Evaluation of the use of non-invasive prenatal testing in Ontario, Canada 2016-2020

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ABSTRACT

Background:

There are few studies on the uptake of non-invasive prenatal screening, but those available suggest substantial variation in uptake in the initial years in which it was offered. There is a need to update the earlier evidence and determine whether there has been any change in usage trends as the number of users have increased. This will help inform policy makers about NIPT uptake under currently existing policies and guidelines which can help inform whether to maintain or refine policies on NIPT.

Objectives:

The primary objective of this thesis was to investigate recent trends in NIPT utilization, and the secondary objective was to identify differences between pregnant individuals aged 40 years and above and/or with a history of previous aneuploidy who opted for first-tier (first-line screening) or second-tier (contingent screening) NIPT and pregnant individuals aged less than 40 years with no history of previous aneuploidy.

Methods:

This retrospective cohort study used a province wide birth registry from Ontario and the population studied comprised pregnant individuals with an expected date of delivery from August 1st, 2016 to March 31st, 2020.

Results:

Of 536,748 pregnant individuals resident in Ontario during the study period, 27,733 were classified as high-risk of giving birth to a baby with a chromosomal aneuploidy and 509,015 were classified as low-risk of giving birth to a baby with a chromosomal aneuploidy. Uptake of NIPT has increased every year since 2016. We found substantial variation in NIPT between regions within the province. Highest uptake was found in urban areas, highest neighbourhood of income and education quintiles, high-risk population, among those with a prenatal care visit in

the first trimester, multiple pregnancy, multigravidity, body mass index within the normal range (18.5-24.9 kg/m^2), and OHIP funding.

Conclusion:

Our results suggest a need to provide more education/training about NIPT and funding eligibility to health professionals and pregnant individuals, including low-risk pregnant individuals in the first-tier (first-line screening) NIPT funding policy, to ensure equitable assess.

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DEFINITIONS

Better Outcomes Registry & Network (BORN) Information System

BORN Ontario is a prescribed registry under the Personal Health Information Protection Act (PHIPA) and collects, interprets, shares, and rigorously protects high quality data essential in Ontario.

BORN Information System (BIS)

The BORN Information System (BIS) contains province-wide data on pregnancy encounters with the prenatal care system.

Chromosomes

Chromosomes are thread-like structures located inside the nucleus of animal and plant cells and is made up of a single deoxyribonucleic acid (DNA) strand tightly coiled many times around proteins called histones that support its structures.

Chromosomal aneuploidy

Chromosomal aneuploidy is the most common type of chromosomal abnormalities and is due to an extra or missing chromosome.

First-tier non-invasive prenatal testing

Non-invasive prenatal testing is the use of NIPT as a primary/first-line prenatal test without using any conventional screening test to screen for chromosomal aneuploidy.

Gravidity

Gravidity refers to the number of times a woman has been pregnant, regardless of whether the pregnancy was interrupted or resulted in a live birth.

Non-invasive prenatal testing

NIPT is a molecular technology for assessing fetal aneuploidy using cell-free fetal deoxyribonucleic acid (cfDNA) from the plasma of pregnant women.

Parity

Parity refers to the number of pregnancies reaching a viable gestation (including live births and still births).

Prenatal genetic testing

Prenatal tests which are done during pregnancy to check a pregnant individual's health and that of the unborn baby, as well as to detect whether the baby has certain genetic disorders.

Prenatal genetic screening testing

Prenatal genetic screening tests are screening tests which are done to determine the chance whether a baby is more or less likely to have certain genetic disorders but is not intended to make a definitive diagnosis.

Prenatal genetic diagnostic testing

Prenatal genetic diagnostic testing are diagnostic tests which are done to determine whether a fetus is having certain genetic disorders.

Second-tier non-invasive prenatal testing

Non-invasive prenatal testing is done after the pregnant individuals has undergone a first-maternal screening and has been identified as a higher risk of having a fetus with chromosomal aneuploidy before making a decision about having genetic diagnostic test.

CHAPTER 1: INTRODUCTION

1.1 Background

Prenatal screening practices differ between jurisdictions, with practices varying with regards to what tests are offered, insurance coverage and the legal situation concerning the option of terminating an affected pregnancy (1). Despite international policies recommending a universal offer of prenatal screening, uptake varies by maternal preferences (2–4), provider practice pattern (5–7) and maternal socio-demographic patterns (8–11). This variation has become even more complex as a result of the introduction of non-invasive prenatal testing (NIPT) in 2011 and its subsequent rapid dissemination such that, by 2014 it was available in sixty countries and six continents (12). NIPT became available in Ontario, Canada in the late 2012 and the province started funding NIPT in 2014 (13).

NIPT was introduced to screen numerical chromosomal aneuploidies, specifically Down syndrome (trisomy 21), Patau syndrome (trisomy 13) and Edward syndrome (trisomy 18), which are the most common types of chromosomal aneuploidies (14). Many studies have found NIPT to have high sensitivity and high specificity to detect these abnormalities (15–17). NIPT is not a diagnostic test and in most countries, it has been introduced as a second-tier test. i.e., it is offered to pregnant individuals who have undergone first-tier maternal screening and have been identified as having high-risk of having a fetus with a chromosomal aneuploidy prior to making a decision about diagnostic testing (18) . However, NIPT is now offered as a first-tier test (i.e., not contingent on the results of one or more other screening test results) to all pregnant women in Belgium (19) and the Netherlands (20,21). In addition, internationally, substantial numbers of pregnant individuals opt for first- tier NIPT on a self -pay basis (22,23)

There are few studies of the uptake of non-invasive prenatal screening, but those available suggest substantial variation in uptake in the initial years in which it has being offered (22,24–28). There is a need to update the earlier evidence and determine whether there has been any change in usage trends as the number of users have increased which will help policy makers to determine whether to change or maintain the existing polices. In Canada, Ontario was the first province in which NIPT was publicly funded (13), is the largest province and is diverse in terms of socio-economic status, ethnicity, and geography (29). It is therefore an excellent setting to investigate changes in patterns of uptake. The aim of my thesis was to evaluate recent trends in the uptake of NIPT in Ontario, including investigation of the differences between pregnant individuals who opted for NIPT as their prenatal screening test for the most common clinically significant fetal aneuploidies and pregnant individuals who did not.

1.2 Research objectives

1. The primary research objective of this thesis was to investigate recent trends in NIPT utilization in Ontario, Canada and to compare NIPT usage across health regions during the period 2016-20.

2. The secondary objective was to investigate, the differences between pregnant individuals aged 40 years and above and/or with a history of previous aneuploidy who opted for first-tier (first-line screening) or second-tier (contingent screening) NIPT and pregnant individuals aged less than 40 years with no history of previous aneuploidy.

1.3 Organization of thesis

This is a monograph thesis which addresses the two research objectives of investigating uptake of NIPT in Ontario and relating this to provincial funding criteria. The thesis starts with a literature review of the biology and epidemiology of chromosomal aneuploidy, prenatal screening and testing and non-invasive prenatal testing in Chapter 2, followed by a description and explanation of the study methods (Chapter 3), and results (Chapter 4). The thesis concludes with a discussion (Chapter 5) of the main findings, considerations of strengths and limitations of my work and implications for prenatal screening policies and research.

CHAPTER 2: LITERATURE REVIEW

2.1 Chromosomal abnormalities

There are many types of chromosomes inside the nucleus of animals and plants. Each chromosome contains a single strand of deoxyribonucleic acid (DNA) tightly coiled many times around proteins called histones that support their structure (30,31). An individual strand of DNA is made up of alternating sugar groups (deoxyribose) as well as purines and pyrimidines (32) and it contains the biological instructions that makes every living thing unique (33). Every cell in the human body contains 23 pairs of chromosomes: thus, a person's diploid number is 46 and haploid number 23 (34). One member of each chromosome pair comes from the mother and the other from the father. The first 22 pairs are called autosomes, which direct many body activities and the final pair comprises the sex chromosomes which determine biologic sex: XX for females and XY for males (35).

A chromosome can be recognized by its number, size, centromere position and banding pattern (36). Autosomes are classified in descending order of karyotypic size with the largest being chromosome 1, the next largest chromosome 2 and so forth (36). Every chromosome carries thousands of genes, which control the body's development, growth, and chemical reactions. Chromosomal abnormalities are mainly classified into two groups: numerical and structural abnormalities (37).

Numerical abnormalities occur when there is an abnormal number of chromosomes in a cell resulting in aneuploidy (31). They include nullisomy, which occurs when there is a non-disjunction during cell division; trisomy which occurs when there is a presence of three copies of a chromosome; and tetrasomy, which occurs when there is a presence of an additional copy of chromosome, resulting in four copies of a chromosome in the cell. Aneuploidies typically occur

following errors during dividing of gametes (meiosis) and error during dividing of other cells (mitosis) (38). Trisomy 21 is the most common autosomal chromosome aneuploidy among live births and its estimated prevalence is 1 in every 800 newborns (25). As pregnant individuals ages their chance of given birth to a baby with a chromosomal aneuploidy increases (39). A summary of different types of numerical chromosomal abnormalities and their prevalence at birth is presented in Table 2.1.

A structural abnormality occurs when large sections of DNA are missing or added to a chromosome. Types of structural anomalies include deletions, translocations, duplications, inversions, isochromosomes, dicentric chromosomes and ring chromosomes (31). Collectively, the prevalence at birth has been estimated as less than 7.4 per 10,000 (40).

Table 2-1. Numerical abnormalities, adapted from Ontario Health Technology Assessment Series; Vol. 19: No. 4, p. 11 (41)

Condition	Common name (s)	Estimated Prevalence in Newborns
Numerical		
Trisomy 21	Down syndrome	• 1 in 800 newborns • Most common autosomal chromosome aneuploidy among live births • Frequency is strongly dependent on maternal age
Trisomy 18	Edwards syndrome	• 1 in 5,000 newborns • More common in fetuses that do not survive to term

		• Frequency increases with maternal age
Trisomy 13	Patau syndrome	• 1 in 16,000 newborns • Frequency increases with maternal age
Monosomy X (45, X)	Turner syndrome	• 1 in 2,000–2,500 newborn females • More common in fetuses that do not survive to term
XXY syndrome (47, XXY	Klinefelter syndrome	• 1 in 500–1,000 newborn males • Most common sex chromosome aneuploidy • Variants are much rarer (e.g., XXXY, XXXXY, XXYY, XX male), occurring in 1 in 50,000 or fewer newborn male
Triple X syndrome (47, XXX	Trisomy X	• 1 in 1,000 newborn females
XYY syndrome (47, XYY)	Jacob's syndrome	• 1 in 1,000 newborn males

2.2 Prenatal genetic screening and prenatal genetic diagnostic testing

Prenatal genetic tests are provided to pregnant individuals to check their health and that of the fetus, as well as to determine whether the fetus has certain genetic disorders (42). The two main types of prenatal genetic testing are screening tests and diagnostic tests (42). Several types of prenatal genetic testing are available depending on the trimester of pregnancy and the type of

genetic condition in question. Genetic screening tests includes serum screening, carrier screening and ultrasound and they are done usually during the first or second trimester to identify whether a baby is more or less likely to have a genetic disorder, but they are not intended to make a definitive diagnosis (43). Conversely, genetic diagnostic tests are procedures intended to make definitive diagnoses about whether a developing baby has a genetic disorder (44). Serum screening tests are blood tests which are done either in the first or second trimester to identify if a fetus has an increased risk for chromosomal abnormalities such as trisomy 21, 13 or neural tube defects. (45). Carrier screening test is a test which is done either before or during pregnancy to determine whether a person carries the gene for certain inherited genetic disorders (46). A fetal ultrasound is an imaging technique that uses sound waves to produce image of a fetus to check the anatomy of the fetus if it is developing well and also look for the presence of soft makers (47). There are several options for prenatal genetic screening tests, including prenatal cell free DNA screening, available depending on the gestational age and the jurisdiction of the pregnant individuals (refer to Table 2-2 for the options for prenatal genetic screening). In Canada, the tests offered vary by province/ territory. In Ontario Enhanced First Trimester Screening, Maternal screening (quadruple screening) and NIPT are the only options available (13).

Types of prenatal genetic diagnostic tests are chorionic villus sampling and amniocentesis. Chorionic villus sampling involves examination of cells collected from the placenta to determine the probability of a fetus being born with trisomy 21 or trisomy 18 (48). Amniocentesis is the examination of fetal cells which are collected from the amniotic fluid to determine whether or not a fetus has trisomy 21, trisomy 18 or, in some jurisdictions, an open neural tube defect (49). A concern with both tests is risk of fetal deaths, regardless of whether the fetus has an anomaly or not (50–52). This has been one of the stimuli for the introduction of NIPT as a contingent test

However, recent evidence syntheses that the risk of fetal loss is substantially less that the previously thought (53,54). A contingent test is a second line screening test which is performed on pregnant individuals who have undergone first-line tests (conventional screening tests) and have been identified as high-risk of giving birth to a baby with a chromosomal aneuploidy and it is done before a decision is made about prenatal genetic diagnostic test (42).

Table 2-2. Types of prenatal genetic screening tests (55–57)

Early prenatal screening (available for pregnant individuals who are 14 weeks pregnant or earlier)
First Trimester Screening (FTS) First trimester screening involves a blood test and nuchal translucency ultrasound between 11 and 14 weeks of pregnancy. Both tests can be done on the same day. The results can be received between 12 and 15 weeks. FTS is 80 to 85% accurate in detecting trisomy 21 and 18 and has a false positive rate of 3 to 9%. The markers analysed are nuchal translucency, pregnancy-associated plasma protein A and free-β subunit of human chorionic gonadotropin.
Enhanced First Trimester Screening (eFTS) Enhanced FTS is a first trimester screening test that uses 4 serum markers plus the nuchal translucency (NT) test between 11 weeks 2 days to 13 weeks 3 days gestation. The results can be received between 7 to 10 days after the test. In addition to pregnancy-associated plasma protein and the free-β subunit of human chorionic gonadotropin which are included in FTS. eFTS includes placental growth factor and alpha -fetoprotein. eFTS is 80 to 90 % accurate in detecting trisomy 21 and 18 and has a false positive rate of 3 to 6%.

Serum Integrated Prenatal Screening (SIPS)

Serum Integrated Prenatal Screening is done if nuchal translucency ultrasound is not available in the jurisdiction in which a pregnant person lives. SIPS involves a blood test between 11 and 14 weeks of pregnancy (first phase) and another between 15 and 20 weeks (second phase). The results can be received between 16 and 21 weeks. SIPS is 80 to 85% accurate in detecting trisomy 21 and 18 and has a false positive rate of 2 to 7%. The marker analysed for the first phase is pregnancy-associated plasma protein A. The markers analysed for the second phase are α-fetoprotein, human chorionic gonadotropin, unconjugated estriol and dimeric inhibin-A.

Non-invasive prenatal testing (NIPT)

NIPT is a molecular technique for assessing fetal aneuploidy using cell-free fetal deoxyribonucleic acid (cfDNA) from the plasma of pregnant women. It is available from 9 or 10 weeks until the end of pregnancy. It is used to screen for trisomy 21,18 or 13. It is 95% to 99% accurate in detecting trisomy 21 and 18 and has a false positive rate of <0.1 to 0.1%.

Late prenatal screening (available for pregnant individuals with gestations of 14 weeks or more)

Maternal Serum Screening (MSS)

A blood test is taken from 14 weeks and 20 weeks 6 days of pregnancy and the results are received between 16 and 21 weeks. Multiple marker screening is 60% to 81% accurate in detecting trisomy 21 and 18 and has a false positive rate of 0.5 to 5%. The markers analysed are α-fetoprotein, human chorionic gonadotropin, unconjugated estriol and dimeric inhibin-A.

2.3 Evolution of prenatal testing

Prenatal testing for birth defects was initially developed in the 1950s and has become increasingly prominent in routine obstetric care (58). Changes in prenatal testing procedures are summarized in Table 2-3.

Table 2-3. Evolution of Prenatal Testing, adapted from the Hastings Center (58).

Year	**Achievement**
1956	Amniocentesis was used to identify genetic disorders.
Late 1960s and early 1970s	Ultrasound was first used clinically to detect and assess the fetus.
1980s	Ultrasound routinely used in early pregnancies for dating, identification of multiple pregnancy, and identification of some major structural malformations.
1983	Second trimester (15-20 weeks) multiple maker maternal blood screen was first used to measure likelihood that a fetus has trisomy 18 or trisomy 21, as well as neural tube defects such as spina bifida and anencephaly. Chorionic villus sampling and percutaneous umbilical blood sampling was first performed.

Early 1990s	Detailed fetal scan at 20 weeks' gestation became part of routine prenatal care in higher income countries.
Early 2000s	First trimester (11-14 weeks) maternal blood tests were first used in combination with ultrasounds to assess likelihood that the fetus has trisomy 13, 18, or 21. Nuchal translucency result could also indicate increased likelihood of a heart defect or rare genetic condition.
2011	Cell free DNA screening tests (also known as "non-invasive prenatal testing or sequencing") were introduced.
2013	Testing companies begin marketing versions of cfDNA tests that assess likelihood that the fetus has other chromosomal conditions, including sex chromosome aneuploidy.
2017 onwards	Ultrasound is still used from the earliest stages of pregnancy to confirm pregnancy, check dates, measure development, and detect structural abnormalities. It is used in combination with other methods for enhanced information, such as a blood test with cfDNA, to calculate the likelihood that the fetus has certain chromosomal conditions, particularly trisomy 21. Invasive tests are still available, but many opt for the "non-invasive" blood tests instead as a secondary screen, hoping for additional information prior to deciding whether to have an invasive prenatal test.

2.4 Non-invasive prenatal testing

NIPT is a molecular technique for assessing fetal aneuploidy using cell-free fetal deoxyribonucleic acid (cfDNA) from the plasma of pregnant women. The presence of cell-free fetal DNA in maternal plasma was discovered in 1997 by Professor Dennis Lo (59). All DNA that is not encapsulated in a cell is termed cell free, and it arises in the bloodstream from apoptotic cells, tumor cells or fetal cells during physiological phases of cell turnover (60). The introduction of NIPT has substantially reduced the need for invasive testing (61) because the procedure for performing NIPT is simple and its test performance for trisomy 21, 18 and 13 is better than conventional combined test based on nuchal translucency, blood tests and maternal age (62), although there are concerns about test failure rates (15). The primary use of NIPT to date has been to identify pregnancies at high risk for one of the common aneuploidies: trisomy 21, trisomy 18, trisomy 13. The possibility of detecting extra or missing copies of X and Y chromosomes, microdeletions/duplications has been offered in some programs (63). There are five types of NIPT (Table 2-4).

Table 2-4. Types of NIPT, based on Institute of Health Economics update (24)**.**

Type	Summary description
Targeted Massively Parallel Sequencing	This method selects and amplifies only those chromosomal regions that are of interest and assesses whether an excess or deficit of a given chromosome is present, based on the relative number of DNA fragment counts for that subset of chromosomes.

Massive Parallel Shotgun Sequencing (MPSS)	This method relies on the identification and counting of large numbers of the DNA fragments in the maternal plasma sample. The approach is referred to as “shotgun” because it relies on sequencing and counting all informative chromosome regions.
Single Nucleotide Polymorphism (SNP)	This method analyzes variations in single nucleotides (A, T, C, or G) and calculates the likelihood that a fetus is euploid or aneuploid.
Digital Polymerase Chain Reaction (PCR)	This method allows for the dilution, amplification, and counting of individual DNA fragments of interest.
RNA-based testing	This method identifies cell-free fetal RNA (cff-RNA) in maternal plasma.

2.5 Availability of NIPT

2.5.1 International

NIPT was first introduced into clinical practice in Hong Kong in August 2011, then soon after was introduced commercially in the USA in October 2011 and it is now available in over 60 countries (12). The introduction of NIPT has resulted in a substantial decrease in invasive prenatal diagnostic testing (64). In Europe 14 countries have assimilated NIPT into a national policy but in the USA although NIPT is widely used there is no national consensus policy on the use of NIPT (65). Publicly funded second-tier NIPT was introduced in December 2019 in Hong Kong to pregnant women who screened positive by the 11-13 week ultrasound (including nuchal translucency) and serum maker screening test for trisomy 21 (66).

2.5.2 Canada

In 2011, the Society of Obstetrics and Gynaecology of Canada (SOGC) recommended that all pregnant women in Canada, regardless of age, should be offered through an informed counselling process, the option of prenatal screening test for the most common clinically significant fetal aneuploidies in addition to a second trimester ultrasound for dating assessment of fetal anatomy and detection of multiple fetuses (67). In 2013, the SOGC recommended NIPT for women at above the average risk for trisomy 13, 18 and 21, with positive results confirmed by invasive diagnostic testing (27). NIPT was first introduced in Ontario, Canada in late 2012 (68) and is now part of prenatal screening tests options available to pregnant women in all provinces in Canada (25). There is funding for NIPT for women who meet certain requirements in British Columbia, Manitoba, Nova Scotia, Nunavut, Prince Edward Island, Yukon, Quebec and Ontario (69,70) The funding covers screening only for trisomy 21,18,13 (13).

2.5.3 Ontario

Ontario was the first province in Canada to introduce NIPT into its prenatal screening tests options (refer to Appendix 1 for prenatal testing process). Funding by the Ministry of Health and Long-Term Care (MOHLTC) for NIPT was introduced in 2014 (25,68). From that time until the declaration of the COVID-19 pandemic emergency in March 2020, the funding criteria from the Ontario Health Insurance Plan (Table 2-5) did not change (71). Testing for women who meet "category one" criteria can be ordered by any physician whereas testing for women who are in "category two" must be ordered by a genetic or maternal-fetal medicine specialist.

Table 2-5.Ontario Health Insurance Plan criteria for funding NIPT (26,72)

Category	Indications
1	• A positive prenatal screening result from multiple marker screening • Maternal age (or age of the egg donor) of 40 years or older at the expected date of delivery • A nuchal translucency measurement equal to, or greater than, 3.5mm. • A personal history of a previous pregnancy or child with trisomy 21, 18 or 13
2	• Findings on ultrasound which are associated with an increased risk of trisomy 21, trisomy 18 or trisomy 13. • Risk of sex-linked genetic condition • Ultrasound shows findings suggestive of a sex chromosome difference or a disorder of sex determination

Multiple marker screening uses a combination of maternal age and two or more biochemical tests, with or without an ultrasound examination, to produce a single result for risk of trisomy 21 or trisomy 18 (67). Nuchal translucency ultrasound measures the fluid accumulation at the back of a baby's neck, if the measurement is thicker than a specific threshold then it means the baby could have an increased risk of aneuploidy or genetic condition (28). In Ontario two laboratories are publicly funded to provide NIPT to detect trisomies 21, 18 and 13- the Dynacare Lab (Harmony test) and LifeLabs (Panorama test). The Panorama test targets 19,488 single nucleotide polymorphisms (SNPs) covering chromosomes 21, 18, 13, X and Y, and uses a patented algorithm called the Next -Generation Aneuploidy Test (73) while the Harmony test uses the digital analysis of selected regions (DANSR^{TM}) assay and FORTE algorithm that specifically targets just the chromosome of interest for deep, directed analysis (74).

2.6 Use of NIPT

In a study in the USA, the introduction of NIPT resulted in a substantial decrease in invasive prenatal diagnostic testing (22). In Canada, British Columbia (75) and Ontario (76) reported substantial decreases in the number of invasive tests performed after public funding of NIPT was introduced. In an analysis of trends in patients submitting samples for NIPT testing in over 65 countries, an increase was observed in the proportion of samples that were submitted from pregnant women below 35 years of age, suggesting increased use of NIPT in lower risk pregnancies (22). These studies suggest increased use of NIPT, in Canada particularly after implementation of funding policies for NIPT, but it is unclear whether NIPT is equally accessible to all pregnant women. Maxwell et al reported disparities in screening uptake and inequity in access to services, particularly for aboriginal, remote and socio-economically

disadvantaged women after conducting a study using prenatal screening data (2005-2006) in Western Australia (77). Also in Australia (Victoria), Hui et al found substantial disparities in screening indications for prenatal diseases according to a region-based measure of socio-economic status (78). In a study in Ontario of pregnant women of 16 weeks' gestation or more in 2007–2009, maternal, provider and regional differences in the uptake of prenatal screening were observed, with discrepancies expected to increase with the emergence of non-invasive prenatal tests paid for out of pocket by many women (79). Almost ten years later, again in Ontario (2016 – 2017), among women who underwent NIPT, one third paid for the procedure themselves, yet more than 10% of these women could have had the procedure publicly funded (26). In summary, the few available studies suggest substantial variation in the uptake of non-invasive prenatal screening in the initial years of it being offered. There is a need to update the earlier evidence and determine whether there has been any change in usage trends as the number of users increased.

2.7 Rationale

Ontario was the first province in which NIPT was publicly funded, is the largest province in Canada and is diverse in terms of socio-economic status, ethnicity, and geography. It is, therefore, an excellent setting to investigate changes in patterns of NIPT uptake. Therefore, the aim of my thesis was to investigate a comprehensive study of recent trends in uptake of NIPT in Ontario, including investigation of differences between pregnant individuals who opted for NIPT as their prenatal screening test for the most common clinically significant fetal aneuploidies and pregnant individuals who did not. This will help inform policy makers about NIPT uptake under currently existing policies and guidelines which can help inform whether to maintain or refine,

policies on NIPT to increase equitable and appropriate use in Ontario and jurisdictions where publicly funded health care system is similar to Ontario.

CHAPTER 3: METHODS

3.1 Study design and study population

We used a population-based retrospective cohort study design for both thesis objectives. For the primary analysis (Objective 1), assessing the uptake of NIPT and how this varied by maternal demographic characteristics, the study population comprised all pregnant individuals who were Ontario residents with an expected date of delivery between August 1st, 2016 and March 31st, 2020 as captured in the Better Outcomes Registry & Network Information System (BIS). For the secondary analysis (Objective two), assessing NIPT uptake in relation to two categories of eligibility for provincial funding, two subpopulations were considered: pregnant individuals 40 years and above and/or with history of previous aneuploidy (i) and pregnant individuals below 40 years with no history of previous aneuploidy (ii).

3.2 Data sources

3.2.1 Better Outcomes Registry & Network (BORN) Information System

BORN Ontario is a prescribed registry under the Personal Health Information Protection Act (PHIPA). The BORN Information System (BIS) contains province-wide data on pregnancy encounters with the prenatal care system, including multiple marker screening, cffDNA screening, labour, birth and early newborn care from all prenatal screening centers, midwifery practices and hospitals in Ontario (76). Dunn et al., conducted a validation study in which perinatal data re-abstracted from a random sample of 100 linked mother and newborn charts from ten selected representative hospitals in Ontario were compared to data entered in the BIS (80). The study found that percentage agreement between the two data sources ranged from 56.9 to 99.8%; three quarters of the data elements (22 of 29) had greater than 90% agreement, 12 of the categorical elements displayed almost perfect (kappa 0.81–0.99) or substantial (kappa 0.61–

0.80) agreement, six elements showed fair-to-moderate agreement (kappa <0.60) and four continuous data elements demonstrated moderate-to-excellent agreement (ICC >0.50) (80). This suggested that prenatal variables collected by BORN were likely to have a level of accuracy that was adequate for this study. From January 2016, cell-free fetal DNA (cffDNA) screening data were directly uploaded to BORN by the two laboratories provincially funded to perform the test in Ontario (81).

3.2.2 Statistics Canada Postal Code Conversion File (PCCF+)

The PCCF+ is a digital file that is distributed by Statistics Canada's Data Liberation Initiative (82) which provides a correspondence between the Canada Post Corporation (CPC) six-character postal code and Statistics Canada's standard geographic areas for which census data and other statistics are produced (83). The first version of the PCCF+ file was released in April 1963 by the Statistical and Geography Division and since then, the file has been updated on regular basis to reflect changes in geographical areas (82). The PCCF+ permits combining of data from various sources through the link between postal coded and standard geographical area (83). In this study, we used PCCF+ version 7C, which uses 2016 census data for neighbourhood income quintile and neighbourhood educational quintile was derived directly through 2011 StatsCanada census data. Both were linked to the BIS data via the residential postal code of the pregnant individuals.

3.3 Covariates

3.3.1 Primary analysis (Objective 1):

The variables that were included for the primary analysis were Local Health Integration Network (LHIN) region of residence of the pregnant individuals, calendar quarter of expected date of delivery, maternal characteristics (neighbourhood household income, neighbourhood level of

education, parity, gravidity, age, history of previous aneuploidy BMI), number of fetuses and whether there was prenatal care visit in the first trimester.

3.3.2 Secondary analysis (Objective 2):

The variables that were used included in the secondary analysis were the same as those in the primary analysis with the addition of whether OHIP funding for NIPT had been provided.

3.4 Outcomes

3.4.2 Primary analyses (Objective 1):

The outcome of interest for our primary analyses was the uptake of NIPT. We ascertained this outcome from the BIS for pregnant individuals with an expected date of delivery between August 1st, 2016 and March 31st, 2020 by linking the maternal delivery records with the NIPT dataset. Pregnant individuals that used NIPT were classified as "yes" for the NIPT variable.

3.4.2 Secondary analyses (Objective 2):

For our secondary analyses, the outcome of interest was the uptake of NIPT as either a first or second-tier NIPT test. We used the NIPT dataset to ascertain this outcome. Pregnant individuals recorded as having OHIP funding for NIPT and as having a positive serum screen were categorized as having a second-tier NIPT, otherwise if they were recorded as having OHIP funded NIPT but no positive serum screen they were categorized as having a first-tier NIPT.

3.5 Analyses

3.5.1 Primary analyses (Objective 1)

3.5.1.1 Descriptive analyses

Characteristics of the study populations were described using frequencies and proportions for categorical variables. We used standardized differences to assess the distribution of each baseline variable by the study outcome. We considered an absolute standardized difference <10% to be indicative of a well-balanced variable. The number of NIPT undertaken were counted in the year of expected date of delivery.

3.5.1.2 Statistical models

We used modified Poisson regression for the analyses. We had originally planned to use a log binomial regression model but encountered problems with model convergence. Such a problem usually occurs when the outcome is low or uncommon (84), and modified Poisson regression is an appropriate approach to estimate relative risk in this situation. The modified Poisson regression model estimates the relative risk by combining a log Poisson regression with a robust sandwich variance estimator to rectify the variance error (i.e., over estimation of the relative risk error) (85).

We assessed the model fit and presence of over-dispersion by calculating the ratios of the Pearson chi-square statistic and the deviance to the model degrees of freedom (86). Data were considered to be over-dispersed when these ratios were greater than one (87). Using modified Poisson regression, we estimated unadjusted and adjusted rate ratios (RR) and 95% confidence interval (CI) for uptake of NIPT among pregnant individuals.

3.5.1.3 Pregnant individuals with complete information of all variables and pregnant individuals with missing information on one or more of the variables

Missing data are defined as data values that are not available for a variable but would be of interest in an analysis if they were available (88). The potential for missing data to introduce bias depends on the pattern missingness and the analytical method applied to address the missingness (89). Data can be categorized as missing completely at random (MCAR), missing at random (MAR) or missing not at random (MNAR) (90). For data MCAR there is assumed to be no systematic difference between the participants with missing data and those with complete data; this is regarded as a strong and often unrealistic assumption (91). MAR means there might be systematic differences between participants with missing and observed values, but these can be entirely explained by other observed variables (92). MNAR means that the probability of an observation being missing depends on unobserved data(93).

We were most concerned about the variables for BMI and prenatal care initiation in the first trimester, as these had the highest proportion of missing data (BMI: 16.0 %; prenatal care initiation in the first trimester: 10.7 %). All other study variables had < 10% missing data. A primary reason for missingness is early pregnancy loss which BORN is unable to capture in the labour/birth dataset. Another principal reason for missingness, especially for BMI can be due to some clinics not able to collect information during prenatal care visit. To evaluate the pattern of missingness we made two comparisons. Firstly, we compared the distribution of baseline characteristics between (i) pregnancies with complete information on all study variables and (ii) pregnancies with missing information on one or more study variables. Secondly, we compared the distribution of baseline characteristics between (iii) pregnancies with complete information on all variables or with missing information only on BMI or first trimester visit (iv) pregnancies

with missing information on one or more study variables other than BMI or first trimester visit. Based on this evaluation, we concluded that a "missing at random" pattern was supported for BMI and first trimester prenatal care visit.

We initially wanted to do multiple imputation to address the missingness but due to the size of the study population, and the limitations of the software in the BORN secure data environment, we were not able to do so. For the missing values for BMI and first trimester visit we created a new category for each called "Missing" and included these categories in our adjusted model in order not to delete those records. We excluded records with missing values for other variables from the adjusted models but kept these records in the descriptive and unadjusted analyses.

3.5.1.4 Sensitivity analyses

Two sensitivity analyses were conducted to assess the robustness of our main findings. In the first sensitivity analysis we did not include either BMI or timing of prenatal care visit in the model. We did this to assess whether excluding either one of these variables (BMI, timing of prenatal care visit) would alter the results or interpretation from the primary analyses. For our second sensitivity analysis we restricted the study population to the subset of pregnant individuals for whom there was complete information on BMI and included all the variables in the model, again to assess whether there was any change in our primary findings.

3.5.2 Secondary analyses (Objective 2)

3.5.2.1 Descriptive statistics

Characteristics of the study populations were described using frequencies and proportions for categorical variables. Among pregnant individuals 40 years and above and/ or with a history of previous aneuploidy, we compared the distribution of baseline characteristics between pregnant individuals that used first-tier NIPT (subgroup 1) and pregnant individuals that used second-tier

NIPT (subgroup 2). We also compared the distribution of baseline characteristics between pregnant individuals that used first-tier NIPT (subgroup 1) and pregnant individuals that did not use NIPT (subgroup 3).

Among pregnant individuals below 40 years with no history of previous aneuploidy, we compared the distribution of baseline characteristics between pregnant individuals that used first-tier NIPT (subgroup 4) and pregnant individuals that used second-tier NIPT (subgroup 5). We also compared the distribution of baseline characteristics between pregnant individuals that used second-tier NIPT (subgroup 5) and pregnant individuals that did not use NIPT (subgroup 6). We considered an absolute standardized difference <10% to be indicative of a well-balanced variable. The number of NIPT were counted in the year of estimated date of delivery.

3.6 Ethical considerations.

Consent was not required for secondary use of personal data under section 45 of Ontario's Personal Health Information Privacy Act. Research ethics board (REB) approval for the analysis of data undertaken for this thesis was obtained from Children's Hospital of Eastern Ontario REB and the University of Ottawa Health Sciences and Science REB. All analysis were carried out using SAS 9.4 (SAS Institute Inc., Cary, N.C) within the secure BORN network environment.

3.7 REporting of studies Conducted using Observational Routinely collected Data (RECORD)

This study was reported according to the RECORD guidelines. These guidelines were created as an extension to the STROBE statement to address reporting items specific to observational studies using routinely collected data (94). The RECORD statement consists of a checklist of 13

items which are related to the title, abstract, introduction, methods, results and discussion section (95). The completed checklist is presented in Appendix B.

CHAPTER 4: RESULTS

4.1 Utilization of NIPT (Objective 1)

4.1.1 Descriptive characteristics

Table 4-1 shows the characteristics of pregnant individuals in this study. There were 536,748 pregnant individuals with an expected date of delivery between August 1st, 2016 and March 31st, 2020 in the BORN registry. The highest proportion lived in the Central region of the province (13.0%). Calendar year quarter two recorded the lowest percentage of pregnant individuals relative to other quarters (20.5%); this is because data for calendar year 2016 started from August 1st (therefore, data for quarter one and two were not available) and calendar year 2020 only included data to March 31st (therefore data for quarters one, two and three were not available). Over half of pregnant individuals were between the ages of 30 to 39 years (58.5%) and the majority had no previous history of a birth or fetus with aneuploidy (99.6%). Most of the pregnant individuals in this study started their prenatal care in their first trimester (91.9%) and most were pregnant with one fetus (98.3%). There was a gradient in distribution of neighbourhood income quintiles, ranging from 15.6% in the highest quintile (5) to 23% in the lowest (1).

Table 4-1. Characteristics of pregnant individuals in Ontario with expected date of delivery from August 1st, 2016 to March 31st, 2020 in Ontario, Canada

	TOTAL	
	N	% [a]
All pregnant individuals	536,748	100
Geographical location (LHINs) of maternal residence		
Erie St Clair and South West Region (1&2)	59,934	11.8
Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	82,160	16.2

Central West (5)	43,735	8.6
Mississauga Halton (6)	42,629	8.4
Toronto Central (7)	49,413	9.7
Central (8)	66,145	13.0
Central East (9)	57,284	11.3
South East and Champlain Region (10&11)	63,505	12.5
North Simcoe Muskoka, North East and North West Region (12-14)	42,724	8.4
Missing [d]	*29,219*	*5.4*
Quarter of the year (expected date of delivery)		
One (January- March)	139,402	26.0
Two (April-June)	110,238	20.5
Three (July- September)	143,690	26.8
Four (October –December)	143,418	26.7
Maternal age (years)		
Less than 20 years	8,195	1.5
20-29 years	188,923	35.2
30-39 years	313,795	58.5
40 years and above	25,835	4.8
Number of fetuses		
One (singleton pregnancy)	510,668	98.3
Two or more (multiple pregnancy)	8,548	1.7
Missing [d]	*17,532*	*3.3*
Parity [b]		
0 (nullipara)	219,187	42.5
1 (primipara)	179,852	34.8
>1 (multipara)	117,204	22.7
Missing [d]	*20,505*	*3.8*

Gravidity [c]		
1 (primigravida)	162,986	31.5
>1 (multigravida)	355,076	68.5
Missing [d]	*18,686*	*3.5*
History of previous aneuploidy		
Yes	2,085	0.4
No	534,663	99.6
Income quintile (neighbourhood)		
1 (lowest)	119,642	23.0
2	109,294	21.0
3	109,358	21.0
4	101,707	19.5
5 (highest)	81,041	15.6
Missing [d]	*15,710*	*2.9*
Educational quintile (neighbourhood)		
1 (lowest)	92,070	18.7
2	108,147	22.0
3	108,135	22.0
4	106,189	21.6
5 (highest)	77,286	15.7
Missing [d]	*44,921*	*8.4*
Body mass index (kg/m^2)		
< 18.5	23,399	5.2
18.5-24.9	229,279	50.8
25.0-29.9	110,702	24.6
≥30	87,606	19.4
Missing [d]	*85,762*	*16.0*

Started prenatal care in the first trimester		
Yes	440,251	91.9
No	39,010	8.1
Missing [d]	*57,487*	*10.7*

[a] *Column percentage.*

[b] *Parity indicates the number of pregnancies reaching a viable gestation (including live births and still births).*

[c] *Gravidity refers to the number of times a woman has been pregnant, regardless of whether the pregnancy was interrupted or resulted to a live birth.*

[d] *Missing values were not included in the denominators for calculating the distribution for each of these variables in turn.*

Table 4-2 presents the uptake of NIPT in the study population. Overall, 55,038 pregnant individuals (10.3%) used NIPT from 2016 to 2020. NIPT uptake was highest among those living in Toronto Central (20.4%) and lowest among those living in Erie St Clair and South West Region (4.7%). NIPT uptake was 59.2% among pregnant individuals who were 40 years and older and 7.8% among those under 40 years of age. Uptake of NIPT by pregnant individuals with no history of previous aneuploidy (10.1 %), or who did not start prenatal care in the first trimester (5.5 %) were low. There was a general pattern of increasing NIPT uptake with maternal residence in neighbourhoods with increasing income quintile (from 7.3% in the lowest to 15.6% in the highest) and educational quintile (from 5.0% to 19.5%).

Table 4-2. Distribution of baseline maternal characteristics of pregnant individuals that used and did not use NIPT and rates of NIPT uptake in Ontario, Canada from 2016 to 2020

	Pregnant individuals that used NIPT		Pregnant individuals that did not use NIPT		SD [b]	NIPT uptake per 100 (95% CI)
	N	% [a]	N	% [a]		
All pregnant individuals	55,038	100	481,710	100		10.2 (10.2-10.3)
Geographical location (LHINs) of maternal residence						
Erie St Clair and Southwest Region (1&2)	2,827	5.7	57,107	12.5	0.2	4.7 (4.6-4.9)
Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	5,960	12.1	76,200	16.6	0.1	7.3 (7.1-7.4)
Central West (5)	3,076	6.2	40,659	8.9	0.1	7.0 (6.8-7.3)
Mississauga Halton (6)	5,107	10.4	37,522	8.2	0.1	12.0 (11.7-12.3)
Toronto Central (7)	10,097	20.5	39,316	8.6	0.3	20.4 (20.1-20.8)
Central (8)	9,816	19.9	56,329	12.3	0.2	14.8 (14.6-15.1)
Central East (9)	4,802	9.7	52,482	11.5	0.1	8.4 (8.2- 8.6)
South East and Champlain Region (10&11)	5,424	11.0	58,081	12.7	0.1	8.5 (8.3-8.8)
North Simcoe Muskoka, North East and North West Region (12-14)	2,230	4.5	40,494	8.8	0.2	5.2 (5.0-5.3)
Missing [c]	*29,219*					
Quarter of the year (expected date of delivery)						
One (January- March)	15,265	27.7	124,137	25.8	0.0	10.9 (10.8-11.1)
Two (April-June)	11,156	20.3	99,082	20.6	0.0	10.1 (9.9-10.3)
Three (July- September)	13,892	25.2	129,798	26.9	0.0	9.7 (9.5-9.8)
Four (October –December)	14,725	26.8	128,693	26.7	0.0	10.3 (10.1-10.4)

Maternal age (years)						
Less than 40	39,741	72.2	471,172	97.8	0.8	7.8 (7.7-7.9)
40 years and above	15,297	27.8	10,538	2.2	0.8	59.2 (58.6-59.8)
Number of fetuses						
One (singleton pregnancy)	48,806	97.7	461,862	98.4	0.1	9.6 (9.5-9.7)
Two or more (multiple pregnancy)	1,152	2.3	7,396	1.6	0.1	13.4 (12.7-14.2)
Missing [c]	*17,532*					
Parity						
0 (nullipara)	19,951	40.4	199,236	42.7	0.1	9.1 (8.9-9.2)
1 (primipara)	18,190	36.8	161,662	34.6	0.1	10.1 (10.0-10.3)
>1 (multipara)	11,284	22.8	105,920	22.7	0.0	9.6 (9.5-9.8)
Missing [c]	*20,505*					
Gravidity						
1 (primigravida)	13,375	26.9	149,611	31.9	0.1	8.2 (8.1-8.3)
>1 (multigravida)	36,301	73.1	318,774	68.1	0.1	10.2 (10.1-10.3)
Missing [c]	*18,686*					
History of previous aneuploidy						
Yes	693	1.3	1,392	0.3	0.1	33.2 (31.3-34.3)
No	54,345	98.7	480,318	99.7	0.1	10.1 (10.1-10.3)
Income quintile (neighbourhood)						
1 (lowest)	8,717	16.3	110,925	23.7	0.2	7.3 (7.1-7.4)
2	9,635	18.0	99,659	21.3	0.2	8.8 (8.7-9.0)
3	10,521	19.7	98,837	21.1	0.0	9.6 (9.4-9.8)
4	11,867	22.2	89,836	19.2	0.2	11.7 (11.5-11.9)
5 (highest)	12,690	23.8	68,351	14.6	0.2	15.6 (15.4 -15.9)
Missing [c]	*15,710*					

Educational quintile (neighbourhood)						
1 (lowest)	4,587	9.1	87,483	19.8	0.3	5.0 (4.8-5.1)
2	7,516	14.9	100,631	22.8	0.2	7.0 (6.8-7.1)
3	10,184	20.2	97,951	22.2	0.1	9.4 (9.3-9.6)
4	12,949	25.7	93,240	21.1	0.1	12.2 (12.0-12.4)
5 (highest)	15,088	30.0	62,198	14.1	0.4	19.5 (19.2-19.8)
Missing [c]	*44,921*					
Body mass index (kg/m^2)						
< 18.5	5,912	4.5	214,187	5.3	0.0	8.2 (7.8-8.5)
18.5-24.9	23,599	55.4	205,680	50.4	0.1	10.3 (10.2-10.4)
25.0-29.9	9,969	23.4	100,733	24.7	0.0	9.0 (8.8-9.2)
≥ 30	7,118	16.7	80,488	19.7	0.1	8.1(8.0-8.3)
Missing [c]	*85,762*					
Started prenatal care in the first trimester						
Yes	40,437	95.0	399,814	91.6	0.1	9.21 (9.1-9.3)
No	2,151	5.1	36,859	8.4	0.1	5.5 (5.3-5.8)
Missing [c]	*57,487*					

[a] *Column percentage.*

[b] *Absolute standardized difference. Shaded cells represent standardized differences >10%.*

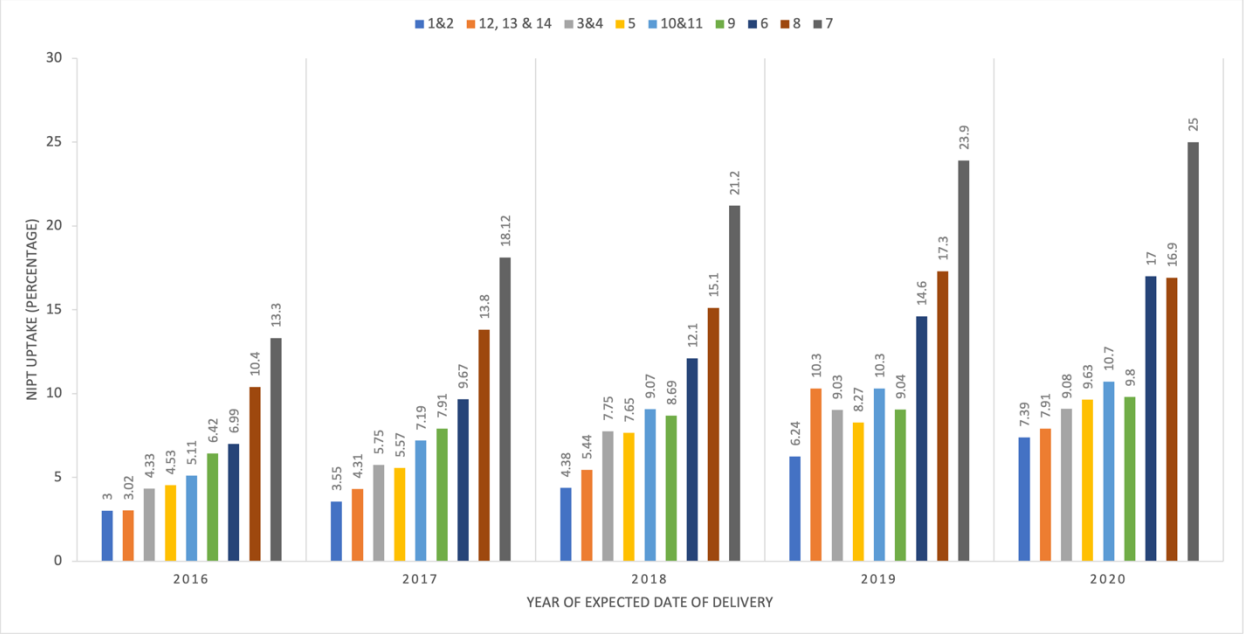
[c] *Missing values were not included in the denominators for calculating the distribution for each of these variables in turn.*

Table 4-3. Proportion of pregnant individuals that underwent NIPT in Ontario, Canada from 2016 to 2020

Year **(Expected year of delivery)**	2016	2017	2018	2019	2020
Number of pregnant individuals	61,728	146,118	146,428	147,245	35,229
Number of pregnant individuals that used NIPT	4,043	12,861	15,546	17,944	4,644
NIPT uptake per 100 (95% CI)	6.5 (6.3-6.8)	8.8 (8.7-9.0)	10.6 (10.4-10.8)	12.2 (12.0-12.4)	13.2 (12.8-13.5)

NIPT was counted in the year of expected delivery.

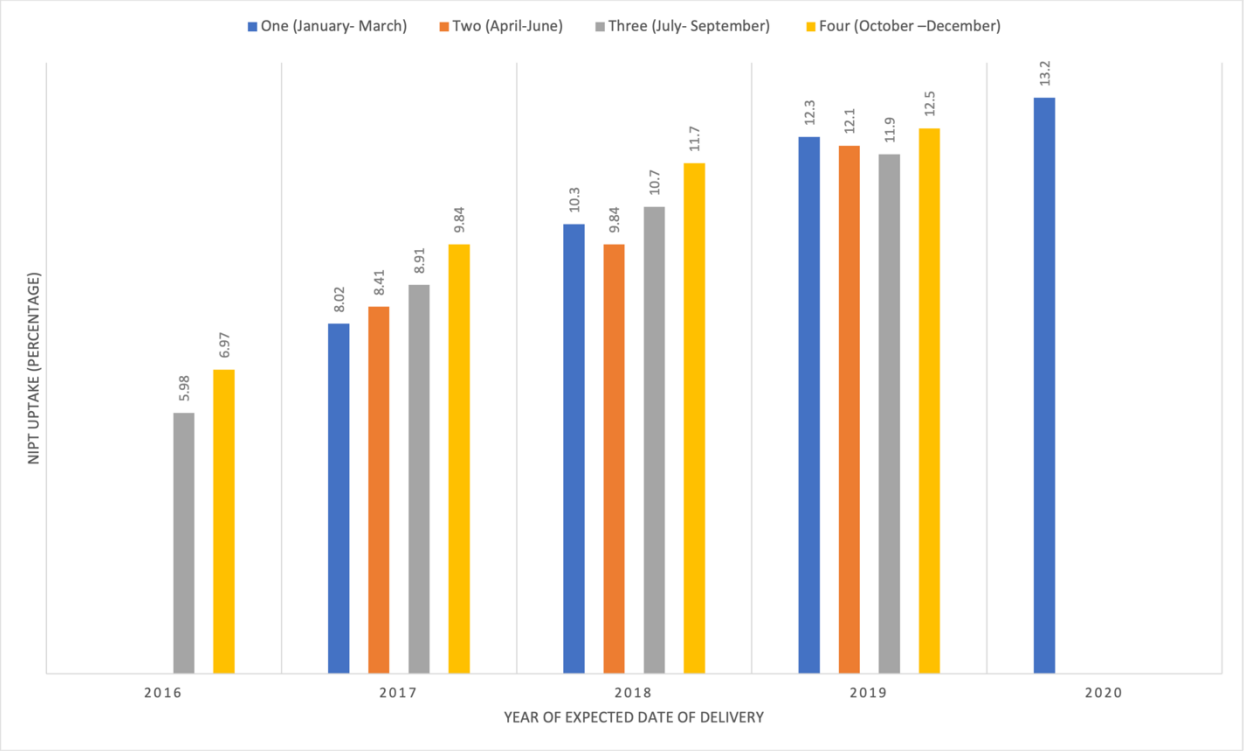
Figure 4-1. Proportion of pregnant individuals that underwent NIPT in Ontario, Canada from 2016 to 2020 by LHINs



NIPT was counted in the year of expected delivery.

1&2 (Erie St Clair and South West Region), 12,13&14 (North Simcoe Muskoka, North East and North West Region), 3&4 (Hamilton, Niagara, Haldeman, Brant Region), 5 (Central West), 10&11 (South East and Champlain Region), 9 (Central East), 6 (Mississauga Halton) 8 (Central), 7 (Toronto Central)

Figure 4-2. Proportion of pregnant individuals that underwent NIPT in Ontario, Canada from 2016 to 2020 by quarter of year



NIPT was counted in the year of expected delivery.

There was an increase in the uptake of NIPT every year from 2016 to 2020, such that it doubled across this period (from 6.5 in 2016 to 13.2 in 2020; **Table 4-3).** This pattern was apparent in each region. In each year, Toronto Central recorded the highest percentage of pregnant individuals that used NIPT and Erie St Clair and South West Region recorded the lowest percentage (**Figure 4-1**). When the data were considered by quarter of expected date of delivery, there was a pattern of steady increase (**Figure 4-2**).

4.1.2 Missing information on one or more of the variables

There was no missing information for quarter of EDD, maternal age or history of previous aneuploidy. The proportion of missing information was 5% or less for location of residence, number of fetuses, parity, gravidity, and quintile of income **(Table 4.1).** There was a higher proportion of missing values for body mass index (16.0%) and whether prenatal care started in the first trimester (10.7%). Evaluation of the patterns of missingness is presented in **Table 4-4.** There was complete information on all variables for 390,537 pregnant individuals (72.8%), while 146,211 pregnant individuals had missing information on one or more study variables (27.2%). There were differences between pregnant individuals with complete information and those missing information on one or more variables, as indicated by a standardized difference > 0.1, in geographical location, number of fetuses, and educational quintile (see standardized differences comparing groups (i) and (ii) in **Table 4-4**).

A total of 472,468 pregnant individuals had complete information for all variables or had missing information only on BMI or first trimester visit. An additional 64,280 pregnant individuals had complete information on BMI and first trimester visit but were missing information for one or more of the other study variables. The baseline characteristics that differed between these two groups, as indicated by a standardized difference >0.1, were geographical location, gravidity and educational quintile (see standardized differences comparing groups (iii) and (iv) in **Table 4-4**).

Table 4-4. Baseline maternal characteristics of pregnant individuals with complete information on all variables and for those with missing information

	(i) Pregnancies with complete information on all variables		(ii) Pregnancies with missing information on one or more study variables		SD [b, c]	(iii) Pregnancies with complete information on all variables or with missing information only on BMI or first trimester visit		(iv) Pregnancies with missing information on one or more study variables other than BMI or first trimester visit		SD [b, d]
	N	**% [a]**	**N**	**% [a]**		**N**	**% [a]**	**N**	**% [a]**	
All pregnancies	**390,537**	**100**	**146,211**	**100**		**472,468**	**100**	**64,280**	**100**	
Geographic location (LHINs) of maternal residence										
Eric St Clair and Southwest Region (1&2)	52,523	13.5	7,411	6.3	0.3	57,034	12.1	2,900	8.3	0.1
Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	68,790	17.6	13,370	11.4	0.2	76,980	16.3	5,180	14.8	0.0
Central West (5)	23,884	6.1	19,851	17.0	0.3	39,195	8.3	4,540	12.9	0.2
Mississauga Halton (6)	29,684	7.6	12,945	11.1	0.1	39,577	8.4	3,052	8.7	0.0
Toronto Central (7)	30,988	7.9	18,425	15.8	0.2	46,092	9.8	3,321	9.5	0.0
Central (8)	49,607	12.7	16,538	14.1	0.0	60,031	12.7	6,114	17.4	0.1
Central East (9)	46,661	11.9	10,623	9.1	0.1	53,735	11.4	3,549	10.1	0.0

South East and Champlain Region (10&11)	52,520	13.5	10,985	9.4	0.1	59,710	12.6	3,795	10.8	0.1
North Simcoe Muskoka, North East and North West Region (12-14)	35,880	9.2	6,844	5.9	0.1	40,114	8.5	2,610	7.4	0.0
Missing [e]			*29,219*	*19.9*				*29,219*	*45.5*	
Quarter of the year (expected date of delivery)										
One (January-March)	100,108	25.6	39,294	26.9	0.0	121,371	25.7	18,031	28.1	0.1
Two (April-June)	80,088	20.7	29,350	20.1	0.0	97,487	20.6	12,751	19.8	0.0
Three (July-September)	105,112	26.9	38,578	26.4	0.0	127,197	26.9	16,493	25.7	0.0
Four (October – December)	104,425	26.7	38,989	26.7	0.0	126,413	26.8	17,005	26.5	0.0
Maternal age (years)										
Less than 40 years	373,624	31.7	39,310	30.8	0.0	450,434	95.3	60,479	94.1	0.1
40 years and above	266,861	68.3	88,215	69.1	0.0	22,034	4.7	3,801	5.9	0.1
Number of fetuses										
One (singleton pregnancy)	384,611	98.5	126,057	97.9	0.0	464,821	98.4	45,847	98.1	0.0

Two or more (multiple pregnancy)	1,490	0.4	2,622	2.6	0.2	7,647	1.6	901	1.9	0.0
Missing [e]			*17,532*	*11.9*				*17,532*	*27.3*	
Parity										
0 (nullipara)	166,783	42.7	52,404	41.7	0.0	198,622	42.0	20,565	46.9	0.1
1 (primipara)	136,332	34.9	43,520	34.6	0.0	165,445	35.0	14,407	32.9	0.0
>1 (multipara)	87,422	22.4	29,782	23.7	0.0	108,401	22.9	8,803	20.1	0.1
Missing [e]			*20,505*	*14.0*				*20,505*	*39.9*	
Gravidity										
1 (primigravida)	123,676	31.7	39,310	30.8	0.0	147,180	95.3	15,806	34.7	1.7
>1 (multigravida)	266,861	68.3	88,215	69.2	0.0	325,288	4.7	29,788	65.3	1.7
Missing [e]			*18,686*	*12.8*				*18,686*	*29.1*	
History of previous aneuploidy										
Yes	1,490	0.4	595	0.4	0.0	1,844	0.4	241	0.4	0.0
No	389,047	99.6	145,616	99.6	0.0	470,624	99.6	64,039	99.6	0.0
Income quintile (neighbourhood)										
1 (lowest)	90,403	23.2	29,239	22.4	0.0	110,444	23.4	9,198	18.9	0.1
2	82,388	21.1	26,906	20.6	0.0	100,095	21.2	9,199	18.9	0.1

3	80,195	20.5	29,163	22.4	0.0	97,921	20.7	11,437	23.6	0.1
4	76,211	19.5	25,492	19.5	0.0	90,112	19.1	11,591	23.9	0.1
5 (highest)	61,340	15.7	19,701	15.1	0.0	73,896	15.6	7,145	14.7	0.0
Missing [e]			*15,710*	*10.7*				*15,710*	*24.4*	
Educational quintile (neighbourhood)										
1 (lowest)	78,144	20.0	13,926	13.8	0.2	89,733	18.9	2,337	12.1	0.2
2	89,181	22.8	18,966	18.7	0.1	104,783	22.2	3,364	17.4	0.1
3	84,257	21.6	23,878	23.6	0.1	103,881	21.9	4,254	21.9	0.0
4	83,174	21.3	23,015	22.7	0.0	101,631	21.5	4,558	23.5	0.1
5 (highest)	55,781	14.3	21,505	21.2	0.2	72,440	15.3	4,846	25.0	0.2
Missing [e]			*44,921*	*30.7*				*44,921*	*69.9*	
Body mass index (kg/m^2)										
< 18.5	19,904	5.1	3,495	5.8	0.1	21,228	5.2	2,171	5.6	0.0
18.5-24.9	196,379	50.3	32,900	54.3	0.1	208,533	50.6	20,746	53.3	0.1
25.0-29.9	96,738	24.8	13,964	23.1	0.0	101,321	24.6	9,381	24.1	0.0
≥ 30	77,516	19.9	10,090	16.7	0.1	80,953	19.7	6,653	17.1	0.1
Missing [e]			*85,762*	*58.7*		*60,433*	*12.8*	*25,329*	*39.4*	
Started prenatal care in the first trimester										
Yes	361,114	92.5	79,137	89.2	0.1	402,568	91.9	37,683	90.8	0.0

No	29,423	7.5	9,587	10.8	0.1	35,195	8.0	3,815	9.2	0.0
Missing [e]			*57,487*	*39.3*		*34,705*	*7.4*	*22,782*	*35.4*	

[a] *Column percentage*

[b] *Absolute standardized difference. Shaded cells represent standardized differences >10%.*

[c] *Standardized difference between groups (i) and (ii).*

[d] *Standardized difference between groups (iii) and (iv).*

[e] *Missing values were not included in the denominators for calculating the distribution for each of these variables in turn.*

4.1.3 Rate of NIPT utilization among pregnant individuals in Ontario

Table 4-5 presents the unadjusted and adjusted rate ratios of NIPT utilization among the pregnant individuals in the study population. Uptake of NIPT was associated with several maternal characteristics.

Compared with Toronto Central, the adjusted rate ratios (aRR) for NIPT use were lower in all other regions, and lowest in women living in Erie St. Clair and South West Region (aRR: 0.39; 95% CI: 0.39-0.43). Uptake was higher in the first quarter of expected date of delivery than in the other three quarters. Uptake decreased with decreasing quintile of income and was 37% lower among pregnant individuals in the lowest neighbourhood income quintile compared with the highest (quintile 1 vs. quintile 5 aRR: 0.63; 95% CI: 0.61-0.65), and 47% lower among pregnant individuals in the lowest neighbourhood education quintile compared with the highest (quintile 1 vs. quintile 5 aRR: 0.53; 95% CI: 0.51-0.55). Compared with those who initiated their prenatal care during the first trimester, NIPT uptake was lower if pregnant individuals did not start prenatal care in the first trimester (aRR: 0.64; 95% CI: 0.61-0.66) but was higher in individuals who were missing this information on their record (aRR: 1.25; 95% CI: 1.21-1.29). NIPT uptake was lower for women whose body mass index was outside the normal range 18.5-24.9 kg/m^2, or if this information was missing. Uptake was increased for pregnant individuals with a history of previous aneuploidy (aRR: 2.49; 95% CI: 2.29-2.79), aged 40 years or more (aRR: 6.82; 95% CI: 6.67-6.97), multigravidae (aRR: 1.19; 95% CI: 1.15-1.23), or with a multiple pregnancy (aRR: 1.20; 95% CI: 1.12-1.27). All magnitudes of these associations were similar to, but somewhat attenuated from, those observed in the unadjusted analysis. There was no evidence of over-dispersion in our model.

4.1.4 Sensitivity analyses

We conducted two sensitivity analyses to assess the robustness of our main findings. In the first, we excluded either BMI or timing of prenatal care visit from the model and in our second sensitivity analysis, we performed a complete case analysis by restricting the study population to only those with complete information on BMI. For all models, the point estimates were very similar to those for the adjusted primary analysis. There was no evidence of over-dispersion for our two models.

Table 4-5. Rate of NIPT utilization among pregnant individuals in Ontario, Canada from 2016 to 2020

	Primary analyses		Sensitivity analyses	
	Unadjusted	**Adjusted**	**Adjusted**	
	Rate ratio (95%CI) **N=536,748** [d]	**Rate ratio (95%CI)** [a] **N=472,468** [d]	**Rate ratio (95%CI)** [b] **N=472,468** [d]	**Rate ratio (95%CI)** [c] **N=412,035** [d]
Geographical location (LHINs) of maternal residence				
Toronto Central (7)	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Erie St Clair and South West Region (1&2)	0.23 (0.22-0.24)	0.41(0.39-0.43)	0.40 (0.39-0.42)	0.42 (0.40-0.44)
Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	0.36 (0.34-0.37)	0.58 (0.56-0.61)	0.57 (0.55-0.60)	0.59 (0.57-0.61)
Central west (5)	0.34 (0.33-0.36)	0.54 (0.52-0.58)	0.54 (0.51-0.56)	0.55 (0.53-0.59)
Mississauga Halton (6)	0.59 (0.57-0.61)	0.68 (0.65-0.71)	0.67 (0.65-0.70)	0.68 (0.65-0.70)
Central (8)	0.73 (0.71-0.76)	0.91 (0.88-0.95)	0.91 (0.88-0.93)	0.92 (0.89-0.95)
Central East (9)	0.41 (0.40-0.42)	0.65 (0.65-0.69)	0.65 (0.63-0.68)	0.66 (0.63-0.68)
South East and Champlain Region (10&11)	0.42 (0.40-0.43)	0.61 (0.58-0.63)	0.59 (0.57-0.62)	0.61 (0.58-0.64)

North Simcoe Muskoka, North East and North West Region (12-14)	0.26 (0.24-0.27)	0.50 (0.47-0.53)	0.49 (0.47-0.52)	0.52 (0.48-0.54)
Quarter of the year (expected date of delivery)				
One (January-march)	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Two (April-June)	0.92 (0.90-0.95)	0.96 (0.93-0.99)	0.94 (0.92-0.97)	0.96 (0.93-0.99)
Three (July-September)	0.88 (0.86-0.90)	0.92 (0.89-0.94)	0.92 (0.89-0.93)	0.91 (0.88-0.93)
Four (October –December)	0.94 (0.92-0.96)	0.94 (0.92-0.97)	0.96 (0.91- 0.97)	0.94 (0.92-0.97)
Income quintile (neighborhood)				
5 (highest)	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
4	0.75 (0.73-0.76)	0.88 (0.86-0.90)	0.88 (0.86-0.92)	0.88 (0.86-0.92)
3	0.61 (0.60-0.63)	0.81 (0.78-0.83)	0.80 (0.78-0.83)	0.80 (0.78- 0.81)
2	0.56 (0.55-0.58)	0.75 (0.73-0.77)	0.75 (0.73-0.76)	0.75 (0.73- 0.76)
1 (lowest)	0.47 (0.45-0.48)	0.63 (0.61-0.65)	0.63 (0.62-0.66)	0.63 (0.62-0.67)
Educational quintile (neighbourhood)				
5 (highest)	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
4	0.63 (0.61-0.64)	0.80 (0.79-0.84)	0.81 (0.79-0.84)	0.81 (0.79-0.83)
3	0.48 (0.47-0.50)	0.74 (0.71-0.73)	0.73 (0.71-0.75)	0.73 (0.71-0.75)
2	0.36 (0.35-0.37)	0.61 (0.60-0.63)	0.61 (0.59-0.63)	0.61 (0.59-0.63)

1 (lowest)	0.23 (0.25-0.26)	0.53 (0.51 -0.55)	0.52 (0.50-0.54)	0.53 (0.50- 0.55)
Started prenatal care in the first trimester				
Yes	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
No	0.60 (0.57-0.63)	0.64 (0.61-0.66)	0.64 (0.61-0.66)	0.66 (0.64-0.69)
Missing category	2.36 (2.31-2.40)	1.25 (1.21-1.29)	1.24 (1.20-1.27)	1.23 (1.19-1.27)
History of previous aneuploidy				
No	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Yes	3.27 (3.03-3.52)	2.49 (2.29-2.79)	2.49 (2.29-2.70)	2.51 (2.28-2.73)
Maternal age (years)				
Less than 40	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
40 years and above	7.61 (7.45-7.76)	6.82 (6.67-6.97)	6.81 (6.67-7.00)	6.95 (6.79-7.11))
Parity				
0 (nullipara)	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
1 (primipara)	1.11 (1.09-1.13)	0.93 (0.91-0.96)	0.93 (0.90-0.96)	0.93 (0.90- 0.96)
>1 (multipara)	1.06 (1.03-1.08)	0.84 (0.81-0.86)	0.84 (0.81-0.86)	0.84 (0.81-0.86)
Gravidity				
1 (primigravida)	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
>1 (multigravida)	1.23 (1.22-1.27)	1.20 (1.15-1.23)	1.18 (1.15-1.22)	1.21 (1.17-1.25)

Number of fetuses				
One (singleton pregnancy)	1.00 (REF)	1.00 (REF)	1.00 (REF)	1.00 (REF)
Two or more (multiple pregnancy)	1.41 (1.33-1.49)	1.20 (1.12-1.27)	1.19 (1.12- 1.26)	1.22 (1.14-1.33)
Body Mass Index (kg/m^2)				
< 18.5	0.79 (0.76-0.83)	0.86 (0.82-0.91)		0.87 (0.83-0.92)
18.5-24.9	1.00 (REF)	1.00 (REF)		1.00 (REF)
25.0-29.9	0.87 (0.85-0.90)	0.93 (0.91-0.96)		0.93 (0.91-0.95)
≥ 30	0.79 (0.77-0.81)	0.93 (0.90-0.96)		0.93 (0.90-0.95)
Missing category	1.40 (1.38-1.44)	0.94 (0.91-0.97)		

[a] *Variables included in the model were geographical location (LHINs) of maternal residence, quarter of the year (expected date of delivery), income quintile (neighbourhood), educational quintile (neighbourhood), started prenatal care in the first trimester, history of previous aneuploidy, maternal age (years), parity, gravidity, number of fetuses and BMI.*

[b] *Variables included in the model were geographical location (LHINs) of maternal residence, quarter of the year (expected date of delivery), income quintile (neighbourhood), educational quintile (neighbourhood), started prenatal care in the first trimester/BMI, history of previous aneuploidy, maternal age (years), parity, gravidity and number of fetuses.*

[c] *Pregnant individuals with complete BMI data were used for the analysis. Variables included in the model were geographical location (LHINs) of maternal residence, quarter of the year (expected date of delivery), income quintile (neighbourhood), educational quintile (neighbourhood), started prenatal care in the first trimester, history of previous aneuploidy, maternal age (years), parity, gravidity, number of fetuses and BMI.*

[d] *Total number of observations used in the model for analysis.*

Pearson statistic: adjusted primary analysis (0.85[a]), adjusted sensitivity analysis (0.87[b], 0.87[c]).

Deviance statistic: adjusted primary analysis (0.36 [a]), adjusted sensitivity analysis (0.35 [b] 0.36 [c])

4.2 Uptake of first-tier or second-tier NIPT (Objective 2)

4.2.1 Descriptive characteristics

Table 4-6 presents the characteristics of pregnant individuals who had a high-risk pregnancy (i.e., pregnant individuals aged 40 years or more and /or with a history of previous aneuploidy) in the study. A total of 27,733 pregnant individuals were classified as having a high-risk pregnancy. More than half of these pregnant individuals used NIPT (15,841; 57.1%), and 93.3% of NIPT users received OHIP funding. The largest number of high-risk pregnant individuals lived in Central Ontario (17.6%), had singleton pregnancies (97.3%) and started prenatal care in the first trimester (91.7%). The number of pregnant individuals who lived in neighbourhoods with the lowest income quintile (22.1%) was somewhat higher than the numbers in the other income quintiles, whereas the number who lived in neighbourhoods with the two lowest educational quintiles were substantially less than in the other educational quintiles.

Table 4-6. Characteristics of pregnant individuals who were 40 years and above and/or with a history of previous aneuploidy with expected date of delivery from August 1st, 2016 to March 31st, 2020 in Ontario, Canada

	TOTAL	
	N	% [a]
All pregnant individuals	27,733	100
Number of pregnant individuals that used NIPT	15,841	57.1%
Received OHIP funding for NIPT		
Yes [c]	14,782	53.3
No [d]	1,059	3.8
Did not undergo NIPT	11,892	42.9
Geographical location (LHINs) of maternal residence		
Erie St Clair and South West Region (1&2)	2,045	8.1

Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	3,304	13.2
Central West (5)	2,188	8.7
Mississauga Halton (6)	2,734	10.8
Toronto Central (7)	3,758	14.8
Central (8)	4,456	17.6
Central East (9)	2,756	10.9
South East and Champlain Region (10&11)	2,877	11.4
North Simcoe Muskoka, North East and North West Region (12-14)	1,174	4.6
Missing [b]	*2,441*	*8.8*
Quarter of the year (expected date of delivery)		
One (January- March)	7,604	27.4
Two (April-June)	5,422	19.4
Three (July- September)	7,060	25.5
Four (October –December)	7,647	27.6
Number of fetuses		
One (singleton pregnancy)	25,220	97.3
Two or more (multiple pregnancy)	697	2.7
Missing [b]	*1,816*	*6.6*
Parity		
0 (nullipara)	6,527	25.4
1 (primipara)	8,667	33.7
>1 (multipara)	10,491	40.8
Missing [b]	*2,048*	*7.4*
Gravidity		
1 (primigravida)	3,471	13.5
>1 (multigravida)	22,342	86.6

Missing [b]	*1,920*	*6.9*
Income quintile (neighbourhood)		
1 (lowest)	5,957	22.1
2	5,320	19.7
3	5,384	20.0
4	5,270	19.5
5 (highest)	5,042	18.7
Missing [b]	*760*	*2.7*
Educational quintile (neighbourhood)		
1 (lowest)	3,227	12.6
2	4,685	18.3
3	5,579	21.7
4	6,450	25.1
5 (highest)	5,729	22.3
Missing [b]	*2,063*	*7.4*
Body mass index (kg/m^2)		
< 18.5	729	3.4
18.5-24.9	10,211	47.1
25.0-29.9	5,920	27.3
≥30	4,833	22.3
Missing [b]	*6,040*	*21.8*
Started prenatal care in the first trimester		
Yes	20,779	91.7
No	1,891	8.34
Missing [b]	*5,063*	*18.3*

[a] *Column percentage.*

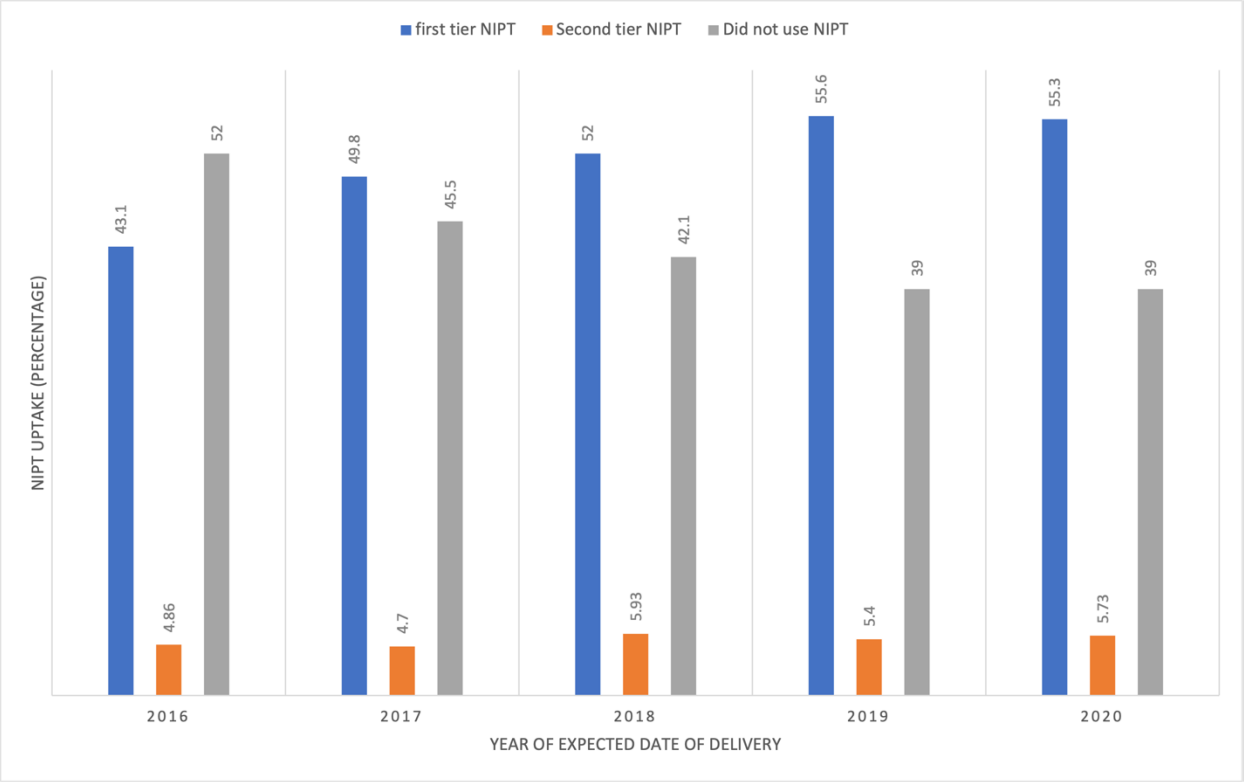
[b] *Missing values were not included in the denominators for calculating the distribution for each of these variables in turn.*

[c] *13313 pregnant individuals used OHIP funding for first-tier NIPT and 1469 used OHIP funding for second-tier NIPT which sums to 14782.*

[d] *1049 pregnant individuals did not use OHIP funding for first-tier NIPT and 10 did not use OHIP funding for second-tier NIPT which sums to 1059.*

The proportion of pregnant individuals that used first-tier NIPT (i.e., did not undergo any prenatal screening) or second-tier NIPT (i.e., contingent on the results of one or more other screening test results) among high-risk pregnant individuals from 2016 to 2020 is presented in **Figure 4-3.** There was a steady increase in the uptake of first-tier NIPT but minimal change in the uptake of second-tier NIPT from 2016 to 2020.

Figure 4-3. Proportion of pregnant individuals that used first-tier NIPT, second-tier NIPT or did not use NIPT among pregnant individuals who were 40 years and above and/ or with a history of previous aneuploidy from 2016 to 2020



NIPT was counted in the year of expected delivery.

Table 4-7 presents the uptake of either first or second-tier NIPT among high-risk pregnancies in the study population. In total 14,362 used first-tier NIPT, 1,479 used second-tier NIPT and 11,892 did not use NIPT. Among pregnant individuals at high-risk, the rate of use of first-tier NIPT was highest among those that lived in the Toronto Central (66.0 %) and lowest in the Erie St Clair and South West (34.1%). Most of the pregnant individuals received OHIP-funding for either first or second-tier NIPT (53.3%). There was a general pattern of increasing NIPT uptake with maternal residence in neighbourhoods with increasing income quintile (from 44.6% in the lowest to 61.7% in the highest) and educational quintile (from 36.9% in the lowest to 64.9% in the highest). Uptake reduced with increasing parity, gravidity, in multiple as compared to singleton pregnancies, with body mass index outside the range of 18.5-24.9 kg/m^2, and for those who had their first prenatal care visit after the first trimester. As indicated by a standardized difference > 0.1, high-risk pregnant individuals that used first-tier NIPT differed from those that used second-tier NIPT in geographical location, quintile of neighbourhood income and education, and OHIP funding for NIPT.

Among high-risk pregnant individuals, uptake of second-tier NIPT ranged between 4.3% and 7.4%. Uptake decreased slightly with decreasing quintile of income and there was no clear pattern across quintiles of neighbourhood education. Uptake was lower in multiple as compared to singleton pregnancies and in those who started prenatal care after the first trimester as compared to those who started during the first trimester. Uptake was somewhat increased for pregnant individuals whose body mass index was outside the range 18.5-24.9 kg/m^2. As indicated by a standardized difference > 0.1, high-risk pregnant individuals that used first-tier NIPT differed from those that did not use NIPT with respect to location of residence, quintile of income and education, parity, or timing of first trimester visit.

Table 4-7. Distribution of baseline maternal characteristics of pregnant individuals that used first-tier NIPT, second-tier NIPT or did not use NIPT and rates of first or second-NIPT uptake among pregnant individuals who were 40 years and above and/or with a history of previous aneuploidy

	Pregnant individuals that used first-tier NIPT		Pregnant individuals that used second-tier NIPT		Pregnant individuals that did not use NIPT		SD [b]	SD [c]	First-tier NIPT uptake per 100 (95% CI)	Second-tier NIPT uptake per 100 (95% CI)
All women	14,362	% [a]	1,479	% [a]	11,892	% [a]				
Geographical location (LLINs) of maternal residence										
Erie St Clair and South West Region (1&2)	698	5.4	97	7.0	1,250	11.4	0.1	0.2	34.1 (32.1-36.2)	4.7 (3.9-5.7)
Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	1,446	11.2	178	12.9	1,680	15.3	0.1	0.1	43.8 (42.1-45.5)	5.4 (4.6-6.2)
Central west (5	858	6.7	161	11.6	1,169	10.6	0.2	0.1	39.2 (37.2-41.3)	7.4 (6.3-8.5)
Mississauga Halton (6)	1,402	10.7	162	11.7	1,170	10.6	0.0	0.1	51.3 (49.4-53.2)	5.9 (5.1-6.8)
Toronto Central (7)	2,481	19.2	168	12.2	1,109	10.1	0.2	0.2	66.0 (64.5-67.5)	4.5 (3.8-5.2)
Central (8)	2,636	20.4	289	20.9	1,531	13.9	0.0	0.2	59.2 (57.7-60.6)	6.5 (5.8-7.3)
Central East (9)	1,440	11.2	142	10.3	1,174	10.7	0.0	0.0	52.3 (50.4-54.1)	5.2 (4.4-6.04)
South East and Champlain Region (10&11)	1,473	11.4	135	9.8	1,269	11.5	0.1	0.0	51.2 (49.4-53.0)	4.7 (4.0-5.5)

North Simcoe Muskoka, North East and North West Region (12-14)	472	3.7	51	3.7	651	5.9	0.0	0.1	40.2 (37.4-43.0)	4.3 (3.3-5.7)
Missing [d]	*2,441*									
Quarter of the year (expected date of delivery)										
One (January-march)	3,986	27.8	416	28.1	3,202	26.9	0.0	0.0	52.4 (51.3-53.5)	5.5 (5.0-6.0)
Two (April-June)	2,898	20.2	254	17.2	2,270	19.1	0.1	0.0	53.4 (52.2-54.8)	4.7 (4.2-5.3)
Three (July-September)	3,556	24.8	386	26.1	3,118	26.2	0.0	0.0	50.4 (49.2-51.3)	5.5 (5.0-6.02)
Four (October –December)	3,922	27.3	423	28.6	3,302	27.8	0.0	0.0	51.3 (50.2-52.4)	5.5 (5.0-6.1)
Income quintile (neighbourhood)										
1 (lowest)	2,659	18.9	373	25.7	2,925	25.5	0.2	0.2	44.6 (43.4-45.9)	6.2 (5.6-6.1)
2	2,651	18.9	320	22.0	2,349	20.5	0.1	0.0	49.8 (48.5-51.1)	6.0 (5.4-6.7)
3	2,707	19.3	276	19.0	2,401	21.0	0.0	0.0	50.3 (48.9-51.6)	5.1 (4.5-5.9)
4	2,934	20.9	263	18.1	2,073	18.1	0.1	0.1	55.7 (54.3-57.0)	5.0 (4.4-5.6)
5 (highest)	3,112	22.1	221	15.2	1,709	14.9	0.2	0.2	61.7 (60.4-63.1)	4.4 (3.8-5.0)
Missing [d]	*760*									
Educational quintile (neighbourhood)										
1 (lowest)	1,191	8.9	167	11.9	1,869	17.3	0.1	0.3	36.9 (35.3-38.6)	5.2 (4.5-6.0)
2	2,072	15.4	270	19.2	2,343	21.7	0.1	0.2	44.3 (42.8-45.7)	5.8 (5.1-6.4)

3	2,837	21.1	345	24.5	2,397	22.2	0.1	0.0	50.9 (49.5-52.2)	6.2 (5.6-6.9)
4	3,646	27.1	362	25.7	2,442	22.6	0.0	0.1	56.5 (55.3-57.7)	5.6 (5.1-6.2)
5 (highest)	3,715	27.6	264	18.8	1,750	16.2	0.2	0.3	64.9 (63.6-66.1)	4.6 (4.1-5.2)
Missing [d]	*2,063*									
Parity										
0 (nullipara)	3,658	28.3	350	25.3	2,519	22.1	0.1	0.2	56.0 (54.8-57.2)	5.4 (4.8-5.9)
1 (primipara)	4,760	36.8	478	34.5	3,429	30.1	0.1	0.1	54.9 (53.9-56.0)	5.5 (5.1-6.0)
>1 (multipara)	4,506	34.9	556	40.2	5,429	47.7	0.1	0.2	43.0 (42.0-43.9)	5.3 (4.9-5.8)
Missing [d]	*2,048*									
Gravidity										
1 (primigravida)	1,860	14.3	201	14.5	1,410	12.3	0.0	0.1	53.6 (51.9-55.2)	5.8 (5.0-6.6)
>1 (multigravida)	11,138	85.7	1,188	85.5	10,016	87.7	0.0	0.1	49.9 (49.1-50.5)	5.3 (5.0-5.6)
Missing [d]	*1920*									
Number of fetuses										
One (singleton pregnancy)	12,779	97.7	1,370	98.4	11,071	96.7	0.1	0.1	50.6 (50.1-51.33)	5.4 (5.2-5.7)
Two or more (multiple pregnancy)	295	2.3	23	1.7	379	3.3	0.0	0.1	42.3 (38.7-46.0)	3.3 (2.2-4.9)
Missing [d]	*1,816*									
Body mass index (kg/m^2)										
< 18.5	353	3.2	41	3.6	335	3.5	0.0	0.0	48.4 (44.8-52.1)	5.6 (4.2-7.5)

18.5-24.9	5,489	50.0	499	44.1	4,223	44.1	0.1	0.1	53.8 (52.3-54.7)	4.9 (4.5-5.3)
25.0-29.9	2,887	26.3	330	29.2	2,703	28.2	0.1	0.0	48.8 (47.5-50.0)	5.6 (5.0-6.2)
≥30	2,256	20.5	262	23.1	2,315	24.2	0.1	0.1	46.7 (45.3-48.1)	5.4 (4.8-6.1)
Missing [d]	*6,040*									
Started prenatal care in the first trimester										
Yes	10,494	94.4	1134	94.0	9,151	88.5	0.0	0.2	50.0 (49.8-51.22)	5.5 (5.6-5.8)
No	629	5.7	72	6.0	1,190	11.5	0.0	0.2	33.3 (31.2-35.4)	3.8 (3.0-4.8)
Missing [d]	*5,063*									
OHIP Funding for NIPT										
Yes	13,313	92.7	1,469	99.3			0.3			
No	1,049	7.3	10	0.7			0.3			

[a] *Column percentage.*

[b] *Absolute standardized difference between pregnant individuals that used first-tier NIPT and pregnant individuals that used second-tier NIPT. Shaded cells represent standardized differences >10%.*

[c] *Absolute standardized difference between pregnant individuals that used first-tier NIPT and pregnant individuals that did not use NIPT. Shaded cells represent standardized differences >10%.*

[d] *Missing values were not included in the denominators for calculating the distribution for each of these variables in turn.*

Table 4-8 presents characteristics of 509,015 pregnant individuals who were low-risk pregnant individuals in the study population (i.e**.,** pregnant individuals who were below 40 with no history of previous aneuploidy). Over 90% of the low-risk pregnant individuals did not use NIPT. The largest number of pregnant individuals lived in the Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (16.4%), had singleton pregnancies (98.4%) and started prenatal care in the first trimester (91.9%). The smallest numbers of low-risk pregnant individuals lived in neighbourhoods with the highest quintiles of income and education.

Table 4-8. Characteristics of pregnant individuals who were below 40 years with no history of chromosomal aneuploidy with expected date of delivery from August 1st, 2016 to March 31st, 2020 in Ontario, Canada

	TOTAL	
	N	% [a]
All pregnant individuals	509,015	100
Number of pregnant individuals that used NIPT	39,197	7.7%
Received OHIP funding for NIPT		
Yes [c]	19,509	3.8
No [d]	19,688	3.9
Did not undergo NIPT	469,818	92.3
Geographical location (LHINs) of maternal residence		
Erie St Clair and South West Region (1&2)	57,889	12.0
Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	78,856	16.4
Central West (5)	41,547	8.6
Mississauga Halton (6)	39,895	8.3
Toronto Central (7)	45,655	9.5

Central (8)	61,689	12.8
Central East (9)	54,528	11.3
South East and Champlain Region (10&11)	60,628	12.3
North Simcoe Muskoka, North East and North West Region (12-14)	41,550	8.6
Missing [b]	*26,778*	*5.3*
Quarter of the year (expected date of delivery)		
One (January- March)	131,798	25.9
Two (April-June)	104,816	20.6
Three (July- September)	136,630	26.8
Four (October –December)	135,771	26.7
Number of fetuses		
One (singleton pregnancy)	485,448	98.4
Two or more (multiple pregnancy)	7,851	1.6
Missing [b]	*15,716*	*3.1*
Parity		
0 (nullipara)	212,660	43.4
1 (primipara)	171,185	34.9
>1 (multipara)	106,713	21.8
Missing [b]	*18,457*	*3.6*
Gravidity		
1 (primigravida)	159,515	32.4
>1 (multigravida)	332,734	67.6
Missing [b]	*16,766*	*3.3*
Income quintile (neighbourhood)		
1 (lowest)	113,685	23.0
2	103,974	21.0
3	103,974	21.0

4	96,433	19.5
5 (highest)	75,999	15.4
Missing [b]	*14,950*	*2.9*
Educational quintile (neighbourhood)		
1 (lowest)	88,843	19.1
2	103,462	22.2
3	102,556	22.0
4	99,739	21.4
5 (highest)	71,557	15.4
Missing [b]	*42,858*	*8.4*
Body mass index (kg/m^2)		
< 18.5	22,670	5.3
18.5-24.9	219,068	51.0
25.0-29.9	104,782	24.4
≥30	82,773	19.3
Missing [b]	*79,722*	*15.7*
Started prenatal care in the first trimester		
Yes	419,472	91.9
No	37,119	8.13
Missing [b]	*52,524*	*10.3*

[a] *Column percentage.*

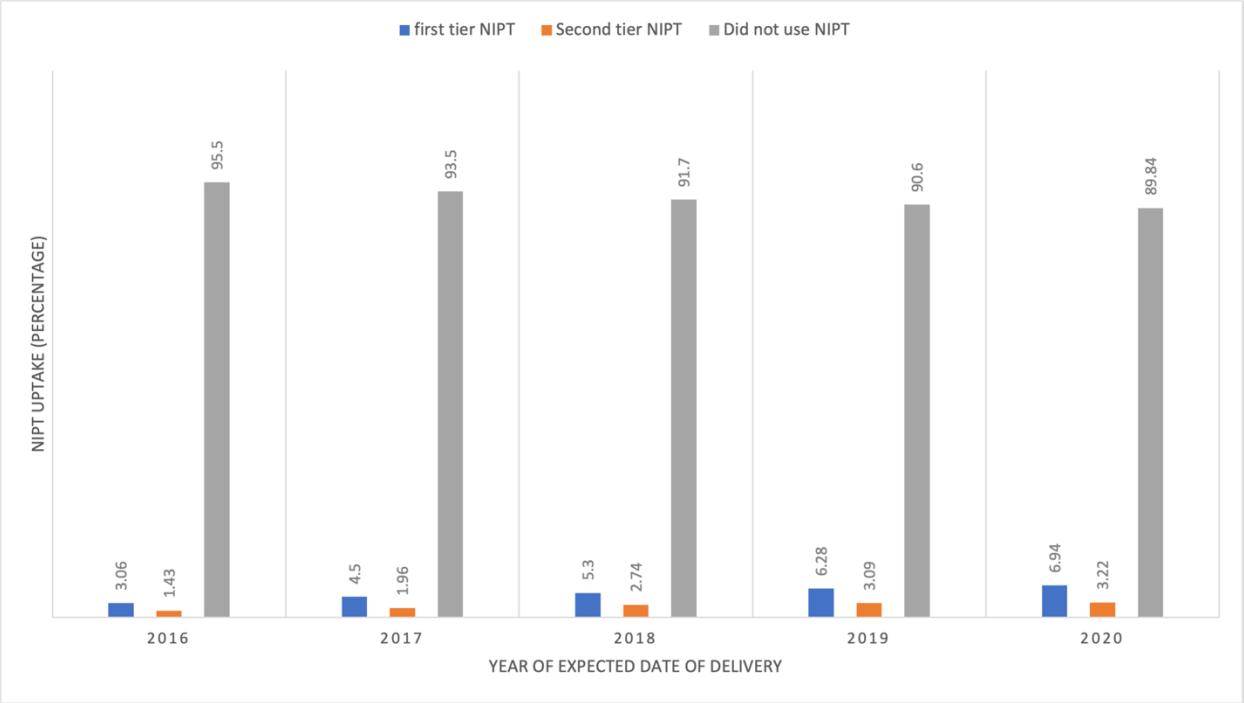
[b] *Missing values were not included in the denominators for calculating the distribution for each of these variables in turn.*

[c] *6,850 pregnant individuals used OHIP funding for first-tier NIPT and 12,659 used OHIP funding for second-tier NIPT which sums to 19,509.*

[d] *19,688 pregnant individuals did not use OHIP funding for first-tier NIPT and 78 did not use OHIP funding for second-tier NIPT which sums to 19,688.*

The percentage of pregnant individuals that used first-tier NIPT (i.e., did not undergo any prenatal screening) or second-tier NIPT (i.e., contingent on the results of one or more other screening test results) among low-risk pregnant individuals from 2016 to 2020 is presented in **Figure 4-4.** There was a small increase in the use of both-first tier and second-tier NIPT among the low-risk pregnant individuals from 2016 to 2020.

Figure 4-4. Proportion of pregnant individuals that used first-tier NIPT, second-tier NIPT or did not use NIPT among pregnant individuals who were below 40 years with no history of previous aneuploidy from 2016 to 2020



NIPT was counted in the year of expected delivery.

Table 4-9 presents the rate uptake of either first or second-tier among low-risk pregnant individuals. In total 26,460 used first-tier NIPT, 12,737 used second-tier NIPT and 469,818 did not use NIPT as prenatal screening. Among low-risk pregnant individuals, the highest rate of uptake of first-tier NIPT was recorded among those who lived in the Toronto Central region (13.4 %), and the lowest in the Erie St Clair and South West Region (1.0%). Uptake increased with increasing quintile of income (9.5% in the highest compared with 2.8% in the lowest) and education (12.4% in the highest compared with 1.8% in the lowest), and in multiple as compared to singleton pregnancies. Uptake decreased with increasing parity, gravidity, with body mass index outside the range of 18.5-24.9 kg/m^2, and in those who started prenatal care after the first trimester as compared to those who started during the first trimester.

The highest rate of uptake of second-tier NIPT among low-risk pregnant individuals was observed among those who lived in the Central region (3.9 %) and the lowest in the Erie St Clair and South West Region (1.5%). The uptake was about twice as high among women who lived in neighbourhoods with the highest quintile of education (3.9%) compared with the lowest (1.8%), a similar but less marked pattern for quintile of income. Uptake reduced in multiple as compared to singleton pregnancies, and for those who started prenatal care after the first trimester as compared to those who started during the first trimester.

As indicated by a standardized difference >0.1, low-risk pregnant individuals that used first-tier NIPT differed from those who used second-tier NIPT in location of residence, quintile of income and education, parity, gravidity, multiplicity of pregnancy, OHIP funding and body mass index. Those who used second-tier NIPT differed from those who did not use NIPT in location of residence, quintile of education and multiplicity of pregnancy.

Table 4-9. Distribution of baseline maternal characteristics of pregnant individuals that used first-tier NIPT, second-tier NIPT or did not use NIPT and rates of first or second-NIPT uptake among pregnant individuals who were below 40 years with no history of previous aneuploidy

	Pregnant individuals that used first-tier NIPT		**Pregnant individuals that used second-tier NIPT**		**Pregnant individuals that did not use NIPT**		**SD [b]**	**SD [c]**	**First-tier NIPT uptake per 100 (95% CI)**	**Second-tier NIPT uptake per 100 (95% CI)**
All women	26,460	% [a]	12,737	% [a]	469,818	% [a]				
Geographical location (LLINs) of maternal residence										
Erie St Clair and South West Region (1&2)	1,144	5.0	888	7.4	55,857	12.5	0.1	0.2	2.0 (1.9-2.1)	1.5 (1.4-1.6)
Waterloo Wellington, Hamilton, Niagara, Haldeman, Brant Region (3&4)	2,642	11.5	1,694	14.0	74,520	16.7	0.1	0.1	3.4 (3.2-3.5)	2.2 (2.1-2.3)
Central west (5	1,005	4.4	1,052	8.7	39,490	8.83	0.2	0.0	2.4 (2.3-2.6)	2.5 (2.4-2.7)
Mississauga Halton (6)	2,385	10.3	1,158	9.6	36,352	8.13	0.0	0.0	6.0 (5.8-6.2)	2.9 (2.7-3.1)
Toronto Central (7)	6,123	26.7	1,325	11.0	38,207	8.54	0.4	0.1	13.4 (13.1-13.7)	2.9 (2.6-3.1)
Central (8)	4,480	19.5	2,411	20.0	54,798	12.3	0.0	0.2	7.3 (7.1-7.5)	3.9 (3.8-4.1)
Central East (9)	1,804	7.85	1,416	11.7	51,308	11.5	0.1	0.0	3.3 (3.2-3.5)	2.6 (2.5-2.7)
South East and Champlain Region (10&11)	2,522	11.0	1,294	10.7	56,812	12.7	0.0	0.1	4.2 (4.0-3.3)	2.1 (2.0-2.3)

North Simcoe Muskoka, North East and North West Region (12-14)	865	3.8	842	67.0	39,843	8.9	0.1	0.1	2.1 (2.0-2.2)	2.0 (1.9-2.2)
Missing [d]	*26,778*									
Quarter of the year (expected date of delivery)										
One (January-march)	7,316	27.7	3,547	27.9	120,935	25.7	0.0	0.0	5.6 (5.4-5.7)	2.7 (2.6-2.8)
Two (April-June)	5,467	20.7	2,537	19.9	96,812	20.6	0.0	0.0	5.2 (5.1-5.4)	2.4 (2.3-2.5)
Three (July-September)	6,827	25.8	3,123	24.5	126,680	27.0	0.0	0.1	5.0 (4.9-5.1)	2.3 (2.2-2.4)
Four (October –December)	6,850	25.9	3,530	27.7	125,391	26.7	0.0	0.0	5.0 (4.9-5.2)	2.6 (2.5-2.7)
Income quintile (neighbourhood)										
1 (lowest)	3,208	12.6	2,477	19.8	108,000	23.7	0.2	0.0	2.8 (2.7-2.9)	2.2(2.1-2.3)
2	4,153	16.3	2,511	20.1	97,310	21.3	0.1	0.0	4.0 (3.9-4.1)	2.4 (2.3-2.5)
3	4,896	19.3	2,642	21.1	96,436	21.1	0.1	0.0	4.7 (4.6-4.8)	2.5 (2.4-2.6)
4	5,941	23.4	2,729	21.9	87,763	19.2	0.1	0.1	6.2 (6.0-6.3)	2.8 (2.7-2.9)
5 (highest)	7,228	28.4	2,129	17.1	66,642	14.6	0.3	0.2	9.5 (9.3-9.7)	2.8 (2.7-2.9)
Missing [d]	*14,950*									

Educational quintile (neighbourhood)										
1 (lowest)	1,619	6.7	1,610	13.6	85,614	19.9	0.2	0.2	1.8 (11.7-1.91)	1.8(1.7-1.9)
2	2,892	12.3	2,282	19.2	98,288	22.8	0.2	0.1	2.8 (2.7-2.9)	2.2 (2.1-2.3)
3	4,213	17.9	2,789	23.5	95,554	22.2	0.1	0.0	4.1 (4.0-4.2)	2.7 (2.6-2.8)
4	5,975	25.3	2,966	25.0	90,798	21.1	0.0	0.0	6.0 (5.9-6.1)	3.0 (2.9-3.1)
5 (highest)	8,895	37.7	2,214	18.7	60,448	14.0	0.4	0.1	12.4 (12.2-12.6)	3.9 (4.0-4.3)
Missing [d]	*42,858*									
Parity										
0 (nullipara)	11,520	50.0	4,423	36.6	196,717	43.2	0.3	0.1	5.4 (5.3-5.5)	2.1 (2.0-2.1)
1 (primipara)	7,935	34.5	5,017	41.5	158,233	34.7	0.1	0.1	4.6 (4.5-4.7)	2.9 (2.7-3.0)
>1 (multipara)	3,560	15.5	2,662	22.0	100,491	22.1	0.2	0.0	3.3 (3.2-3.3)	2.5 (2.4-2.6)
Missing [d]	*18,457*									
Gravidity										
1 (primigravida)	8,161	35.3	3153	30.0	148,201	32.4	0.2	0.1	5.1 (5.0-5.3)	2.0 (1.9-2.1)
>1 (multigravida)	14,979	64.7	8996	74.1	308,759	67.5	0.2	0.1	3.3 (4.4-4.6)	2.7 (2.7-2.8)
Missing [d]	*16,766*									

Number of fetuses										
One (singleton pregnancy)	22,547	96.7	12,110	99.4	450,791	98.5	0.2	0.1	4.6 (4.6-4.7)	2.5 (2.5-2.5)
Two or more (multiple pregnancy)	759	3.3	75	0.6	7,017	1.5	0.2	0.5	9.7 (9.0-10.)	0.9 (0.7 1.2)
Missing [d]	*15716*									
Body mass index (kg/m^2)										
< 18.5	1,058	5.3	460	4.4	21,152	5.3	0.0	0.0	4.7 (4.4-5.0)	2.0 (1.9-2.2)
18.5-24.9	12,321	61.5	5,290	50.6	201,457	50.5	0.2	0.0	5.6 (5.5-5.7)	2.4 (2.4-2.5)
25.0-29.9	4,153	20.7	2,599	24.9	98,030	24.6	0.1	0.0	4.0 (3.9-4.1)	2.5 (2.4-2.6)
≥30	2,495	12.5	2,105	20.1	78,173	19.6	0.2	0.0	3.0 (2.9-3.13)	2.5 (2.4- 2.6)
Missing [d]	*79,722*									
Started prenatal care in the first trimester										
Yes	18,310	95.3	10,499	95.0	390,663	91.6	0.01	0.1	4.4 (4.3-4.4)	2.5 (2.5-2.6)
No	897	4.7	553	5.0	35,669	8.4	0.01	0.1	2.4 (2.3-2.6)	1.5 (1.4-1.6)
Missing [d]	*52,424*									
OHIP Funding for NIPT										
Yes	6,850	25.9	12,659	99.4			2.3			

No	19,610	74.1	78	0.6			1.9			

[a] *Column percentage.*

[d] *Absolute standardized difference between pregnant individuals that used first-tier NIPT and pregnant individuals that used second-tier NIPT. Shaded cells represent standardized differences >10%.*

[c] *Absolute standardized difference between pregnant individuals that used second-tier NIPT and pregnant individuals that did not use NIPT. Shaded cells represent standardized differences >10%.*

[d] *Missing values were not included in the denominators for calculating the distribution for each of these variables in turn.*

CHAPTER 5: DISCUSSION

In our study of 536,748 pregnant individuals in Ontario during the period 2016-2020, we observed an increase in uptake of NIPT from 6.5% for pregnancies with an expected due date in 2016 to 13.2% for expected deliveries by the end of the study period in early 2020. There was substantial variation in NIPT uptake between regions within the province, but increased uptake was observed in each region over the study period. We also observed variability in uptake according to maternal characteristics and behaviour; specifically, lower uptake with decreasing quintile of income and education, lower uptake among pregnant individuals who did not start prenatal care in the first trimester, and lower uptake among individuals with a pre-pregnancy body mass index outside the normal range. Conversely, pregnant individuals with a history of previous aneuploidy, aged 40 years or more, multigravidae or with a multi-fetal pregnancy had higher NIPT uptake.

Our findings that the highest NIPT uptake was in the predominantly urban Toronto Central Region and the lowest in the largely rural North Simcoe Muskoka, North East and North West Regions are consistent with two previous studies conducted in Ontario. In the period 2014-16, Hung et al. (62) found substantial regional variation in the uptake of NIPT, with 64.5% uptake in the Toronto Central Region as compared to 24.2% in the North West Region among high-risk pregnant individuals. In an assessment of utilization of provincially funded cfDNA screening and adherence to the criteria laid out in Ontario prenatal screening guidelines, Bellai-Dussault et al. (16) also found the highest uptakes of NIPT were in urban areas of the province. The two Northern LHINs (i.e., North East and North West) dominate more than 80% of the Ontario land mass but only add 6% of the Ontario's population, with a population density of less than one

person per square kilometre as opposed to 4100 people per square kilometre in Toronto Central (96). This makes healthcare providers face challenges in delivery of care to residents in Northern regions due to the larger areas and accessibility to healthcare facilities becomes a problem due to the distance to the facilities. Our findings of a steady year-on-year increase is also consistent with the increase over 2016-17 documented in that study and also in other studies conducted in other jurisdiction (59,97,98).

Our findings of the highest uptake of NIPT among high-risk pregnant individuals, that is pregnant individuals who were aged 40 years and above and /or with a history of previous anomalies, is consistent with the results of Larion et al. who reported that in a large centre in the USA, NIPT was quickly adopted by the high-risk patient populations (99). There are several possible explanations to this observed result. Firstly, cost of NIPT; in Ontario and many other jurisdictions, funding for NIPT started with high-risk pregnant individuals. As a result, NIPT use remains highest for this group (i.e., pregnant individuals aged 40 years and above and/or with a history of previous aneuploidy). Pregnant individuals who do not fall in this group have to pay for their NIPT screening or undergo a second-tier NIPT if they want funding for it, which may create a financial burden that will limit selection for NIPT especially for low-risk pregnant individuals. A survey conducted in Canada by Birko et al., identified the cost of NIPT affecting heath care professionals decisions to offer NIPT to pregnant individuals (100). Secondly, many high-risk pregnant individuals opt for NIPT over amniocentesis or chorionic villus sampling to avoid perceived increased risk of pregnancy loss irrespective of whether the fetus is unaffected or not (18,101,102). Thirdly, there are the perceived psychological benefits of NIPT by health professionals. Many health professionals believe that a major benefit of NIPT is that it helps in reducing psychological burden in high-risk pregnant individuals because of its early detection of

fetal conditions (103). This perceived psychological benefit by health professionals may increase their decision to offer the NIPT to high-risk pregnant individuals.

We found that uptake increased with increasing quintile of neighbourhood income and education. This is consistent with an earlier study of variation in multi-marker screening uptake by a rurality index in Ontario (65) and with studies of neighbourhood measures of socio-economics status in Australia, before (104) and after (105) the introduction of NIPT. In our study, multigravidae had a higher uptake of NIPT than primigravidae, as also observed in a previous study in Toronto (Ontario) (82) and one in Belgium (83). However, we observed low NIPT uptake among multiparous pregnant individuals, and this is consistent the study of multiple marker screening in Ontario (42) and a study conducted in 2000 in two centres in the UK (84). Both multipara and multigravidae might be expected to have good knowledge about prenatal tests because of experience from previous pregnancies (106). Therefore, it might have been expected that the uptake of NIPT should have been higher in multiparous pregnant individuals like that of multigravida but a study conducted by Laberge et al. observed that knowledge about NIPT alone does not influence pregnant individuals decision to opt for NIPT; it also involves factors such as the partners input and the influence of health professionals (107). Another study conducted by Montgomery et al. also identified the influence of societal perception of NIPT in opting for NIPT or not (108). Our observation that having the first prenatal care visit in the first trimester was associated with higher uptake of NIPT is similar to that observed for multiple marker screening in Ontario by Hayeems et al. (109).

In our second objective relating to 27,733 high-risk pregnant individuals and 509,015 low-risk pregnant individuals, there was a steady increase in the use of first-tier NIPT among the high-risk pregnant individuals as compared to a small increase in first-tier NIPT use among the low-risk

pregnant individuals. There was no change in use of second-tier NIPT use among the high-risk pregnant individuals as compared to a small increase in the use of second-tier NIPT in low-risk pregnant individuals.

NIPT was introduced to screen for fetal aneuploidies such as trisomy 21, 13 or 18 and in most jurisdictions, funding for NIPT was only introduced for high-risk pregnancies (110,111). Our study confirmed this as most of the high-risk pregnant individuals received OHIP-funding for either first-tier or second-tier NIPT. Conversely, among low-risk pregnant individuals, it is only the second-tier NIPT users where most of them received OHIP-funding, the majority of the first-tier NIPT users paid out of pocket. The introduction of the funding for high-risk pregnant individuals in many jurisdictions led to the increase in the use of NIPT among high-risk pregnant individuals when compared with low-risk pregnant individuals as seen in the results of our study. Our finding of a steady increase of the use of NIPT among high-risk pregnant individuals is also in consistent with Huang et al. study conducted in Ontario during the period of 2014-16. Their study identified a dramatic increase of NIPT uptake in high-risk pregnancies after implementation of NIPT funding from 854 in 2013 to 6298 in 2015. In the Huang et al. (76) study, which is from 2014-16, only 7.2% of high-risk pregnant individuals used first-tier NIPT and in our study from 2016-20 there was an increase in the use of first-tier NIPT from 7.2% to 51.8%. The Netherlands is the first country in Europe to offer funding for first-tier NIPT to pregnant individuals; the Dutch TRIDENT (Trial by Dutch laboratories for Evaluation of NIPT) study conducted by Jan-Galjaard et al. to evaluate the uptake of first-tier NIPT in pregnant individuals after the implementation of the policy observed a nationwide uptake of first-tier NIPT of 40% (112).

Our findings also identified a proportion of pregnant individuals who were eligible for OHIP-funding but paid out pocket. There are several potential explanations for these results. Firstly, it could be the result of lack of healthcare provider education to their pregnant patients on eligibility requirement for NIPT. When funding for NIPT was introduced there was no province-wide education/training for prenatal health care providers which resulted in gaps in their knowledge and counselling confidence regarding who should be offered funding for NIPT (26). Wilson et al. observed significant gaps in health professionals knowledge about NIPT and its funding eligibility requirements (113). Another study survey conducted in Europe by Benachi et al. also observed that lack of patient education by physicians have been one of the major barriers in the uptake of NIPT (114). Secondly, it may be the pregnant individuals own decision to pay out of pocket for NIPT; a study conducted by Sacco et al. in England to assess pregnant individuals choices in NIPT for aneuploidy found 516 pregnant individuals out of 7939 accessed first-tier NIPT in the private sector (115). Many pregnant individuals opt for self-paid NIPT as a result of its early detection to screen for any aneuploidies and this helps in reducing psychological stress among the pregnant individuals (106,116). Reasons also associated for the uptake of NIPT among the pregnant individuals includes accuracy and safety of NIPT (61,117,118) and absence of physical risk to the fetus (118,119). Understanding the underlying reasons for variability in NIPT use is important is important for identifying inequalities and to developing and implementing policies to address them and thus improve equitable access.

5.2 STRENGTHS AND LIMITATIONS OF THE STUDY

A strength of this study is that it was a large population-based study using data from a province-wide maternal-newborn registry. Ontario being the largest province in Canada, and its diversity in terms of socio-economic status, ethnicity, and geography provided an excellent setting to investigate changes in patterns of NIPT uptake. Secondly, the BORN Information System captured information on all prenatal testing in Ontario, and a validation study conducted by Dunn et al showed that perinatal variables collected by BORN are likely to have a high level of accuracy (80).

One limitation of our study is the ability to capture all NIPT information for pregnant individuals living in Ontario. Those who did not meet the specific criteria for OHIP-funding and chose to self-pay or pay through private insurance may have accessed other laboratories besides the two laboratories that process OHIP-funded NIPTs (71). Also pregnant individuals who obtained NIPT outside Canada through self-pay (107) were not captured in the study. We expect this to have resulted in a small underestimation of the estimated NIPT uptake by pregnant individuals in Ontario. The variables for body mass index and prenatal care visit starting in first trimester had high levels of missing values. We created a missing category for both them and included them in adjusted our model. The missing values for the other variables were not included in our adjusted model, in order to not exclude their records; however, we acknowledge there is misclassification of information on these variables as a result.

5.3 IMPLICATIONS/CONCLUSION FOR PRACTICE

NIPT is a fast-moving field, with pressures to introduce in first trimester as replacement for multi-marker screening, as well as broaden the range of screened conditions. The results of our study may provide insight for Ontario, other provinces in Canada and other international jurisdiction whose health systems are similar to the Canadian health system. Our findings of lowest uptake of NIPT among low-risk pregnant individuals and those living in rural areas suggest a need to provide more education/training about NIPT and OHIP-funding eligibility criteria for health professionals and pregnant individuals. Also, policy makers need to think about including low-risk pregnant individuals in the first-tier NIPT funding policy to ensure equitable assess.

We observed that the uptake of NIPT has increased every year in Ontario since 2016, and uptake was highest among pregnant individuals living in urban areas, neighbourhoods with highest family income and educational quintiles, high-risk pregnant individuals, pregnant individuals receiving OHIP-funding and pregnant individuals who started their prenatal care in the first trimester and lowest among pregnant individuals living in rural areas, neighbourhoods with lowest income and educational quintiles and low-risk pregnant individuals. In future works we recommend exploring the possible reasons uptake of NIPT is lowest among the categories which had the lowest uptake of NIPT and also assessing the level of knowledge about NIPT among health professionals and pregnant individuals in order to develop guidance on how to provide NIPT education to the health professionals and pregnant individuals.

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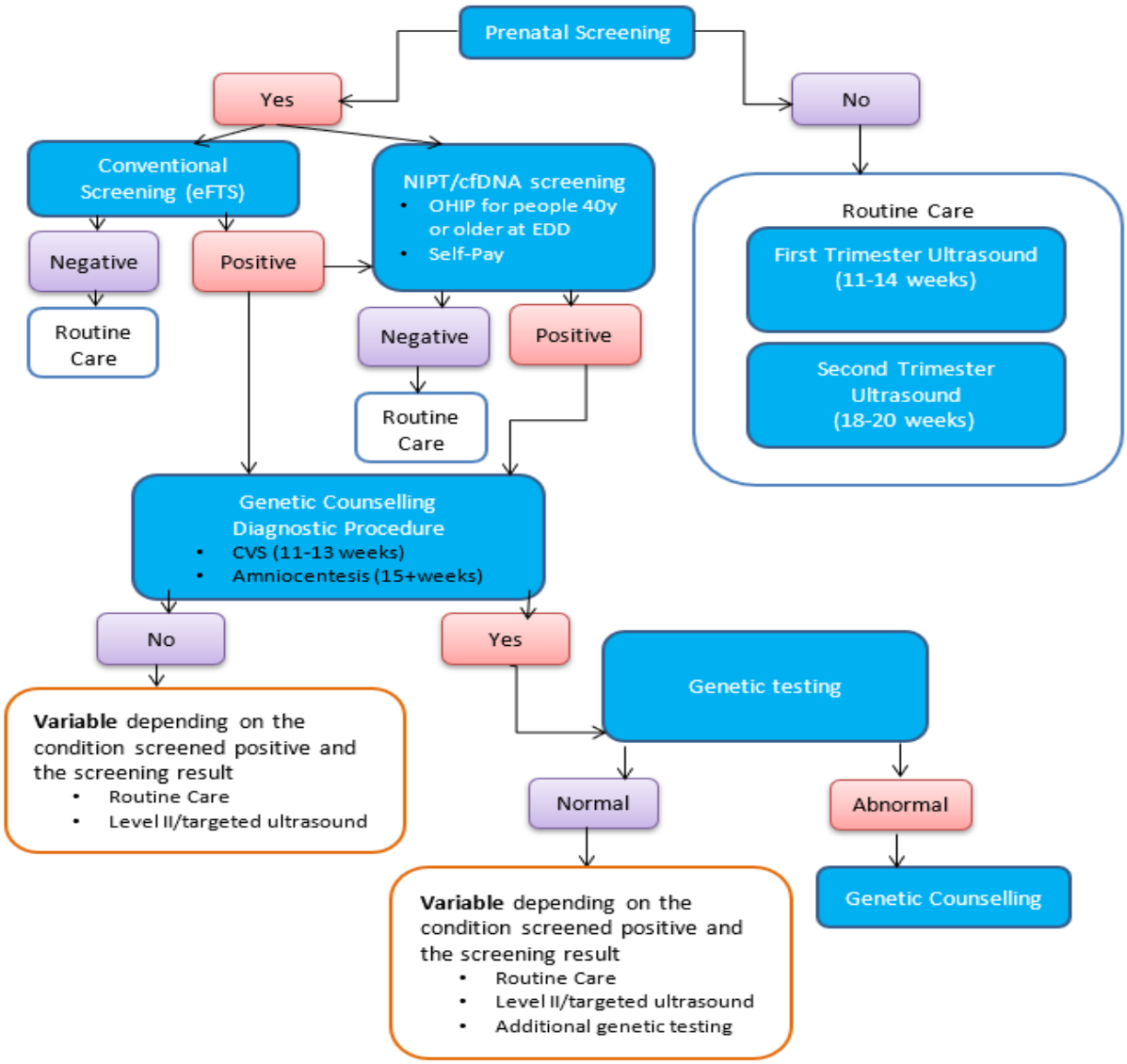
APPENDICES

Appendix A. Prenatal testing process in Ontario, Canada



BORN. What is prenatal screening_ - Prenatal Screening Ontario [Internet]. 2018 [cited 2020 Jun 7]. Available from: https://www.prenatalscreeningontario.ca/en/pso/about-prenatal-screening/about-prenatal-screening.aspx#Types-of-prenatal-screening

Appendix B. RECORD Checklist.

The RECORD statement – checklist of items, extended from the STROBE statement, that should be reported in observational studies using routinely collected health data.

	Item No.	STROBE items	Location in manuscript where items are reported	RECORD items	Location (page) in manuscript where items are reported
Title and abstract					
	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found		RECORD 1.1: The type of data used should be specified in the title or abstract. When possible, the name of the databases used should be included. RECORD 1.2: If applicable, the geographic region and timeframe within which the study took place should be reported in the title or abstract. RECORD 1.3: If linkage between databases was conducted for the study, this should be clearly stated in the title or abstract.	II
Introduction					
Background rationale	2	Explain the scientific background and rationale for the investigation being reported			1, 17

Objectives	3	State specific objectives, including any prespecified hypotheses			2
Methods					
Study Design	4	Present key elements of study design early in the paper			19
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection			19
Participants	6	*(a) Cohort study* - Give the		RECORD 6.1: The methods of study	19

		eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study* - Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study* - Give the eligibility criteria, and the sources and methods of selection of participants *(b) Cohort study* - For matched studies, give matching criteria and number of exposed and unexposed *Case-control study* - For matched studies, give matching criteria and the number of controls per case		population selection (such as codes or algorithms used to identify subjects) should be listed in detail. If this is not possible, an explanation should be provided. RECORD 6.2: Any validation studies of the codes or algorithms used to select the population should be referenced. If validation was conducted for this study and not published elsewhere, detailed methods and results should be provided. RECORD 6.3: If the study involved linkage of databases, consider use of a flow diagram or other graphical display to demonstrate the data linkage process, including the number of individuals with linked data at each stage.	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable.		RECORD 7.1: A complete list of codes and algorithms used to classify exposures, outcomes, confounders, and effect modifiers should be provided. If these cannot be reported, an explanation should be provided.	20

Data sources/ measurement	8	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group			19
Bias	9	Describe any efforts to address potential sources of bias			23-24, 36, 74

Study size	10	Explain how the study size was arrived at			
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why			20 (quintiles of neighbourhood education and neighbourhood income derived from PCCF+)

Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study* - If applicable, explain how loss to follow-up was addressed *Case-control study* - If applicable, explain how matching of cases and controls was addressed *Cross-sectional study* - If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses			22-24
Data access and cleaning methods		..		RECORD 12.1: Authors should describe the extent to which the investigators had access to the database population used to create the study population. RECORD 12.2: Authors should provide information on the data cleaning methods used in the study.	19

Linkage		..		RECORD 12.3: State whether the	20 (PCCF+ to BIS), 21 (NIPT dataset to BIS)
				study included person-level, institutional-level, or other data linkage across two or more databases. The methods of linkage and methods of linkage quality evaluation should be provided.	
Results					
Participants	13	(a) Report the numbers of individuals at each stage of the study (*e.g.*, numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed) (b) Give reasons for nonparticipation at each stage. (c) Consider use of a flow diagram		RECORD 13.1: Describe in detail the selection of the persons included in the study (*i.e.,* study population selection) including filtering based on data quality, data availability and linkage. The selection of included persons can be described in the text and/or by means of the study flow diagram.	25-28, 48-51, 58-60

Descriptive data	14	(a) Give characteristics of study participants (*e.g.*, demographic, clinical, social) and information on exposures and potential confounders (b) Indicate the number of participants with missing data for each variable of interest (c) *Cohort study* - summarise follow-up time (*e.g.*, average and total amount)			25-43 48-68
Outcome data	15	*Cohort study* - Report numbers of outcome events or summary measures over time *Case-control study* - Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study* - Report numbers of outcome events or			29-32, 51-53, 61-63
		summary measures			

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period			36-47
Other analyses	17	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses			46-47, 48-68
Discussion					
Key results	18	Summarise key results with reference to study objectives			69-73
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias		RECORD 19.1: Discuss the implications of using data that were not created or collected to answer the specific research question(s). Include discussion of misclassification bias, unmeasured confounding, missing data, and changing eligibility over time, as they pertain to the study being reported.	74

Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence			69-73, 75
Generalisability	21	Discuss the generalisability (external validity) of the study results			75
Other Information					
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based			75
Accessibility of protocol, raw data, and programming code		..		RECORD 22.1: Authors should provide information on how to access any supplemental information such as the study protocol, raw data, or programming code.	

*Reference: Benchimol EI, Smeeth L, Guttmann A, Harron K, Moher D, Petersen I, Sørensen HT, von Elm E, Langan SM, the RECORD Working Committee. The REporting of studies Conducted using Observational Routinely-collected health Data (RECORD) Statement. *PLoS Medicine* 2015; in press.