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EDITORIAL

Diagnostics of CFTR-negative patients with congenital bilateral absence of vas deferens: which mutations are of most interest?

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Male infertility is one of the best examples of multifactorial diseases in which the genetic contribution is substantial [1]. Male reproductive function, including spermatogenesis, testicular development, endocrine regulation of testicular function, and sperm fertilizing ability are under the control of thousands of genes [2–7]. Basic and clinical research sustained by new technologies for genetic analysis (whole-genome association studies, arrays, next-generation sequencing, and exome sequencing) suggested that a very high number of genetic tests could potentially be introduced in the clinical practice to correctly identify male infertility of genetic origin [1,5,7]. However, only a few genetic analyses are currently recommended in standard clinical practice so far [1].

In this context, it is of primary importance to correctly assess infertile males with an adequate clinical workup, in order to clearly address the appropriate genetic tests in a personalized way [1]. Therefore, experts in reproductive medicine and andrology should strictly cooperate with clinical geneticists to perform the correct tests in the correct patient. Only the correct identification of subjects to be tested and the right application of genetic tests based on clear clinical data could be useful in the management of the infertile couple and genetic counseling, especially when assisted reproduction techniques should be used to overcome infertility.

Basically, genetic tests recommended in clinical practice for male infertility [1] could be grouped on the basis of the clinical diagnosis in those recommended for 1) primary testicular failure (karyotype, analysis of Y chromosome long arm microdeletions, and eventually analysis of TEX11 and NR5A1 gene mutations); 2) endocrine dysfunction (panel of genes for hypogonadotropic hypogonadism, androgen receptor gene mutations); 3) obstructive forms (CFTR gene mutations for congenital absence of vas deferens); 4) sperm morphological and functional defects (dynein genes DNAI1, DNAH5, DNAH11 for asthenozoospermia, DPY19L2 gene for globozoospermia, AURKC gene for macrocephaly).

Azoospermia with low seminal volume and acid pH, normal testicular volumes, and normal plasma levels of FSH, LH, and testosterone clearly suggest an obstructive form [1], which can be due to congenital bilateral absence of vas deferens (CBAVD) in up to 25% of cases [8,9]. The diagnosis of non-palpable vas deferens should be confirmed by scrotal ultrasound to confirm the absence of vas deferens and transrectal ultrasound to detect agenesis of the seminal vesicles, which is associated with CBAVD in about half of the cases. In other cases, hypoplasia of seminal vesicles could be found, and the degree of hypoplasia probably reflects the severity of the genetic abnormalities [10]. CBAVD could occur as an isolated symptom or as an atypical symptom of cystic fibrosis, one of the most frequent autosomal recessive conditions. More than 2000 mutations have been identified in CFTR gene (Cystic Fibrosis Mutation Database, CFMD, 2017) and classified in severe and mild based on their functional and phenotypic effect. Two severe mutations lead to cystic fibrosis, whereas two mild mutations or one severe plus one mild mutation lead to CBAVD. Nearly all patients with cystic fibrosis have CBAVD, and nearly all patients with isolated CBAVD have a mutation in the CFTR gene [9,11,12].

Genetic analysis of CFTR gene is therefore recommended in CBAVD patients. However, standard protocols of analysis allow detection of at least one CFTR mutation in 'only' about 80% of cases, with less than 50% of patients found to carry mutations in both alleles (compound heterozygotes) [1,9]. Therefore, a not negligible percentage of CBAVD patients are classified as CFTR negative. When two mutations are found, the diagnosis is made, but when just one mutation is found we cannot distinguish between the carrier status (1/30 subjects in western countries) from a condition actually related to CFTR but in which the second mutation is not found. This latter condition could be due to several factors, such as inadequate selection of mutations to be tested in the panel, incomplete gene coverage, or mutations in genes other than CFTR. Furthermore, in CBAVD patients' large rearrangements and deletions in the CFTR gene have been described, which could be detected only by novel molecular techniques of analysis [13–15]. For example, by applying different techniques (reverse dot-blot analysis, multiple ligation-dependent probe amplification assays, denaturing high-performance liquid chromatography) in 15 CBAVD patients in whom a single CFTR mutation was found after screening for 36

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mutations and the 5T allele (from a cohort of 23 patients), the second CFTR mutation was detected in six patients, which increased the final detection rate to 60.8% [16].

Generally, first-level CFTR gene mutation screening in patients with CBAVD should be performed as the targeted variant panel that includes causing variants based on geographic and ethnic origin of the patient. The panel usually includes 30–50 mutations able to detect >80% of carriers of classic cystic fibrosis mutations and should also include the 5T allele, a variant typically associated with CBAVD, and TG(n) repeat polymorphism, which influences its penetrance and clinical effect. When no mutation or one mutation is found, second step analysis should be performed by Sanger sequencing of exons, 5′ flanking regions and intron-exon boundaries or, preferably, by next-generation sequencing of the entire gene. Also, after this in-depth analysis, about 20% of CBAVD patients remain undiagnosed (no mutations or one mutation detected). However, the application of Next Generation Sequencing analysis in the diagnosis of CFTR mutations in CBAVD patients will likely improve our ability to identify both CFTR mutations, and as a consequence, the number of CFTR real negative CBAVD will decrease.

To this regard, it has to be noted that the main difference between typical cystic fibrosis and CBAVD is the identification of different and rare CFTR mutations and variants in high frequency in individuals with CBAVD as compared to the typical cystic fibrosis forms, and only a few typical cystic fibrosis mutations, such as the p.F508del, are found in individuals with CBAVD. The panels used for CFTR gene screening have been developed for the identification of the most frequent mutations associated with cystic fibrosis, whereas there is no consensus as to whether a CBAVD-specific panel could have better diagnostic potential.

Finally, it has been suggested that CFTR-negative CBAVD patients could harbor mutations in ADGRG2, an X-linked gene, for which therefore the identification of only one causative mutation is sufficient for genetic diagnosis. Four studies reported screening for ADGRG2 gene mutations in CFTR-negative CBAVD patients: the original study [17] was performed on 26 patients and replication studies have been performed only in 18 Chinese men [18], 17 Chinese men [19] and in a Pakistani family [20]. Altogether, these studies suggested that ADGRG2 gene mutations could account for about 10–15% of the CBAVD patients who are CFTR negative. Although these results are very promising, unless further studies will be performed in a higher number of patients of different ethnic origin, we suggest that screening for ADGRG2 gene mutation should be limited to research purpose and cannot be suggested yet in the routine clinical management of men with CBAVD.

Congenital absence of vas deferens could also be unilateral (CUAVD) as a consequence of mild CFTR mutation. This condition is rare, with a 1:100 proportion with CBAVD. In CUAVD cases the clinical suspicion could be extremely difficult. In fact, these cases might present with mild oligozoospermia and even normozoospermia (if the contralateral testis is normally functioning), and seminal volume and pH can be unaffected [1]. The only way to have a correct diagnosis is performing scrotal ultrasound, but it is evident that a large number of patients remain undiagnosed. A proportion of CUAVD patients has an ipsilateral absence of the kidney, but this condition is not related to CFTR.

Expert opinion

In conclusion, the detection rate of CFTR gene mutations in patients with CBAVD (and CUAVD) could be ameliorated if the test is performed in the correct patient after a complete diagnostic workup, including, for example, also abdominal ultrasound to exclude patients with renal agenesis, a condition not related to CFTR. We recently showed that CFTR gene mutation screening in almost 500 azoospermic men identifies 3.1% of patients with two mutations, further highlighting the importance of correct and comprehensive clinical evaluation of infertile males in selecting patients who really need genetic analysis [21]. The detection rate could also be higher if next-generation sequencing of the entire gene (including analysis of Copy Number Variants) is used at first step analysis, allowing the detection of both mutations. However, genetic counseling for the couple and calculation of the risk of transmission are not affected and we should consider the cost of such analysis. ADGRG2 gene screening could be proposed to patients who are CFTR negative after in-depth analysis and without renal anomalies, however not yet as a routine procedure, and it has to be determined whether this test could also be useful in CBAVD patients with only one CFTR mutation detected and in CUAVD patients.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Ferlin A, Dipresa S, Delbarba A, et al. Contemporary genetics-based diagnostics of male infertility. Expert Rev Mol Diagn. 2019;19:623–633.
• **Practical review on current diagnostics for male infertility.**
2. Ferlin A, Raicu F, Gatta V, et al. Male infertility: role of genetic background. Reprod Biomed Online. 2007;14:734–745.

3. Ferlin A, Foresta C. New genetic markers for male infertility. Curr Opin Obstet Gynecol. 2014;26:193–198.
4. Pizzol D, Ferlin A, Garolla A, et al. Genetic and molecular diagnostics of male infertility in the clinical practice. Front Biosci (Landmark Ed). 2014;19:291–303.
5. Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol. 2018;15:369–384.
6. Cannarella R, Condorelli RA, Duca Y, et al. New insights into the genetics of spermatogenic failure: a review of the literature. Hum Genet. 2019;138:125–140.
7. Oud MS, Volozonoka L, Smits RM, et al. A systematic review and standardized clinical validity assessment of male infertility genes. Hum Reprod. 2019;34:932–941.

• **A comprehensive review of genetics of male infertility with indications of potential new genes to be included in the clinical practice based on evidence.**

8. Yu J, Chen Z, Ni Y, et al. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. Hum Reprod. 2012;27:25–35.
9. de Souza DAS, Faucz FR, Pereira-Ferrari L, et al. Congenital bilateral absence of the vas deferens as an atypical form of cystic fibrosis: reproductive implications and genetic counseling. Andrology. 2018;6:127–135.

• **Comprehensive review on mutations of CFTR associated with CBAVD.**

10. Casals T, Bassas L, Egozcue S, et al. Heterogeneity for mutations in the CFTR gene and clinical correlations in patients with congenital absence of the vas deferens. Hum Reprod. 2000;15:1476–1483.
11. Casals T, Bassas L, Ruiz-Romero J, et al. Extensive analysis of 40 infertile patients with congenital absence of the vas deferens: in 50% of cases only one CFTR allele could be detected. Hum Genet. 1995;95:205–211.
12. Chillon M, Casals T, Mercier B, et al. Mutations in the cystic fibrosis gene in patients with congenital absence of the vas deferens. N Engl J Med. 1995;332:1475–1480.

•• **The first large description of CFTR mutations in CBAVD.**

13. Ratbi I, Legendre M, Niel F, et al. Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements enriches the mutation spectrum in congenital bilateral absence of the vas deferens and impacts on genetic counselling. Hum Reprod. 2007;22:1285–1291.
14. Taulan M, Girardet A, Guittard C, et al. Large genomic rearrangements in the CFTR gene contribute to CBAVD. BMC Med Genet. 2007;8:22.
15. Trujillano D, Ramos MD, Gonzalez J, et al. Next generation diagnostics of cystic fibrosis and CFTR-related disorders by targeted multiplex high-coverage resequencing of CFTR. J Med Genet. 2013;50:455–462.
16. Giuliani R, Antonucci I, Torrente I, et al. Identification of the second CFTR mutation in patients with congenital bilateral absence of vas deferens undergoing ART protocols. Asian J Androl. 2010;12:819–826.
17. Patat O, Pagin A, Siegfried A, et al. Truncating mutations in the adhesion g protein-coupled receptor G2 gene ADGRG2 cause an X-linked congenital bilateral absence of vas deferens. Am J Hum Genet. 2016;99:437–442.

•• **First description of possible involvement of ADGRG2 gene mutations in CBAVD.**

18. Yang B, Wang J, Zhang W, et al. Pathogenic role of ADGRG2 in CBAVD patients replicated in Chinese population. Andrology. 2017;5:954–957.
19. Yuan P, Liang ZK, Liang H, et al. Expanding the phenotypic and genetic spectrum of Chinese patients with congenital absence of vas deferens bearing CFTR and ADGRG2 alleles. Andrology. 2019;7:329–340.
20. Khan MJ, Pollock N, Jiang H, et al. X-linked ADGRG2 mutation and obstructive azoospermia in a large Pakistani family. Sci Rep. 2018;8:16280.
21. Ferlin A, Garolla A, Ghezzi M, et al. Sperm count and hypogonadism as markers of general male health. Eur Urol Focus. 2019 Aug 16: S2405-4569(19)30210-X. [Epub ahead of print] PubMed PMID: 31427194. DOI:10.1016/j.euf.2019.08.001.

•• **The largest study on etiology of male infertility and its association with metabolic parameter, hypogonadism and osteoporosis.**