Outcome of intracytoplasmic sperm injection for a couple in which the man is carrier of CFTR p.[R74W;V201M;D1270N] and p.P841R mutations and his spouse a heterozygous carrier of p.F508del mutation of the cystic fibrosis transmembrane conductance regulator gene

Florence Brugnon, M.D., a Frederic Bilan, Ph.D., b Marie-Christine Heraud, M.D., c Genevieve Grizard, Ph.D., a Laurent Janny, M.D., a and Isabelle Creveaux, M.D., Ph.D. d

a CHU Clermont-Ferrand, Biologie du Développement et de la Reproduction, CECOS, Hôtel Dieu, Clermont Ferrand; 
b CHU Poitiers, Laboratoire de Génétique Cellulaire et Moléculaire, Université de Poitiers, UFR Médecine-Pharmacie, Poitiers; 
c CHU Clermont Ferrand, Pédiatrie A, Hôtel Dieu; and d CHU Clermont Ferrand, Laboratoire de biochimie médicale et biologie moléculaire, Faculté de Médecine, Clermont Ferrand, France

Objective: To document the phenotype associated with the p.[R74W;V201M;D1270N] and p.P841R mutations of cystic fibrosis transmembrane conductance regulator (CFTR) gene.

Design: Case report.

Setting: Biology and medicine of reproduction in a university hospital.

Patient(s): A couple in which the man is carrier of the triple mutant p.[R74W;V201M;D1270N] allele in trans to p.P841R mutation and his spouse a heterozygous carrier for the severe p.F508del mutation of the CFTR gene, who became pregnant after intracytoplasmic sperm injection (ICSI) with twins.

Intervention(s): Genetic counseling; CFTR gene sequencing; ICSI; children’s follow-up.

Main Outcome Measure(s): First report of a male phenotype associated with the p.P841R mutation.

Result(s): The triple mutant p.[R74W;V201M;D1270N] allele associated with the unknown p.P841R mutations were detected in this man with congenital bilateral absence of the vas deferens, which may presume p.P841R as a severe mutation. After genetic counseling, the couple preferred prenatal diagnosis after ICSI than preimplantation genetic diagnosis, which revealed that the boys were both carriers of p.[R74W;V201M;D1270N] and p.F508del mutations. They are now 4 years old and show normal growth without nutritional deficiency.

Conclusion(s): This case report documents for the first time a male phenotype associated with the p.P841R mutation and underlines the difficulties in counseling a man with congenital bilateral absence of the vas deferens carrying uncommon mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene before ICSI.


Treatment by assisted reproductive technology (ART) of infertile men with congenital bilateral absence of the vas deferens (CBAVD) associated with mutations of the CFTR gene changed after the introduction of intracytoplasmic sperm injection (ICSI) with epididymal or testicular spermatozoa (1). Sequencing of the CFTR gene made it possible to demon-
p.[R74W;V201M;D1270N] allele was previously reported in men with CBAVD (3), but never in symptomatic patients with CF. This case report underlines the difficulties in genetic counseling for infertile couples with rare mutations of the CFTR gene before ICSI.

CASE REPORT
A 31-year-old man presented in our center with a 2-year history of infertility in a primarily infertile marriage. The patient’s past medical history was unremarkable. He was healthy and had never required any medical attention, in particular for respiratory pathologies. His brother and two sisters were fertile and healthy. A complete physical examination with particular focus on the genitals revealed normal volume of both testicles. The vas deferentia were not palpable bilaterally. A semen analysis, performed at our institution according to World Health Organization (WHO) criteria (4), revealed a volume of 0.8 mL, pH 6.5, and azoospermia. This azoospermia was confirmed by a second sample 3 months later at our institution after complete pellet analysis of the ejaculate. Biochemical analysis of the seminal plasma revealed considerably decreased concentrations of fructose (below the detection limit of the assay) and alpha-gluco- sidase (10 mU/ejulate, normal range >35 mU/ejulate). Plasma values of FSH and T were normal (FSH: 5.0 UI/L, normal range 1.0–8.0 UI/L; LH: 1.7 UI/L, normal range 2.0–12.0 UI/L; T: 9.7 nmol/L, normal range: 8.7–30 nmol/L). The karyotype was 46,XY. Renal ultrasonography showed the presence of two normal kidneys. Transrectal ultrasonography confirmed the bilateral absence of seminal vesicles. Given the man’s high probability of being a mutation carrier, CFTR mutation analysis was performed by complete gene analysis using denaturing gradient gel electrophoresis and sequencing (5) for the couple. Results revealed that the man was a compound heterozygous carrier of the triple mutant p.[R74W;V201M;D1270N] allele and the unknown p.P841R mutation (Fig. 1) and his spouse was a heterozygous carrier for p.F508del. Segregation analysis of the man’s parents revealed that his father transmitted the CFTR p.P841R mutation and his mother, the complex allele p.[R74W;V201M;D1270N]. To our knowledge the significance of CFTR p.P841R has never been described to date, and its functional significance is unknown. Consequently it was a complex task to estimate the risk of CF or CBAVD inheritance.

After genetic counseling, both prenatal diagnosis and pre-implantation genetic diagnosis were suggested to the couple because the couple’s risk of having a child with CF was high. Although the risks were clearly explained, the couple preferred prenatal diagnosis and refused preimplantation genetic diagnosis. At the fourth ICSI attempt with thawed epididymal sperm, two embryos were transferred and a twin dichorionic pregnancy occurred. The outcome of this pregnancy was supervised by a gynecologist independent of our center. At 22 weeks gestation, prenatal diagnosis revealed that the two male fetuses carried not only the triple mutant allele p.[R74W;V201M;D1270N] inherited from their father but also p.F508del from their mother. After 34 weeks of gestation, the pregnancy resulted in the birth of healthy twin boys, weighing 1,760 g and 1,540 g, with 1-minute Apgar score of 9 and a 5-minute score at 10 at for both children. Four days after birth, one boy suffered from neonatal

FIGURE 1

Mutation analysis. Analysis of cystic fibrosis transmembrane conductance regulator gene was performed by denaturing gradient gel electrophoresis and sequencing in the male patient with congenital absence of vas deferens. Electrophoregrams of the sequencing products obtained in the patient showing heterozygous profiles for the p.D1270N, p.R74W, and the newly identified p.841R mutations (indicated by red arrow in each figure). (a) p.D1270N mutation (3940 G>A); (b) p.R74W mutation (352 C>T); (c) p.P841R mutation (2854 C>G).

Brugnon. Rare CFTR mutations and ICSI. Fertil Steril 2008.
intestinal occlusion, surgical treatment of which revealed a sigmoidal stenosis. Histologic analysis of the sigmoidal tissue did not reveal an accumulation of mucus in the sigmoidal glands or glandular distension. This boy presented an abnormal trypsin immunoreactive test (74 μg/L, normal <65 μg/L) and a normal sweat chloride concentration (14 mmol/L, normal <40 mmol/L). His twin brother had a normal trypsin immunoreactive test value (57 μg/L) and a borderline sweat test chloride concentration (45 mmol/L). The boys have been the subject of pediatric counseling every 3 months since birth. Currently, they are 4 years old and do not present any major health issue. They are pancreatic sufficient and they do not have any respiratory symptoms.

DISCUSSION

At present most of the alterations identified in the CFTR gene are compiled in an electronic database maintained by Toronto Sick Children’s Hospital (http://www.genet.sickkids.on.ca/cftr/app). Nevertheless, it is extremely difficult to predict the risks of inheritance of uncommon or newly identified mutations whose phenotype has never been described, such as the p.P841R mutation. The p.P841R mutation leads to the substitution of a proline for an arginine in exon 14a. This amino acid is located in the intracytoplasmic domain near the CFTR regulatory domain. This proline is highly conserved in mammalian species. The change is predicted to be probably damaging using prediction tools (Polyphen http://coot.embl.de/PolyPhen/, panther database http://www.pantherdb.org/tools/csnpscoreForm.jsp). The CBAVD phenotype of this infertile patient could indicate that this mutation may be severe or mild, as the triple mutant p.[R74W;V201M;D1270N] is considered as a mild one, and as it was described in CBAVD patients in trans with severe mutations.

Since the beginning of population screening for CF carriers, new data have demonstrated that the prevalence of certain complex alleles like p.[D1270N;p.R74W] is high (6). These missense mutations are thought to affect the expression of the phenotype by modulating the effect of a mutation (3). Thus p.D1270N was found more frequently (6) in carrier screening than in patients with CF (frequency 14% vs. 0.068%). Initially p.R74W and p.D1270N were described in isolation but they have since been found in association in many men with CBAVD (3, 7). Structure function analysis demonstrated that when they are expressed in HeLa cells, mutants p.R74W, p.D1270N, and p.[R74W;D1270N] do not affect CFTR processing. However, lower cyclic adenosine 3’5’ monophosphate (cAMP) responsive anion conductance was observed with the double mutant p.[R74W;D1270N]. This study suggested that mutant p.R74W alone could be considered as a polymorphism, p.D1270N alone could generate a CBAVD phenotype, whereas the complex allele p.[R74W;D1270N] may produce a more severe phenotype because p.R74W could enhance the effect of p.D1270N (8). However, these results have never been confirmed by other studies. Compound heterozygote for p.D1270N has been identified in asymptomatic adults (6), whereas individuals carrying p.D1270N associated with p.R74W on the same allele (p.[R74W;D1270N]) have not been found among asymptomatic (3) or among men with CBAVD (7). In addition, the combination of this double mutant complex allele with p.508del in the other allele was described (9) in a man with CBAVD, recurrent respiratory infection, rhinitis, and high sweat test results (100 mEq/L). The alteration found for our patient is a triple mutant allele p.[R74W;V201M;D1270N]. The additional mutation (p.V.201M) is a missense in exon 6a that leads to the substitution of a valine for a methionine in the third transmembrane domain of CFTR. The CBAVD phenotype described in homozygous p.[R74W;V201M;D1270N] patients (3) suggests that we can consider this triple mutant allele as mild.

Intracytoplasmic sperm injection is an effective therapeutic intervention used worldwide to assist conception but the risks of transmitting damaged genes most commonly found in obstructive azoospermia, particularly mutations of CFTR gene (10), to the offspring are of major concern (11, 12). During genetic counseling before ICSI, the couple was given explanations concerning the high risk of CF for the children if both p.F508del and p.P841R were inherited, respectively, from their mother and father, and the risk of CBAVD if p.[R74W;V201M;D1270N] and p.F508del were inherited. Preimplantation genetic diagnosis or prenatal diagnosis was proposed to the couple. Preimplantation genetic diagnosis needs a high number of good quality embryos for the biopsy and only heterozygous or healthy embryos would be transferred. The couple, therefore, preferred the higher chance of pregnancy using the alternative of prenatal diagnosis, although preimplantation genetic diagnosis may involve less psychological and physical stress (13). If the fetus were found to be a carrier of p.P841R and p.F508del, the couple was offered therapeutic abortion, given the high risk of CF for the offspring. Although the couple and their gynecologist were well-informed, the prenatal diagnosis was performed late because the parents decided to keep this precious pregnancy whatever the genetic diagnosis. The situation at present is that CBAVD is not excluded in these boys, but the diagnosis will be considered at puberty. Because the twin boys are carriers of this complex association of mutations and that the double complex allele p.[R74W;D1270N] associated with p.F508del has been described before as being associated with a pauci-symptomatic form of CF (9), they are monitored regularly.

In conclusion, this case report underlines the difficulties in counseling an infertile man carrying rare alterations of the CFTR gene before ICSI and the need to have a documented phenotype and outcome associated with these uncommon mutations to evaluate the risks of inheritance for the offspring more accurately.

Acknowledgments: The authors thank Alain Kitzis, Christine Francannet, Jean-Luc Pouly, and Hilde van de Velde for their critical reading; Monique Petit, Marlène Baudis, Marie Claude Pasquet for their technical assistance in analyzing CFTR mutations; and Elisabeth Petit for revising the English of the manuscript.
REFERENCES


