Arrays in daily practice: promises and pitfalls
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Arrays in prenatal diagnosis

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Considerations based on 181 cases (156 ongoing pregnancies and 25 abortions) + cases from the literature for a total of about 700 cases

181 cases (from 2009 to 2011; array platform: in most of the cases 180k, Agilent):

• 72 fetal malformations
• 95 chromosomal abnormalities with or without fetal malformations
• 14 previous child with a pathogenic CNV or maternal anxiety

RESULTS at array-CGH

49 imbalances:

36 in fetuses with chromosome abnormalities
8 in fetuses with ecographic malformations
5 in fetuses with previous affected child

All but 2 were ongoing pregnancies at first examination; Autopsy done in most of the abortions
GENERAL CONSIDERATIONS I:

Among 50 de novo reciprocal translocations, only 1 with a causative deletion (fetus with ecographic malformations)

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Among 6 complex apparently balanced rearrangements, 4 had one or more pathogenic deletions

In Warburton 1991: 6.1% of de novo translocations detected prenatally has an abnormal phenotype at postnatal follow up

In de Gregori et al 2007: 40% of de novo translocations detected in abnormal subjects has a cryptic deletion
GENERAL CONSIDERATIONS II:

Among 12 sSMCs, a duplication was visible in 9
37-year-old primigravida, referred at 15+5 weeks of gestation for septated cystic hygroma, fetal hydrops, anhydramnios

**Normal karyotype**

aCGH 180k:
3 Mb DiGeorge deletion

[Diagram showing aCGH results with 22q11.21 deletion highlighted]
Severe anhydramnios at 32.1 weeks of amenorrhea

atrioventricular canal at 15 weeks of gestation, normal male karyotype, FISH with probes 22q11.23: normal signals

2.7Mb deletion
GATA4: a transcriptional activator: haploinsufficiency: cardiac abnormalities and diaphragmatic hernia; duplication of the entire region: very mild phenotype apparently not related to GATA4.
33-year-old primigravida, referred at 21 weeks of gestation for a congenital heart defect (ultrasound)

at 12 weeks of gestation, nuch. translucency: 1.7 mm (50th cent);
at 20 weeks, significant right ventricle prominence with fetal anatomy and measurements normal for gestational age;
at 21 weeks, dominance of the right ventricle and mild reduction of aortic diameter

**normal karyotype**

**FISH analysis with a 22q11 probe and a 22q13 control probe**
Autopsy:
Retrognathia
Varus feet
Right diaphragmatic hernia
Pulmonary lobation defect (bilobated right lung)
Cardiovascular heart defects with augmented right ventricular wall thickness
In fetal heart abnormalities, it doesn't make any sense to request FISH for the 22q11 region and the array must be performed because several other imbalances can mimic the heart defect associated with the diGeorge/VCF syndrome and these imbalances can be associated with a phenotype involving not only heart defects.
Ecography (12 weeks of gestation): nuchal fold: 7.1 mm
Later ecographies: partially empty stomach, atrio-ventricular septal defect, DIV, single umbelical artery. Karyotype: 46,XY,t(6;11)(q13;p15)de novo

del16q24.1
1,295 Mb
84039234
84065951
85361587
85388535

Not present in DGV

In Decipher: at least two overlapping losses in patients with heart defects
Ecographic findings (16w): cerebellar vermis agenesis plate face with micrognatia, hypertelorism

Autopsy: complete agenesis of cerebellar structures
4q23 de novo deletion of 1.48 Mb

Not reported in the Database of Genomic Variants

Not similar cases reported in Decipher (at that time)

No known disease genes in the region

**Dilemma?**

Is this deletion responsible for the fetal abnormalities??

Tetraspanin-5 (Tm4sf9) mRNA expression parallels neuronal maturation in the cerebellum of normal and L7En-2 transgenic mice.


Pattern of expression of the tetraspanin Tspan-5 during brain development in the mouse.

INDICATION TO INVASIVE PRENATAL DIAGNOSIS:

MATERNAL AGE + PREVIOUS BROTHER 46,XX WITH AMBIGUOUS EXTERNAL GENITALIA, SRY-

Fetus: normal ecography at 12 weeks of gestation; karyotype: 46,XX
SOX9: 67,628 Mb

66.306 N
66.322 D
67.111 D
67.121 N
Duplicated father and grandfather: completely normal

:duplic in the desert region upstream to SOX9

46,XY

46,XX: male/AmbGen; SRY-
Minimal duplication region brachydactyly-anonychia (four families in the literature)
46,XY

46,XY

46,XX: male/AmbGen; SRY-

TOP because of testes detected at 21 weeks of gestation

:duplic in the desert region upstream to SOX9
Minimal duplication region in two XX males brothers, SRY negative

Minimal duplication region brachydactyly-anonychia (four families in the literature)
de novo supernumerary marker chromosome in 70% of metaphases
Array-CGH, 180k. hg18

chr1:83061240-84663025
1.6 Mb  Log2ratio 1.08 (trip)

last norm  82999282
first trip  83061240
last trip   84663025
first norm  84713307
FISH with probe(s) of the unbalanced region demonstrated that the triplicated material was located on a small marker chromosome.
Is the genetic material contained within the de novo marker chromosome associated with any “at risk” phenotype??
From OMIM: urate oxidase is presumably a nonessential enzyme in humans. Although lack of this enzyme may contribute to the development of hyperuricemia and gout in adult life, most humans do not develop the disease except in conjunction with other factors. No information for increased dosage of the gene from the database of genomic variants.
DECIPHER: two cases with larger deletions; no cases with duplication

**Patient 248394**

**Phenotype:**
- Asymmetric face
- Auricular tags
- Club foot, valgus
- Cryptorchid testes
- Fetal finger pads
- Gynaecomastia
- High palate
- Hypotonia (non-myopathic)
- Inguinal hernia
- Joint laxity
- Mental retardation/developmental delay
- Mouth, general abnormalities
- Palpebral fissures slant down
- Pectus excavatum
- Scoliosis
- Small ears/microtia
- Toes, general abnormalities

**Patient View:** More details on patient 248394 at DECIPHER.

**Patient 250905**

**Phenotype:**
- Autism/autistic behaviour
- External ears, general abnormalities
- Lower limbs, general abnormalities
- Macrostomia
- Mental retardation/developmental delay
- Pointed chin
- Triangular face
- Wide forehead

**Patient View:** More details on patient 250905 at DECIPHER.
FUNCTION: Polyglutamylase which preferentially modifies beta-tubulin. Involved in the side-chain initiation step of the polyglutamylation reaction rather than in the elongation step. Required for neurite growth (By similarity).

SUBCELLULAR LOCATION: Cell projection, cilium (By similarity). Cell projection, cilium basal body (By similarity). Cell projection, dendrite (By similarity). Perikaryon (By similarity). Note=In cells with primary cilia, found in both cilia and basal bodies. In neuronal cells, found in dendrites and perikaryon (By similarity).

TISSUE SPECIFICITY: Highly expressed in the nervous system including spinal cord, thalamus, hippocampus, hypothalamus and cerebellum.

SIMILARITY: Belongs to the tubulin--tyrosine ligase family.

FUNCTION: Mediates cAMP-dependent signaling triggered by receptor binding to GPCRs. PKA activation regulates diverse cellular processes such as cell proliferation, the cell cycle, differentiation and regulation of microtubule dynamics, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, as well as regulation of intracellular transport mechanisms and ion flux.

CATALYTIC ACTIVITY: ATP + a protein = ADP + a phosphoprotein.

COFACTOR: Magnesium.

ENZYME REGULATION: Activated by cAMP.

SUBUNIT: A number of inactive tetrameric holoenzymes are produced by the combination of homo- or heterodimers of the different regulatory subunits associated with two catalytic subunits. cAMP causes the dissociation of the inactive holoenzyme into a dimer of regulatory subunits bound to four cAMP and two free monomeric catalytic subunits.

SUBCELLULAR LOCATION: Cytoplasm (By similarity). Nucleus (By similarity). Note=Translocates into the nucleus (monomeric catalytic subunit) (By similarity). The inactive holoenzyme is found in the cytoplasm (By similarity).

TISSUE SPECIFICITY: Isoform 1 is most abundant in the brain, with low level expression in kidney. Isoform 2 is predominantly expressed in thymus, spleen and kidney. Isoforms 3 and 4 are only expressed in the brain.

Description: sterile alpha motif domain containing 13 isoform

Description: deoxyribonuclease II beta isoform 1 precursor

RefSeq Summary (NM_021233): The protein encoded by this gene shares considerable sequence similarity to, and is structurally related to DNase II. The latter is a well characterized endonuclease that catalyzes DNA hydrolysis in the absence of divalent cations at acidic pH. Unlike DNase II which is ubiquitously expressed, expression of this gene product is restricted to the salivary gland and lungs. The gene has been localized to chromosome 1p22.3 adjacent (and in opposite orientation) to the uricase pseudogene. Two transcript variants encoding different isoforms have been described for this gene. [provided by RefSeq].
When 18-year-old, mild mental retardation + Carney Complex*

lentiginosis and mixomas
+ mild mental retardation

*: tumors susceptibility, syndrome, autosomal dominant, in 60% of the cases loss-of-function mutations of **PRKAR1A**

*The proband is normal for both PRKAR1A and STK11*
PRKAR1A: loss of function mutations of regulatory subunit of protein kinase A
Triplication of the PRKACB cAMP-dependent protein kinase catalytic subunit

Same effect of PRKAR1A mutation?
Altogether, in our experience and taking into account the parental array we were unable to say if the CNV was causative in two case out of 181.

Taking into account the data from the literature and all CNVs databases, we detected causative imbalances associated with a normal karyotype:

- in 8 fetuses with ecographic malformations
- in 5 fetuses with previous affected child

Is it acceptable?
Do molecular karyotyping results need a confirmation??

Quality assessment of the experiment must be carefully evaluated and only experiments which pass quality control steps should be considered.

From our data in postnatal (2800) and prenatal cases (158) we could confirm (by microsatellites analysis, FISH, qPCR) the imbalances (828) in all but two cases.

Both were small duplications considered positive with three consecutive spots and a $\log_2$ ratio of +0.5.
Further considerations

How to handle CNVs of unknown clinical significance (less than 1% with the 60k targeted platforms)

It depends from the orientation of the couple: in pre-testing counseling this point has to be clearly explained

Do we need to use a filter?

According to Agilent Software, one can set a filter excluding calls made by less than three consecutive spots

What to report

It depends from the orientation of the couple: do they want to know any deletion/duplication known to be associated with late-onset diseases??

Should parents always be analyzed at the same time?

Parents blood has to be collected at the same time of fetal sampling and analyzed later if necessary
What we have acquired:

molecular karyotype necessary in case of major ultrasound anomalies or multiple soft markers in presence of a normal conventional karyotype

- nuchal translucency >3mm before 15gw or nuchal fold ≥ 6mm between 14 and 20 gw with normal karyotype

- association of two or more minor echographic markers of aneuploidy
  - choroid plexus cysts, intestinal hyperechogenicity, renal pielectasy, single umbilical artery, hyperecogenic cardiac foci;

- amniotic fluid volume alteration and/or IUGR associated with major structural abnormalities (congenital cardiac abnormalities, diaphragmatic hernia, CNS abnormalities)
What we have acquired:

Molecular karyotype necessary in presence of

abnormal cytogenetic findings

of dubious significance

including

marker chromosomes and de novo reciprocal translocations

(40% of those identified in subjects with abnormal phenotype have cryptic deletions at bps or elsewhere)
The better platform is a genome-wide one possibly enriched in oligomers containing dosage-sensitive genes and with a backbone not inferior to 500 kb (3 consecutive spots x 500= 1500 kb).

Targeted with 500 kb backbone and a total of 60k

With a 60k platform from 3 (in the backbone) to 5 consecutive spots with the same log₂ ratio should identify a deletion; 5 to 7 a duplication

For reciprocal translocations and marker chromosome we’ll continue to use the 180k
Conventional cytogenetics and FISH are necessary in parents of unbalanced fetuses to assess the risk of recurrence.

CNV (both losses and gains) may be inherited from a parent carrying a balanced or an insertional translocation increasing the recurrence risk.
Unbalanced translocation der(7q)t(3p;7q) lost by conventional cytogenetics negative prognosis for the fetus (SHH included in the deletion)

to test the risk of recurrence: subtelomeric FISH in parental chromosomes; fetal chromosomes are not necessary
FISH with subtelomeric probes of 3p and 3q shows that the mother is a balanced carrier of the t(3;7)
Who pays??
Amniocentesis because of **advanced maternal age**

15q+

Painting FISH: all chromosome 15
SNRPN (PWS/AS region): normal

15q+ is inherited from the normal mother
the 15q11 region is variable in copy numbers containing a cassette of pseudogenes;

one disease gene, associated with hematological disorders in translocation;

the 15q11 amplification does seem to correlate with any consistent phenotype.

18657447   First abnormal
20079994   Last abnormal

amplified at least 7 times (1.4 Mb x 7 = 9.8 Mb)
What about BoBs?!

The idea behind is to detect CNVs related to:
- 100% penetrant diseases
- not showing echographic signs

- First release: 9 target loci plus chromosomes 13, 18, 21, X and Y

### Constitutional BoBs target regions:

<table>
<thead>
<tr>
<th>Chromosomes</th>
<th>13, 18, 21, X and Y</th>
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<tbody>
<tr>
<td>4p16.3</td>
<td></td>
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<tr>
<td>5p15.3-p15.2</td>
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<tr>
<td>7q11.2</td>
<td></td>
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<tr>
<td>8q23-q24</td>
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</tr>
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<td>10p14</td>
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<td>15q11-q12</td>
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<td>17p13.3</td>
<td></td>
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<tr>
<td>17p11.2</td>
<td></td>
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<tr>
<td>22q11.2</td>
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</tbody>
</table>
The frequency of the target disorders is lower than that of many other deletions/duplications/mutations not associated with ecographic anomalies.

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prader-Willi Syndrome</td>
<td>1:25,000</td>
</tr>
<tr>
<td>Angelman Syndrome</td>
<td>1:12,000</td>
</tr>
<tr>
<td>DiGeorge Syndrome</td>
<td>1:5,000</td>
</tr>
<tr>
<td>Williams Syndrome</td>
<td>1:20,000</td>
</tr>
<tr>
<td>Dravet Syndrome</td>
<td>1:10,000</td>
</tr>
<tr>
<td>Rett Syndrome</td>
<td>1:9,000 females</td>
</tr>
<tr>
<td>MEF2C dels/mutations</td>
<td>1:3,300</td>
</tr>
<tr>
<td>FXS</td>
<td>1:2,500 males</td>
</tr>
</tbody>
</table>
Non-invasive prenatal assessment of trisomy 21 by multiplexed maternal plasma DNA sequencing: large scale validity study

WHAT IS ALREADY KNOWN ON THIS TOPIC

Non-invasive prenatal detection of fetal trisomy 21 is achievable by massively parallel sequencing of maternal plasma DNA.

Its diagnostic performance and practical feasibility in the clinical setting has not been tested on a large scale.

WHAT THIS STUDY ADDS

Among high risk pregnancies clinically indicated for invasive prenatal diagnosis, non-invasive detection of fetal trisomy 21 can be achieved with the use of multiplexed massively parallel sequencing of maternal plasma DNA at 100% sensitivity and 97.9% specificity, giving a 96.6% positive predictive value and 100% negative predictive value.

The sequencing test could be used to rule out trisomy 21 among high risk pregnancies before proceeding to invasive diagnostic testing to reduce the number of cases requiring amniocentesis or chorionic villus sampling.

Dennis Lo BMJ 2011