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Teste pré-natal não invasivo para detecção de
doenças genéticas utilizando sequenciamento
de nova geração

*Noninvasive prenatal test for detection of
genetic diseases using next-generation
sequencing*

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Chapter 1

General Introduction

Cell-free DNA and its applications

Cell-free DNA (cfDNA) is naturally found fragmented within the blood at a very low concentration (1000-10000 genomes/ml plasma), originated from apoptosis and/or tissue necrosis. Its half-life is approximately 16 minutes, which makes it a very interesting potential biomarker for dynamic monitoring of diseases associated with “exogenous” DNA (i.e., derived from other sources, such as fetus, cancer or transplanted organ) (LO et al., 1998b, 1997, 1999; STROUN et al., 1989; JAHR et al., 2001; SUZUKI et al., 2008).

Presence of “exogenous” cfDNA was first described for cancer (LEON et al., 1977; STROUN et al., 1989), and later described for fetal DNA (LO et al., 1997), and for transplanted organs (LO et al., 1998a). Use of cfDNA for analysis of non-solid biological tissue is called liquid biopsy, and it has been increasingly used as a diagnostic and monitoring tool for diseases, having the advantage of being non-invasive, as described below.

- ***Cancer Detection***

In general, cancer possesses a strong genetic component (STRATTON; CAMPBELL; FUTREAL, 2009; GARRAWAY; LANDER, 2013; TOMASETTI; LI; VOGELSTEIN, 2017; VOGELSTEIN et al., 2013). The gold standard for cancer monitoring is biopsy analysis; however, other than being invasive, expensive and not

always available, it does not contemplate inter and intratumoral heterogeneity, and it is specific to the moment of punctum, which might take too long to translate into clinical action.

The discovery of circulating tumor DNA (ctDNA) (LEON et al., 1977; STROUN et al., 1989) opened possibilities to perform real-time disease monitoring through the diverse disease stages, and can be used not only for early detection of the disease, but also for prognosis and disease treatment, such as predicting response to treatments (CROWLEY et al., 2013; HUANG et al., 2012; ROTHÉ et al., 2014). It also addresses the limitations of biopsy when it comes to assessing inter and intratumoral diversity because ctDNA fragments are derived from tumors across the organism. The ctDNA fraction varies between 0.01 and 93%, and it is correlated with tumor burden, early relapse and treatment response (CROWLEY et al., 2013; DIEHL et al., 2005; JAHR et al., 2001; THIERRY et al., 2010) (Figure 1).

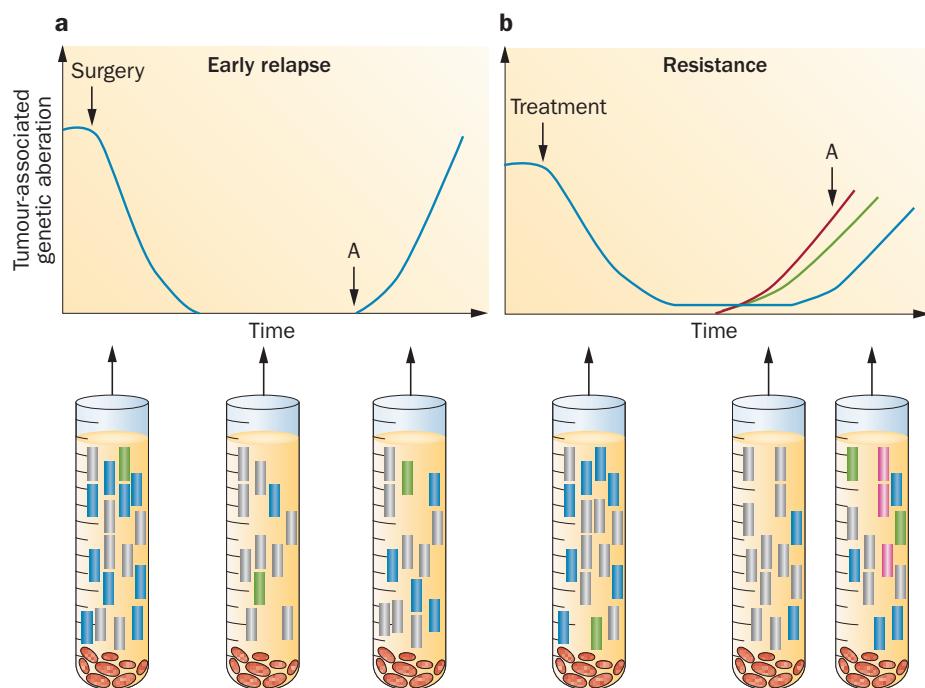


Figure 1: Monitoring tumor-specific aberrations to detect A) relapse after surgery; B) resistance to treatment. 'A' represents the clinically detectable time of relapse. Blue represents an early occurring mutation present in the tumor and reflects overall tumor burden. Red and green represent mutations associated with development of the tumor, as resistant clones. Adapted from CROWLEY et al., 2013.

Detection of tumor-specific mutations in ctDNA helps performing a real-time diagnosis and monitoring of the disease. Although mutation profile varies among patients, cancer types and disease stages (HAN; WANG; SUN, 2017; HUANG et al., 2012; SCHWARZENBACH; HOON; PANTEL, 2011), there are some genes in which mutations are already well characterized for some types of cancer, for instance, *BRAF* in melanomas and *EGFR* for several types of cancer, such as colorectal, breast, thyroid and lung (DAVIES et al., 2002; DOUILLARD et al., 2014; HAN; WANG; SUN, 2017; KRISHNAMURTHY et al., 2017; UCHIDA et al., 2015). The advent of next-generation sequencing (NGS) has permitted simultaneous investigation of such genes of interest and the identification of rare mutations in complex DNA mixtures, due to increased sensitivity of this method (FORSHEW et al., 2012; KINDE et al., 2011; LEARY et al., 2010). Although it does not cover all possible scenarios, tracking mutations in such genes can be used as a screening strategy for early detection of certain types of cancer. Another use for this approach is to provide clues to the etiology of the disease when there is a cancerous mass present, but direct biopsy is too complicated due to technical issues or patient frailty.

It has been shown that liquid and tumor biopsies have a concordance of >70%, and liquid biopsies have the additional benefit of detecting genetic information of tumors from metastatic sites of the body, making it a great potential biomarker (DOUILLARD et al., 2014; KRISHNAMURTHY et al., 2017; PATEL; TSUI, 2015; ROTHÉ et al., 2014). Although validation is still required for most cases of cancer, liquid biopsy has already been used for some cancers, such as lung cancer, which recently had the first FDA-approved liquid biopsy (cobas EGFR Mutation Test v2 – Roche Molecular Systems, Inc.). It relies on screening for *EGFR* (epidermal growth factor receptor) mutations to indicate therapeutic decisions regarding the use of EGFR tyrosine kinase inhibitors (TKIs) (KRISHNAMURTHY et al., 2017; UCHIDA et al., 2015). This is particularly important for lung cancer due to the difficulty in obtaining biopsies for some cases.

- ***Transplanted organ follow up (Allograft Rejection Detection)***

Incidence of acute allograft rejection varies depending on the organ type, being as high as 55% in intestinal transplant (BECK et al., 2015; COLVIN-ADAMS et al., 2015; GIELIS et al., 2015; VALAPOUR et al., 2015). The gold standard for transplant rejection diagnosis is allograft biopsy, an invasive procedure that has pitfalls, such as: variability of grading scale due to different observers, and, for small biopsies, chance of missing the acute rejection due to the inhomogeneous nature of graft damage (ARCASOY et al., 2011; MARBOE et al., 2005; SARAIVA et al., 2011). Therefore, a noninvasive method has been pursued to eliminate discomfort and to overcome these pitfalls.

The discovery of donor cfDNA (cfDNA) in the receptor's blood (LO et al., 1998a) was an important step for the development of a noninvasive method for diagnosis of transplant rejection, since during rejection there is an increase of organ cell death, which will translate into an increased cfDNA in the receptor's blood. This method basically consists on monitoring the levels of cfDNA as a rejection signature (BLOOM et al., 2017; DE VLAMINCK et al., 2014; SCHÜT et al., 2017; SIGDEL et al., 2013; SNYDER et al., 2011; ZHANG et al., 1999). This monitoring can be performed with the use of Y-chromosome sequences, however it works only in 25% of cases, in which the receptor is a woman and the donor is a man. More recently, a universal method (regardless of gender) called GTD (Genomic Transplant Dynamics) was described, and it uses single nucleotide polymorphisms (SNPs) to quantify cfDNA and therefore detect the onset of rejection (BECK et al., 2015; BLOOM et al., 2017; DE VLAMINCK et al., 2014; GIELIS et al., 2015; SNYDER et al., 2011) (Figure 2).

Although there are other methods for SNP detection (such as digital droplet PCR), the use of NGS (targeting a panel of informative SNPs) is advantageous because it has been shown to detect not only organ rejection, but also transplant-related infections simultaneously, through detection of non-human sequence reads used for identification of infectious agents (BECK et al., 2013; DE VLAMINCK et al., 2014, 2015; HIDESTRAND et al., 2014).

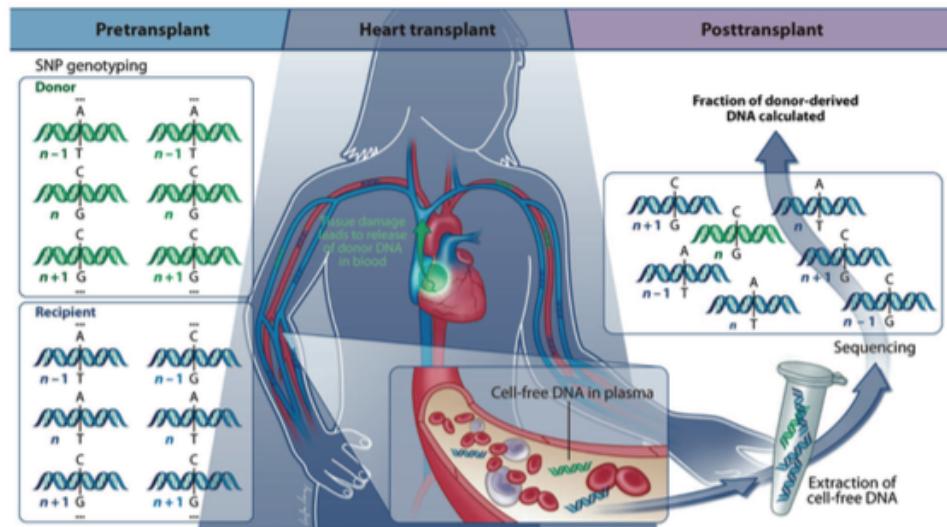


Figure 2: GTD principle: genotyping recipient and donor before the transplant. Sequencing of plasma performed after transplant to detect single bases alleles that were distinct between donor and recipient, allowing discrimination of donor and recipient derived sequences (positions n, but not n-1 and n+1). Adapted from DE VLAMINCK *et al.*, 2014.

The GTD method has shown that the basal cfDNA fraction is variable and organ-specific, and rejection detection can be achieved months before diagnosis by biopsy (up to 5 months in heart transplant), therefore preventing graft damage (BECK *et al.*, 2013; DE VLAMINCK *et al.*, 2014; SCHÜT *et al.*, 2017). An improvement of the GTD method has been recently accomplished, obviating the need of genotyping the donor or the receptor: it uses a panel of informative SNPs, taking into account different degrees of relatedness between recipient and donor (GRSKOVIC *et al.*, 2016).

Recently, the first commercially available allograft rejection monitoring using cfDNA and NGS was launched in the US (AlloSure – CareDx, Inc.) (WOODWARD *et al.*, 2015).

▪ **Prenatal Genetic Disease Diagnosis**

Chromosomal anomalies are responsible for 50% of spontaneous abortions, about 6% of congenital anomalies and 5.6% to 11.5% of perinatal deaths (MOORE;

PERSAUD, 1998; RAI; REGAN, 2006; SANSEVERINO, 2006). Aneuploidies constitute the most common chromosomal anomalies, and the most frequent are autosomal aneuploidies of chromosomes 21 (Down Syndrome), 18 (Edwards Syndrome) and 13 (Patau Syndrome) (HYDE; SCHUST, 2015; NUSSBAUM, 2007; SKRZYPEK; HUI, 2017). Therefore, screening for these aneuploidies is routinely offered in prenatal care (BENN et al., 2013).

Conventional prenatal screening methods for aneuploidy and other birth defects include maternal serum screening and ultrasound imaging in the first and/or second trimester. However, these approaches have their own limitations and suffer from high false positive rates (5% on average), leading to unnecessary invasive diagnostic procedures (BIANCHI et al., 2014; RUSSO; BLAKEMORE, 2014; SHAMSHIRSAZ; BENN PETER; EGAN, 2010; SKRZYPEK; HUI, 2017). If the screening tests indicate that a fetus has an increased risk of aneuploidy, the indication is to proceed with invasive tests, like chorionic villus sampling (CVS) or amniocentesis for diagnosis, offered around 10-13 and after 15 weeks of gestational weeks, respectively. Invasive tests entail, besides discomfort for the mother, risks for the pregnancy as miscarriage and fetal abnormalities (AKOLEKAR et al., 2015; CAUGHEY; HOPKINS; NORTON, 2006; MUJEZINOVIC; ALFIREVIC, 2007).

Discovery of cell-free fetal DNA (cffDNA) led to significant advances in prenatal diagnosis (LO et al., 1997). This DNA of fetal origin ranges between 133 and 166bp in size. It is released into the maternal bloodstream through apoptosis of trophoblastic cells (which form the placenta), being rapidly eliminated after birth, which makes it a promising pregnancy-specific biomarker (BISCHOFF; LEWIS; SIMPSON, 2005; FLORI et al., 2004; LICHTENSTEIN et al., 2006; LO et al., 1999, 2010; SMID et al., 2003). Maternal cfDNA is derived mainly from apoptosis and active secretion of cellular DNA (GAHAN; ANKER; STROUN, 2008; VAN DER VAART; PRETORIUS, 2008).

Since its discovery, cffDNA has been widely used for detection of qualitative traits not present in the mother, such as fetal sex determination (chromosome Y detection), Rh factor (only possible for Rh- mothers), and the presence of *de novo* or paternally inherited monogenic disease mutations (BUSTAMANTE-ARAGONÉS et al., 2012; CHITTY et al., 2011; FAAS et al., 1998; LO et al., 1997, 1998c). With the

advent of next-generation sequencing, it has been possible to detect quantitative traits, such as aneuploidies (CHIU et al., 2008; FAN et al., 2008).

Noninvasive prenatal testing (NIPT) using next-generation sequencing

▪ **Aneuploidy detection**

Fan and colleagues (2008) demonstrated for the first time the utility of NGS to reliably detect fetal quantitative traits through read count. After several efforts in 2011, noninvasive prenatal testing (NIPT) became clinically available in the United States and China, changing the prenatal care field (CHIU et al., 2010; EHRICH et al., 2011; FAN et al., 2008; FAN; QUAKE, 2010; LO et al., 2010; PALOMAKI et al., 2011; SEHNERT et al., 2011; SPARKS et al., 2012a, 2012b; ZIMMERMANN et al., 2012).

NIPT tests have high sensitivity (true positive rate) and specificity (true negative rate) values, especially for chromosome 21 (GIL et al., 2015; KOUMBARIS et al., 2016). Bianchi and colleagues (2014) demonstrated that, for detection of trisomy of chromosome 21 (T21) and chromosome 18 (T18) in general obstetric population, NIPT shows lower false positive rates than standard screening (false positive rates: 0.3% vs. 3.6% for T21, $P<0.001$, and 0.2% vs. 0.6% for T18, $P=0.03$) (BIANCHI et al., 2014).

Two approaches have been developed and implemented for NIPT commercially: SNP-based NIPT and read count-based NIPT (NGS and targeted NGS).

➤ **SNP-based NIPT**

This approach selectively sequences SNPs with high heterozygosity level and compares cfDNA SNPs with parental genotypes. Thousands of SNPs are used for each chromosome of interest, and only informative *loci* are used to determine aneuploidy. The aneuploidy risk is calculated through SNP distribution, taking into

account different hypotheses of the fetus being monosomic, disomic or trisomic. It then compares the expected and observed allele distributions to determine the most likely scenario (LIAO *et al.*, 2011a; ZIMMERMANN *et al.*, 2012; RYAN *et al.*, 2016).

➤ *Read count-based NIPT*

Next-generation sequencing can be performed as whole genome sequencing (WGS), or as targeted sequencing, which sequences only some parts of the genome (exome and/or a panel of genes of interest).

For trisomy detection, both approaches rely on detecting an increase of the read count originated from the trisomic chromosome. Therefore, women carrying an trisomic fetus will have a higher proportion of read count on maternal plasma DNA (pool of maternal and fetal DNA) than women carrying euploid fetus, and this increase will be proportional to the cffDNA fraction (fetal fraction - FF) present in maternal blood, as shown at Figure 3.

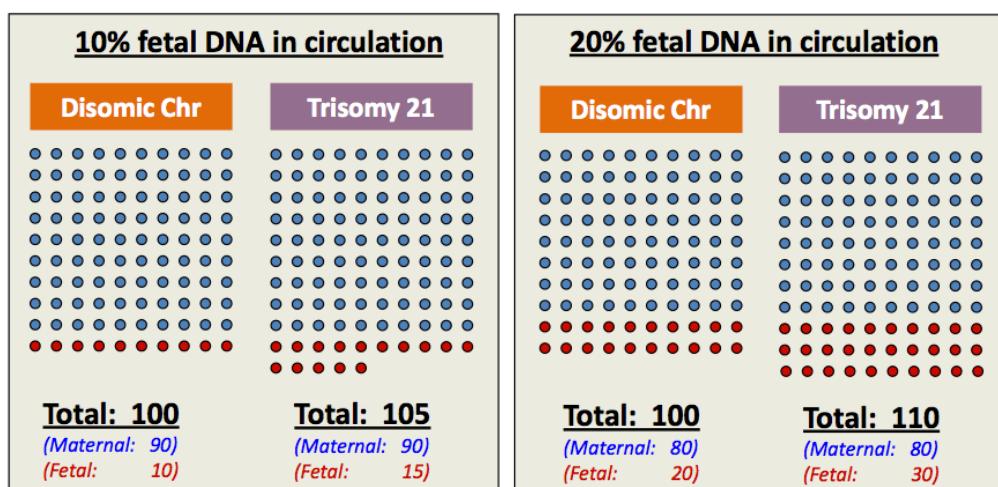


Figure 3: Representation of chromosomal increase in presence of a trisomic fetus for chromosome 21, proportional to fetal fraction. Blue: maternal cfDNA; Red: fetal cfDNA. Adapted from SPARKS; STRUBLE; et al., 2012.

As mentioned above, targeted sequencing is performed for only part of the genome, and the coverage required for detection of trisomies of chromosomes 21, 18 and 13 for targeted sequencing is less than 5% of the coverage required for WGS. Therefore, targeted sequencing is a great strategy to reduce costs and increase coverage, and it was applied for NIPT by Sparks and colleagues (2012) for the first time (ASHOOR et al., 2012a; KOUMBARIS et al., 2016; LIAO et al., 2011; NORTON et al., 2012; SPARKS et al., 2012a, 2012b).

NGS and targeted NGS uses amplification of maternal plasma cfDNA (mix of maternal and fetal cfDNA), and fetal chromosome copy number is determined by comparing the ratio of reads from the chromosome of interest against mean and standard deviation of ratio of a reference disomic dataset, expressed as Z-Score or NCV (normalized chromosome value) (CHIU et al., 2011; SEHNERT et al., 2011; SPARKS et al., 2012a). Z-score is regularly used in the literature and a Z-score higher than 3 is the threshold used to detect trisomy (meaning the result is 3 standard deviations above the mean of the reference dataset, or Z-score < 3 means the result is 99% within the normal expected z-score, i.e. unaffected) (CHIU et al., 2008). The Z-score increases with FF, since samples with higher FF have more fetal cfDNA to be detected (EHRICH et al., 2011; PALOMAKI et al., 2011; SPARKS et al., 2012b). Therefore, estimating FF is a great quality control for NIPT, although not all tests contain this measurement.

Although already offered for patients in Brazil, NIPT technology has not been implemented in our country. The laboratories that commercialize the test outsource the technology or the test itself. Besides decreasing the amount of sequencing required, another advantage of targeted NGS is the ability to detect SNPs, which allow estimation of fetal fraction and detection of monogenic diseases (although all these tests are not performed altogether thus far). Therefore, targeted sequencing is a great option for a clinical scenario.

- ***Monogenic disease detection by NIPT***

Most NIPT initially focused on detection of autosomal dominant traits, which are present only in the fetus and are caused by *de novo* or paternally inherited mutations, such as fetal sex and RH status (BUSTAMANTE-ARAGONES et al., 2008; CHITTY et al., 2011; FAAS et al., 1998; GONZÁLEZ-GONZÁLEZ et al., 2002; LO et al., 1997, 1998c). Identification of fetal sex is useful for instance in cases where couples are at risk of having a child affected by an X-linked disease, as well as conditions where there is possibility of clinical intervention during pregnancy, such avoiding virilisation of affected females in CAH (congenital adrenal hyperplasia) cases by administration of maternal dexamethasone early in pregnancy (RIJNDERS et al., 2001).

Detection of *de novo* or paternally inherited gene disorders is already performed for some diseases, such as skeletal dysplasia (SD) (CHITTY et al., 2011, 2015; DAN et al., 2016; VERHOEF et al., 2016), where detection of the pathogenic mutations confirms fetal diagnostic. On the other hand, mutation detection when the mother carries (or may carry) the mutation, such as autosomal recessive or X-linked diseases is more challenging. For these cases, approaches such as detection of relative haplotypes or mutation dosages have been developed (LAM et al., 2012; LUN et al., 2008b; MA et al., 2014; PARKS et al., 2016). Although most tests for monogenic diseases focus on specific diseases, the feasibility of constructing a fetal genome map using maternal plasma deep sequencing and parental genotype information has already been demonstrated as a proof of concept (FAN et al., 2012; KITZMAN et al., 2012; LO et al., 2010).

There are about 5,000 known monogenic disorders (AMBERGER et al., 2015), and only a few of those are currently being detected through NIPT in clinical settings (BUSTAMANTE-ARAGONÉS et al., 2012; LENCH et al., 2013; SKRZYPEK; HUI, 2017; VERHOEF et al., 2016). Even for those, at least maternal genotype information is required in addition genotype information from plasma sequencing, increasing price and turnaround time for test results. The ideal clinical scenario would be a NIPT relying only on maternal plasma sequencing information.

- **Cell-free fetal DNA fraction (Fetal fraction)**

Fetal fraction estimation is important for detection of monogenic diseases and aneuploidies, since FF is proportional to the allele fraction (AF – proportion of the mutation in respect with the reference allele) for monogenic diseases, as is proportional with the increase of the reads from the trisomic chromosome for aneuploidy detection (as shown above in Figure 3).

FF increases through pregnancy, corresponding to about 10% of total cfDNA in maternal blood at about the 10th gestational week, varying between patients (LUN et al., 2008a; WANG et al., 2013). There are several factors associated with fetal FF, such as maternal weight (inversely proportional) and maternal ethnicity; however, these factors have not been assessed in the Brazilian population (ASHOOR et al., 2012b; KINNINGS et al., 2015; LO et al., 1998b; POON et al., 2013; ZHOU et al., 2015). FF is an important factor that influences the accuracy of NIPT. The currently accepted FF threshold for NIPT is 4%, and lower FFs may yield false negative results for the test (EHRICH et al., 2011; PALOMAKI et al., 2011; SPARKS et al., 2012a, 2012b; TAKOUDES; HAMAR, 2015). There are several ways of measuring FF, however, most of them are only applicable to pregnancies of male fetuses (real-time PCR or the ratio between the Y chromosome and autosomes' reads) (FAN et al., 2008; LO et al., 1998b) or are performed with the analysis of fetal-specific alleles, requiring genotype information of the mother, at least (CHU et al., 2010; LO et al., 2010).

As explained above, while performing noninvasive prenatal testing using NGS, some groups use targeted sequencing and estimate the FF from the retrieved SNP information, while groups performing low-coverage whole-genome sequencing are not able to retrieve SNP information (LAU et al., 2014; SPARKS et al., 2012b; ZIMMERMANN et al., 2012). For FF estimation without the use of parental genotypes, a maximum likelihood estimation using the binomial distribution is employed to determine the most likely FF based upon measurements from several informative SNP *loci* (JIANG et al., 2012; SPARKS et al., 2012b).

For the clinical scenario, the ideal approach is to be able to estimate FF for any pregnancy, regardless of fetal gender, and independently of the genotype

information of either parent. Any additional laboratory step increases the turnaround time and cost of the exam. It is important to note that this approach can be extrapolated to other fields that use cfDNA as a diagnostic tool, such as cancer detection and transplant rejection (DE VLAMINCK et al., 2014; ROTHÉ et al., 2014).

To our knowledge, there is no NIPT test integrating all analyses (fetal fraction, fetal sex, trisomies and monogenic disease), which would be the ideal clinical scenario, because it diminishes cost and turnaround time, as already discussed above. At the present time we do not have a NIPT test developed in Brazil, since the tests offered in our country outsource the technology or the test itself. Development of such technique is important for the scientific development of our country, as well as for the development of novel tests for the population.

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Chapter 3

General Discussion and Conclusions

Implementing these novel techniques in Brazil is of great importance, so we can be competitive at the research world scenario as well as open the opportunity to develop better cost-effective tests to be offered to our population. We are located at the Human Genome and Stem Cell Research Center (HUG-CELL), a reference center of genetic diseases in Brazil at Universidade de São Paulo. An important feature of this work is that it is a scalable test for monogenic diseases. This means that once the gene panel is ready and the workflow established, it is possible to test virtually any disease there are genes on the panel for, and with the advent of new technologies and analyses, we have a great potential of developing new noninvasive tests (without need of any parental genotype) for other diseases with variants difficult to be detected currently, such as for recessive disorders (indicated by slight increase in AF, as discussed previously).

Also, the ability to incorporate new techniques enables translating them into new fields, increasing the impact of our research within the country, for instance permitting us to apply the knowledge obtained in this work in areas such as cancer and transplant rejection monitoring, already performed abroad. In addition, it diminishes our dependence on sending genetic tests abroad, which mean we have a possible additional source of revenue for our country, and we are able to offer that for our patients at university extension-basis, as well as transfer the technology for companies, like spin-offs from Universities.

The methodology used here can be more easily applied to allograft rejection than for cancer monitoring in the short term for 2 main reasons: 1) detection of allograft rejection is less complex than cancer, since its goal is to detect increase of the cdDNA fraction (which is performed similarly as we did for fetal fraction), while for

cancer the knowledge of the etiology is often needed, and the cancer genome may change during disease progression, due to metastasis and/or therapy resistance acquire; 2) For cancer there is a need for a higher coverage (since cfDNA fraction can be as low as 0.01%), and this amount of sequencing is not financially feasible with the current technology applied by us.

The main novelty of this work was to be able to integrate several analyses (fetal fraction, fetal sex, trisomies and monogenic diseases), without needing parental genotype. In this scenario, having the fetal fraction estimated is an important quality control of the test, since we are able to differentiate true negatives from samples with a low FF (which is a limiting factor for detection). Besides, having the FF information helps to detect monogenic disease variants, since we can detect variants within the expected AF range. However, most of groups that do perform the FF estimation use genotype information from at least the mother, which increases cost and turnaround laboratory time. In this work we describe a new model for estimate fetal fraction using only plasma sequencing data, overcoming these issues.

The development of this estimation model was performed in collaboration with the research group of Dr. Iwijn De Vlaminck, at Cornell University, a reference group at analyzing variants from cfDNA sequencing data. The group of Dr. De Vlaminck utilizes the sequencing data of cfDNA from plasma of organ receptors to detect rejection of the transplanted organ. Setting these kind of collaborations is very important for at least 2 reasons: 1) increasing the quality of the research being performed in Brazil, therefore being competitive 2) having contact with new research lines, being able to translate the knowledge and mindset to our environment.

As mentioned, HUG-CELL is a reference center in our country, and has a multidisciplinary team to attend hundreds of patients and their families. Translational projects like the one described here have the potential to bring better care for our patients and offer exams not available for them before. Thus, the purpose of this study is in line with the objective of the Ministry of Science Technology and Innovation Ministry and the National Council for Scientific and Technological Development (CNPq) designed to promote science, technology and innovation in the country.

RESUMO

Desde 2011 a área de diagnóstico pré-natal sofreu uma revolução com a introdução de teste pré-natal não-invasivo (NIPT) de doenças genéticas, que se baseia na análise de DNA fetal livre de células presente no plasma materno. Apesar de estar disponível no Brasil, nós dependemos em terceirizar a tecnologia desenvolvida no exterior, ou o teste em si. Sendo assim, nosso objetivo foi desenvolver e implementar um teste NIPT abrangente utilizando sequenciamento de nova geração de alta cobertura *targeted* para: 1) estimar fração fetal; 2) determinar sexo fetal; 3) detectar trissomia; 4) detectar doença monogênica.

Nós desenvolvemos um modelo robusto e preciso ($r^2= 0.994$, p-value < 2.2e-16) para estimativa de fração fetal baseado na distribuição da fração alélica de SNPs. Nós utilizamos Z-score para determinação de sexo fetal (100% de precisão) e detecção de trissomia dos cromossomos 21 (T21) e 18 (T18), atingindo uma sensibilidade de 100% (95% IC: 63.06% - 100.00%) e especificidade de 98.53% (95% IC: 92.08% - 99.96%) para T21, e 40% (95% IC: 5.27% - 85.34%) e 98.59% (95% IC: 92.40% - 99.96%) para T18. Para detecção de doença monogênica (displasia esquelética) nós realizamos análise de variante, com uma taxa de detecção de 71% (5/7). Até onde sabemos, este é o primeiro trabalho a integrar todas as análises em um único teste, e a realizar detecção de doença monogênica sem utilizar genótipo parental.

Nós mostramos neste trabalho que é possível implementar essas técnicas no nosso país, utilizando recursos disponíveis e/ou desenvolvendo colaborações com grupos referência internacionais. Isto demonstra o potencial de desenvolver tecnologias internas, e aplicá-las à outras áreas não-invasivas, como diagnóstico e monitoramento de câncer e rejeição de transplante.

ABSTRACT

Since 2011 the prenatal diagnosis field has undergone a revolution with the introduction of a noninvasive prenatal test (NIPT) for genetic diseases relying on analysis of fetal cell-free DNA present in maternal plasma. Although available in Brazil, we rely on outsourcing the technology developed abroad or the test itself. Therefore, our objective was to develop and implement a comprehensive NIPT using high-coverage targeted next-generation sequencing to: 1) estimate fetal fraction; 2) determine fetal sex; 3) detect trisomy; 4) detect monogenic disease.

We developed a robust and accurate model ($r^2 = 0.994$, p-value < 2.2e-16) for fetal fraction estimation based on distribution of SNP minor allele fraction (MAF). We used Z-score for fetal sex determination (100% accuracy) and trisomy detection of chromosomes 21 (T21) and 18 (T18), achieving a sensitivity of 100% (95% CI: 63.06% - 100.00%) and a specificity of 98.53% (95% CI: 92.08% - 99.96%) for T21, and 40% (95% CI: 5.27% - 85.34%) and 98.59% (95% CI: 92.40% - 99.96%) for T18. For monogenic disease detection (skeletal dysplasia) we performed variant analysis, with 71% (5/7) of detection rate. To our knowledge, this is the first work to integrate all analysis in one single test, and to perform monogenic disease detection without using parental genotype.

We showed in this work that it is possible to implement such techniques in our country, using available resources and/or engaging in collaboration with reference research groups abroad. It shows the potential of developing internal technologies, and applying it to other noninvasive fields, such as cancer and organ rejection diagnostic and monitoring.

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