MICROCHIP FOR DETERMINATION OF THE SYNDROMES RELATED TO INCREASED NUCHAL TRANSLUCENCY

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INTRODUCTION

In the first trimester of pregnancy there is a subcutaneous collection of fluid in the fetal neck that is vizualized by ultrasonography as nuchal translucency (NT).
Increased NT refers to a measurement above the 95th centile- 2,7 mm at crown-rump lenght (CRL) of 84 mm (1)
Increased NT between 11 and 14 weeks' gestation is a common phenotypic expression of chromosomal abnormalities. The risk of major chromosomal abnormalities is very high and increases from about 20% for NT of 4.0 mm to 65% for NT of 6.5 mm or more (1).
In the absence of aneuploidy it is associated with adverse perinatal outcome due to foetal malformations and genetic syndromes, including some monogenic disorders. Most of the foetal malformations associated with increased NT can be diagnosed on songraphic investigation performed in 14 to 16 and/or 20 to 22 week of gestation.
In pregnancies where foetal nuchal edema remains unexplained there is a 10% risk of evolution to hydrops and perinatal death or a livebirth of a child with a genetic syndrome (1)
Genetic syndromes such as congenital adrenal hypoplasia, Noonan syndrome, Smith-Lemli-Opitz syndrome and spinal muscular atrophy appear to be substantially higher than in the general population in the cohort of foetuses with increased NT.
Therefore arrayed primer extension (APEX) microarray assay was developed to analyse conditions which are related to increased NT in case aneuploidy is excluded- table 1.

TABLE 1. LIST ON THE CONDITIONS AND GENES INVESTIGATED WITH INCREASED NT CHIP

Disorder	Gene	No of mutations detected on APEX array	Detection rate
21-hydroxylase deficiency	*CYP21A2*	25	~95%
Smith-Lemli-Opitz syndrome	*DHCR7*	140	~98%
Noonan syndrome	*PTPN11*	57	~70%
	SOS1	24	
	KRAS	10	
	RAF1	14	
	MEK1	1	
Del22q11.2 syndrome	SNPs in the deleted region		Additional investigations should follow

OBJECTIVE

To evaluate the detection rate of the prenatal diagnostic test in case of increased NT in the foetuses with normal karyotype.

MATERIAL and METHODS

We investigated 220 DNA samples isolated from the foetal chorionic cells or amniocytes.

Methods:

- APEX array for simultaneous detection of 267 variations in 15 different genes.
- Restriction for detection of 30 kb deletion in the *CYP21A2* gene.

REFERENCES

1. Souka, A. P. et al "Increased nuchal translucency with normal karyotype" – Am J Obstet Gynecol 2005: 192:1005-21
2. Pergament, E. et al "Genetic assessment following increased nuchal translucency and normal karyotype" – Prenat Diagn 2011: 31:307-310

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RESULTS

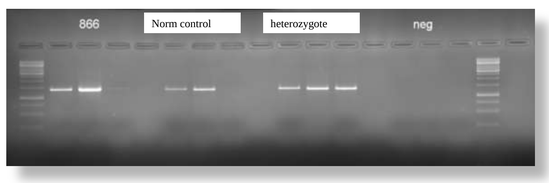
Genetic changes were detected in 38 DNA.
Test was diagnostic in 20 fetuses from our cohort (table 2).
-Noonan syndrome was diagnosed in 17 cases
-21-hydroxylase deficiency was diagnosed in 3 cases
-Smith-Lemli-Opitz syndrome was diagnosed in 1 fetus.
In one foetus homozygosity of the signals in locus 22q11.2 was observed and deletion in this region was suspected.
In 17 cases heterozygosity for autosomal recessive disease was found (table 3).
-Heterozygous mutations in *CYP21A2* gene related to 21-hydroxylase deficiency were found in 13 cases.
-Heterozygous mutations in *DHCR7* gene related to Smith-Lemli-Opitz syndrome were found in 4 cases.

TABLE 2. OVERVIEW OF THE GENOTYPES OF THE AFFECTED FETUSES DETERMINED BY NT CHIP

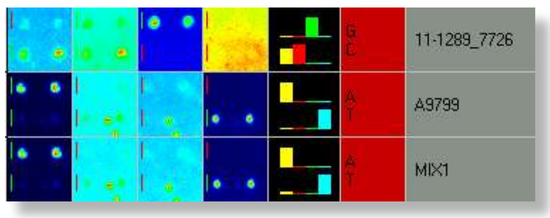
Syndrome	Gene	Genotype	No of cases
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	*CYP21A2*	Homozygous IVS-13A/C>G	3
		Heterozygous p.Y63C	1
		Heterozygous p.A72G	1
Noonan syndrome	*PTPN11*	Heterozygous p.T73I	1
		Heterozygous p.D106A	1
		Heterozygous p.F285S	2
		Heterozygous p.N308D	1
		Heterozygous p.I309V	3
		Heterozygous p.S502L	1
		Heterozygous p.T553M	1
	SOS1	Heterozygous p.P655L	3
	RAF-1	Heterozygous p.S259F	1
Smith-Lemli-Opitz syndrome	*DHCR7*	Homozygous p.W151X	1
TOTAL			**20**

TABLE 3. OVERVIEW OF THE HETEROZYGOUS MUTATIONS IN *CYP21A2* AND *DHCR7* DETERMINED BY NT CHIP

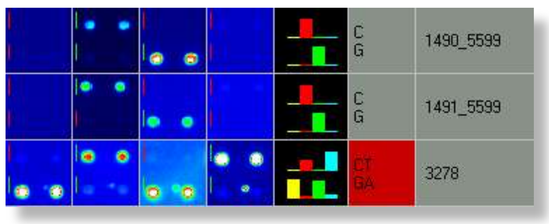
Syndrome	Gene	Mutation	No of cases
Congenital adrenal hyperplasia (21-hydroxylase deficiency)	*CYP21A2*	p.V281L	9
		p.P453S	3
		IVS6+63	1
Smith-Lemli-Opitz syndrome	*DHCR7*	p.V326L	2
		p.R467H	1
		p.469H	1
TOTAL			**17**



Picture 1. Large (30 kB) deletion in CYP21A2 gene associated with CAH. The two first band correspond to healthy genotype, third band in the gel indicates the heterozygous genotype



Picture 2. Homozygotic mutation in the DHCR7 gene associated with Smith-Lemli-Opitz syndrome 452G>A (W151X). The A signal in sense strand and T signal in antisense strand correspond to wild-type genotype, G signal in sense and C signal in antisense strand point to the mutation



Picture 3. Heterosygotic mutation in the PTPN11 gene associated with Noonan syndrome 218C>T (T73I). The C signal in sense strand and G signal in antisense strand correspond to wild-type genotype, T signal in sense and A signal in antisense strand point to the mutation

CONCLUSIONS

According to our preliminary data the detection rate of our diagnostic test is 9,1% in the cohort of the foetuses with NT>3mm and normal karyotype.
In combination with fetal karyotyping and sonography increased NT testing panel has a potential for reducing the risk of the birth of a child with genetic syndrome.
The high incidence of heterozygous foetuses detected needs to be explained in the future.