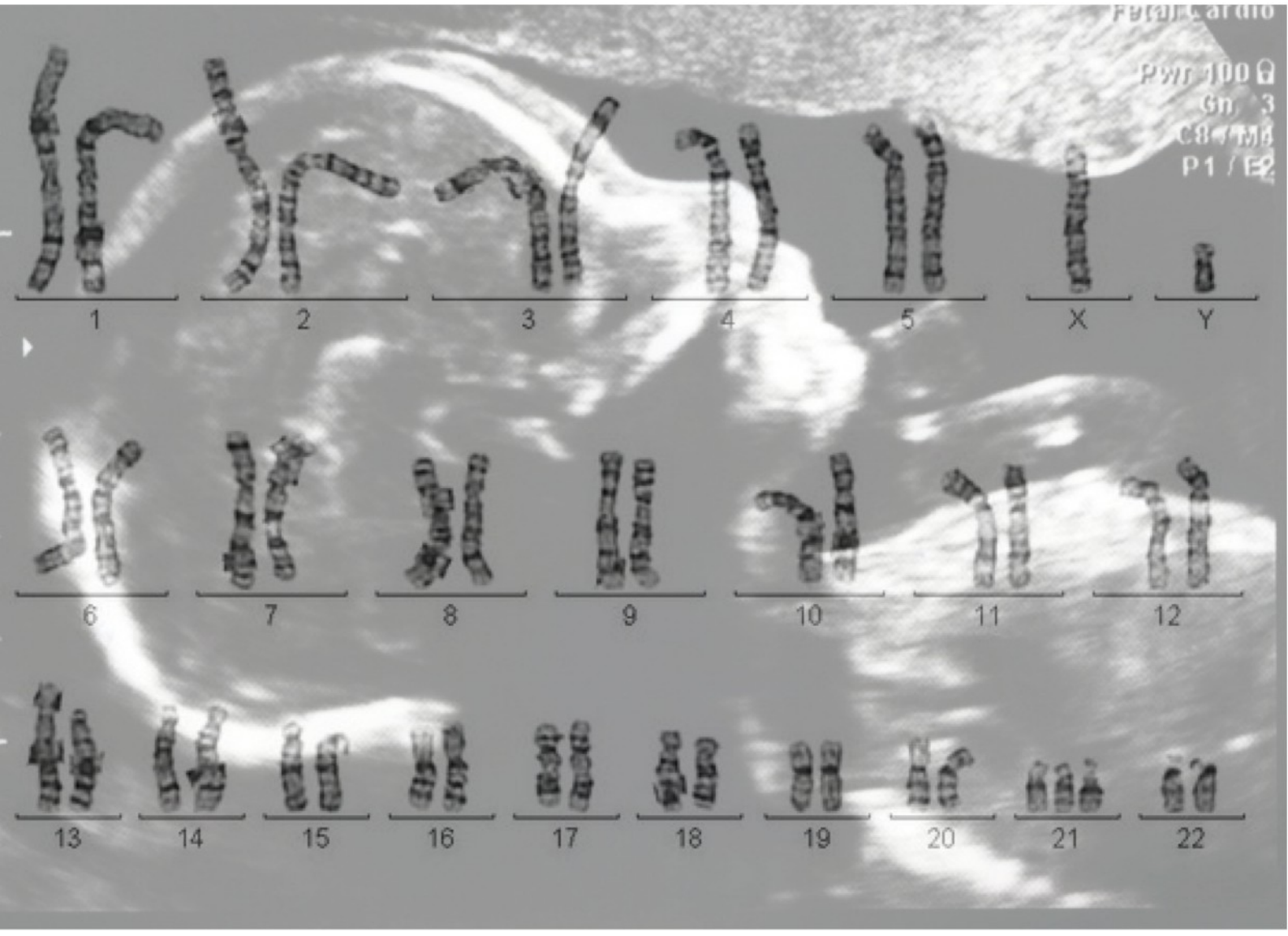
PrenaTest®

- Fighting the Down Syndrome -



A Term Paper by
Rebekka Hosch & Sophia Egli
February 2015

1. Preface

People affected by Down Syndrome have been present in our society for ages. They are recognizable by their expressive traits, their movements and their open and emotional nature. But still many people do not accept them as a part of the society, they are outsiders for them. Not only the way of treatment but also the rate of abortion are indicators for this. Over 90% of the pregnancies with a diagnosis of Down Syndrome are terminated. This circumstance is quite surprising if one respects that their opportunities are getting better and better; concerned people and their family get support from consultants, help from the state is offered, organizations (journalism-, musician- and sport projects) for affected people are founded and the chance to get an education grows. There is even a person that reached a degree at the university with help of his environment. Furthermore the diversity in jobs increases. However most people decide to take the easier way- not to use the opportunities and not to have their child.

This amount of people that decide to abort their pregnancy made us think a lot, when we read about it. What are the motivations of a mother to abort a child that differs from other children? Is it unbearable to live under these conditions? Probably most parents are afraid of not being accepted in the community, of having to fend for the child a whole life. The trouble caused by these, not even born, individuals makes them panic.
But on the other hand, is it tolerable, that all these children will never get the chance to see the world just because they are different? Can they not live a happy live?

However, not only the behavior of people with the Down Syndrome and ethical aspects are interesting. Also the biological practices are worth to know. So we decided to write our term paper about those practices with the main interest on one procedure: the PrenaTest®.

In the following pages we will dive deeper into this subject of this test. The question we ask ourselves are the following: What is the PrenaTest®? How does its procedure look like? How does it work on the genetic level? What makes people do the test? What are its consequences? What are its advantages and how do the drawbacks look like? What are possible future steps?

2. Introduction

People affected by the Down usually have development and cognitive delays. Nevertheless, phenotype as well as mental abnormalities can differ widely between each other. Causes for those disabilities are either an extra copy of chromosome 21 in every cell (called Trisomy 21), an additional chromosome 21 that is attached to another chromosome (called Translocation Down Syndrome) or an extra copy of chromosome 21 that does only occur in few body cells (called Mosaic Down Syndrome).

Now some words to the history of the PrenaTest®: In 1997, scientist Lo and his team (Anglo-Chinese) found out, that in the serum of a pregnant woman cell free fetal DNA (cffDNA) is present. This was the base for the development of the PrenaTest®, a non-invasive prenatal test (NIPT), performed earliest in the ninth week of pregnancy and using the massively parallel sequencing screening method. Using this method DNA fragments of the maternal and the fetal cell free DNA are analyzed and assigned to the chromosomes. In this way, it is possible to obtain information whether chromosome 21 has more copies by simply comparing numbers of information analyzed with information of an unaffected person. Therefore this test gives a clear positive or negative result for the Down Syndrome.

PrenaTest® is a Sequenom-licenced technology, thus PrenaTest® is the brand name of Lifecodexx for this form of examination.

Since August 2012 this testing method is available in many countries of the world. The PrenaTest® is available mainly in Europe and most densely in Germany and Switzerland. Only 20 milliliters of blood are needed for Lifecodexx in Konstanz - the only place in the world the PrenaTest® is completed- to perform the testing and to send back the results within ten working days. Recently, a new opportunity has opened – an express version of the PrenaTest® has been launched, taking only six working days.
Still, health insurances deny to pay for the testings, so it is quite expensive (800 CHF plus 100 CHF for express version) for private individual. The certain circumstances that favor the performing, and preselect are for example: age of mother over 35 years, cases of Down Syndrome in the family history or conspicuous signs in the ultrasound.

Besides the PrenaTest® other methods exist: they are divided into invasive-, for example the analysis of the placenta, and non-invasive testings, including ultrasound and pregnancy hormones in the blood. The problem is, that in the former the probability of miscarriage is relatively high and in the second the accuracy is not a hundred percent.

A completely different method, called XIST, is right at the moment examined by Jeanne Lawrence. She and her research group found out, that every X-chromosome of a woman carries the X-inactivation gene that silences the whole chromosome if activated. The idea now is to silence one chromosome of the three chromosomes 21 in order to have only two chromosomes 21 expressed in the cells and one silent chromosome 21, which is the normal amount expressed chromosomes 21. This would be the only treatment, discovered so far, nevertheless it is not in use yet. However, this method would need an invasion right after the fertilization, as the development of the Down Syndrome starts in this early stage.

3. Analysis of the PrenaTest® in the Laboratory

As already mentioned earlier, the PrenaTest® uses the analysis of cell free DNA (cfDNA) circulation in the pregnant woman's blood in order to give a feedback on whether or not the unborn child is a affected by Trisomy 21. How this method works will be discussed in the following spread.

The cell-free DNA is found in the mother's blood as a mixture of approximately 90% cfDNA from the mother and 10% from the child (also called cell-free fetal DNA (cffDNA)) and can be assigned to the different chromosomes.
Assuming that you have a sample of which a hundred fragments are chromosome 21 specific, you can say that, in the case where no Down Syndrome is present, 10 of those cfDNA fragments are from the child and 90 are from the mother (this is the amount of the comparison sample). If you now have the data from a mother carrying a child that has the Down Syndrome, you will find a hundred and five chromosome 21 specific fragments of cfDNA. In this case, still 90 originate from the mother, however 15 come from the fetus (fifty percent more than in the comparison sample). The two samples, the comparison sample and the tested sample, are compared and the ration between the amount of chromosome 21 specific fragments is made, which would be in this case 1:1.05. With those ratios, the PrenaTest® gives a reliable result.

If a pregnant woman decides to take the PrenaTest®, this will be the course from blood to result: The medical practitioner takes 20 milliliters of blood and sends the sample to Lifecodexx in Germany where the test is carried out.
In a first step, the plasma is isolated from the blood and in the second step, the cell- free DNA is separated from the rest. However, fetal and maternal cfDNA cannot be split from each other, so both are scanned in the following test.
Important for the successful testing is a minimum amount of cffDNA out of the cfDNA is four percent, or eight percent in a twin pregnancy. To make that sure, the QuantYfeX™ technology is applied. It uses the fact that maternal DNA is said to be hypomethylated and fetal DNA is hypermethylated[1]. The cfDNA is then scanned (using special light) and evaluated by the LightCycler 480 II Instrument which distinguishes hypomethylated and hypermethylated cfDNA. If the amount of cffDNA is high enough, the following procedure will be applied.

In order to have more DNA fragments, the present fragments are cloned in a genomic library, for example by using a bacteria colony. It is necessary, since it is easier to analyze a big sample instead of a small one. Once done, the random massively parallel sequencing method is applied, using the IlluminaHiSeq2000 next generation sequencing technology, deciphering the DNA into approximately 15 million sequences. One example of this deciphering can be seen in Figure 1, where the DNA (in this case the cfDNA) is translated using a laser and a fluorescent detector in the sequence of the bases. Figure 1 is a simplification of the process, excluding all calculations and further detections, the machine does.

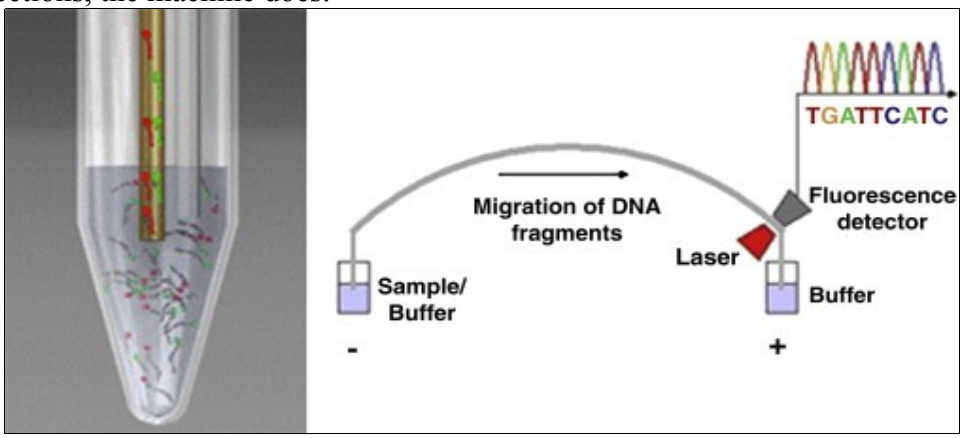


Figure 1: Analysis of cfDNA sequences using a laser and a fluorescence detector.

1 Definition: if DNA is methylated, this refers to methyl-groups on the cytosine which are present in huge amounts in embryonic DNA, but rare in DNA of older people, those groups are lost during development.

From this 15 million sequences that are deciphered, 6 millions are selected in a further step. The criteria for this selection is the specificity for one chromosome . Roughly two thirds are sorted out because they could, according to the information they carry belong to different chromosomes.

Using a human reference genome, those selected sequences then are matched with the corresponding chromosomes. (see Figure 2)

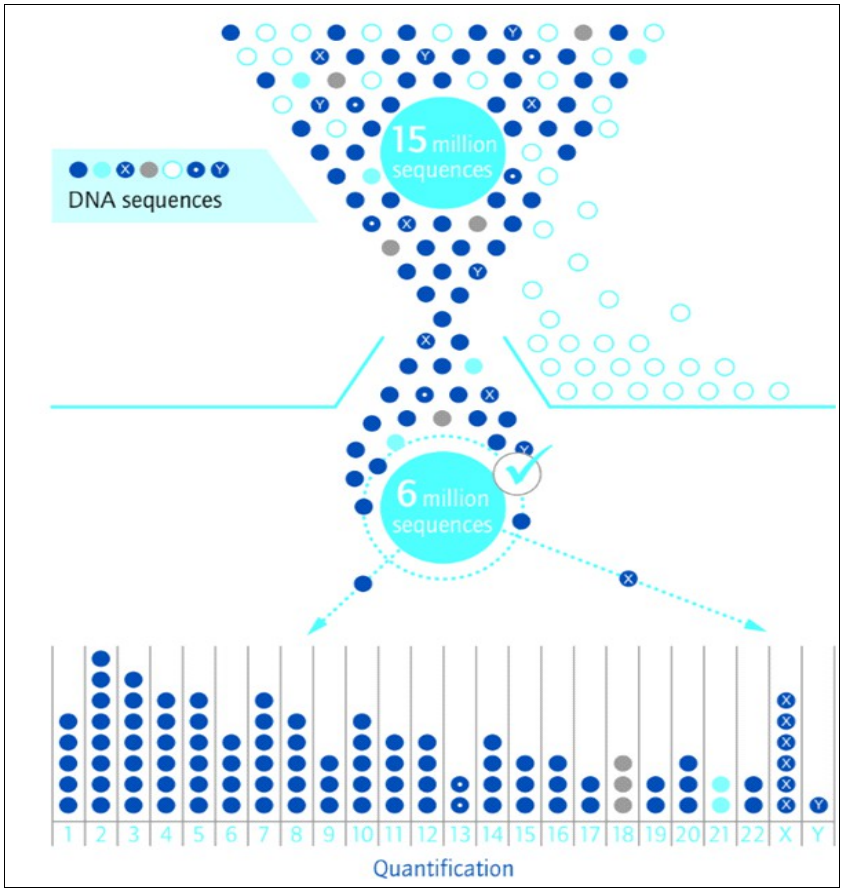


Figure 2: Quantification of the DNA sequences during the PrenaTest.

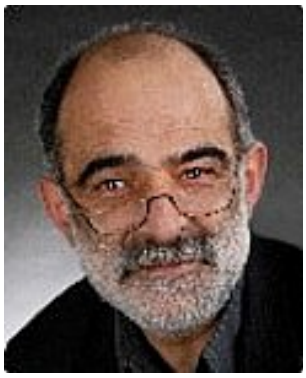
To calculate whether an abnormality is present, the so called z-score is introduced. It indicates the amount of sequences belonging to the chromosome 21 (or what ever chromosome is tested) minus the amount of the same sequences from the chromosome 21 in a reference sample where no trisomy is present and needs to be divided by a constant, which is defined as 0.0069. If the z-score is higher than three or exactly three, after the Gaussian rules, the sample is defined as positive for the Down Syndrome. If its lower than three, the result is negative for the Down Syndrome.

To show the calculation clearer, an easy example:

- In the **reference sample**, out of 6'000'000 sequences, 76'000 can be assigned to the chromosome 21, which equals a percentage of **1.27%**
- In the **examined sample**, out of 6'000'000 sequences, 79'000 can be assigned to the chromosome 21, which equals in this case **1.32%**
- The calculation for the z-score looks now as follows: $z\,(Chr21) = \frac{1.32-1.27}{0.0069} = 7.25$

  → Since 7.25 is by far higher than three, this sample absolutely must be classified as affected by Trisomy 21!

4. Interview with Mr Miny and impressions of the laboratory



We had the pleasure to meet Prof. Dr. Med. Peter Miny, who is the supervisor of the department for medical genetics at Universitätsspital Basel. We are very thankful that he devoted time to us and our questions. The interview was originally in German and afterwards translated into English.

Mr Miny, you work on the specific field of medical genetics. What does that include?
Medical genetics is quite a young discipline. The doctors examine children with birth deformities or such syndromes under consideration of their genes. Furthermore, a big field is the prenatal examination which is often in cooperation with other doctors such as gynecologists.
The PrenaTest® is a quite new practice to discover Down Syndrome in a prenatal state.
Yes, that's true. Actually the whole subject of noninvasive prenatal testing (NIPT), to which the PrenaTest® belongs is quite recent. PrenaTest®, which is by the way a brand name like CocaCola, is one of those tests. It is offered by Lifecodexx in Konstanz, who bought a license from the inventor company, Sequenom (USA). Beside Lifecodexx and Sequenom, there are a few more companies in Europa, China and the USA that feature this test, but the procedure is identical. As you realize, the whole examination doesn't belong to any academic institution.
So the test can basically be done everywhere?
Absolutely. But the problem is that it doesn't belong to the general patient care. So the patient has to pay the test on his own, which is quite a big amount of money. Even in Switzerland, health assurance denies to pay for it but there are currently discussions about changing this.
But is it not even payed when the child before has had Down Syndrome or other circumstances indicate a higher risk?
No, not that kind of tests. There are established checks, their expenses are borne by the health insurance. After the law, every women in Switzerland over the age of 35, or with a provable high risk as inheritable diseases, has the right, to sort the probability of a chromosomal disorder out. This is due to the fact, that a 35 year old woman, or older, has a rick of 3% to get pregnant with a challenged child, however, the PrenaTest® doesn't belong belong to that sort of testings provided.
In the recent years, one started not only to look at the age, but to do a so called first-trimester-screening. This method is available for everyone. The medical contains a ultrasonic testing of the neck, an amniocentesis and a blood-check of the mother. This has led to a detection rate of about 90% of chromosomal disorders; earlier, when only the age was considered, about 25% were discovered.
How many pregnant women are actually older than 35?
In earlier times, only about 5-10% were over 35 years old. But during the last few years this number dramatically increased – about 30% of expectant mothers are nowadays older than 35 years old.
So NIPTs, like the PrenaTest®, are only done after a first-trimester-screening with a result of higher risk?
Well, that's what one is thinking about at the moment. You may not forget, that the PrenaTest® is only done since about 2 years in Switzerland. So it is a very young and rapid development. But at the moment it is really as you said: first the first-trimester-screening, afterwards the NIPT.
You said that the NIPT is way more expensive. But what are in your opinion the advantage of this procedure in contrast to other procedures?
You don't run a risk to lose your child, like in invasive procedures and is more precise than first trimester screening, still the PrenaTest® may not discover all types of chromosomal disorders– only 21, 18 and 13.

What is the general attitude towards the PrenaTest® (or other NIPTs)?
That is difficult to say. We don't even have good numbers for Switzerland concerning who is interested in prenatal diagnostic. Always if a new procedure is released, there are critical voices, that are e.g. afraid that there would be no more children with Trisomy on the planet, despite the fact that it seems, that the amount of people with Down Syndrome has doubled in the last ten years. This most likely refers to the increasing age of mothers. The capabilities of the prenatal diagnostic have improved at the same time, but for a significant part of the population, it is due to religious or ethical reasons no opportunity. So you have to consider many movements. Both first-trimester-screening and PrenaTest® have the impact that today much less interventions are considered. We realized that too – we did amniocentesis and other examinations. That drastically declined. So we think, that in many cases in in Switzerland these noninvasive examinations are done. But we don't know how many. We only know that worldwide many hundred thousand test were done in the last few years. Only commercial companies are involved, which do a lot of publicity, for example on the internet. This is a very new situation, because the market is now controlled by private concerns.
We would like to come to the process of the PrenaTest®. From which week on can it be performed?
Today, all experts recommend not to do the test as early as possible, which would be from ninth week on, but the chances of a wrong result are higher, the chance of a miscarriage exist (so the test would not have been necessary) and there is comparison to other tests like ultrasonic screening during that time. In addition there are some controversies, because during the test you get to know the sex of your child. During that period of the pregnancy, you may still abort the child without giving a reason. People want to prevent abuse of the test to abort a child with the sex you don't want, so the test is recommended from 12th week on.
What is the exact process when a patient visits the hospital to do the test?
First a ultrasonic screening is done. If there Is nothing special, the patient may do a first-trimester-screening or choose to do the PrenaTest® which she has to pay. In the second case, 20 mg of blood are taken and sent to either a company in Switzerland or, as most frequent, to the USA because some specific computers are needed to analyze the material. During the procedure, one has to pay attention, so that the maternal cells in the blood don't break apart and spread out there DNA.
How long does it take to analyze the sample?
At the moment it takes about two weeks, but the next step will be, that these American companies will open branches in Europe. So the time will become shorter and shorter.
Can you explain the method to us on genetic level? What exactly do you analyze?
It is a dream since decades, and we have already worked on it for 25 years, to identify infantine DNA in the maternal body and to use this DNA for genetic investigations. One knows since a long time that cells from a child, or better to say from the placenta, exist in the blood circulation of the mother. In the 80s, scientist tried to find those cells, of which the pathologist knew already in the 19th century.
In 1997, famous Mr. Lo identified the fetal DNA in the blood of the mother. In addition the possibility of “next generation sequencing”, which was developed around 2010, was important for the method. This procedure is used to sequence the whole, or at least parts of the gene.
And what happens there exactly?
The maternal blood plasma contains cell free DNA (cfDNA). 90% of that DNA belong to the mother, 10% belong to the child and are called cell free fetal DNA (cffDNA). It isn't possible to isolate the cffDNA, all the examinations are done with the whole plasma.
There are basically two technical approaches to do so: the first one you makes a complete sequencing of the genome. If a child had now three chromosomes 21, the amount of the specific DNA for chromosome 21 would be higher. So it is compared to a plasma with two copies. But one may not forget, that 90% DNA belong to the mother, who doesn't carry three chromosomes. The difference will thus be very little percent, but it is reliable.
A familiar approach is the “massive parallel shotgun sequencing”. There, everything is sequenced

that has a value, but it is also possible to sequence only the chromosomes of interest.The second approach doesn't work with the amount of specific DNA, but with the genotyping of snips. Snips are single nucleotide polymorphisms. A third copy of the child can be discovered when maternal and infantile snips are genotyped. This approach has the advantage, that triploidies may be discovered too.
The problem is, that all those results aren't diagnostic. Consequently, if something is found, a amniotic fluid test has to be done to prove it. There wrong positive reports and wrong negative reports. The error source can be on a technical- or on a biological level. The biological reason is that the cells don't really belong to the child but to a part of the placenta – the cytotrophoblast. This cytotrophblast doesn't always represent the childish DNA. In about 1% of the cases, there is a discrepancy.
Furthermore it may happen that a chromosomal disorder disappears on its own during cell division. But in the placenta such that trisomal cell line may sometimes still be found. This phenomena is called mosaic Down Syndrome.

We made some research on other practices too and realized that there is actually none that treat the disease. Do you think this will be a field of research in the future?

Actually, there is one approach called XIST, however until now it only worked in a cell culture and whether that's going to work with human beings is questionable. This is a very interesting development: since years we have been searching therapies for a number of diseases, but all hopes balked until now with a few exceptions. The problem is that from the moment when there is an additional chromosome, the whole development goes wrong. So the transplantation would have to be as early as possible. This is also the reason why one tries to diagnose the chromosomal disorder as early as possible. But this is an example that could maybe work one day.

Does the Universitätsspital research too?

Yes, of course! We were always interested in prenatal diagnostic. We try to identify genes that cause diseases. Our new principal is also interested in genetic aspects of psychological maladies.

What do you think would be the consequences for the society if there were no more people with Down Syndrome (out of an ethical perspective)?

That's a difficult question. I often hear those fears, but this is, as often proved, nonsense. It may happen that a disease disappears, but not in the case of Down Syndrome. Down Syndrome is so to say the cliché of a handicapped person, whose right and worth to exist is discussed in the general public. No one would discuss whether it makes sense to exterminate a disease, but that's a different situation. When looking at statistics, this fear is completely baseless.

So the test doesn't actually exist to extinct the disease, but to give everyone the opportunity to decide on his own whether he/she wants the child...?

Exactly! It's finally all about giving a pregnant woman autonomy in her decisions. Many people may decide much better than doctors or lawyers, because they have personal experiences. And I think this freedom is a symbol of our western values.

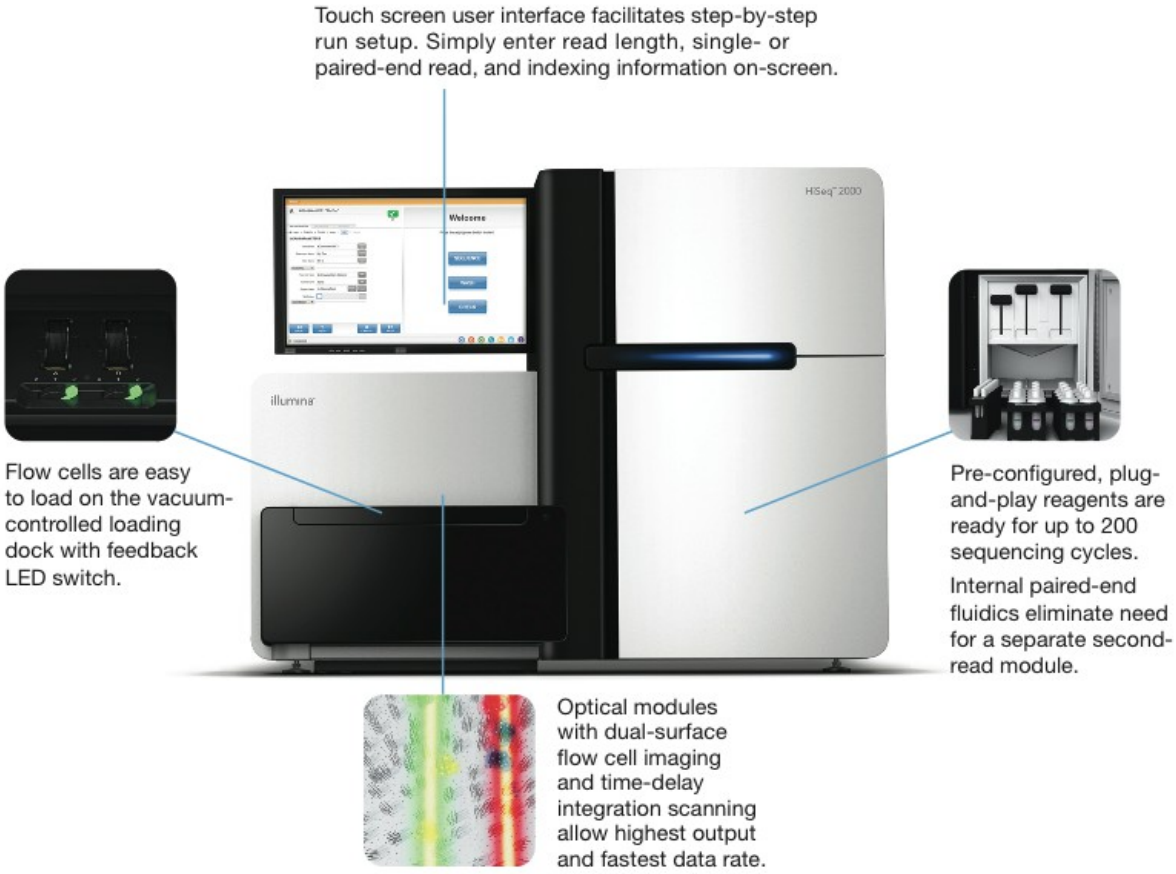
Do many decide to abort when they get a positive result?

Yes, I think the realistic estimations are around 90%. But that aren't many, because that are only those who go to the doctor and make the examinations. The reason why many don't even take the path of testing, is, that a termination is no opportunity for them. So the abortion is finally the ethical problem why many pregnant women don't take prenatal diagnostic into account.

Since the PrenaTest® uses specific instruments and computers to analyze the blood and since Lifecodexx is located in Konstanz we were unable to a visit a laboratory. The laboratories of the Universitätsspital Basel wouldn't have added anything here. However we found some pictures of the instruments in use on the Internet.

Picture 1: Blood samples are taken and sent to Lifecodexx, where it is analyzed.

Picture 2: The blood is analyzed with the help of an instrument called IlluminaHiSeq2000 in the laboratory.



Picture 3: Explanation of the HiSeqTM Sequencing Systems

5. Discussion

The PrenaTest® and and other non-invasive prenatal diagnostic methods were a big step in science and medicine. Experts were able to find genetic information of individuals who hadn't even been born. Furthermore they could use these information to calculate chromosomal disorders. So it is a huge step towards human's ability to predict characteristics of offspring by easy methods, in this case by just analyzing blood.
But why is that actually necessary to do the PrenaTest® or other non-invasive prenatal tests? It is more expensive, compared to invasive methods (which are payed by health insurance) , not reliable without the proof by a invasive test and furthermore it can not detect mosaic Down Syndrome. What are the advantages of the PrenaTest® then? Lifecodexx arguments as follows: even though an analysis of the placenta is very precise, it bears the risk (0.2 -1%) of loosing your child. The PrenaTest® is completely sure, no risk is present. Ultrasonic examinations don't bear this risk too, but they are less precise than a result given by the PrenaTest®. For PrenaTest®, Lifecodexx states that 99% of all results are correct (for Trisomy 21, 18 and 13). So comparing all the three of them, the PrenaTest® would be the best solution. A further advantage is, that it gives clear yes-or-no-answer, not an assessment of risk. The test may also consult pregnancies of twins and pregnancies based on egg donation. An other benefit named by Lifecodexx is the short waiting time.

People with Trisomy often, like other disabled people, are not fully accepted by the surrounding society and therefore it has been goal of many researchers to find a method of how to prevent the Down Syndrome and there will be even more in the future. There are many approaches, trying to prevent but none of them actually fights the Down Syndrome in a healing way. As the field of identification of genes is already investigated quite a lot, scientist might now step over to find such a treatment. A good example would be XIST, but exact application still needs to be sorted out. An other interesting goal is probably to be able to find any type of Down Syndrome with an accuracy of 100%.

Out of an ethical perspective the whole subject looks a bit different. Who decides whether such tests maybe placed on the market? What is the legitimation for parents to decide whether a child may be born or not? And what are the advantages of having less handicapped people for the society?

An advantage is for sure the opportunity of an autonomous decision. As there is no danger for the disease to become extinct, why shouldn't we open this possibility to everyone if we have it? Maybe the ethical problem isn't the fight against Down Syndrome as a disease, but abortion. Or better to say the abortion of an individual just because it is abnormal, just because it doesn't fit into our system.

An other plus factor which should be mentioned, although it sounds rude in our opinion, is the financial aspect. The education and health care of disabled people costs the state a lot of money each year, invested in persons that will not be able to work as good as “normal” people in our achievement-oriented society. But not only the state may gain money in preventing Down Syndrome – all the companies selling those products make a big effort out of the business.

However, there are also negative sides to the PrenaTest®. First of all, doing the PrenaTest®, the parents get to know the sex of the child, so some of the people might abuse this, as they are only interested in this information by doing the testings. Since the test can be performed very early in pregnancy, parents are free to abort and don't have to legitimate themselves.

In addition to that, the PrenaTest® cannot detect all chromosomal abnormalities, not even all forms of the Down Syndrome (f.e the Mosaic Down Syndrome not). So a negative result, could lead to a bad surprise after the child is born and has an abnormality.

Furthermore, a positive result of the PrenaTest® leads in most cases to abortion - probably because the parents are scared to have a different child. And this leads to a next ethical problem: abortion is not accepted by most of the religions and could lead to banishing from society.

Finally, there is the danger for a mother, not to ever again be able to give birth to a child if she aborts!

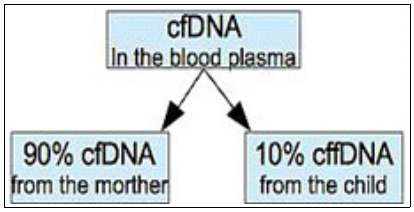
In our opinion, the negative aspects overweigh the positive ones. To us it doesn't make to much sense, to test whether the child has an abnormality or not, since we personally think all children should be accepted as they are and everybody should be given a chance to live.
What's more is that such tests put an enormous pressure on to parents, and they are almost nonautonomous if they receive a positive result. To us it seems like a vicious circle: once you decide in favor of the PrenaTest®, you do it and if you get a positive result back, you are automatically led towards abortion, as humans usually don't take risks on them.
If parents however decide to do the test, this is perfectly alright because it is their own decision.

However, we don't think that the test should be payed by health insurances. If parents want to do the test, they should be able to pay the test. Otherwise they can use traditional treatments. Paying for the test on your own has, in our opinion, the good side effect, that parents think twice about doing the test.

6. Summary

Down Syndrome is a well known disease with development and cognitive delays. Since decades, medical genetics deals with treatments to prevent all forms of Trisomy (most common are Trisomy 21, 18, 13 and the mosaic Down Syndrome). There are different ways to discover Down Syndrome before birth – investigations of pregnancy hormones in the blood, analysis of the placenta and ultrasonic examinations. Since 2012, parents may furthermore decide to do a so called PrenaTest®.

The PrenaTest® is a non-invasive prenatal test (NIPT) released by Lifecodexx in Konstanz, that bought the license for the technology from Sequenom (USA). To perform such a test, the cell free DNA (cfDNA) in the blood plasma of a pregnant woman is isolated. 90% of this cell free DNA originate from the mother, 10% belong to the unborn child (cffDNA). To achieve a precise result, at least 4% (for twins 8%) of the whole sample of cfDNA must be cffDNA. Using the massive parallel sequencing, fragments of the whole cell free DNA, as cfDNA and cffDNA can't be separated, is analyzed. The sequences are matched to the corresponding chromosomes and the amount of sequences for chromosome 21 is compared to a a proved negative result. The difference between a positive and a negative result however is very small (1:1.05) due to the fact that 90% of the chromosomes belong to the mother who doesn't carry the Down Syndrome. It takes Lifecodexx between 8 and 10 workdays to communicate the result to the patient.



It isn't known how many PrenaTest®'s are performed in Switzerland each year since only commercial companies are involved. But as Prof. Dr. Med. Peter Miny from the Universitätsspital Basel states, amniocentesis and other examinations drastically declined in the last years. Therefore is is assumed that the PrenaTest® is frequently performed. However, the amount of children affected by the Down Syndrome has increased. This causes Peter Miny to think that there is no danger/ possibility for the disease to disappear.

The PrenaTest® contains, compared to other examinations, the following advantages:

- The test is non-invasive, meaning it doesn't bring the risk of a miscarriage with it.
- It is more precise than for example an ultrasonic examination, 99% of all results are correct
- The amount of a wrong positive report are about 0.5%. Therefore it is important to make sure that no children are aborted due to a mistake.
- It can be performed in twin pregnancies and pregnancies based on egg donation.
- It is analyzed in a short time.

Still it contains a few disadvantages for the parents:

- The PrenaTest® is in most cases not payed by health insurances.
- The result is not diagnostic.
- They get to know the sex of the child, which they might not want to.

Out of an ethical point of view, the prenatal testings are a controversial issue. On one hand side, our western values state that people should be able to make autonomous decisions. On the other hand side, shouldn't every single person have the right on life?
If Down Syndrome was a stomach flu, no one would discuss whether it should be eradicated. If the treatments acted in a healing way, no one would discuss them either. But since the discovery of Down Syndrome most often leads to an abortion, there are many critical voices on the treatments. Probably the ethical problem isn't whether Down Syndrome should be discovered, but whether people that could lead a happy life should be aborted.

7. References

Cover picture: http://www.bio-pro1.de/magazin/thema/00182/index.html?artikelid=%2Fartikel%2F03055%2Findex.html&lang=de&image=NHzLpZeg7t,lnp6I0NTU042l2Z6ln1acy4Zn4Z2qZpnO2Yuq2Z6gpJCDdnt3fGym162bpYbqjKbNpKCYlq7p (last visit:1/2/2015)

http://polpix.sueddeutsche.com/bild/1.1383262.1355303067/860x860/ultraschallbild-bluttest-downsyndrom-kuerze-markt.jpg (last visit:1/2/2015)

1. Picture: http://www.katholisch.de/de/katholisch/themen/gesellschaft/121227_interview_downsyndrom.php (last visit 1/2/2015)

   Information: http://www.focus.de/wissen/natur/bildung-die-unmoegliche-karriere_aid_198391.html (last visit 1/2/2015)

   http://ohrenkuss.de/ (last visit 1/2/2015)

   http://www.zeit.de/2009/12/M-Down-Syndrom (last visit 1/2/2015)

   Statistics: Handout from P. Ruggle (lesson of the 12/22/2014)

2. Information about the Down Syndrome:

   http://ndsccenter.org/worpsite/wp-content/uploads/2012/03/dsBrochure.pdf (last visit 01/01/2015)

   http://www.nature.com/news/researchers-turn-off-down-s-syndrome-genes-1.13406 (last visit 01/01/2015)

   About PrenaTest:

   http://praenatal-zuerich.ch/wp-content/uploads/2014/06/Praenatest_Arzt_d.pdf (last visit 01/01/2015)

   http://praenatal-zuerich.ch/wp-content/uploads/2014/06/akademos-cme-314-HTL-3984.pdf (last visit 12/31/2014)

   http://www.nzz.ch/wissen/wissenschaft/es-aendert-sich-nicht-viel-1.17445942
   http://lifecodexx.com/fileadmin/lifecodexx/pdf/Patientinnen-Infobroschuere_2014/WM-1102-EN-001_PraenaTest_Informationen_und_Aufklaerung_fuer_Schwangere_Screen.pdf (last visit 01/01/2015)

3. Procedure in the laboratory:

   http://lifecodexx.com/fileadmin/lifecodexx/pdf/Monografie_2013/WM-1103-EN-001_Information_for_professionals.pdf (last visit 01/25/2015)

   http://www.b3cnewswire.com/20130710926/improved-prenatestr-provides-early-knowledge-for-high-risk-pregnancies.html (last visit 01/25/2015)

   http://www.mdpi.com/2077-0383/3/3/679/htm (last visit 01/25/2015)

   http://www.biogen.com.tr/prenatest_uzman_doktor_e.asp (last visit 01/25/2015)

   definitions (footnote 1) from: http://www.whatisepigenetics.com/dna-methylation/ (last visit 01/27/2015)

Figure 1: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2725244/figure/fig1/
(last visit 01/27/2015)

Figure 2: http://lifecodexx.com/fileadmin/lifecodexx/pdf/Monografie_2013/WM-1103-EN-001_Information_for_professionals.pdf (last visit 01/27/2015)

4. Portrait of Prof. Dr. Med. Peter Miny:

https://forschdb2.unibas.ch/inf2/images/profiles/495e6c1dea68e_tmp1.jpg
(last visit01/31/2015)

Picture 1:
http://media0.faz.net/ppmedia/aktuell/3408879159/1.1862498/article_multimedia_overview/der-praena-test-gilt-als-individuelle-gesundheitsleistung-die-kosten-traegt-die-schwangere-selbst.jpg (last visit 2/1/2015)

Picture 2:http://www.bcgsc.ca/platform/037_AS_Lab_n_5438.jpg/image (last visit 2/1/2015)

Picture 3: http://www.illumina.com/Documents/systems/hiseq/datasheet_hiseq_systems.pdf
(last visit 2/1/2015)

5. Information:
https://www1.sequenom.com/press/lifecodexx-launches-praenatest®-in-germany,-austri/
(last visit 01/31/2015)

http://lifecodexx.com/fileadmin/lifecodexx/pdf/Patientinnen-Infobroschuere_2014/WM-1102-DE-002_PraenaTrest_Information_und_Aufklaerung_fuer_Schwangere_online.pdf
(last visit 01/31/2015)

http://www.downsyndromenipt.info/nipt-DE.html (last visit 01/31/2015)