Introduction – The Clinical Need

Prenatal diagnosis of birth defects

Birth defects are innate developmental errors, encompassing structural or functional irregularities that impact physical, mental, and social wellbeing [1]. They pose a significant and growing public health issue globally, affecting an estimated 8.5 million newborns annually with a prevalence of **4-8 per 100 live births** [2,3]. In the US, they incur around $3 billion in annual healthcare costs [4], besides substantial lifelong personal and societal expenses. Birth defects are a major cause of infant mortality in the US, accounting for 20% of deaths in the first year of life [5]. Early detection can significantly improve or save lives, and advances in diagnosis, care, and prevention have led to a 46% reduction in infant mortality from birth defects in high-income countries between 1980 and 2001 [3].

Prenatal diagnosis detects fetal anomalies leading to birth defects, intersecting multiple medical specialties like obstetrics and gynecology, genetics, and pediatrics. It typically involves methods such as ultrasound and blood marker analysis for risk assessment. However, definitive diagnosis requires **invasive procedures with inherent miscarriage risks**, like amniocentesis (amniotic fluid test), done at 15-20 or 30-32 weeks, and chorionic villus sampling (CVS), done at 10-13 weeks.

DNA from these procedures is analyzed using karyotyping or CMA (i.e., the genetic chip) technologies, which can reveal whole-chromosome conditions like Down syndrome or smaller aberrations like DiGeorge syndrome. Mutations in a single DNA building block are detected through next-generation sequencing (NGS), either whole genome or exome sequencing (WGS, WES), or targeted panels, often comparing the DNA of the parents and fetus; this includes both point mutations (single nucleotide variants, SNVs) and insertions-deletions of several building blocks (indels), causing single-gene disease.

Noninvasive prenatal diagnosis

Over the past decade, noninvasive prenatal tests (NIPT) have emerged as a safe, accurate option for detecting fetal anomalies, relying on cell-free DNA (cfDNA) in maternal blood, which includes fetal DNA. By the end of the first trimester, the fetal fraction of cfDNA reaches about 10%. **Initially, NIPT was used for detecting chromosomal anomalies like trisomy 21, which leads to Down syndrome**, showing high accuracy in both high-risk and, later on, general populations [6]. It's also used for determining fetal sex, helping to anticipate X-linked disorders.

NIPT now also identifies significant sub-chromosomal changes, which are more common in pregnancies than aneuploidies [7,8]. However, **NIPT does not yet detect point mutations and small indels, which cause up to 30% of genetic birth defects** (Figure 1) [9]. Moreover, an increasing number of birth defects, previously of unknown cause, are now recognized as genetically determined only through advanced diagnostics, i.e., NGS [10].

The future of prenatal diagnosis is expected to be largely noninvasive, with cfDNA-based fetal screening expanding beyond high-risk cases to become the standard screening tool in the wide population. The main hurdle to a fully noninvasive approach is detecting small structural variation, point mutations and small indels, which is the focus of Identifai's work [11].

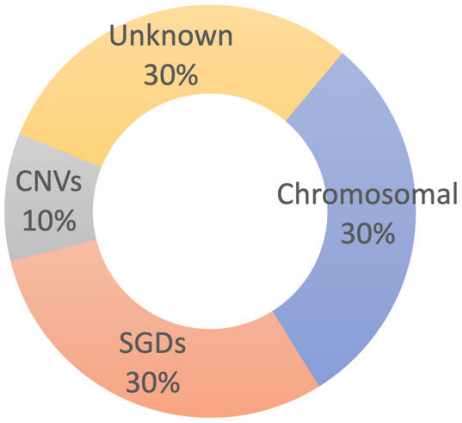

Figure 1. Out of all genetic birth defects an estimated 30% are a result of chromosomal abnormalities, which are only partially detected with current NIPT approaches. Up to 10% of genetic birth defects are estimated to be a result of insertions-deletions of varying sizes, which cause copy number variations (CNV) and can be detected by chromosomal microarray (CMA). **Most cases (an estimated 60%) are undetected using current NIPT solutions**, and up to 50% of these undetected cases are estimated to be a result of point mutations that lead to single gene disorders (SGD) [9].

Identifai's Solution

Design

Identifai's proprietary solution provides an **early, risk-free, "one stop shop" test** for detecting a wide range of genetic mutations, from chromosomal to specific point mutations. With numerous prenatal tests available, each with its pros and cons, selecting the most suitable one can be overwhelming. These tests vary in terms of the anomalies they detect (i.e., diagnostic yield), associated risks, costs, accessibility, and availability along a pregnancy. For instance, while CMA can identify chromosomal abnormalities, it's invasive, poses risks to the pregnancy, and is limited to a specific time window in pregnancy. Conversely, chromosomal NIPT is safe but limited in scope. This plethora of partial solutions often leads to "decision fatigue" among expectant couples, complicating decision-making. The ideal prenatal test would be **accessible at all pregnancy stages, safe, comprehensive, continuously updated, focused on clear and meaningful findings, cost-effective, and conveniently available as a single solution.**

Technology and IP

Identifai's approach begins with a blood sample drawn from the mother. Maternal DNA is extracted from blood cells, while the cfDNA, containing both fetal and maternal DNA, is extracted from the plasma. All DNA samples are sequenced using WGS. To decipher the fetal genome, maternal DNA is analyzed together with plasma cfDNA by proprietary statistical and AI algorithms and pipeline [12,13]**.** Similar to invasive tests, paternal DNA can improve accuracy, if available.

Addressing the complexity of harmful genetic variations involves segmenting the issue into more manageable parts, leveraging our expertise in genetics and medicine. There is no "silver bullet", i.e., a single algorithm that is suitable for all types of genetic changes. Part of Identifai's expertise is in defining which aspects of the comprehensive NIPT challenge can be isolated and solved. Single-gene diseases, for instance, can be categorized into autosomal recessive (AR) and autosomal dominant (AD) disorders, based on Mendelian inheritance principles. AR conditions require two mutated gene copies, one from each parent, which could be identical (homozygous mutation) or different (compound heterozygosity) within the same gene. **The inheritance patterns of single-gene disorders necessitate distinct computational strategies.** For this reason, we designed a meticulous IP strategy, which consists of our main patent

developed in academia, along with multiple supporting patent families that focus on enhancing accuracy and providing solutions for additional mutation types.

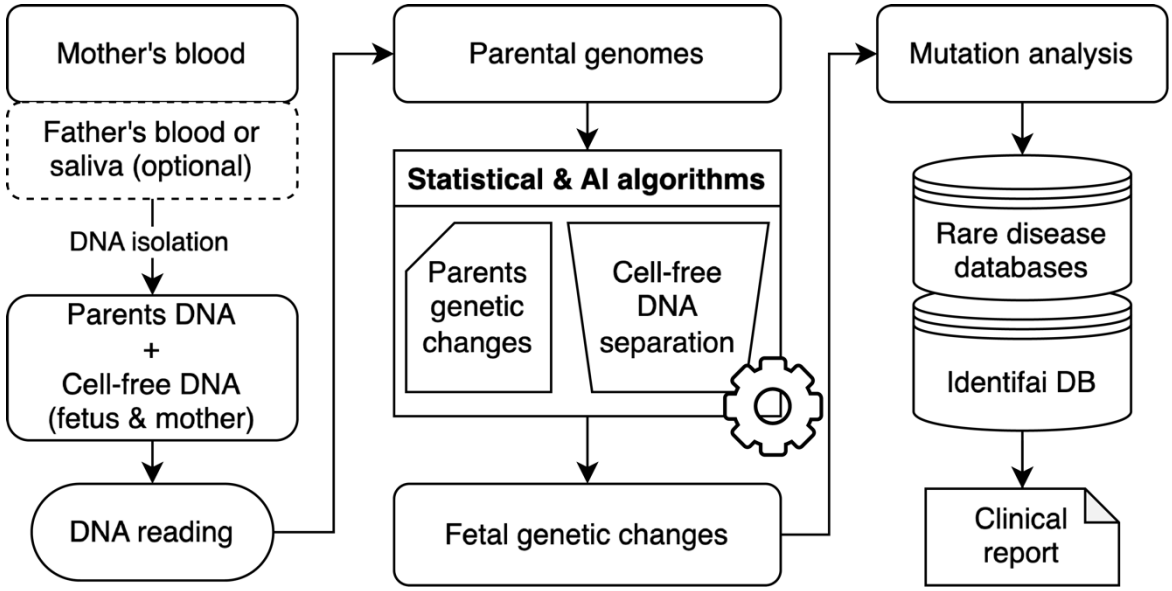

Figure 2. The general workflow of Identifai's approach. Our proprietary algorithm assembles the fetal genome using an AI-enhanced Bayesian statistical algorithm and detects fetal mutations.

Detection of point mutations

Unlike AD disorders, in which one mutated copy of a gene will typically cause a disease, AR conditions are of great interest for screening tests, since healthy people can carry a mutation. Predicting the inheritance of AR conditions is different between compound heterozygous cases and homozygous mutations. For the former, ruling out a disease requires ruling out either the paternal or the maternal mutation, which enables high accuracy. For a high-risk (positive) result, however, it is required to predict the inheritance of both mutations. Testing the paternal mutation (as well as *de novo* mutations) is straightforward and based on the detection of a non-maternal, unique paternal mutation in maternal plasma. **Testing the maternal mutation inheritance requires far more sophisticated algorithms**, which are based on imbalances between the normal copy and the mutation within the plasma. Over representation of the mutation implies its inheritance. **To detect mild imbalances, our proprietary algorithm assesses each DNA fragment separately and searches for maternal and fetal features and signatures.** A similar high-resolution method is used in cases of homozygous mutations, in which both parents carry a mutation.

Analyzing genomes using domain knowledge, statistics, and AI

Identifai's approach originated in academia, in a laboratory that specialized for years in NGS analysis and genomics, and detection of Mendelian diseases (the lab's methods have also lead to industrial solutions and have spawned biotech companies). Using this knowledge and experience, we realized that NIPT of point mutations requires a variant caller, i.e., a bioinformatics algorithm that separately assesses each DNA fragment, to detect mutations. Today, our solution consists of several **statistical and AI algorithms**, creating a wide portfolio of six patent families. In the core of our method there is an AI-based **fetal-maternal cfDNA classifier**, i.e., a machine learning method that predicts which fragments are most likely

derived from the fetus. Then, inspired by the longstanding standard in NGS analysis, **we developed a Bayesian algorithm that intelligently integrates all the evidence at each genomic position to detect a mutation**. Rather than strictly filtering out fragments, which can cause loss of valuable information and introduce bias, we assign each fragment a score denoting the probability of it being fetal.

Furthermore, after assessing each potential variant independently, we incorporate another layer of information, utilizing nearby variants to improve prediction accuracy. Specifically, we rely on genetic linkage and haplotypes, i.e., genomic loci that are physically proximate on the same copy of the chromosome and tend to be inherited together. Our algorithm assembles haplotypes based on overlapping DNA fragments and uses these haplotypes to improve upon our genotype predictions.

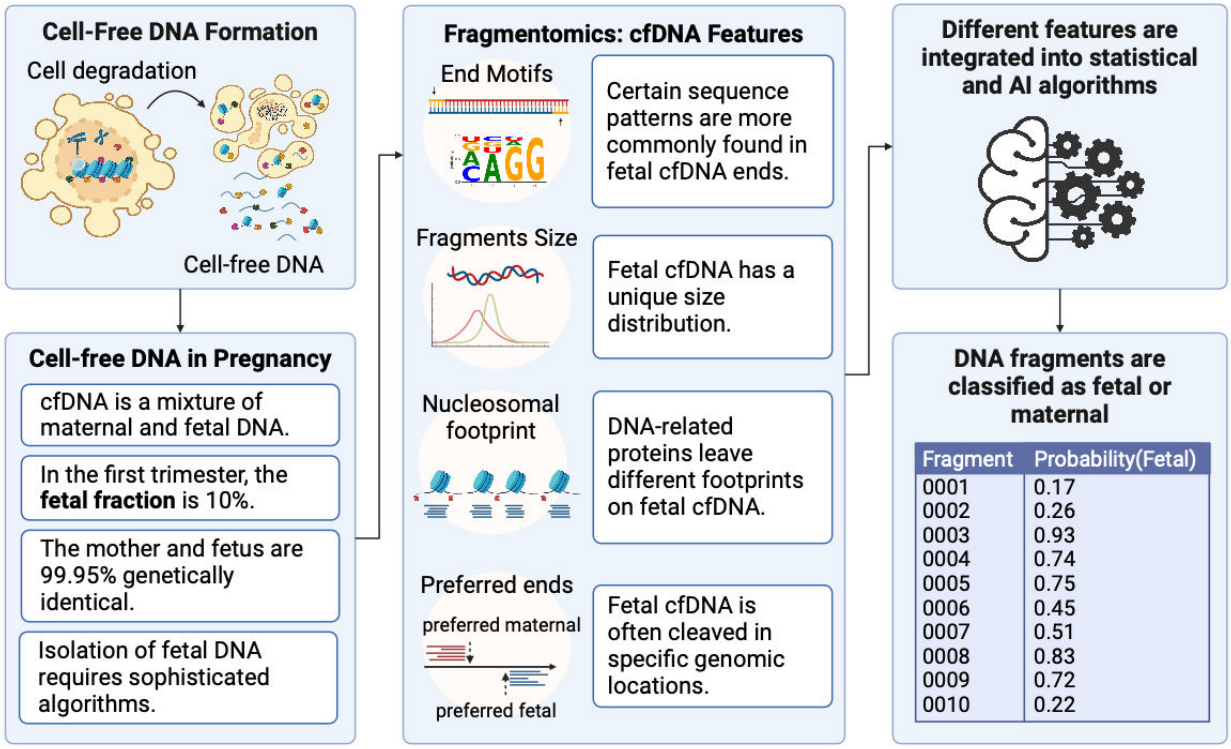

Figure 3. Fetal and maternal cell-free DNA in the maternal plasma differ in various features that are used in the fetal-maternal cell-free DNA fragment classifier.

Finally, we leverage even more available information: both population information and Identifai's **unique, ever-growing family database are leveraged by an AI layer that corrects prediction errors.**

Our combination of methods enables the **complete coverage of point mutations** across the genome, using a custom-fit solution for each category. By solving this missing part of the puzzle, we managed to develop a **safe, early, up-to-date technology**, that **covers all genetic disorders** across the genome and enables a **convenient and cost-effective test**.

Results

In our preliminary study, in conjunction with Rabin Medical Center's Beilinson Hospital in Israel, we collected DNA from 18 families with various mutations [14,15]. Initially we focused on reaching a clinical level accuracy in first trimester pregnancies. Since then, we sought to expand our test to pregnancies with low

fetal fractions, considered more challenging. To ascertain that accuracy is not compromised, we used a statistical no-call threshold, such that low-confidence results are excluded.

As seen in the below figure, across a range of fetal fractions, a negative result (low risk of being affected) in our test is correct in 99.9% of the cases for the common scenario of compound heterozygous mutations, and 99.5% in the rare homozygous mutation case, thus sparing the need for invasive procedures. This is achieved while keeping a low no-call rate, i.e., a small number of variants are filtered out. A positive result in our test is also highly accurate, especially for a screening test

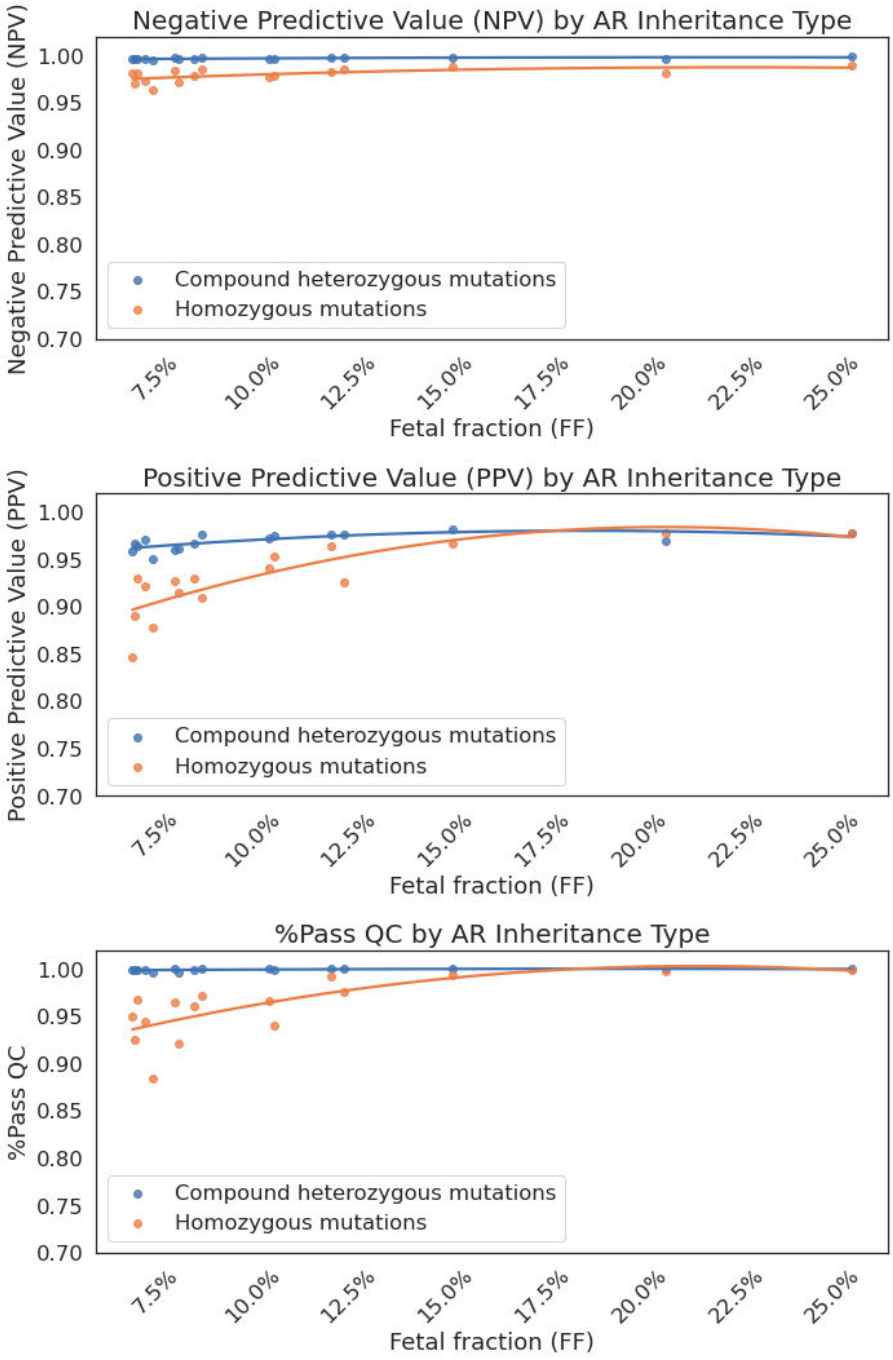

Figure 4. Identifai's algorithm capabilities in a cohort of 18 first trimester cases for heterozygous and homozygous mutations. Each dot represents the average of millions of variants from one fetus. Compound heterozygous mutations causing AR diseases are the more common case, while homozygous mutations are rare and typically associated with genetic relatedness between parents.

Conclusions

Identifai's proprietary technology, which addresses the challenge of noninvasive prenatal screening, has demonstrated promise in a preliminary study in Israel. It is currently being further evaluated in a multicenter study in the USA, conducted in conjunction with Columbia University.

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