Cytogenetic analysis and SRY gene detection in four Moroccan brothers with Disorder of sex development

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SUMMARY
The objective of this study is to determine the genetic cause of the disorder of sex development on Moroccan family. Indeed, the genomics and human genetics laboratory at the Pasteur Institute of Morocco recruited four brothers with ambiguous genitalia. Cytogenetic analysis of the first brother revealed the presence of a chimeric karyotype 46, XX/46, XY with the presence of the SRY gene. The other three brothers present a normal female karyotype 46, XX with the presence of the SRY gene.

Introduction
Disorder of sex development (DSD) are defined as congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical. To date, several studies on mouse models have revealed numerous causes of DSD and the percentage of patients with a confirmed molecular diagnosis remains disappointing (around 25%) (Yang et al. 2013; 2014; Farwell et al. 2015). In contrast, the molecular diagnosis of patients with classic steroidogenesis disorders can be successful in most situations (Achermann et Hughes 2016).

Material and Methods
Case family
The genomics and human genetics laboratory at the Pasteur Institute of Morocco has recruited a family with four brothers with ambiguous genitalia. This study is approved by the local research ethics committee of the Pasteur Institute of Morocco.
Cytogenetic analysis

For routine cytogenetic analysis, peripheral blood (2-3 ml) was collected in heparinized tubes and incubated in RPMI-1640 solution in the presence of phytohemagglutinin for 72 h at 37 °C. Colchicine was added two hours before the end of the culture. After centrifugation, the recovered pellet was treated with a hypotonic solution (0.075 M KCl) and fixed with the carnoy fixative (acetic acid / methanol 1/3). The fixed cell suspensions were spread on glass slides using a Pasteur pipette. These slides were immersed in Berger Fixative and then in Earle's Denaturing Medium. Giemsa stain was subsequently performed and the slides were read by the G-band technique using a microscope connected to a computer via a camera. At least 20 metaphases were counted for each sample. The chromosomes have been classified into several groups and numbered according to the international system of human cytogenomic nomenclature.

Detection of the sex-determining region (SRY) by PCR

Genomic DNA extraction was performed using the PureLink® Genomic DNA Mini Kit Invitrogen. The detection of the SRY gene in patients with DSD was carried out from lymphocytic DNA of peripheral blood by PCR, using specific sequences (STS) for this region according to the recommendations of the European Academy of Andrology (EAA): the primers sY14 (470 bp) used are: SY14 F (Forward): 5′GAATATTCCCCTCCTCCGGA 3′ and SY14 R (Reverse): 5′GCTGGTGCTCCATTCTTGAG 3′. The PCR was carried out in a total volume of 15μL, containing 3μL of genomic DNA (~15 to 25ng), 1.2μL of dNTPs (deoxyribonucleotides) (2.5mM), 0.3μL of each primer (20mM), 0.45μL of MgCl2 (50mM), 0.05μL of Taq polymerase (5U / µL) and 1.5μL of PCR buffer (10x). It took place under the following conditions: an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles, each comprising a denaturation at 94 °C for 40s, a hybridization at 61 °C for 40s and an extension at 72 °C for 50s. At the end of the 35 cycles a final extension was carried out for a period of 7 min.

The PCR product was checked by electrophoresis on 3% agarose gel in the presence of a size marker of 100bp. This is to verify, under UV rays, the presence of the fragment of the SRY gene, the size of which is 472 bp.

Results and discussion

In Morocco, the identification of the genetic cause of patients suffering from an abnormality of sexual development remains very difficult to achieve to this day, because of the weak genotype-phenotype correlation of the patients, and because of the difficulty of carrying out an exome analysis.

In this study, we performed cytogenetic analysis of four brothers with genital ambiguity and identified the presence of the SRY gene. Thus, we determined that the first child has a 46, XX/46,XY karyotype with the presence of the SRY gene, while the 3 other brothers have a 46, XX female karyotype with the presence of the SRY gene.

The SRY gene is involved in male sexual differentiation. Indeed, at the 7th week of embryonic development, the gonads are undifferentiated and have an identical structure.
in both sexes. In fact, SRY gene expression activates SOX9 gene expression which upregulates AMH expression leading to regression of Mullerian ducts. On the other hand, testosterone will have a direct action on the growth of the wolf ducts, then allowing the differentiation of these ducts into the epididymis, vas deferens, seminal vesicles and ejaculatory ducts. In the absence of a Y chromosome, and therefore expression of SRY and SOX9, the production of AMH and testosterone is not triggered, resulting in the persistence of Mullerian ducts and regression of Wolff ducts. However, the translocation of SRY gene on an XX embryo and 46,XX/46,XY karyotype may be genetic causes of ovotesticular disorder of sex development. Ovotesticular DSD is a rare condition that has been reported in approximately 500 people worldwide. It occurs in about 1% of newborns who have consulted because of their atypical genitalia. It occurs in patients with karyotype 46,XX/46,XY; patients with karyotype 46, XX and patients with karyotype 46, XY (Achermann et Hughes 2016). Sexual differentiation varies in cases of ovotesticular DSD. Most patients have genital ambiguities or hypospadias. Cryptorchidism is very common, and in most cases a gonad is palpable and located in the inguinal region. A hemi-uterus is often present on the side of the ovary or ovotestis (Achermann et Hughes 2016). The diagnosis of ovotesticular DSD is considered in all patients with ambiguous genitalia. The 46,XX/46,XY karyotype strongly supports the diagnosis, but the presence of a normal karyotype (46, XX or 46, XY) does not exclude the diagnosis, especially in a 46, XX newborn who presents genital asymmetry. Pelvic imaging or magnetic resonance imaging (MRI) is useful for viewing the internal genitalia. The presence of testicular tissue can be detected by measuring basal testosterone, AMH and inhibin B in the first months of life, and by measuring basal AMH thereafter. The presence of ovarian tissue is very difficult to detect in early childhood. Nevertheless, laparoscopy can provide more detailed information on the structure of the internal genitalia and can confirm the diagnosis of ovotesticular DSD when other forms of DSD have been excluded (Steinmetz et al. 2009; Steven et al. 2012; Moriya et al. 2014).

The management of ovotesticular DSDs depends on the age of diagnosis, genital development, internal structures, and reproductive capacity. Individuals with an uterus and a 46, XX karyotype are likely to have functional ovarian tissue, and female gender assignment may be appropriate. Potentially functional testicular tissue is removed before puberty and monitored postoperatively by measuring serum AMH and testosterone levels. A male may be more suitable if there is normal phallic development, testicular tissue, absence or malformation of the Mullerian ducts. Ovarian tissue and Mullerian structures are usually removed (Achermann et Hughes 2016).

Conclusion

In this study, we analyzed the cytogenetic profile and the presence of the SRY gene in four brothers from the same family, all presenting genital ambiguity. the first child presents a 46, XY/46,XX karyotype with the presence of the SRY gene and the other three brothers have a normal female karyotype 46,XX with the presence of the SRY gene.

Conflicts of Interest:

There is no conflict of interests

References

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