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Genetic forum

# Karyotype is not dead (yet)!

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ABSTRACT

*Background:* While array-comparative genomic hybridization (a-CGH) and next-generation sequencing (NGS or exome) technologies have swiftly spread throughout the medical field, karyotype has gradually lost its leading role among genetic tests. Several international guidelines recommend starting with a-CGH screening then going on with exome analysis when investigating a patient with intellectual disability (ID) and no precise clinical diagnosis. A-CGH and whole exome sequencing increase etiologic diagnoses rate up to 30% in case of ID. However, physicians have to deal with the lack of qualitative information of the genome. Especially, exome and a-CGH analysis fail to detect chromosomal rearrangements because breakpoints are either located in introns or not associated with a gain or loss of genetic material. If these technologies cannot easily identify chromosomal translocations or inversions which sometimes split a gene, karyotype can.
*Discussion:* For the 5 cases described, karyotype provided the right diagnosis for a Mendelian disease while molecular analysis remained unsuccessful. We conclude that when a Mendelian disease is strongly suggested clinically, if molecular analysis is normal, it could be very useful to carry out a karyotype in order to demonstrate a chromosomal rearrangement involving the targeted gene. If this gene is disrupted, the physician can confirm the suspected disease and give appropriate genetic counseling.
*Summary:* This article aims at keeping in mind that karyotype, this old-fashioned genetic tool, can still remain powerful and useful within some genetic issues. Even in this modern period of whole exome sequencing, young geneticists should know that karyotype remains a powerful and cheap technology, available throughout the world and can still do a lot for families.


## 1. Background

While array-comparative genomic hybridization (a-CGH) and next-generation sequencing (NGS or exome) technologies are swiftly spreading throughout the medical field, karyotype has gradually lost its leading role among genetic tests. In many rare disease areas, this technological evolution has deeply impacted both clinical strategy and laboratory practices. In case of a child with intellectual disability (ID) or autism spectrum disorder, without any specific sign leading to a precise clinical diagnosis, several international guidelines recommend starting with a-CGH

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screening (South et al., 2013) (Verloes et al., 2012). Generalized use of a-CGH increases etiologic diagnoses rate up to 15% in case of ID. Hence, if a-CGH is normal, NGS or exome may be carried out in order to demonstrate the genetic causing-disease variant in another 15% cases (de Ligt et al., 2012). These technologies are definitely major steps in the etiological process of ID. However, several disadvantages should be discussed: First, these technologies are often available in Western countries and some wealthy countries; majority of humans do not have access to these expensive modern approaches. Second, physicians have to deal with some difficulties as these pan-genomic analyses may show one variant of unknown signification (VOUS) or incidental findings. These unexpected variants make genetic counseling harder than previous, and have psychological impacts for families. Another point hardly mentioned in literature is the lack of qualitative information of the genome. Especially, NGS and a-CGH fail to detect chromosomal rearrangements not associated with a gain or loss of genetic material. If these quantitative technologies cannot identify chromosomal translocations or inversions which sometimes split a gene, karyotype can. 30 years ago, when karyotype showed a chromosomal translocation, research could at once focus on specific loci and genes involved in a significant pathway. This was one of the former strategies in order to identify a gene causing disease, and sometimes still currently reported (Moyses-Oliveira et al., 2015).

In this article, we show that karyotype can still help the physician towards diagnosis in some circumstances. For the 5 cases described here, karyotype provided the right diagnosis for a Mendelian disease while gene molecular analysis remained unsuccessful.

## 2. Patients and methods

### 2.1. Patients

Clinical data and biological samples from patients were obtained after all patients or their parents gave informed consent for genetic testing to determine the cause of their disease.

**Patient 1**: A 25-year-old male showed Duchenne muscular dystrophy when seen for the first time at the genetics clinic for familial genetic counseling. Although the diagnosis was doubtful on clinical history and muscular biopsy, an extensive molecular screening of *DMD* gene (dystrophin) failed to identify any DNA variant. A fibroblast culture on skin biopsy was then carried out to induce myoblast and keep on genetic research. By chance, a systematic karyotype showed a pericentric X chromosome inversion, inherited from his mother. His healthy brother has normal karyotype.

**Patient 2**: When first seen at genetics clinic, this 30-year-old female showed typical hypohidrotic ectodermal dysplasia (HED - OMIM # 305100) signs from birth. She wears a wig and complete dentures. She wanted to know the underlying molecular cause in order to get clear genetic counseling for her family (which was unremarkable). X-inactivation was completely biased and lead us to an X-linked form. However female carriers of the X-linked form of HED are usually asymptomatic or have a milder phenotype. As *ECTD1* gene (involved in the HED X-linked form) screening was normal, we suggested performing a karyotype from a blood sample showing X-autosomal translocation.

**Patient 3**: Patient 3 is the second child of non-consanguineous healthy Caucasian parents. Pregnancy was remarkable as high chromosomal risk on maternal blood screening lead to fetal karyotype. It showed a balanced translocation with chromosomal breakpoints t (2; 10) (p23.1; q22.1) de novo. In the absence of ultrasound detection the pregnancy was carried to term resulting in a male newborn with macrosomia (weight: 4.340 kg) and macrocephaly (HC: 39 cm). He grew up with a language delay. At the age of 2.5 years he showed 3 febrile seizures, a head circumference of 57 cm (+3.5DS) with a prominent forehead. Despite absence of cutaneous, thyroid or gastrointestinal symptoms, a Cowden disease (OMIM # 158350) linked to *PTEN* on 10q22 is suspected. An a-CGH screening showed only one 176 kb deletion on 4q22.1, inherited from his father therefore considered as a benign variant.

**Patient 4**: The first pregnancy of a 34 years old woman with no family or personal medical problems is marked by nuchal translucency at 4 mm for 54.5 mm crown-lump length. An amniocentesis, performed at 13 SG, shows a de novo t(3; 5)(p14.3; q11). An a-CGH screening is normal. FISH refinement of breakpoints ended in a 1.3 Mb interval in 3p14.3 and a 2.6 Mb interval in 5q11.2. The only well known syndrome-associated gene in both intervals is *WNT5A*, causing autosomal dominant Robinow syndrome (OMIM #180700). Since further ultrasounds were all normal, including male genitalia, pregnancy was carried to term. At birth the baby showed dysmorphic features compatible with Robinow syndrome, two gingival cysts which fell a few days later, normal genitalia. On four limbs were found hypoplastic distal phalanx with hypoplastic nails. On both feet a small preaxial polydactyly (nubbin) was found. X rays showed no costo-vertebral abnormality but several absent distal phalanxes. At 6 months of age, he is developing well.

**Patient 5**: Patient 5 is the first child of non-consanguineous parents. The mother has supravalvular aortic stenosis, which was discovered during the pregnancy. At birth the baby needed an echocardiography showing a valvular aortic and pulmonary stenosis for which cardiac surgery was performed. She showed neither dysmorphic features nor developmental delay. An a-CGH was carried out and was normal, ruling out Williams-Beuren syndrome. At the same time, one maternal cousin was seen because of valvular and peripheral artery pulmonary stenosis without any dysmorphic features or developmental delay. Her mother (the twin sister of patient 5's mother) had also a heart murmur but to date no echocardiography was performed. In this family, the cytogenetic investigations show a balanced reciprocal translocation involving the 7q11.23 locus.

## 3. Methods

### 3.1. Karyotypes

Conventional R-banded karyotypes were performed according to standard protocols with a resolution of more than 450-band level. Prenatal karyotypes were obtained from *in situ* cultures of the amniotic fluid. Post-natal karyotypes were obtained from peripheral blood lymphocytes culture. Parental studies were performed when available.

## 4. A-CGH

Oligonucleotide a-CGH was performed using the Agilent Human Genome CGH microarray 180K (Agilent Technologies, Santa Clara, CA, USA) according to the protocol provided by the manufacturer. Identification of probes with a significant gain or loss was based on the log2 ratio plot deviation from 0 with cut-off values of 0.5–1 and -0.5 to 1 respectively.

### 4.1. Determination of the breakpoints

FISH was carried out using commercial clones or BAC clones (RPMI11) mapping the breakpoints of the chromosomal inversion or translocations. Clones were selected on the public databases UCSC Genome Browser. The selected clones were obtained from the