Gene dosage effects in 46, XY DSD: usefulness of CGH technologies for diagnosis

S. Jaillard · A. Bashamboo · L. Pasquier ·
M. A. Belaud-Rotureau · K. McElreavey ·
S. Odent · Célia Ravel

Received: 18 July 2014 / Accepted: 30 October 2014 / Published online: 12 November 2014
© Springer Science+Business Media New York 2014

Introduction

In 46, XY individuals, the testicular development is initiated by the expression of *SRY*, which sets into motion a chain of signal transduction events leading to development of the male external and internal genitalia. In the absence of *SRY*, ovarian development occurs, associated with a female phenotype [1]. Disorders of Sex Development (DSD) are a range of congenital conditions of atypical gonadal development leading to discordance between chromosomal, gonadal and phenotypic sex. According to the Chicago consensus [2], DSD are divided in three categories based on karyotype: 46, XX DSD, 46, XY DSD and sex

Capsule We report the usefulness of a combined approach (karyotype, FISH and array-CGH) to deliver an accurate molecular diagnosis in patients presenting with 46, XY DSD in a clinical setting and describe the results in two women 46, XY DSD with complete or partial gonadal dysgenesis.

S. Jaillard · M. A. Belaud-Rotureau
CHU Rennes,
Service de Cytogénétique et Biologie Cellulaire, F-35033 Rennes,
France

S. Jaillard · C. Ravel

Univ Rennes1, Faculté de médecine, Histologie et Embryologie,
F-35043 Rennes, France

A. Bashamboo · K. McElreavey
Institut Pasteur, Unité Génétique du Développement Humain,
Paris, France

L. Pasquier · S. Odent
CHU Rennes, Service de Génétique Clinique, F-35033 Rennes,
France

C. Ravel (✉)
CHU Rennes, Service de Biologie de la Reproduction-CECOS,
F-35033 Rennes, France
e-mail: celia.ravel@chu-rennes.fr

chromosome DSD. Although there have been considerable advances in our understanding of the genetic factors and mechanisms involved in gonadal differentiation in the last 10 years [3], a molecular diagnosis is made in only around 20 % of cases of DSD, except in cases where the biochemical profile indicates a specific steroidogenic block [2]. In 46, XY DSD with disorders of gonadal development, individuals with complete gonadal dysgenesis are phenotypically females with streak gonads and are often diagnosed because of primary amenorrhea whereas, in individuals with partial gonadal dysgenesis, the phenotype is more ambiguous. Approximately 15 % of all cases of gonadal dysgenesis carry inactivating mutations in *SRY*, with the majority localized within the HMG-domain [4]. Rare cases of gonadal dysgenesis with small interstitial deletions outside the *SRY*-open reading frame have been described [5]. When *SRY* function is normal, other genes are known to be involved in the occurrence of 46, XY DSD gonadal dysgenesis, with a loss-of-function (for example *SOX9*, *WT1*, *NR5A1/SF1*, *DMRT1*, *MAP3K1*) or with a gain-of-function (for example *WNT4* and *NR0B1/DAX1*) [1, 6]. Nevertheless, the underlying genetic basis of complete gonadal dysgenesis is unknown in about 50 % of the patients [7]. As many of the genes involved in gonadal development have a dosage effect, DSD may be caused by copy-number variations (CNVs) corresponding to deletions or duplications of these genes [8]. Conventional karyotype may not be adequate to detect these changes but array-CGH has been emerging as a powerful tool to identify submicroscopic CNVs on a genome wide scale.

Material and methods

Clinical evaluation

Patient 1 A phenotypically normal 18-year-old female presented with primary amenorrhoea, without any other medical

history. There was no significant familial history. She had normal external genitalia and presented delayed puberty and especially a lack of breast development. Ultrasonogram of abdomen and pelvis showed uterine hypoplasia (36 mm) with no visualization of the ovaries. Endocrine profile showed hypergonadotropic hypogonadism and there was a delayed bone age with great height (1.81 m).

Patient 2 A phenotypically normal 17-year-old female presented with primary amenorrhea, associated with a Basedow syndrome. There was no significant familial history. Hypertrophy of the clitoris was noted. Ultrasonogram of abdomen and pelvis showed uterine hypoplasia. A bilateral gonadectomy was performed and showed a dysgenetic testis on one side and a streak gonad on the other, with some internal Müllerian derivatives. She then had hormonal therapy and consulted at 27-year-old to benefit from oocyte donation. At this time, external genitalia were normal and the womb was compatible with a pregnancy.

Both patients gave an informed consent for a genetic analysis of their infertility according to French law. Ethical approval was given by a local ethic committee.

Cytogenetic analyses

Karyotype

Conventional R-banded karyotypes were performed on spread metaphases prepared from PHA-stimulated cultured peripheral blood cells according to standard protocols.

Array-CGH

Oligonucleotide array-CGH was performed using the Agilent Human Genome CGH microarray 180 K ISCA (patient 1) and 105 K (patient 2) (Agilent Technologies, Santa Clara, CA, USA) according to the protocol provided by the manufacturer. The 180 K ISCA design allows the study of the pseudoautosomal regions 1 and 2 (PAR1 and PAR2) and is enriched in genes of interest like *SRY*. 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 to 1 and −0.5 to −1 respectively.

Fluorescence in situ hybridization (FISH)

FISH was performed using the following probe:

- For patient 1: *SRY* at Yp11.31 (Vysis® Abbott Molecular Inc., Downers Grove, IL, USA)
- For patient 1 and 2: BAC clone mapping the chromosomal abnormality detected by array-CGH (RP11-155 F12 at Xp22.33/Yp11.32p11.31 and RP11-122 N14 at Xp21.2). Clone was selected on public databases UCSC Genome Browser (<http://genome.ucsc.edu>) and was obtained from

the BACPAC Resource Center (Children's Hospital Oakland Research Institute, Oakland, CA, USA, <http://bacpac.chori.org>). Bacterial culture was performed in LB medium supplemented with chloramphenicol (12.5 µg/mL) and DNA from the clone was isolated using a Nucleobond® PC 20 kit (Macherey Nagel, Düren, Germany). BAC DNA was labelled with Cyanine3TM-dCTP (Amersham Biosciences, Buckinghamshire, UK), by random priming using the Bioprime® Array CGH Genomic Labeling System (InvitrogenTM, Carlsbad, CA, USA).

FISH was performed according to standard procedures. Metaphase slides were analysed with an epifluorescent microscope and FISH signals were examined both on metaphase chromosomes and interphase nuclei. Control probes were used for chromosomal identification.

Results

For both patients, karyotype was 46, XY. Patient 1 and 2 were then classified 46, XY DSD, with complete gonadal dysgenesis for patient 1 and partial gonadal dysgenesis for patient 2.

For patient 1, FISH analysis using the *SRY* probe showed a normal hybridization pattern with a signal at Yp11.31 (Fig. 1). Array-CGH analysis revealed a 827 kb deletion at Yp11.32-Yp11.31 (deletion start: 1,840,929 and deletion end: 2,667,856, hg19) involving the part of PAR1 and *SRY* (Fig. 2). The deletion was confirmed by FISH

Fig. 1 Chromosome Y profile on FISH for patient 1 Red : *SRY* probe, Aqua : *DYZ1* probe, Green : *DXZ1* probe A normal hybridization pattern is observed for these probes

using the RP11-155 F12 probe and by PCR targeting *SRY*. The discrepancy was explained by the design of the probe which targets *SRY* and the centromeric neighboring region whereas the deletion involves *SRY* and the telomeric neighboring region.

Discussion

be due to point mutations in gonadal genes, particularly in *SRY*. Like many developmental processes, human gonadal development is sensitive to gene dosage effects that are sometimes difficult to determine, owing to functions of such genes earlier in gonad development or functional redundancy. Thus, 46, XY DSD gonadal dysgenesis may also be caused by CNVs involving sex determining genes. FISH can be used to detect such chromosomal imbalances. However, the use of FISH for clinical diagnosis is driven by an a priori assumption of the involvement of a specific locus, based on the phenotype. Additionally, there are technical limitations that may restrict the number of probes that could be used simultaneously and FISH analysis may fail to identify small duplications even on interphase nuclei. As the number of genes of interest for gonadal dysgenesis is growing, it becomes easier to use a genome-wide approach such as array-CGH to detect rearrangements affecting known gonadal genes or their regulatory sequences [7, 9, 10]. In recent studies, these CNVs mainly concerned *NR0B1/DAX1*, *SOX9*, *GATA4* and *DMRT1* [7, 9, 11, 12]. In our study, CNVs involving such known gonadal genes were detected. In patient 1, array-CGH showed a deletion of *SRY* and the telomeric neighboring region in PAR1, which was not detected by FISH due to the design of the FISH

Fig. 3 Array-CGH profile using Agilent 105 K microarray for patient 2. The 3.78 Mb interstitial duplication at Xp21.2-Xp21.1 involves ten genes including *NR0B1/DAX1* and *DMD*

probe. In patient 2, a duplication of *NR0B1/DAX1* was detected. As in the study of Barbaro et al. [11], this duplication is associated with partial gonadal dysgenesis. Nevertheless, a difference in the severity of the phenotype seems to exist and it can be explained by the involvement or not of the 5' regulatory region, the inter-individual variations of the factors interacting with *NR0B1/DAX1* or by a positional effect. Transmission by unaffected females as been reported suggesting that this duplication could be more frequent than previously thought [11]. Apart from the duplication of the gene, a deletion upstream *NR0B1/DAX1*, probably leading to a loss of regulatory sequences and position effect and then to the upregulation of *NR0B1/DAX1* expression, has also been described [13].

Aside from the detection of rearrangements of genetic factors known to be involved in gonadal development, array-CGH offers the possibility to detect new genes involved in this process [8]. The difficulty is then to distinguish between causative CNVs and benign CNVs. The studies generally focus on genes known to be involved in gonadal development irrespective of whether or not they overlap with previously reported benign CNVs in Database of Genomic Variants (<http://www.tcag.ca>) [7, 9, 12, 14]. Furthermore inherited CNVs are not excluded as gonadal development in males and females involves distinct pathways and gene dosage alteration can act

in a sex-chromosome dependant way [7, 14]. These novel candidate regions for 46, XY DSD gonadal dysgenesis are scattered throughout the genome and concern coding or non-coding sequences that may disturb gene regulation [7, 9].

Array-CGH has become an indispensable tool for clinical setting, allowing the identification of CNVs particularly in developmental disorders, and facilitating patient management and counselling of families. Investigations of dosage imbalances for known genes should be recommended in all patients with 46, XY gonadal DSD when no mutations in the *SRY* gene have been detected [11]. Array-CGH can detect submicroscopic CNVs across the whole genome but it cannot detect balanced chromosomal rearrangements. Therefore using a combined approach, including both karyotype, FISH and array-CGH should be required to deliver an accurate genetic diagnosis for patients presenting with 46, XY DSD in a clinical setting. Next generation sequencing technologies are now emerging and will offer novel insights into the genetics of DSD [8, 15].

Contributors SJ and CR wrote the protocol, designed the trial and did the genetic analysis. AB and KME provided additional scientific advice, guidance, and reviewed the protocol. MAB and SO coordinated the study. LP recruited the patients to the study. The report was written by CR, SJ and AB with contribution, review, and approval from all authors.

Conflicts of interest None.

References

1. Wilhelm D, Palmer S, Koopman P. Sex determination and gonadal development in mammals. *Physiol Rev*. 2007;87:1–28.
2. Hughes IA. Disorders of sex development: a new definition and classification. *Best Pract Res Clin Endocrinol Metab*. 2008;22:119–34.
3. Sekido R, Lovell-Badge R. Sex determination involves synergistic action of SRY and SF1 on a specific Sox9 enhancer. *Nature*. 2008;12:930–4.
4. Ravel C, Chantot-Bastaraud S, Siffroi JP. Molecular mechanisms in sex determination: from gene regulation to pathology. *Gynecol Obstet Fertil*. 2004;32:584–94.
5. Ravel C, Lakhil B, Elghezal H, Braham R, Saad A, Bashamboo A, et al. Novel human pathological mutations. Gene symbol: SRY. Disease: XY sex reversal. *Hum Genet*. 2009;126:333.
6. Ono M, Harley VR. Disorders of sex development: new genes, new concepts. *Nat Rev Endocrinol*. 2013;9:79–91.
7. White S, Ohnesorg T, Notini A, Roeszler K, Hewitt J, Daggag H, et al. Copy number variation in patients with disorders of sex development due to 46, XY gonadal dysgenesis. *PLoS ONE*. 2011;6:e17793. doi:10.1371/journal.pone.0017793.
8. Bashamboo A, Ledig S, Wieacker P, Achermann JC, McElreavey K. New technologies for the identification of novel genetic markers of disorders of sex development (DSD). *Sex Dev*. 2010;4:213–24.
9. Ledig S, Hiort O, Scherer G, Hoffmann M, Wolff G, Morlot S, et al. Array-CGH analysis in patients with syndromic and non-syndromic XY gonadal dysgenesis: evaluation of array CGH as diagnostic tool and search for new candidate loci. *Hum Reprod*. 2010;25:2637–46.
10. Larson A, Nokoff NJ, Travers S. Disorders of sex development: clinically relevant genes involved in gonadal differentiation. *Discov Med*. 2012;14:301–9.
11. Barbaro M, Cicognani A, Balsamo A, Löfgren A, Baldazzi L, Wedell A, et al. Gene dosage imbalances in patients with 46, XY gonadal DSD detected by an in-house-designed synthetic probe set for multiplex ligation-dependent probe amplification analysis. *Clin Genet*. 2008;73:453–64.
12. Tannour-Louet M, Han S, Corbett ST, Louet JF, Yatsenko S, Meyers L, Shaw CA, Kang SH, Cheung SW, Lamb DJ. Identification of de novo copy number variants associated with human disorders of sexual development. *PLoS One*. 2010 26; 5:e15392. doi: 10.1371/journal.pone.0015392.
13. Smyk M, Berg JS, Pursley A, Curtis FK, Fernandez BA, Bien-Willner GA, et al. Male-to-female sex reversal associated with an approximately 250 kb deletion upstream of NR0B1 (DAX1). *Hum Genet*. 2007;122:63–70.
14. Norling A, Lindén Hirschberg A, Iwarsson E, Persson B, Wedell A, Barbaro M. Novel candidate genes for 46, XY gonadal dysgenesis identified by a customized 1 M array-CGH platform. *Eur J Med Genet*. 2013;56:661–8.
15. Arboleda VA, Lee H, Sánchez FJ, Délot EC, Sandberg DE, Grody WW, et al. Targeted massively parallel sequencing provides comprehensive genetic diagnosis for patients with disorders of sex development. *Clin Genet*. 2013;83:35–43.

Web Resources

UCSC Genome Browser: <http://genome.ucsc.edu>
 BACPAC Resource Center: <http://bacpac.chori.org>
 The Centre for Applied Genomics, Database of Genomic Variants: <http://www.tcag.ca>
 OMIM: <http://www.ncbi.nlm.nih.gov>