CFTR analysis should not be offered to all patients with unexplained azoospermia in the presence of normal gonadotropin levels

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CFTR analysis should not be offered to all patients with unexplained azoospermia in the presence of normal gonadotropin levels

Sir,

The paper 'Andrological findings in infertile men with two (biallelic) cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations: results of a multicentre study in Germany and Austria comprising 71 patients' by Rudnik-Schöneborn *et al.* (2021) recently published in *Human Reproduction* recommends CFTR analysis in all men with unexplained azoospermia in the presence of normal gonadotropin levels. We have concerns whether this conclusion is correct based on the reported data.

Firstly, the authors selected patients with two variants in the CFTR gene, but do not provide information on what grounds and how many patients with azoospermia were screened for CFTR mutations to detect the described 71 patients. In the first group, the variants are both pathogenic or likely pathogenic. In the second group of just 15 men, only one pathogenic mutation in combination with a variant of unknown significance was detected which does not support the claim this is the origin of the azoospermia. Furthermore, most variants are suspected but not proven to be biallelic; for one combination of variants, it is even stated they often occur on one allele. Thus, it is doubtful whether these variants are the cause of the azoospermia.

Furthermore, while andrological variables are evaluated, none of them were assessed in the complete group of 71 patients. The most important information for evaluation of azoospermia in congenital bilateral absence of the vas deferens (CBAVD) (ejaculate volume, pH, physical examination and transrectal ultrasound of the seminal vesicles) was available in only 19 patients. It is unclear how the authors corrected for this substantial amount of missing data, and whether some information was missing in many patients, or many information parameters were missing in some patients.

In the patients whose seminal vesicles were examined 21% had normal seminal vesicles. This is contradictory to the finding that less than 5% of the men had semen pH, semen volume and semen fructose levels that are compatible with normal seminal vesicles.

Currently, the European Association of Urology (2020) only recommends CFTR mutation diagnostics when patients are diagnosed with CBAVD (3% of the azoospermic patients).

The authors are right that it is sometimes hard to confirm CBAVD due to the clinical variations. Therefore, the combination of semen parameters together with the other clinical parameters such as laboratory findings and ultrasound assessment will select those patients where CFTR mutation testing is relevant rather than to recommend this analysis for all patients with an unexplained azoospermia in the presence of normal gonadotropin levels.

The fact that one in 30 middle European men carry one CFTR mutation, screening of all men with unexplained azoospermia in the presence of normal gonadotropin levels will certainly lead to detection of CFTR mutations not causal to the clinical picture. This will result in pendency for the patients and unnecessary costs for the society.

Therefore, we oppose the proposition made by the authors that CFTR analysis should be offered to all patients with unexplained azoospermia in the presence of normal gonadotropin levels, but should be performed only in those men who have azoospermia combined with other clinical aspects indicating CBAVD.

Conflict of interest

None declared.

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Reply: CFTR analysis should not be offered to all patients with unexplained azoospermia in the presence of normal gonadotropin levels

Sir,

We thank Cantineau *et al.* (2021) for their critical comments on our recent paper (Rudnik-Schöneborn et al., 2021). The authors suggest that the evaluation of andrological variables was incomplete in our study group of 71 patients. We agree that in an ideal world all possible parameters would be assessed in all patients. However, in the real world—the routine diagnostic setup—information often remains incomplete. In our retrospective data from several large fertility centers in Germany and Austria, only sperm count and FSH levels were inclusion criteria for all cases. In contrast, ultrasound of the seminal vesicles is only rarely performed in the andrological workup. Nevertheless, the data available in normal clinical practice can assist in deciding on cystic fibrosis transmembrane conductance regulator (*CFTR*) genotyping. Nearly all probands (62 of 64, 97%) with presumed *CFTR*-related infertility had at least two semen abnormalities suggestive of obstructive azoospermia, and genetic analyses are recommended in these individuals. On the other hand, normal semen or imaging parameters do not completely rule out CFTR-related azoospermia, and genetic analyses might be considered in these cases. It is interesting to note that in our cohort three out of the four men with reportedly normal seminal vesicles had further evidence of obstructive azoospermia (data not shown).

We thoroughly analyzed and classified the pathogenic effect of the *CFTR* mutations in our cohort. Although most mutations were not confirmed to be *in trans* by segregation studies, we are very familiar with the *CFTR* allele from cystic fibrosis genotyping. We pointed out that the combination of one well-known pathogenic *CFTR* mutation and one (rare) variant of unknown significance (VUS) most likely can be interpreted as biallelic. The classification as VUS mostly applies to catagories defined for severe cystic fibrosis. Functional studies of milder *CFTR* variants with regard to congenital bilateral absence of the vas deferens (CBAVD) or obstructive azoospermia are limited. In the context of our study only a single relevant *cis* double mutant constellation—discussed by us—is known in our populations. Thus a biallelic constellation can be assumed to be present in at least the great majority of our patients. In addition, the andrological phenotype was strikingly homogeneous in our patient group, and we did not find significant clinical differences between individuals with confirmed and postulated CFTR deficient genotypes. Clearly, we cannot rule out a small number of individuals in whom the presence of two *CFTR* variants is unrelated to the azoospermia phenotype. Nevertheless, we are confident that the andrological abnormalities observed in our study correctly reflect the phenotype of men with *CFTR*-related azoospermia.

The authors of the letter are correct that previous guidelines recommend to restrict *CFTR* mutation analysis to patients with CBAVD, but they also admit that it can be hard to confirm CBAVD. Our study was initiated because of our clinical experience that the andrological manifestation spectrum of *CFTR*-related azoospermia is broader than previously reported. This was indeed confirmed by our data. Science advances and recommendations need to be updated accordingly.

The authors of the letter also point out that increased *CFTR* genotyping will result in further detection of VUS and of heterozygous carriers, and they are concerned of concomitant unnecessary costs to the health system. We agree that this is a highly relevant issue but would like to emphasize the importance of making correct diagnoses—particularly in *CFTR*-related azoospermia—for clinical management decisions and the assessment of offspring genetic risks. Any medical activity requires a balance between potential benefits, risks, and cost. Considering the rapidly falling costs of genetic analysis and the improved understanding of genetic variants and their relevance, we believe that one should not underestimate the value of genetic diagnostics in clinical care.

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