% of the children were obese at last assessment, which is down from a rate of 89% observed in the NextGen cohort from 2003-2008.[3] This decrease in obesity may be attributed to an expansion of recruitment of the cohort. Many of the original children in NextGen were at high risk of obesity because they were either siblings of children with T2DM or siblings of children previously identified at high risk. This original group was therefore easy to identify. With the addition of children who were sought out in the community and not identified due to their high risk family history, the high obesity may have been blunted since 2008. This rate still far exceeds the rate seen in a similar population in 2000. Pediatric overweight and obesity was observed in 27.7% of boys and 33.7% of girls, age 2-19 years, in the Oji-Cree speaking community of Sandy Lake in Northwestern Ontario.[5] In part, the high rates of obesity in these children can be attributed to decreases in physical activity and alterations of their diet from their traditional hunter-gatherer lifestyle, resulting in sedentary behaviour and high energy intakes.[6] It is interesting, that despite a high variation in BMI z-score at younger ages, most of the children in NextGen converge to an average BMI z-score of 2.0 at roughly 6 years old. This would suggest that regardless of weight status at younger ages, the children tend to end up at a similar weight status, an observation not seen in the original NextGen report and to this author's knowledge not in any other report. [3] In addition, the convergence around a BMI z-score of 2.0 suggests this level of obesity somehow represents equilibrium between environmental influences and the population genetic potential, or possibly between environmental influences and social pressures.

Again, despite our prediction, children of mothers did not have a significantly increased prevalence T2DM than children of mothers. Despite the fact that every affected child with T2DM in this cohort was born to a mother, there was no association between developing T2DM and gender of parent ($p=0.17$). Our findings are unlike others reported which demonstrated children of mothers with pre-pregnancy T2DM were more likely to develop T2DM than children of fathers with T2DM, children of mothers without T2DM, children of mothers with gestational diabetes, or children of the same mother but born before her development of T2DM.[11, 13, 15] Although we expected to see a significant predominance of maternal transmission of T2DM, conclusions must be reserved until we can recruit more children from fathers into NextGen.

Since the original description of this population covering the years 2003-2008, the number of children diagnosed with T2DM has increased from 7 (25.0%) to 13 (22.0%) of those over 7 years old, but this prevalence has not changed significantly over the years ($p=0.70$).[3] Based on CDA screening criteria (≥10 years old), 34.4% of this cohort eligible for CDA screening have been diagnosed with T2DM, and using ≥7 years old as the criteria for screening, there is a 22% prevalence of pediatric T2DM. In this high risk population the CDA criteria do not seem to be appropriate as 5 children in NextGen would have been missed. Comparisons between children with T2DM and children without are summarized in table 3. Boys demonstrate a significantly lower prevalence of T2DM than girls ($p=0.02$). Similar to the NextGen children, Pima Indian girls had a higher prevalence of pediatric T2DM than boys in the decade of 1987-1996, however it was not reported whether the 2 were significantly different from each other.[14] Also, children from an urban setting are more likely to develop T2DM than those living in a

remote setting. This may be due to the fact that the children who go on to develop T2DM are from high risk families who have relocated to Winnipeg to be close to the tertiary health care centres in the province.

As a testament to the intense efforts by the multi-disciplinary team at DER-CA to alter lifestyle behaviours in children with T2DM, there was a significant (p=0.01) change in BMI z-score from time of diagnosis (2.1) to BMI z-score at last assessment (1.8), and this was also demonstrated in the significant difference (p=0.05) in BMI z-score velocity between those children with T2DM (-0.1) and those without T2DM (0.0). This result is even more striking considering haemoglobin A1C did not change from diagnosis to last assessment (p=0.06). It is clear that children in NextGen have a very high prevalence of overweight and obesity, but there is no demonstrable difference between those children with T2DM and those without (table 3). This may suggest that although obesity may contribute to the high risk of this population to developing T2DM, there are other factors which push some children to clinical T2DM. This fact is evident when comparing T2DM rates from this cohort to those seen in an obesity clinic. The Yale Obesity Clinic in New Haven, Connecticut demonstrates a T2DM rate of 0% in children under 10 years old, 4% in children above 10 years old, and a total T2DM rate of 2%, compared with rates of 1%, 36% and 10% in the respective age categories in the NextGen cohort (figure 4).[25] These factors in the NexGen population may be exposure to an intrauterine environment complicated by T2DM or possession of the G319S allele. There is a strong trend demonstrating an increased prevalence of the G319S allele in those with T2DM, as would be expected as this allele has been shown to be a risk factor for developing T2DM in Oji-Cree children (p=0.07). [7-8]

The NextGen cohort also demonstrates a reduction of the frequency of the S319 allele since 2003-2008. In this report, we observed that 86.5% of children possess the G319S allele, whereas the previous NextGen report demonstrated a frequency of 91%.[3] This may be due to a selection bias in the earliest members of the cohort who are offspring of the first youth diagnosed with T2DM in Manitoba in the 1980's. The allele frequency in NextGen (0.516) was significantly higher than that previously reported by Sellers *et.al.*(0.294) (p=0.007).[7] This report was a cross sectional study in which HNF-1α genotypes were described from all youth with T2DM being followed at the DER-CA who were of all ethnicities, and may or may not have had parents with pediatric-onset T2DM. [7] This same report also described an S allele frequency of 0.639 in the children with T2DM from the Oji-Cree communities of Northeastern Manitoba, which was not significantly different from the S allele frequency in NextGen (p=0.33). The NextGen children have a similar S319 allele frequency to those children who have developed T2DM from the communities with the highest prevalence of pediatric diabetes in Canada, emphasizing the risk the children in NextGen have for developing T2DM.[4] The trend demonstrating that newborns with the S/S genotype are more likely to be born smaller (AGA) than the newborns with the G/S phenotype (LGA), suggests that the S/S children experience less hyperinsulinemia in the face of maternal hyperglycemia than G/S children and the relative lack of this potent intrauterine growth factor prevents the macrosomia commonly seen with pregnancies complicated by maternal T2DM.

In conclusion, this population demonstrates several environment influences which increase the risk of T2DM. As such, counselling during pregnancy and in the neonatal period is required to increase the rates of breastfeeding, to decrease the rates of tobacco smoke exposure, and also to increase folic acid supplementation, to reduce the use of hypoglycaemic agents, and

to reduce excess weight gain in pregnancy to reduce the high rates of congenital anomalies. Although the rate of obesity in this population has decreased since 2003-2008, this population still demonstrates a high rate of obesity. We predicted that children of mothers would demonstrate higher levels of obesity and T2DM. The fact that this was not shown may be due to other risk factors which blunt the effect of the intrauterine T2DM environment on the outcomes of these children, such as high allele frequency of G319S. However, the number of children of fathers maybe still too small to show statistical significance. One important implication of this study is the importance of first trimester screening to identify mothers with pre-pregnancy T2DM and subsequent optimal glycemic and weight control during the pregnancy. Future research must evaluate the impact of maternal and paternal T2DM on the risk of T2DM in the offspring in multiple populations. This research must inform interventional studies, particularly interventions in pregnancies complicated by T2DM that focus on maternal weight reduction, improved maternal glycemic control, and increased breastfeeding.

The authors recognize a number of limitations with this study. Due to the uniqueness of this population, socially, geographically, and genetically, a broad generalization of our findings to other populations is not possible. However, individual insights into the *in utero* influence on childhood outcomes, effects of smoking, and effects of breastfeeding can add to our understanding of the complex world of pediatric diabetes. Secondly, due to the difficulty in staying in contact with this mobile population data collection was sometime difficult and current information on birth history, genetic analysis, and smoking history is somewhat incomplete. Lastly, fasting blood glucose measurements were preferentially collected blood by venipuncture but occasionally, due to limitations in the community health centres, capillary blood collection was performed.

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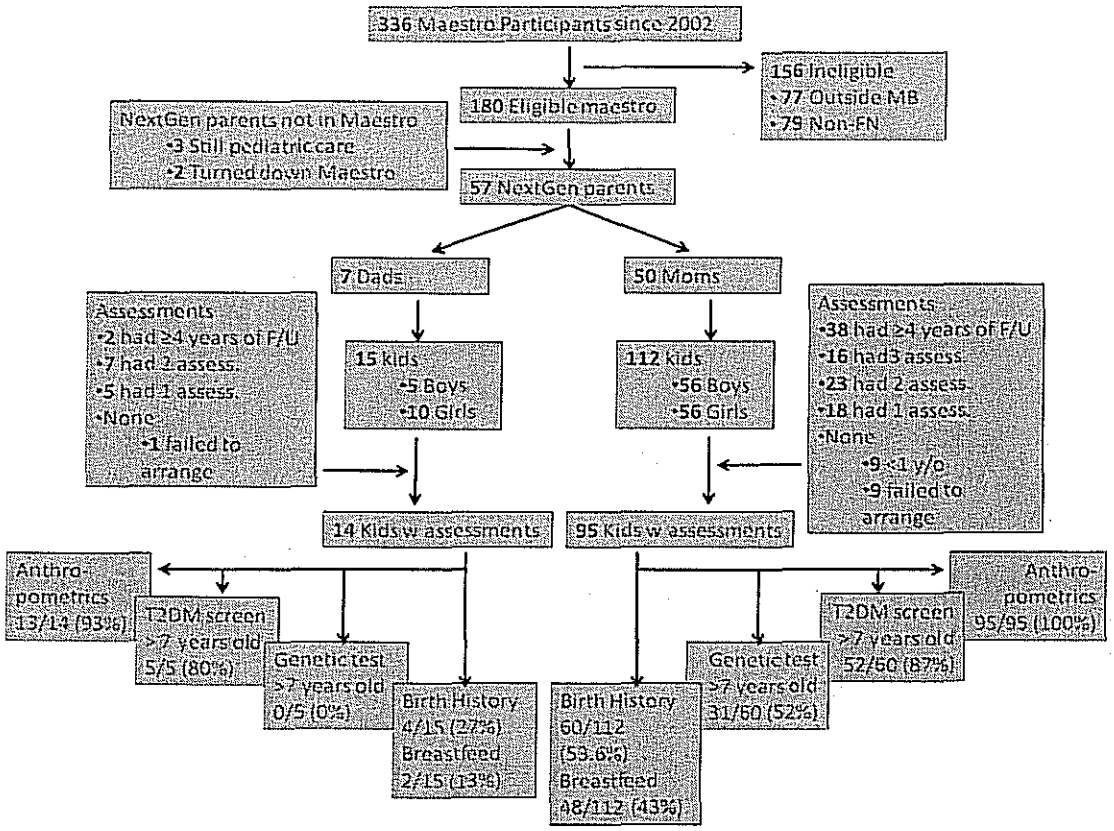

Figure 1: Enrolling and assessing the Next Generation Cohort.

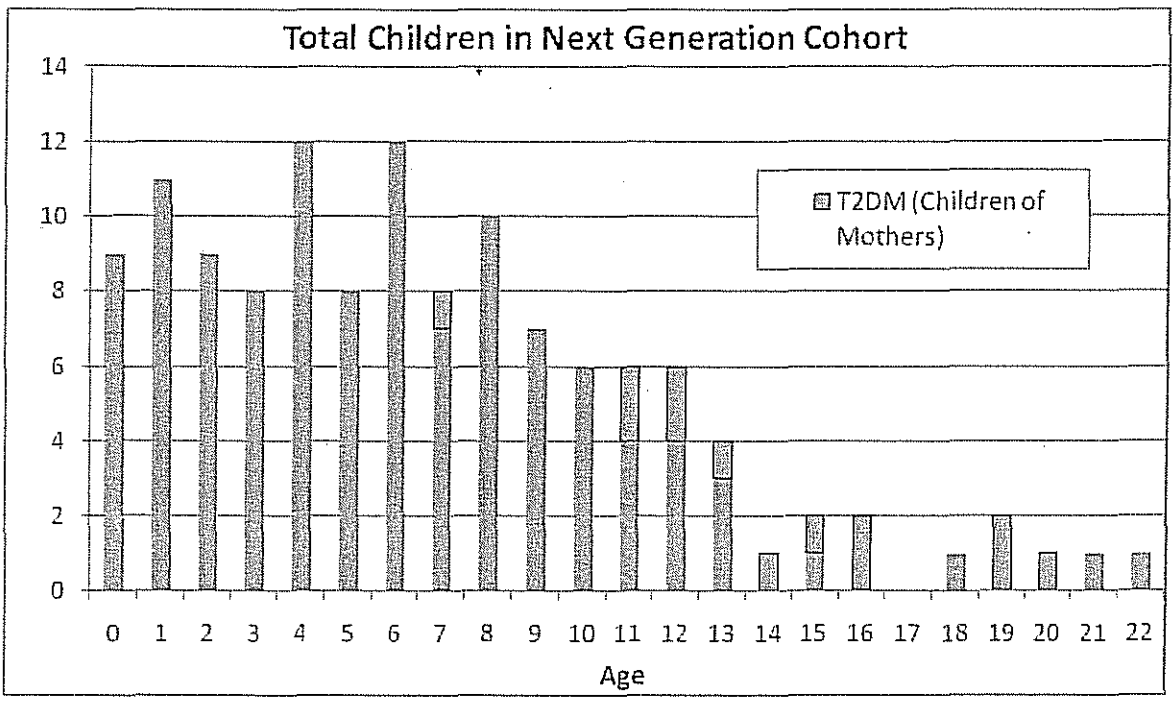

Figure 2: Age distribution of the children in the Next Generation Cohort. Red represents the children of fathers, blue the children of mothers, and purple the children with T2DM who are all children of mothers.

Table 1: Characteristics of the Next Generation Cohort compared by gender of parent and gender of child.

	N	Children of Fathers	N	Children of Mothers	p-value		N	Boys	N	Girls	p-value
Age	15	5.2 (1.3-10.3)	112	7.6 (0.3-22.1)	p=0.08		61	7.3 (0.4-22.1)	66	7.4 (0.3-19.7)	p=0.86
Age at Recruitment	15	4.3 (0.2-9.2)	112	3.3 (0.1-13.9)	p=0.26		61	3.3 (0.1-13.9)	66	3.5 (0.1-13.3	p=0.77
Duration of follow-up	9	3.3 (0.7-10.3)	77	4.6 (0.3-11.3)	p=0.15		37	4.3 (0.3-11.3)	49	4.7 (0.6-10.3)	p=0.50
Mean interval between follow ups	14	2.1 (0.2-7.1)	256	1.4 (0.0-6.4)	p=0.03		108	1.5 (0.1-6.4)	162	1.4 (0.0-7.1)	p=0.73
Children of Fathers							61	5 (8.2%)	66	10 (15.2%)	p=0.28
Boys	15	5 (33.3%)	112	56 (50%)	p=0.28						
Urban	15	0 (0%)	112	34 (30.4%)	p=0.02		61	17 (27.9%)	66	17 (25.6%)	p=0.85
Breastfeed	2	2 (100%)	48	19 (39.6%)	p=0.09		26	10 (38.5%)	24	11 (45.9%)	p=0.60
Smoke during pregnancy	8	6 (75%)	17	8 (47.1%)	P=0.19		8	6 (75%)	17	8 (47.1%)	P=0.19
Smoke exposure	9	2 (22.2%)	19	11 (57.9%)	p=0.08		9	4 (44.4%)	19	9 (47.4%)	p=0.89
Congenital anomaly	11	0 (0%)	76	9 (11.8%)	p=0.23		38	6 (15.8%)	49	3 (6.1%)	p=0.15
SGA	4	0 (0%)	54	1 (1.9%)	p=1.00		24	0 (0%)	34	1 (2.9%)	p=0.40
AGA	4	3 (75%)	54	27 (50%)	p=0.61		24	11 (45.8%)	34	19 (55.9%)	p=0.46
LGA	4	1 (25%)	54	26 (48.1%)	p=0.62		24	13 (54.2%)	34	14 (41.1%)	p=0.33
BMI z-score at last visit	13	2.0 (1.0)	83	1.9 (1.0)	p=0.74		45	1.9 (1.1)	51	1.8 (0.9)	p=0.50
85th to 94th percentile	13	2 (15.4%)	83	11 (13.3%)	p=0.84		45	5 (11.1%)	51	8 (15.7%)	p=0.52
>95th percentile	13	8 (61.5%)	83	55 (66.3%)	p=0.74		45	32 (71.1%)	51	31 (60.8%)	p=0.29
BMI z-score velocity	8	-0.6 (-2.7-+0.6)	60	0.0 (-1.0-+0.8)	p=0.18		28	-0.1 (-2.9-+0.6)	40	0.0 (-1.0-+0.8)	p=0.60
Acanthosis nigricans	13	0 (0%)	88	15 (17.0%)	p=0.21		49	6 (12.2%)	52	9 (17.3%)	p=0.58
≥1 HNF-1α G319S allele	0	N/A	32	27 (84.4%)	N/A		14	10 (71.4%)	18	17 (94.5%)	p=0.08
T2DM	0	N/A	13	13 (100%)	N/A		61	2 (3.3%)	66	11 (16.7%)	p=0.02
Age at diagnosis	0	N/A	13	10.8 (1.8)	N/A		2	9.8 (0.2)	11	11.0 (1.9)	p=N/A

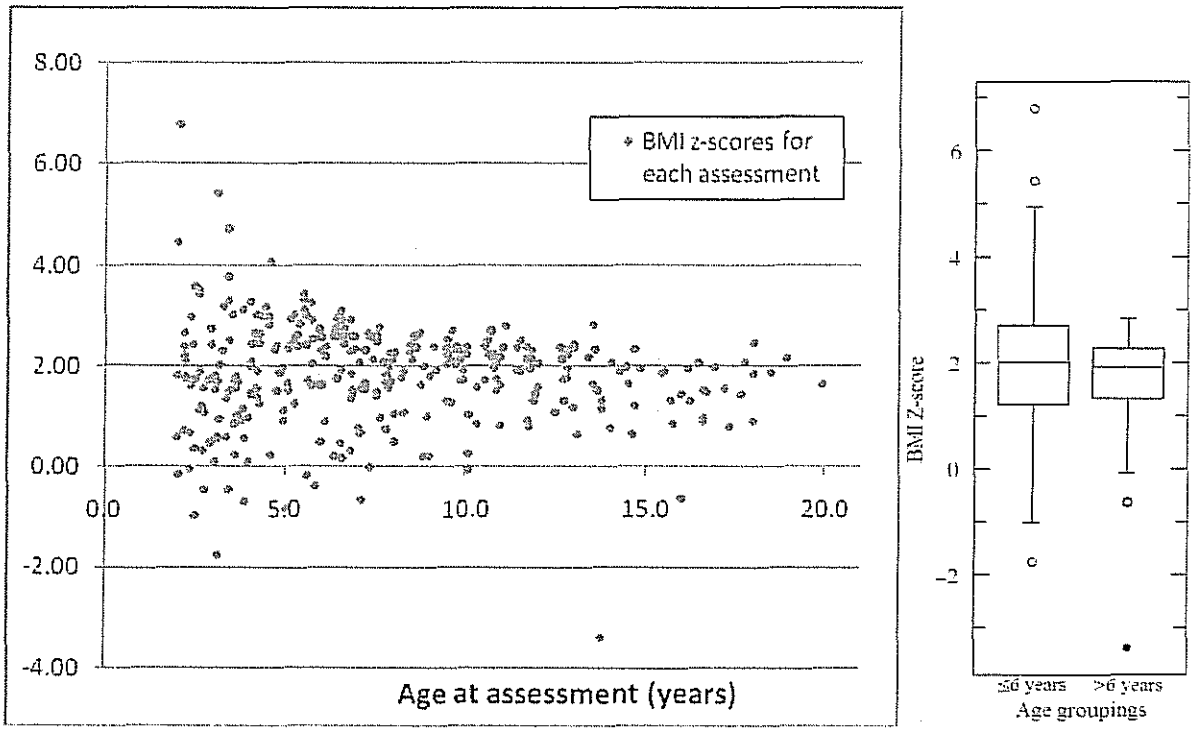

Figure 3: Distribution by age of the BMI z-scores at each NextGen assessment

Table 2: Characteristics of the Next Generation Cohort compared by HNF-1α G319S genotype.

	N	G/G	N	G/S	N	S/S	p-value (G/G vs G/S)	p-value (G/S vs S/S)	p-value (G/G vs G/S and S/S)
SGA	3	0 (0%)	13	0 (0%)	3	0 (0%)	p=1.00	p=1.00	p=1.00
AGA	3	2 (66.7%)	13	5 (38.5%)	3	3 (100%)	p=0.38	p=0.06	p=0.60
LGA	3	1 (33.3%)	13	8 (61.5%)	3	0 (0%)	p=0.38	p=0.06	p=0.60
BMI z-score at last visit	5	1.6 (1.3)	21	1.9 (0.8)	6	1.7 (0.8)	p=0.49	p=0.67	p=0.52
85th to 94th percentile	5	0 (0%)	21	3 (14.3%)	6	0 (0%)	p=0.37	p=0.33	p=0.41
>95th percentile	5	4 (80%)	21	16 (76.2%)	6	4 (66.7%)	p=0.86	p=0.64	p=0.29
BMI z-score velocity	4	0.2 (-0.2-+0.6)	19	0.0 (-0.2-+0.5)	6	-0.1 (-0.3-+0.1)	p=0.33	p=0.16	p=0.23
AN	5	0 (0%)	21	8 (38.1%)	6	3 (50%)	p=0.10	p=0.61	p=0.08
T2DM	5	0 (0%)	21	9 (42.9%)	6	4 (66.7%)	p=0.07	p=0.31	p=0.07
Age at diagnosis	0	N/A	9	11.8 (3.3)	4	9.1(2.4)	N/A	p=0.13	N/A
A1C at diagnosis	0	N/A	9	9.6 (1.7)	4	7.8 (0.6)	N/A	P=0.07	N/A

Table 4: Child characteristics in the Next Generation Cohort compared by T2DM status.

	N	Non-T2DM	N	T2DM	p-value
Children of Fathers	114	15 (13.2%)	13	0 (0%)	p=0.17
Boys	114	59 (51.8%)	13	2 (15.4%)	p=0.02
Urban	114	29 (25.4%)	13	5 (38.5%)	p=0.31
Breastfeeding	42	17 (40.5%)	8	4 (50%)	p=0.71
Smoke during preg	24	14 (58.4%)	1	0 (0%)	P=0.44
Smoke exposure	22	8 (36.4%)	6	5 (83.4%)	p=0.07
Congenital anomaly	76	9 (11.9%)	13	0 (0%)	p=0.35
SGA	49	1 (2.1%)	9	0 (0%)	p=1.00
AGA	49	25 (51.1%)	9	5 (55.6%)	p=1.00
LGA	49	23 (47%)	9	4 (44.5%)	p=1.00
BMI z-score at last visit	83	1.9 (1.1)	13	1.8 (0.6)	p=0.66
85^{th} to 94^{th} percentile	83	12 (14.4%)	13	1 (7.7%)	p=1.00
$>95^{th}$ percentile	83	54 (65.1%)	13	9 (69.2%)	p=1.00
BMI z-score velocity	55	0.0 (-2.7-+0.8)	13	-0.1 (-0.3-+0.1)	p=0.05
AN	94	6 (6.4%)	9	4 (44.5%)	p=0.005
≥1 HNF-1α G319S allele	19	14 (73.7%)	13	13 (100%)	p=0.07
S allele frequency	38	0.421	26	0.654	p=0.08
Age at diagnosis	0	N/A	13	10.8 (1.8)	N/A

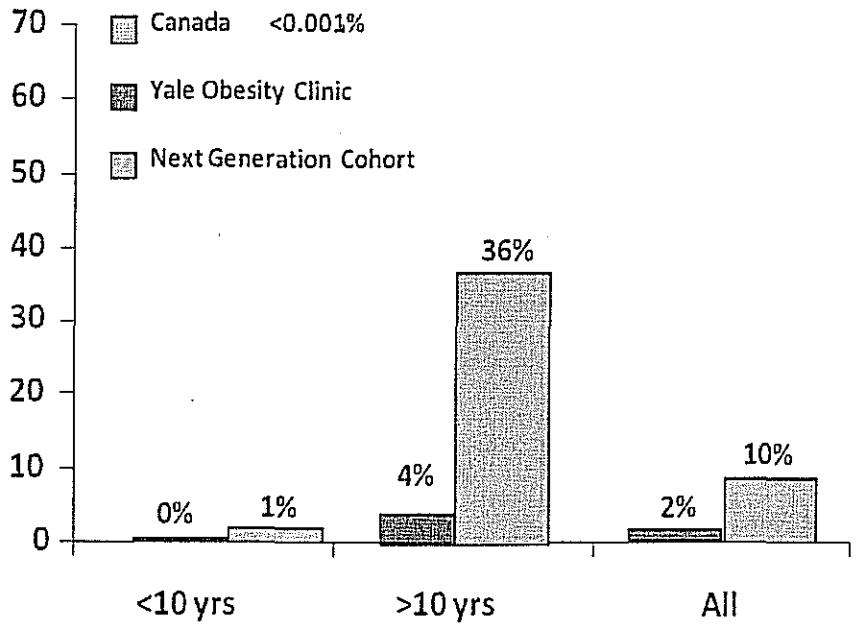

Figure 3: Prevalence of T2DM in obese children from a pediatric obesity clinic and in offspring of mothers with pre-gestational T2DM (Next Generation Cohort)