

# The *InTheKnow* Genetics Program

By Dr. Bruce Cohen

## INTRODUCTION

### ***Disability due to genetic diseases and structural malformations is common.***

In a study performed by the U.S. Census Bureau, approximately 6% or 1 in 17 of 50 million school aged children in the United States were classified as being disabled. Disabilities included mental retardation, autism spectrum disorders, significant visual, hearing and learning impairments, brain injuries and severe mental and physical handicaps that require assistance. These disabilities confer an immeasurable burden upon the affected individuals and their families. The cost of caring for these disabled citizens borne by the United States Federal and State Governments is approximately 260 billion dollars annually, which is more than is spent on the Welfare and Food Stamp programs combined.

### ***With standard screening, only a small minority of fetal malformations that result in physical and mental disability will be diagnosed prenatally.***

Guidelines published by the American Congress of Obstetricians and Gynecologists (ACOG) recommend offering screening or invasive testing for chromosomal abnormalities, Cystic Fibrosis screening, screening for neural tube defects as well as ultrasound for major fetal structural malformations. However, it appears likely that our current testing detects less than a quarter of the genetic and structural malformations that we are capable of detecting prenatally using our most up-to-date technologies. Our national medical societies have been cautious in endorsing some of these most recently introduced technologies due to concern over their lack of proof of efficacy and potential for providing inaccurate or inconclusive results. It is certainly true that no amount of testing can guarantee prospective parents a healthy baby. In some cases, there will be false positives, which can lead to invasive testing that may place the pregnancy at risk for complications. There may also be false negatives where the fetus screens negative, but actually has the disease in question. More often, there may be equivocal findings, where our inexperience and/or lack of knowledge does not permit us to determine whether the finding is truly an abnormality or results in which the degree of accuracy is not yet sufficiently established scientifically. Finally, there can be genetic findings in a fetus that may result in variable outcomes that range from no disease to severe disability. All of these shortcomings of genetic testing can lead to patient anxiety during a pregnancy and have slowed the introduction of our latest technologies into standard clinical practice.

***InTheKnow offer patients the greater opportunity to detect genetic and structural abnormalities that state-of-the art testing offers while providing prospective parents with the necessary counseling and support to minimize anxiety and the necessary education to properly interpret the results.***

With rapidly evolving genetic and ultrasound technologies, Chromosomal abnormalities, Single Gene defects, Copy Number Variants and structural malformations may be detected with far greater probability than in years past. Moreover, new early intervention strategies, including fetal surgery and medical treatments, have provided families with a wealth of clinical management options beyond that of pregnancy termination. The InTheKnow program offers genetic serum screening, genetic amniotic fluid testing and ultrasound examinations that meet and exceed the highest standards established by the American Institute of Ultrasound in Medicine for 2-Dimensional and 3-Dimensional Ultrasound. These services provide prospective parents with a far more comprehensive assessment of their fetus. With a highly knowledgeable staff and an affiliation with the Center for Human Genetics, patients have the opportunity to explore the genetic and structural well-being of their baby in depth early in their pregnancies. We also understand that the complexity of genetic testing and sonographic assessment is vast. As a result, we believe that each prospective parent should receive education, counseling and support in helping them to decide on the genetic testing that most suits their needs and desires. In addition, as some of these tests may not be covered by insurance, understanding the financial implications of Genetic Screening and Testing is imperative. Quality Counseling is also provided to interpret the meaning and potential implications of the screening or testing results.

***InTheKnow does provides provides prospective parents with ongoing management services once abnormal or potentially abnormal results are discovered.***

In some instances, genetic and/or sonographic assessment of a fetus may yield results that are abnormal or are suspicious for a fetal genetic and/or structural malformation. In these instances, our Medical Staff will work in conjunction with experts in the field of genetics, pediatrics, pediatric surgery, family planning and developmental pediatrics to support you in making difficult management decisions. InTheKnow has an affiliation with the Center for Human Genetics and works closely with Massachusetts General Hospital and Children's Hospital to provide the full compliment of expert patient care.

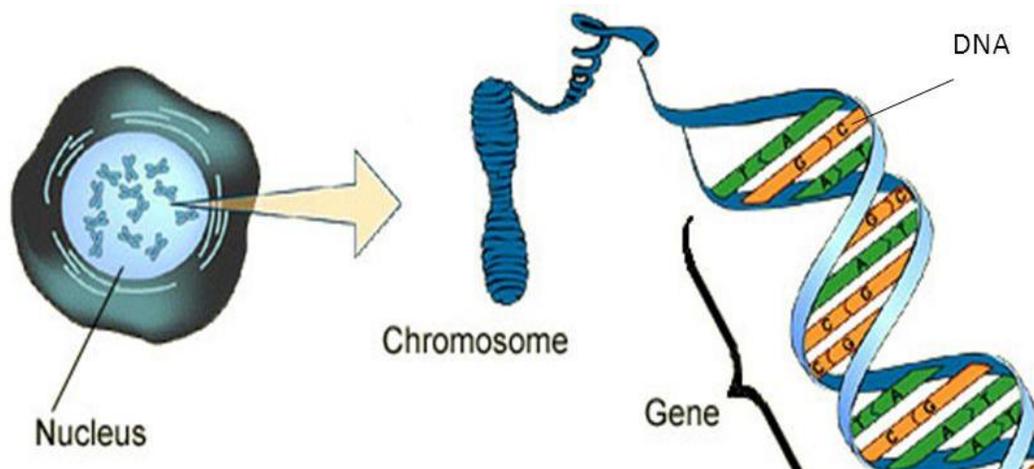
***InTheKnow is about providing prospective parents with knowledge and management options.***

There is an abundance of data to suggest that many parents both desire and benefit from the knowledge gained from prenatal diagnosis. For some parents, the abnormalities detected may lead them to elect to terminate the pregnancy. We believe that termination of pregnancy should be performed safely and with great empathy regarding the parent's loss. However, the benefits of prenatal knowledge extend far beyond the option of pregnancy termination. Many conditions, such as the Autism Spectrum Disorders have been conclusively demonstrated to benefit from early postnatal intervention. Other conditions are now being treated in utero with promising results or may benefit from delivery in a tertiary center where immediate intervention may be provided. For some parents, just having the knowledge regarding their child's condition ahead of time, gives them the opportunity to adjust and make appropriate arrangements for the optimal care of their child.

# ***BASIC GENETICS AND PRENATAL DIAGNOSIS***

## ***The Biology of DNA: Nucleotides, Genes and Chromosomes***

The most basic unit of human inheritance from a parent to his or her offspring is a gene. A gene is a cluster of units known as nitrogenous bases which combine in a predictable way to code for the proteins which form the structure of our bodies and guide its function. Millions of nitrogenous bases, organized into individual gene segments, extend along a double helical structure in the nuclei of each of our cells known as Deoxyribonucleic Acids, or DNA. The DNA is so highly coiled that, although microscopic in nature, each of us has more than 6 meters of DNA in our bodies. Large quantities of DNA, containing millions of genes, are organized into 46 chromosomes in most humans. We receive 23 of these chromosomes from our mother and 23 from our father.

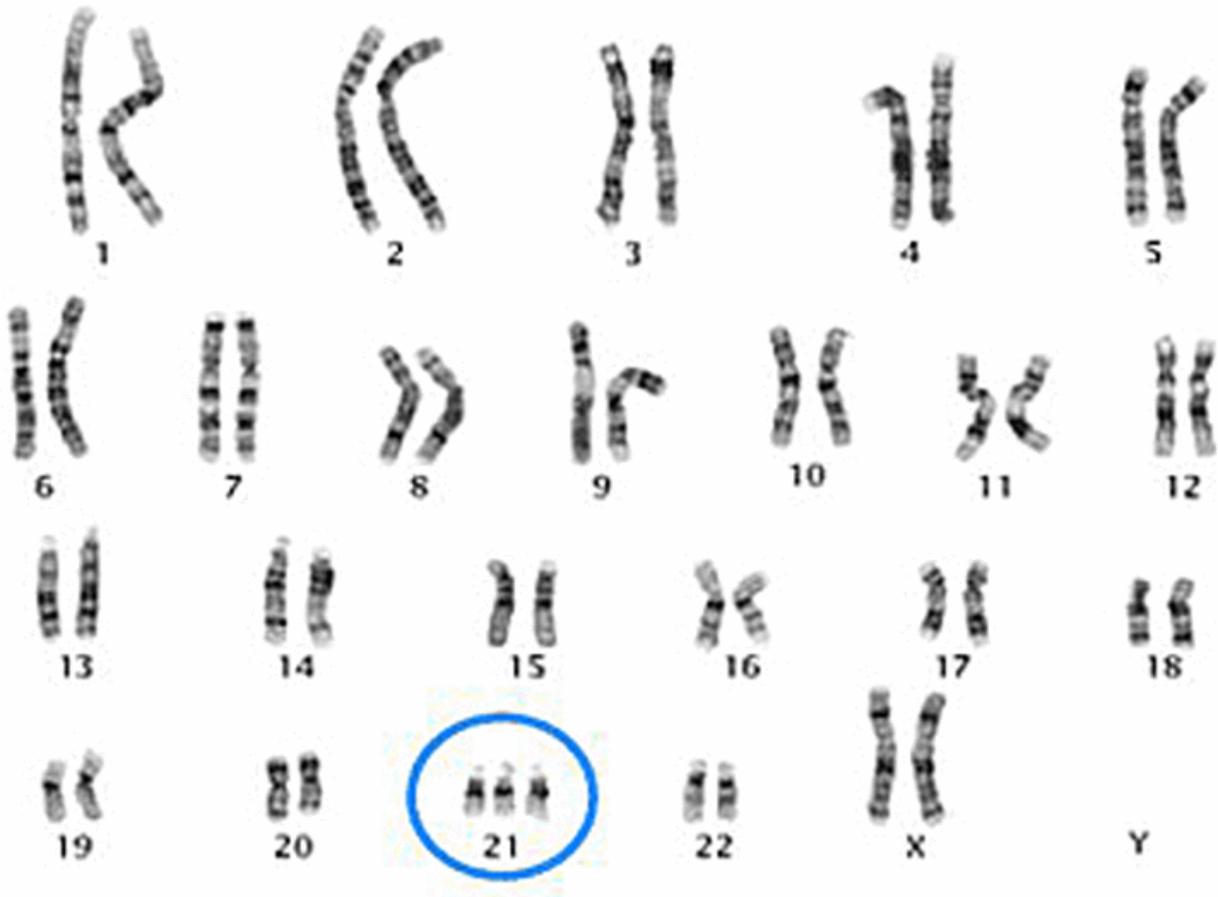


## ***The Three Categories of Genetic Disease***

Genetic disease in humans exists at all levels of the organization of our DNA, ranging from huge defects involving the number of chromosomes to tiny defects which involve a single nitrogenous base within an individual gene. Currently, however, genetic diseases are divided into three main categories:

**Chromosomal malformations** are defined as abnormalities in the number of chromosomes or defects within chromosomes that are so large as to be able to be seen under a microscope. These latter defects involve deletions, duplications and inversions of massive DNA fragments within the chromosomes and have to be larger than 5 million pairs of nitrogenous bases. Notice in the picture below that the chromosomes have been organized under the

microscope into 23 pairs. However, if you look closely, you will see one extra chromosome number 21. This is the chromosomal pattern or “karyotype” of a person with trisomy 21 or Down Syndrome.



You may also notice that there are two X chromosomes and no Y chromosome. This suggests that the person is female. If there was one X chromosome and one Y chromosome, this person with Down Syndrome would be a male. The most common chromosomal abnormality in liveborn babies is Trisomy 21 or Down Syndrome, which results in mental retardation, characteristic facial features and an increased risk for heart defects, early Alzheimer’s disease and certain cancers. Other common chromosomal abnormalities in liveborn babies include Trisomy 18 (Edward’s Syndrome) and Trisomy 13 (Patau’s Syndrome). These syndromes, which involve one too many chromosome 18 and chromosome 13 respectively usually result in the death of the child within the first months of life if not prior to birth. Also common are sex chromosome malformations involving the X and Y chromosomes. These include commonly, Turner’s Syndrome (a female with only one X chromosome and no Y), Klinefelter’s Syndrome (a male involving two X chromosomes and one Y chromosomes) and Triple X Syndrome (a female with three X chromosomes). An important characteristic of Chromosomal abnormalities is that the features of the diseases are predictable based upon the appearance of the chromosomes (i.e. all children with one too many chromosome 21 will have the characteristic set of features

of Down Syndrome.). One problem in predicting the outcome of a patient found to have a chromosomal abnormality, however, is mosaicism. Mosaicism is the presence of a chromosomal abnormality in some cells of the body, while other cells have the normal number of chromosomes. Persons with mosaicism may demonstrate a variable amount of the features of a chromosomal syndrome depending upon their proportion of abnormal cells.

## **Single Gene Defects**

Single gene defects represent the smallest abnormalities inherent in DNA and may be defined as a malformation involving less than 1000 nucleic acid base pairs. Over 4,000 single gene defects have been identified and represent a wide range of disease states. While chromosomal defects are usually “accidents” of cell division during an early stage of the dividing embryo, single gene defects are often “passed down” from generation to generation. In some cases, however, single gene defects are not inherited from parents, but arise *de novo*, due to mutations or small alterations in the DNA replication process.

Single Gene Defects often arise from predictable patterns of inheritance, which include:

***Autosomal Recessive Disorders:*** To have an autosomal recessive disease, the person must inherit two copies of the abnormal gene. In most cases, one abnormal gene comes from the person’s mother and the other from the person’s father. Therefore, both the mother and the father must at least be *carriers* (have just one copy) of the abnormal gene, even though they don’t have the disease and most often they don’t know that they are carriers. The worldwide carrier frequency is small for all autosomal recessive disorders, but it tends to be concentrated in populations that are reproductively isolated. For example, ethnic groups such as the Ashkanazi Jews, have reproduced largely within their limited group for many centuries and tend to have a high incidence of rare autosomal diseases such as Gaucher’s and Tay Sachs disease.

Many autosomal recessive diseases are difficult to diagnose because many different alterations in a gene or different genes may yield the same disease. For example, there are more than 100 different variants of the gene that yields the disease, Cystic Fibrosis. For most autosomal recessive diseases, we know only some of the variants and therefore we may not absolutely rule out the carrier state in an individual. For example, we can never say that a person is not a carrier for Cystic Fibrosis; only that they are not a carrier for the most common mutations which we test. This yields the concept of residual risk or a person’s remaining risk for being a carrier after the common mutations for a given disease are ruled out.

***Autosomal Dominant Disorders:*** To have an Autosomal Dominant disease, a person only needs one copy of the abnormal gene. A person with one of these diseases may inherit the abnormal gene from one of his or her parents (who themselves will be affected with the disease) or it may arise from a new “*de novo*” mutation from two unaffected parents. Autosomal Dominant conditions are characterized by *incomplete penetrance*. This means that, although a person has the abnormal gene, they may or may not show signs of the disease. Autosomal Dominant conditions are also characterized by *variable expressivity*. This means that

we may not be able to predict whether a person who is found to have the abnormal gene will have a very mild or severe form of the disease. Therefore, while a person with an extra copy of chromosome 21 in all of their cells will predictably demonstrate the familiar features of Down Syndrome, a person with an abnormal Neurofibromatosis gene may demonstrate a range extending from what appears as a freckle under the person's armpit to severe, debilitating tumors throughout their body. Other challenges involved in predicting the *phenotype*, or outward characteristics of a person from *genotype*, or the genetic make-up of the person include *genetic imprinting* and *Uniparental Disomy*. Genetic imprinting is a phenomenon in which the phenotype depends upon which parent donated the abnormal gene. In Huntington's Disease, for example, the disease severity is much worse if the abnormal gene comes from one's father as opposed to one's mother. Uniparental disomy is a subset of genetic imprinting in which two imprinted genes derive from either the same maternal or paternal chromosome and there is a deletion of the gene from the other parent's chromosome. A final important feature of Autosomal Dominant inheritance is the potential for late-onset diseases. Huntington's Disease, for example, is silent until a patient reaches their 30s or 40s. At that time, the person begins a profound process of mental and physical deterioration that leads to extreme disability and death within 10-15 years. While some people would wish to be informed as to whether or not they have Huntington's Disease to plan for their future, many others prefer not to know that their future involves this terribly debilitating disease.

***X-Linked Disorders:*** These are single gene defects involving the X chromosome and reflect a unique pattern of inheritance due to its specific features. In women, who inherit an X chromosome from their mother and an X chromosome from their father, there is X-chromosome inactivation. X-chromosome inactivation is a random process where in each cell of a woman's body, either the paternal X chromosome or the maternal X chromosome is shut off. This means that if there is a gene abnormality of the X chromosome, it will exist in less than 100% of the cells, because 0-100% of the abnormal gene will be inactivated on a random basis. For this reason, women rarely are affected by X-linked disorders. However, males have only one X chromosome (and one Y chromosome). Males have no X inactivation and 100% of their cells will feature the abnormal X chromosome. Because the abnormal gene lies on the X-chromosome, X-linked disorders are passed down from generation to generation by unaffected carrier females (who have a 50% chance of passing down their abnormal gene) or by affected males.

**Trinucleotide Repeat Sequence Disorders:** Trinucleotide Repeat Sequence Disorders are a subgroup of 17 autosomal dominant, autosomal recessive and x-linked disorders that increase in severity from generation to generation. The genetic basis for these diseases are repeat sequences of groups of three nucleotides within a gene. The more the number of repeat sequences, the greater the chance of having the disease phenotype. This subgroup of diseases, including Fragile X syndrome and Myotonic Muscular Dystrophy were first identified in the 1990s.

### **Copy Number Variants**

While Chromosomal Malformations are abnormalities involving regions of DNA comprised of greater than 5 million nucleotide pairs and Single Gene Defects are comprised of single genes involving less than 1,000 base pairs of DNA, Copy Number Variants are all of the genetic defects that involve more than a single gene of 1,000 base pairs and less than a microscopically-visible chromosomal defect involving more than 5 million base pairs. The identification of copy number variants and appreciation of their importance as a category of genetic disease resulted from the completion of the Human Genome Project in 2003. Although our work on and understanding of Copy Number Variants is in its infancy, it appears likely that this category of disease will ultimately be discovered to account for more human disability than Chromosomal Abnormalities and Single Gene Defects combined.

Copy Number Variants, often referred to as chromosomal “microdeletions” or “microduplications” have recently been identified as the basis for known genetic syndromes such as DiGeorge Syndrome. Many Copy Number Variants are now being linked with intellectual disability, some Autism spectrum disorders, structural malformations such as congenital heart defects and severe psychiatric diseases such as Bipolar Disorder and Schizophrenia. Moreover, Copy Number Variants appear to play a key role in disease susceptibility, drug resistance and cancer biology.

The field of Copy Number Variant Genetics is currently exploding both with regard to research and clinical applications. Experts in Genetics and Maternal-Fetal Medicine, along with the Professional Societies, however, are being wisely cautious with regards to introduction of this field into clinical practice. For most testing of copy number variants, we do not know the sensitivity of our tests to detect disease, nor the positive predictive value when a copy number variant is identified. Since the completion of the human genome project, many copy number variants have been distinguished as being “pathogenic” or commonly leading to disease or “benign” and not leading to disease. However, there remain some copy number variants that are classified as “Variants of Unknown Significance (VOUS).” It is currently unknown whether these variants are pathogenic or benign. Like autosomal dominant disorders, Copy Number Variants are subject to incomplete penetrance and variable expressivity where the person’s genetic make up may be unpredictably associated with the outward appearance and functioning of the individual.

## ***Genetic Disease and Genetic Testing***

### **Targeted vs. Non-Targeted Approaches to Prenatal Diagnosis**

Our traditional approach to genetics and prenatal diagnosis has been a targeted approach. That is, we attempted to obtain a person’s “genetic history” to determine what diseases their fetus would most likely inherit and what tests to perform. For example, it makes sense to test the fetus of a 40-year old pregnant woman for Down Syndrome as this fetus has a 1:90 risk for this disorder. It makes less sense to perform the same test on a 19-year old who has a 1:1500 risk. Similarly, it makes better sense to screen an Ashkenazi Jewish person for Cystic Fibrosis (carrier

rate of 1:25) than a person from Japan. However, there are major flaws with the logic of targeted screening and testing both on a population and individual level. Because of the age at which most women conceive, the majority of children with Down Syndrome are born to mothers in their twenties. On an individual level, is anyone really Ashkenazi Jewish? I recently had my DNA analyzed and found out that I am 96.5% Ashkenazi Jewish, with a serving of Asian and Native American DNA mixed in! That being said, targeted screening and testing remains a cornerstone of Genetic practice.

The Human Genome Project set out to read the sequence of nucleotides in the DNA of a single human being. Using a technique called Sanger Sequencing, it took 13 years (1990-2003) to read the entire sequence. Today, using Next Generation Sequencing, we can read the entirety of a person's DNA in one day. And thus the field of Genomic Medicine is born. Genomic medicine, utilizing variations of two recent breakthroughs in scientific technique, Next Generation Sequencing and Comparative Genomic Hybridization, provides us with the ability to analyze the entirety of a person's DNA. These modalities are now making great contributions to the fields of prenatal diagnosis, developmental pediatrics, pharmacology and oncology. Of course, Non-targeted screening and testing carries all of the risk for inaccuracy, unanticipated diagnosis, unwanted diagnosis, indeterminate diagnosis, confusion and anxiety for patients and their families.

## **Genetic Screening vs. Diagnostic Testing**

### **GENETIC SCREENING**

A screening test is a test that seeks to identify those who are at risk for having or developing a specific pathologic condition. Screening tests have certain characteristics that are important for patients to understand:

**Sensitivity:** The Sensitivity of a screening test is the likelihood that a patient will test positive if they have the disease. For example, if a screening test for Down Syndrome is 90% sensitive, then 90% of fetuses with Down Syndrome will have a positive screening test.

**Positive Predictive Value:** The positive predictive value of a screening test is the percentage of patients with the disease who screen positive out of all of the patients who screen positive for the disease. For example, if a screening test for Down Syndrome has a positive predictive value of 25%, then the likelihood that you have Down Syndrome if you have a screening test that is positive is only 1:4.

**Negative Predictive Value:** The negative predictive value of a screening test is the percentage of patients who don't have the disease who screen negative on their testing divided by all of those people who screen negative. For example, if a screening test for Trisomy 18 has a 99.8% negative predictive value, then if you screen negative, you have a 99.8% chance of not having the Trisomy 18.

**False Positive Screening Rate:** This is the percentage of people who have a positive screening test for a disease who don't have the disease.

**False Negative Screening Rate:** This is the percentage of people who have a negative screening test for a disease who actually have the disease.

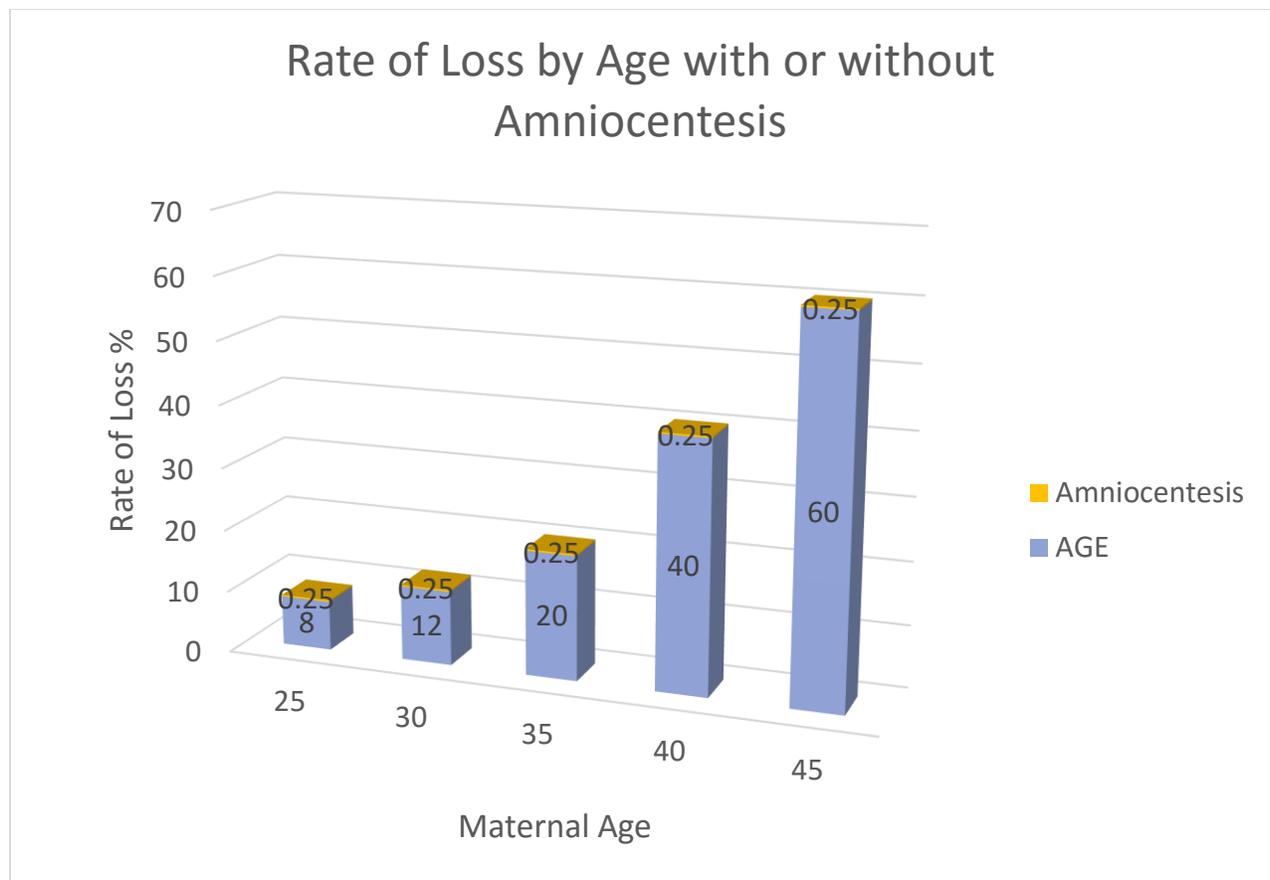
**The Relationship between Predictive Value and Prevalence:** A positive screening test for gonorrhea is more likely to be truly positive in a population of IV drug abusers and prostitutes than the same test performed on a group of nuns in a convent (at least I think that is true!). Therefore, while the sensitivity of a test is a fixed characteristic of the test, the predictive values of a screening test depend upon the population being studied. This is very important in the prenatal diagnosis of chromosomal abnormalities such as Down Syndrome, where the prevalence (and thus the positive predictive value) of fetuses with Down Syndrome is much higher in older pregnant women.

### **GENETIC TESTING**

While genetic screening identifies those at higher risk than the general population for a specific disease, genetic diagnostic testing tells us whether or not a person actually has the disease in question. Often diagnostic testing requires more invasive or costly techniques which is the rationale for the development of a screening modality. An example of a screening and diagnostic testing pair are serum screening for chromosomal abnormalities and an amniocentesis with a fetal karyotype analysis. The serum screening identifies individuals at high risk for having a fetus with Down Syndrome, while the amniocentesis and fetal karyotype identifies whether or not the fetus actually *has* Down Syndrome.

### **INVASIVE TESTING**

Prenatal diagnostic testing involves analysis of fetal cells that may only be obtained through the procedure of amniocentesis. While Chorionic Villous Sampling may be considered diagnostic, it analyzes placental cells. Rarely, there can be a chromosomal abnormality confined to the placenta that is not evident in the fetus (confined placental mosaicism) or vice versa. While amniocentesis provides fetal cells and thus the optimal opportunity for genetic analysis of fetal DNA, the procedure is associated with a small but real risk for fetal loss. The American Congress of Obstetrics and Gynecology suggests that the risk for pregnancy loss with amniocentesis is approximately 1:300-1:500 pregnancies or about .25%. For some patients, this is an unacceptably high risk while for others, this is a tiny risk given the amount of useful information that the procedure provides. Certainly, amniocentesis is a small cause of fetal loss in the context of the natural rate of fetal loss associated with maternal age.



For example, in a 35 year-old woman, it is 80 times more likely that the pregnancy will miscarry due to age (natural causes) than due to the performance of an amniocentesis.

Invasive testing with amniocentesis provides diagnostic testing for chromosomal abnormalities, copy number variants and single gene defects. The two major methods of testing of amniotic fluid include fetal karyotyping and comparative genomic hybridization.

Karyotyping has been performed since the 1970s. It detects chromosomal abnormalities including chromosomal mosaicism. It can not detect copy number variants or single gene defects.

Comparative Genomic Hybridization(CGH) is a new technique that can detect not only chromosomal abnormalities, but also copy number variants. It does have the limitation in that it may not detect mosaicism, triploidy or balanced translocations.

Comparative Genomic Hybridization and Fetal Karyotype analysis may be offered jointly or in tandem using the same amniotic fluid sample. Together, their detection rate for significant genetic abnormalities extends from approximately 1.7% in a 25 year old to approximately 6.7% in a 45 year old. This means that approximately 7 cases of genetic abnormalities will be

detected for every pregnancy lost by amniocentesis in a 25 year-old and nearly 27 cases will be detected in a 45 year-old.

Single gene testing of amniotic fluid involves molecular biology techniques including restriction endonucleases, polymerase chain reaction, Southern Blotting Gel Electrophoresis and Sanger Sequencing. Increasingly, Next Generation Sequencing is being used to assess single gene defects in lieu of the older techniques due to its high accuracy and performance capabilities. Single gene diagnostic testing of fetal DNA is performed only after the fetus is found to be at risk for a targeted disease based upon carrier screening of the parents or a family history for a specific disease.

### ***Noninvasive Fetal Testing***

Noninvasive fetal testing involves the testing of the maternal serum to screen for fetal genetic disease. Although serum testing involves screening rather than diagnostic testing, it offers several advantages over its invasive counterparts. First of all, the testing can be performed as early as 9 weeks gestation compared to 16 weeks for much of invasive testing. Secondly, non-invasive testing does not encounter the 1:300-1:500 risk of pregnancy loss associated with amniocentesis.

Noninvasive serum screening for chromosomal abnormalities traditionally involved assessment of serum proteins that correlated with risk. Using this method, sensitivities for detection of Down Syndrome of up to 95% were achieved, with a false positive rate of 5%. In 2011, the first cell-free DNA testing was introduced to the United States. This testing makes use of the fact that small amounts of the placental DNA float freely in the maternal serum. Using comparative genomic hybridization and next generation sequencing, these tests detect 99.9% of Down Syndrome and are now being used to detect several copy number variants as well. One major drawback of these tests are that they are assessing placental cells instead of fetal cells. If there is a confined placental mosaicism (an abnormal placenta and a normal fetus), there will be a false positive test. In practice, however, the rate of false positive testing is .1% compared to 5% for the traditional serum screening. Limitations with this testing also include the possibility of not having a high enough fraction of fetal DNA to perform the test. Other challenging situations for this type of testing include twin gestation, donor egg recipients, maternal transplant recipients and pregnancies complicated by an early twin demise. Some of these test have yielded unexpected and unintended results such as the presence of cancer or chromosomal abnormalities in the mother.

Single gene non-invasive screening is now making good use of Next Generation Sequencing. Although, single gene screening has traditionally focused on single targeted genes based upon family history (eg. Cystic Fibrosis, sickle cell anemia), now panels screening multiple genes for the presence of the carrier state in potential parents are being used.

# **PRENATAL SCREENING AND DIAGNOSTIC MENU**

## ***Single Gene Carrier Testing: Targeted, Semi-Targeted and Non-Targeted***

In patients with a family history of known genetic disease, targeted Carrier serum testing of the responsible gene may be performed. If a patient has no known family history of genetic disease, but is in a high risk group for being a carrier of a single gene mutation (i.e. Ashkenazi Jews, African Americans), they may have semi-targeted testing based upon their ancestry. Patients with no known genetic history or others may wish to have non-targeted single gene testing in which the carrier state of more than one hundred single gene-based diseases are tested. Approximately .8% of live born children have a single gene defect.

## ***Nuchal Translucency Screening***

Performed between 11-14 weeks gestation, this is a sonographic examination of the fetus involving measurement of a layer of fluid between the fetal spine and the skin on the back of the neck. A large fetal nuchal translucency increases the risk for Down Syndrome and other chromosomal abnormalities as well as the risk for congenital heart disease and miscarriage.

## ***First Trimester Serum Analyte Screening for Chromosomal Abnormalities***

Also performed between 11 and 14-weeks gestation, analyte screening is a fingerprick for 4 drops of blood. This test analyzes the maternal serum levels of the hormones Human Chorionic Gonadotropin and Estriol as well as the level of Pregnancy-Associated Polypeptide A. This “traditional” serum screening test has an 85-90% sensitivity for Trisomy 21 when combined with Nuchal Translucency Screening and a 5% false positive rate. If a serum alpha fetoprotein is added to this combination between 15-20 weeks gestation (the Integrated Screening Test), the sensitive for trisomy 21 reaches 95%. These serum tests are covered by most insurance plans.

## ***Cell-Free DNA Screening for Chromosomal Abnormalities with or without assessment for Copy Number Variants***

Cell-free DNA screening can be performed after 9-weeks gestation and involves a simple blood draw which examines placental cells within the maternal bloodstream. These tests have a sensitivity for Trisomy 21 in excess of 99% and very high sensitivities for Trisomy 18, Trisomy 13 and other chromosome abnormalities. In addition, the false positive rates for these tests are .1%; far lower than Serum Analyte Screening. Many patients enjoy knowing the gender of the baby as early as 10-weeks gestation which these tests make possible. Some of the cell-free DNA tests can also screen for as many as seven Copy Number Variant diseases at the time of this writing. The number of evaluable copy number variants is expected to rise dramatically over the next several months. There is variable insurance coverage for these new, high quality screening tests, although the out-of pocket expense is usually fairly low. The incidence of chromosomal abnormalities is .65% and the incidence of pathogenic Copy Number Variants is 1-1.7%.

### ***Detailed Fetal Anatomical Ultrasound Survey***

In the United States a standard ultrasound assessment of the fetal anatomy is performed at 18-weeks gestation. Numerous studies have demonstrated that the detection rate of fetal malformations ranges widely from approximately 40% in the Community setting to greater than 90% in high quality academic and subspecialty centers. Beginning in 2014, the American Institute of Ultrasound in Medicine established guidelines for a higher level fetal anatomical survey, outlining the specifics of a high level and detailed fetal examination. The standard fetal anatomical survey will be covered by insurance while a detailed fetal anatomical survey may or may not be covered depending upon a patient's risk for fetal anomalies. Structural abnormalities of the fetus occur in 2-3% of pregnancies.

### ***Invasive Genetic Testing with Amniocentesis: Fetal Karyotype, Microarray Analysis, Targeted Single Gene Testing and Amniotic Fluid Alpha-Fetoprotein Assessment***

The prenatal diagnostic testing of Genetic disease depends upon the direct analysis of the fetal DNA within cells found floating in the amniotic fluid. Chorionic Villous Sampling is considered diagnostic; however, like Cell-Free DNA testing, it analyzes placental, rather than fetal cells. This may lead to inaccurate results in certain settings. Invasive amniocentesis and chorionic villous sampling are associated with fetal loss. Amniocentesis is associated with a 1:300-1:500 risk of loss. Whether or not CVS confers an increased risk above that of amniocentesis is controversial.

Until recently, fetal Karyotyping was the standard prenatal diagnostic test for chromosomal malformations. Microarray Analysis, also known as Comparative Genomic Hybridization, is now largely replacing Karyotyping because Microarray detects both chromosomal abnormalities and copy number variants with great accuracy. However, unlike fetal karyotyping, microarray analysis can not detect balanced translocations, triploidy or mosaicism. Additionally, comparative genomic hybridization may detect variants of undetermined significance (VOUS). These are variants that remain unclassified as to whether or not they are pathogenic (associated with known disease states.)

When both potential parents are carriers for an identical single gene defect, amniocentesis with targeted sequencing of the specific gene can distinguish whether the fetus is affected with the disease, a carrier for the disease, or completely unaffected.

When serum alpha fetoprotein (AFP) screening for neural tube defects indicates an increased risk for an open spina bifida, amniocentesis may increase the sensitivity of disease detection. In practice, however, a high quality ultrasound, combined with AFP screening detects approximately 90% of open spina bifida. Many patients do not wish to risk amniocentesis in order to achieve a 99% sensitivity of detection.

### ***Fetal Echocardiography***

Congenital heart defects occur in .8% of the population and this constitutes 40% of major fetal structural malformations. Fetal Echocardiography is an advanced ultrasound technique focusing

on a far more comprehensive evaluation of the fetal heart than one would undergo with either a standard or detailed fetal survey. In general, insurance plans will cover fetal echocardiography in patients with a family history or other risk for having a child with congenital cardiac disease

## **GENETIC TESTING INFORMED CONSENT**

I \_\_\_\_\_ have read and understood the written materials entitled “The InTheKnow Genetics Program at One Brookline Place,” written and provided to me by Dr. Bruce Cohen and his staff. In addition, Dr. Cohen and his staff have counseled me regarding genetic and sonographic screening and testing and have answered my questions. I understand that despite the efforts of Dr. Cohen and his staff, it is impossible to completely educate patients regarding Genetics and Prenatal Diagnosis given the time constraints, vast amount of available information, rapidly advancing knowledge and complexity of these scientific disciplines.

Dr. Cohen and his staff have counseled me that every pregnant patient is at risk for having a fetus with a genetic or structural abnormality. I have learned that there are specific tests, using my serum, placental tissue or the amniotic fluid surrounding my fetus that may screen for or diagnose genetic abnormalities prior to my birth. Advantages of the knowledge of fetal abnormalities from prenatal testing include the ability of prospective parents to plan for a potentially abnormal child, ability to arrange for delivery in an appropriate obstetrical center, opportunity for termination of pregnancy in accordance with State laws, opportunity for in utero interventions with certain anomalies and the opportunity for early childhood intervention.

I have learned from the aforementioned written materials and counseling that genetic testing and prenatal diagnosis can not identify all abnormalities which may affect my fetus. The etiology of some abnormalities may be unknown or we may lack the technical means of identifying certain disease states. I also understand that the current screening and testing may lead to results that are falsely positive, falsely negative or indeterminate. I learned that with some genetic diseases, there can be a wide range of severity that can not be predicted by identifying the abnormal gene or genes in question. I have been informed that there is a chance that fetal genetic testing may suggest that I have a genetic abnormality or a medical condition. It is my understanding that it is possible that I could learn that I or my fetus have a genetic disease that will present later in life, even if I am healthy at present. Finally, I understand that screening tests identify those who are at high risk for an adverse outcome or disease, while diagnostic testing may, for some specific disease states, identify the presence or absence of the disease.

I have been counseled that I have the option of declining all prenatal screening and testing. Alternatively, I may choose a targeted or non-targeted approach to Genetic Screening and/or diagnosis.

I understand that I will be billed for genetic services, screening and testing and that my insurance company may or may not cover the cost of genetic testing.

I understand that the proper use of ultrasound in the assessment of fetal abnormalities has not been associated with an increase risk of fetal malformations, fetal developmental abnormalities or maternal complications. However, sonographic examination of the fetus is limited in its ability to detect fetal structural and genetic malformations, accurately predict fetal growth and well-being and assess the risk of pregnancy complications.

I was also informed that the technique of amniocentesis, used to obtain amniotic fluid as part of invasive genetic testing, is associated with fetal loss in 1:300 to 1:500 procedures according to the American Congress of Obstetrics and Gynecology. Invasive testing may rarely result in harm to a fetus or a premature or pre-viable delivery.

I understand that blood drawing, with trained personnel, is not generally associated with significant maternal risk.

Based upon my understanding of the risks and benefits of Genetic Testing and Prenatal Diagnosis, as well as my option to have no testing, I agree to undergo the specific testing indicated below:

- Nuchal Translucency Screening
- First Trimester Serum Analyte Screening
- The Integrated Screening Test
- Single Gene Carrier Testing: Targeted
- Single Gene Carrier Testing: Non-Targeted Panel
- Cell-Free DNA Screening for Certain Chromosomal Abnormalities
- Cell-Free DNA Screening for Certain Chromosomal Abnormalities and Copy Number Variants.
- Detailed Fetal Anatomical Ultrasound Survey
- Invasive Genetic Testing using amniocentesis: Karyotype
- Invasive Genetic Testing using amniocentesis: Microarray Analysis
- Alpha Fetoprotein Screening
- Fetal Echocardiography

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Patient Signature

Date

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Provider Signature

Date