Involvement of *CATSPER 2* mutation in a familial context of unexplained infertility and fertilization failure associated with hearing loss: a case report

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Objective: To explore the functional implications of a homozygous *CATSPER 2* (cation channel for sperm) deletion within the acrosome reaction pathway during fertilization in 2 brothers, who have unexplained infertility and hearing loss.
Design: Case report.
Patients: Two twin brothers aged 30 years with hearing loss and unexplained infertility.
Exposure or Intervention: Molecular genetic diagnosis of deafness. Evaluation of the acrosome reaction and calcium mobilization assays after induction by progesterone and ionomycin on spermatozoa of the *CATSPER 2*-mutated patient and on fertile controls.
Main Outcome Measures: Fertilization rate during conventional in vitro fertilization. Molecular genetic test. Percentage of acrosome-reacted spermatozoa with peanut agglutinin lectin staining. Recording of progesterone and ionomycin-induced intracellular calcium signals with a fluorescent probe.
Results: Mr. S and his brother have normal, conventional sperm parameters. Both brothers have had repeated intrauterine insemination failures and one fertilization failure after conventional in vitro fertilization. Mr. S obtained 2 healthy babies after intracytoplasmic sperm injection. Genetic analysis found a homozygote deletion of the *STRC* (stereocilin) gene (NM 153700: c.1-? 5328+?del) that removes the *CATSPER 2* gene. Mutation of the *STRC* gene is known to be associated with hearing loss. Sperm functional tests revealed an inability of progesterone to activate intracellular calcium signaling and to induce acrosome reaction.
Conclusion: We demonstrate the absence of a calcium signal and acrosome reaction after progesterone in our patient with a *CATSPER 2* mutation. We emphasize the importance of the male medical interview and of the genetic investigation of hearing loss. We show that in vitro fertilization–intracytoplasmic sperm injection is necessary, even where normal sperm parameters are present. (Fertil Steril Rep® 2024;5:114–22. ©2023 by American Society for Reproductive Medicine.)
Key Words: *CATSPER*, fertilization, hearing loss, spermatozoa, calcium channel, case report

Unexplained infertility is a disorder that has a relatively high incidence (between 10% and 25%), and that can be attributed to factors from both male and female partners seeking to conceive (1). The diagnostic tools that are currently available often fail to explain the origin of infertility, and conventional semen examination cannot always differentiate between fertile and infertile men (2). It is well recognized that the causes of male infertility are present in over half of infertile couples (3, 4) and spermatozoa defects, often poorly explored, seem to play a significant role in a number of cases of unexplained

Received August 23, 2023; revised and accepted December 15, 2023.
Supported by DEFE and IRSD unit funding for materials, consumables, and reagents. S.G.'s doctoral salary was supported by the Medical Research Foundation (FRM – FDT202204014931) and the National Research Institute for Agriculture, Food, and Environment (INRAE).
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Fertil Steril Rep® Vol. 5, No. 1, March 2024 2666-3341

https://doi.org/10.1016/j.xfre.2023.12.003

infertility in which current approaches have failed to reveal a cause (5, 6).

The lack of a clear cause for infertility in a couple, combined with the lengthy treatment involved in assisted reproductive techniques (ART), often results in considerable psychological distress because of the utilization of inappropriate methods. It is, therefore, crucial to have a greater understanding of the origins of male infertility.

This report focuses on the case of a young couple, Mr. and Mrs. S, whose infertility, following the examination of both of them, remains unexplained. After 18 months of infertility, this couple was first treated with intrauterine insemination (IUI; 6 unsuccessful cycles) and then with conventional IVF in vitro fertilization (IVF), resulting in a complete failure of fertilization before having 2 children after IVF–intracytoplasmic sperm injection (ICSI). After treatment, our patient, Mr. S, contacted us again to report a similar infertility in his heterozygous twin brother, being infertility associated with hearing loss. Mr. S's brother was diagnosed with a deletion of chromosome 15 at position 15q13.3. This deletion involved the *STRC* (stereocilin) gene, which causes autosomal recessive deafness, and the *CATSPER 2* gene, which has been implicated in cases of male infertility (7, 8). After conducting another interview with Mr. S, we discovered that he had also experienced hearing loss, which had not been mentioned during the interviews.

STRC and *CATSPER 2* are 2 contiguous genes located at the locus 15q15.3. The *STRC* gene encodes a protein called stereocilin, an extracellular structural protein expressed in the outer hair cells of the inner ear that is crucial for normal hearing. Causative alterations in the *STRC* gene include copy number variations, single nucleotide variants, and small insertions/deletions (indels) (9). The *STRC* deletions are frequently accompanied by the deletion of the contiguous *CATSPER 2* gene accounting for sperm functions. The genotype, characterized by deletions including both *CATSPER 2* and *STRC*, is associated with slight to moderate hearing loss and infertility syndrome in both men and women (10). Although variations in gap junction protein beta 2 (*GJB2*) gene are the most common factor for recessive deafness (50%), *STRC* gene mutations are the second-most prevalent genetic cause of bilateral mild to moderate hearing impairment (11, 12). The prevalence of *STRC* gene mutations in patients with hearing loss after the exclusion of the *GJB2* mutation was 11.10% (95% CI: 0.07–0.17) (9). The prevalence of hearing loss associated with infertility (deafness infertility syndrome DIS) was 36.75% (95% CI: 0.21–0.56) in overall pooled *STRC* gene mutations. The phenotype of hearing loss is variable. Variants in the *STRC* gene significantly contribute to mild to moderate hearing impairment (9).

Major sperm cell processes needed for fertilization, such as chemotaxis, capacitation, and acrosome reaction, are regulated by intracellular calcium signaling (13). These oscillations in $[Ca^{2+}]_i$ (i.e., free intracellular calcium concentration) can be mediated by release from intracellular stores or via the opening of transmembrane calcium channels. *CATSPER* (cation channel for sperm) is the only channel exclusively expressed in spermatozoa (14). This channel is pH-sensitive, weakly voltage-dependent, and primarily permeable to calcium but also, to a lesser extent, to other ions such as sodium, cesium and, barium (14, 15). *CATSPER*, a heterotetrameric pore-forming complex comprising 4 α subunits (*CATSPER 1–4*) and 6 auxiliary subunits (*CATSPER* β, γ, δ, ε, ζ, and *EFCAB9*), is primarily situated in the principal piece of the flagellum in mature spermatozoa (16, 17). Each subunit is encoded by a distinct gene. By virtue of its function, the *CATSPER* channel regulates a large number of physiological processes of the spermatozoa that require calcium, including hyperactivation and chemotaxis to the ovocyte (15, 18–20). There is still no consensus regarding the role of *CATSPER* in the acrosome reaction, but it is thought to be responsible for the initial and transient increase in Ca^{2+} that leads to a calcium elevation in the sperm head via a release of Ca^{2+}, depending on the intracellular store (21). A study in murine spermatozoa has, however, suggested that the increase in $[Ca^{2+}]_i$ mediated by *CATSPER* did not appear to be required for acrosome reaction (22). Either way, since its discovery in 2001, *CATSPER* has been known to play an essential part in male fertility (23). Indeed, a study of mice found that, even in those with normal semen parameters, those lacking *CATSPER* were infertile (24). The scientific understanding of the importance of *CATSPER* in human spermatozoa considers the clinical identification of abnormal cases. Several cases of genetic abnormalities of *CATSPER* in infertile men have been reported and can be characterized according to 2 types of *CATSPER*-related male infertility: nonsyndromic and syndromic male infertility (7, 25) (Table 1) (8, 26–30). Syndromic male infertility involves mutations of the genetic locus including the *CATSPER 2* gene as well as contiguous genes that are responsible for deafness phenomena, whereas nonsyndromic infertility involves mutations in the *CATSPER 1* and *3* subunits but which are not associated with hearing loss.

Only a few cases of infertility linked to *CATSPER* mutations are described in the literature and, to our knowledge, only one other case of *CATSPER 2* mutation linked to idiopathic infertility has been reported to date (8), whereas most of the *CATSPER* mutations are associated with abnormal conventional sperm parameters (Table 1) (8, 26–30). Moreover, the originality of our case is to give a complete genetic, molecular, and clinical description of our patient with a *CATSPER 2* mutation.

As well as providing a description of the clinical and biological background, our study sought to explain the pathophysiology of infertility in twin brothers with unexplained infertility associated with hearing loss. We assessed the functional impact of a homozygous deletion of *CATSPER 2* on acrosome reaction and intracellular calcium response induced by 2 different stimuli: progesterone and ionomycin.

MATERIALS AND METHODS

Patients

GERMETHEQUE BioBank (BB-0033-00081), site of Toulouse, provided samples of frozen spermatozoa from the patient, Mr. S, and from fertile controls with normal sperm parameters, as well as their associated data. Germethèque obtained consent from each patient to use their samples (CPP 2.15.27). The Germethèque pilotage committee approved the study design the

TABLE 1

Overview of clinical and biological literature data from infertile patients with CATSPER mutation.

	CATSPER 1 mutation		CATSPER 3 mutation
Reference	**Avenarius et al. [29], 2009**		**Wang et al. [30], 2021**
Genetic abnormality	Exon 1 insertion (c.539-540insT)	Exon 1 insertion (c.948-949insATGGC)	Homozygous Variant (NM_178019.3: exon5:c.707T>A,p.L236*)
Clinical description	Nonsyndromic infertility	Nonsyndromic infertility	Nonsyndromic infertility
Conventional sperm parameters	Semen volume: 1 mL Sperm conc.: 12 M/mL Progressive motility: 50%	Semen volume: 0.4 mL Sperm conc.: 10.4 M/mL Progressive motility: 0% Typical forms: 20%	Semen volume: 4.2 mL Sperm conc.: 17.1 M/mL Progressive motility: 54.6% Typical forms: 5%
ART outcomes	Unknown	Unknown	5 Intrauterine insemination failures 1 cIVF: 6 oocytes, total fertilization failure 1 IVF-ICS: 6 meta II oocytes, 4 day-5 embryos. Ongoing pregnancy after 2 frozen embryo transfers

	CATSPER 2 mutation							
Reference	**Avidan et al. [26], 2003**		**Zhang et al. [27], 2007**			**Luo et al. [8], 2019**	**Jaiswal et al. [28], 2014**	
Genetic abnormality	15q15.1-15.3 Deletion		15q15.1-15.3 Deletion			Variability of copy number: area 43894500-43950000 of chromosome 15	15q15.3 Deletion	
Clinical description	Syndromic infertility: asthenoteratozoospermia, deafness		Syndromic infertility: asthenoteratozoospermia, deafness			Idiopathic nonsyndromic infertility	Nonsyndromic infertility	
Conventional sperm parameters	NP	Volume: N Sperm conc.: N Motility: 3% Typical forms: 9%	NP	Volume: 4 mL Sperm conc.: 78 M/mL Motility: 5% Typical forms: 11%	Volume: 2.2 mL Sperm conc.: 73 M/mL Motility: 10% Typical forms: 7%	Volume: 1 mL Sperm conc.: 60 M/mL Motility: 15% Typical forms: 11%	Volume: 2 mL Sperm conc.: 75 M/mL Motility: 75.7% Vitality: 82.3% Typical forms: 12%	Severe oligozoospermia <2 M/mL	Oligozoospermia <5 M/mL
ART outcomes	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	1 IVF cycle (18 oocytes) = cIVF (6 oocytes): fertilization failure; ICSI (12 oocytes): 7 embryos 1 transferred embryo, 1 pregnancy	Unknown	Unknown

ART = assisted reproductive technologies; cIVF = conventional in vitro fertilization; ICSI = intracytoplasmic sperm injection; IVF = in vitro fertilization; N = normal; NP = not performed.

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25/08/2022 referenced under the number 20220807. The BioBank has a declaration DC-2021-4820 and an authorization AC-2019-3487. Conventional sperm parameter analysis and acrosome reaction assessments were performed on fresh samples from the patient. The measurement of intracellular calcium $[Ca^{2+}]_i$ was performed on frozen samples from the same patient that had been stored in the Germètheque BioBank.

Genetic Diagnostics

We employed quantitative MLPA (multiplex ligation-dependent probe amplification) analysis (kil P461 IVIRC-Holland) of the *STRC* gene. MLPA was performed to detect the deletion within the *STRC-CATSPER2-OTOA* genes, which causes deafness-infertility syndrome in the homozygous form, in accordance with the manufacturer's protocol (kit P461; MRC-Holland, The Netherlands). The presence of this mutation was confirmed by MLPA analysis of the parents who carried the heterozygous form.

Conventional Sperm Parameters

Fresh semen evaluation (spermiogram) was performed in accordance with the standard methodology proposed by the World Health Organization (WHO) guidelines (31) and sperm morphology was evaluated using modified David's classification after Schorr staining.

Acrosome Reaction Assessment

After liquefaction for 30 minutes at 37°C, semen was prepared on a density gradient. Sperm preparation was then incubated for 3–4 hours for capacitation. Acrosome status was assessed before and after the acrosome induction on addition of 10 mM progesterone or ionomycin at a concentration of 10 μM (Sigma-Aldrich, St-Quentin-Fallavier, France) with a minimum count of 200 cells per patient. To do so, acrosomal content was labeled with fluorescein-isothiocyanate (FITC)-conjugated peanut agglutinin (PNA)-FITC (Sigma-Aldrich, St-Quentin-Fallavier, France) (25 μg/mL), which is lost after acrosome reaction (32). The inner acrosomal membrane was labeled before membrane permeabilization with a mouse anti-CD46 monoclonal antibody linked to Alexa Fluor 647 (Santa Cruz Biotechnology, Santa Cruz, CA) (10 μg/mL). This labeling is acquired after the acrosomal reaction. The acrosome reaction is therefore associated with both the loss of PNA labeling and the acquisition of CD46 labeling.

Measurement of Intracellular Calcium $[Ca^{2+}]_i$

Frozen sperm samples from the patient of interest (Mr. S) and from fertile control donors were used for this study. Sperm samples were thawed and prepared for capacitation as described above. Just after thawing, the total cell concentration and the viability of each sample were quantified by eosin staining. We needed to perform calcium response analysis with both control and patient sperm on the same day, to ensure that both sperms were exposed to the exactly same experimental procedure. In preliminary tests, we have compared freshly harvested and frozen sperms using fertile sperm from donors. Our experiments showed that the calcium response could be measured in both freshly harvested and frozen sperm (data not shown). However, we needed to use twice more spermatozoids when using a frozen sample, to obtain approximately the same calcium profile. From these experiments, we determined that the optimal number of spermatozoids to be used from frozen sperm was 4 million (C) and we used this number for both control and patient analysis.

For all the following incubation and washing steps, a ratio of 100 μL of liquid for 4 million cells was systematically applied. After capacitation, the appropriate volume of semen was centrifuged for 5 minutes at 300 g. The supernatant was removed and the remaining cell pellet was incubated for 30 minutes at 37°C with a Ca^{2+}-sensitive fluo-8 probe (Screen QuestTM Fluo-8 calcium AAT bioquest®) prepared following the manufacturer's recommendations. Stained spermatozoa were then centrifuged for 5 minutes at 300 g and washed once using HBSS (Hanks' Balanced Salt solution, Gibco) after fluo-8 solution removal. After a final centrifugation (5 minutes at 300 g), spermatozoa were placed in HBSS that, in some cases, contained and, in others, did not contain Ca^{2+} ions. The cells were then transferred in a dark 96-well plate (Greiner Bio-One, 655090) at the rate of 100 μL per well. Calcium signals were recorded on the addition of 10 mM progesterone or ionomycin at 10 μM, using a Novostar plate reader (BMG Labtech). Cell fluorescence (λex = 485 nm; λem = 520 nm) was recorded over 230 seconds with a baseline measurement for 30 seconds before cell stimulation. The calcium mobilization kinetics were expressed in normalized values by dividing each fluorescence value for a given time by the baseline value (F/baseline). For some experiments, spermatozoa were pre-incubated with Mibefradil dihydrochloride hydrate (M5441-5MG, Sigma-Aldrich, St-Quentin-Fallavier, France), a T-type calcium channel antagonist, or its vehicle (0.5% water diluted in HBSS) 5 minutes before stimulation.

Statistics

Statistical analyses were performed with Prism software (GraphPad Software, La Jolla, CA). Percentages of spermatozoa with reacted acrosome were compared using the Chi-square test, with the significance level fixed at $P < .05$.

RESULTS

Initial Fertility Check-Up

Normal conventional sperm parameters were found for both Mr. S and his brother (Table 2).

Both Mrs. S and the partner of Mr. S's brother, 30 and 32 years old respectively, had regular cycles and a normal hormonal balance. The exploration of the fallopian tubes by hysterosalpingography revealed permeable fallopian tubes for both female partners.

ART Outcomes

Data concerning ART outcomes have been summarized in Table 2.

TABLE 2

Clinical and biological data from our two brothers with idiopathic infertility, hypoacusis, and CATSPER2 mutations.

	Patient S	Patient S' brother
Genetic abnormality	NM 153700: c.1-? 5328+?del	
Clinical description	Idiopathic infertility with hypoacousia	
Conventional sperm parameters	Semen volume: 5.6 mL Sperm concentration: 43 M/mL Progressive motility: 55% Typical forms: 27%	Semen volume: 3.5 mL Sperm concentration: 25 M/mL Progressive motility: 30% Typical forms: 29%
ART outcomes	6 Intrauterine insemination failures 1st cIVF: total fertilization failure on 12 meta II 1st ICSI: 7 meta II oocytes, 6 day-2 embryos, 1 fresh embryo transfer and 2 frozen embryo transfers: no pregnancy 2nd ICSI: 2 meta II oocytes, 2 day-2 embryos: 1 fresh embryo transfer: no pregnancy 3rd ICSI: 10 meta II oocytes, 10 day-2 embryos: 1 fresh embryo transfer and 2 frozen embryo transfers: live birth of healthy baby girl 1st ICSI bis: 3 meta II oocytes, 2 day-2 embryos, 1 fresh embryo transfer: no pregnancy 2nd ICSI bis: 8 meta II oocytes, 5 day-2 embryos, live birth of a healthy baby boy after fresh embryo transfer	5 Intrauterine insemination failures 1st cIVF: total fertilization failure on 11 meta II 1st ICSI : 10 meta II oocytes, 5 E, 1 fresh embryo transfer at day 3 : no pregnancy 2nd ICSI: 6 meta II oocytes, 6 E with normal morphology: 1 fresh embryo transfer and 2 frozen embryo transfers at day 3: no pregnancy Stop medical care for personal reasons

ART = assisted reproductive technologies; cIVF = conventional in vitro fertilization; ICSI = intracytoplasmic sperm injection; Meta II = metaphase II oocyte.

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In both couples, IUI were unsuccessful, and conventional IVF failed to result in fertilization. Fertilization rates during IVF–ICSI were normal for both couples.

Mrs. S initially received 6 IUIs with sperm from Mr. S. However, no pregnancy was achieved (Table 2).

The couple was subsequently managed in conventional IVF, with all 12 mature retrieved oocytes leading to total fertilization failure.

They then underwent 3 attempts using IVF–ICSI, resulting in a pregnancy and the birth of a healthy child in 2015, and 2 supplementary courses of ICSI, producing a second healthy baby in 2019. The fertilization rate was normal: 86% (6/7), 100% (2/2), and 100% (10/10) in 2015; and 66% (2/3) and 62.5% (5/8) in 2019 (Table 2).

In another IVF clinic, Mr. S's brother and his partner received five courses of IUI for idiopathic infertility, but this did not result in pregnancy. The couple then underwent conventional IVF, resulting in a total fertilization failure. After this, they underwent 2 courses of ICSI, both of which gave normal fertilization rates (50% [5/10] and 100% [6/6]), but no pregnancy was obtained after 2 fresh embryo transfers and 2 frozen embryo transfers (Table 2). This couple stopped medical care for personal reasons.

Genetic Testing

As in his brother, a molecular genetic test found a homozygote deletion of the *STRC* gene in Mr. S (NM 153700: c.1-? 5328+?del) with autosomal recessive transmission. This deletion also removes the *CATSPER 2* gene.

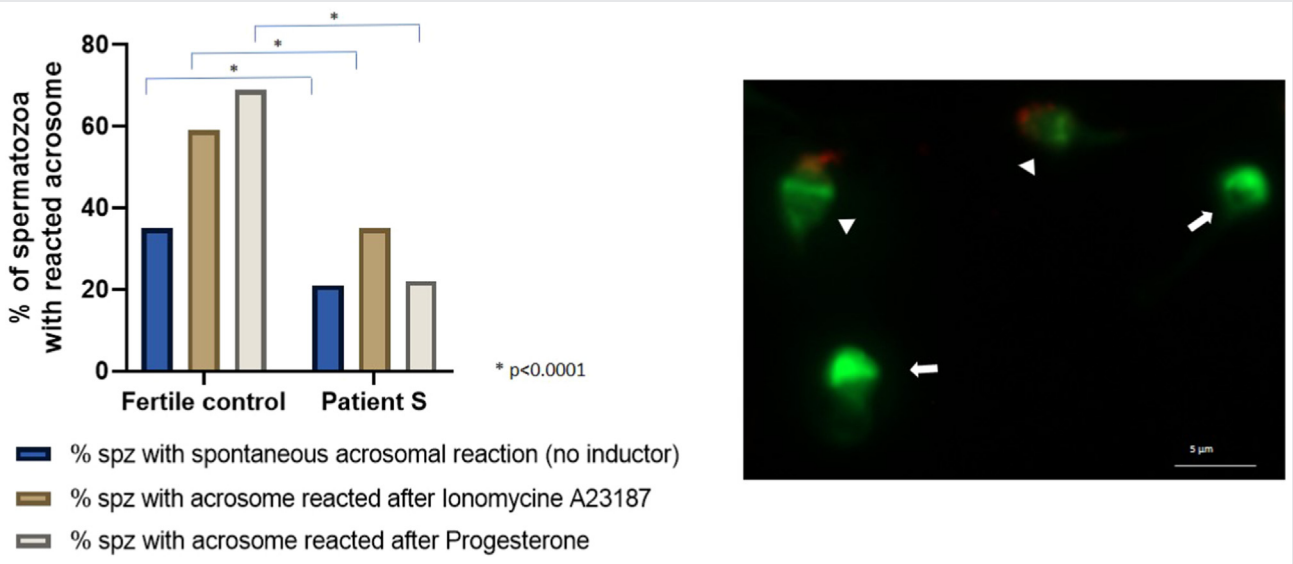
Acrosome Reaction Assessment with PNA Labeling and CD46 Antibody

In the absence of an inducer, the fertile control patient showed a spontaneous acrosome reaction of 35% (194/549). This spontaneous acrosome reaction was lower in Mr. S's sperm compared with in the control: 21% (107/511) ($P < .0001$). After induction with ionomycin, Mr. S and the control had 35% (298/858) and 59% (625/1057) of reacted spermatozoa ($P < .0001$), respectively (Fig. 1). After progesterone stimulation, Mr. S had 22.2% (117/527) of reacted spermatozoa compared with 69% (232/338) for the control ($P < .0001$). In summary, Mr. S's rate of spontaneous and progesterone-induced acrosome reaction was low and fairly similar and was slightly increased by ionomycin (Fig. 1).

Measurement of Intracellular Calcium $[Ca^{2+}]_i$ Flux

To investigate the functional consequences of *CATSPER* deficiency in Mr. S, we monitored the intracellular calcium influx in response to different agonists. We first validated the ability of a control sperm sample obtained from a fertile donor to generate calcium signals (Fig. 2A). As expected, progesterone, a physiological *CATSPER* agonist, elicited a clear and transient $[Ca]_i$ elevation that was almost completely abolished in the absence of extracellular Ca^{2+}, confirming the involvement of a transmembrane calcium channel in this response. The progesterone-induced response was also prevented by 40 μM mibefradil, a T-type calcium channel antagonist that has previously been described as a *CATSPER* signaling blocker (19). In keeping with previous findings, although

FIGURE 1



On the left, % of spermatozoa with acrosome-reacted (spontaneously or after inductor ionomycin or progesterone). At the right, peanut agglutinin (PNA) lectin (green labeling) and anti-CD46 immunocytochemistry (red labeling) in Mr. S. Acrosome-reacted spermatozoa (head arrows) are CD46 positive and have PNA labeling of the equatorial plate. Spermatozoa with intact acrosome (arrows) are CD46 negative and have PNA labeling of the whole acrosome.

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inhibiting the early calcium response induced by progesterone, mibefradil alone induced a low and delayed calcium mobilization in spermatozoa, but with a totally different kinetic compared with progesterone.

We then compared the calcium response obtained using the semen of the control patients and that of Mr. S. As shown in Figure 2B, spermatozoa from the infertile patient, Mr. S, were completely unable to respond to progesterone unlike those from the control donors. Interestingly, semen from both Mr. S and the fertile donors were responsive to ionomycin treatments (Fig. 2C). However, the calcium response to ionomycin was slightly lower in Mr. S's semen compared with that of the fertile donor.

Taken together, these results show that the genetic deletion of the *CATSPER 2* locus in Mr. S is associated with hyposensitivity to acrosomal reaction inducers and a complete inability of progesterone to activate intracellular calcium signaling in his spermatozoa.

DISCUSSION

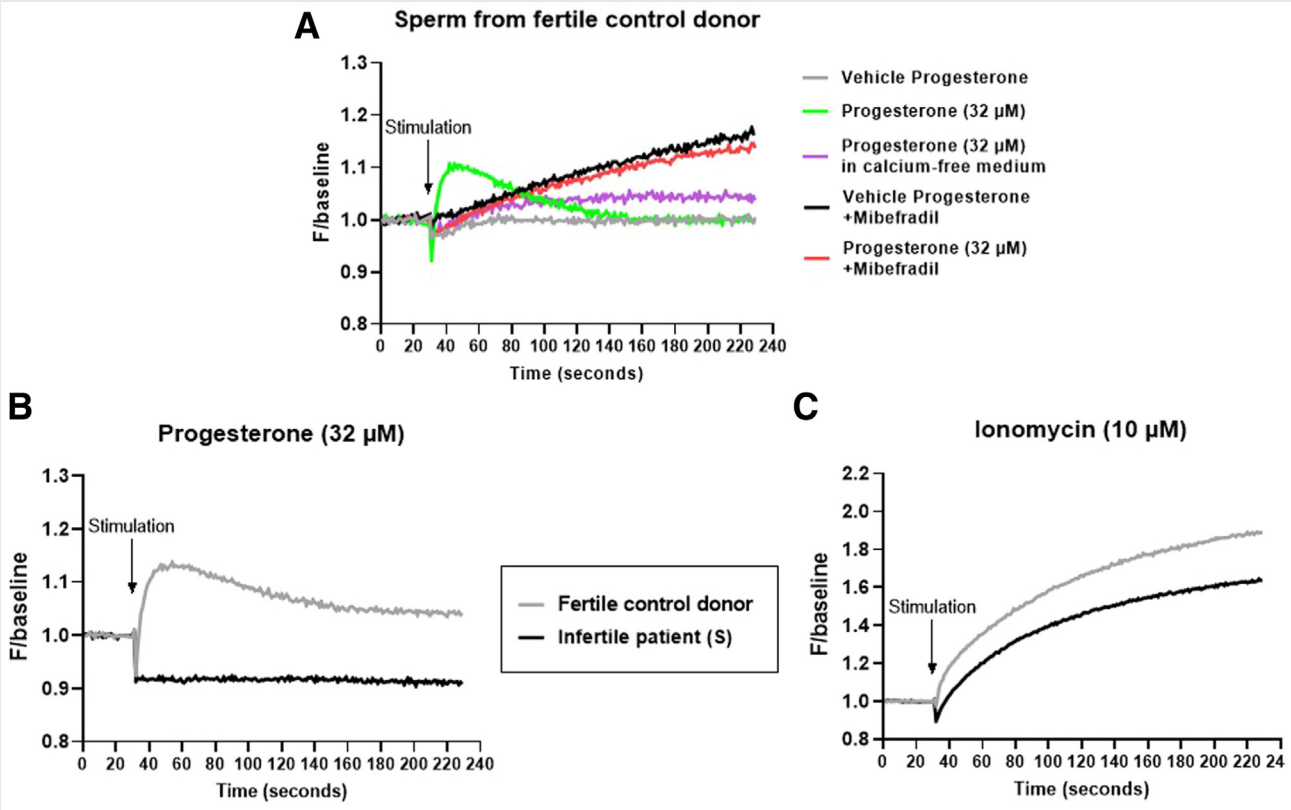
Scientific data, in particular from animals, show the importance of the *CATSPER* channel in essential sperm functions such as hyperactivation, capacitation, and acrosome reaction (14, 16, 23, 33, 34). Only a few cases of patients with *CATSPER* mutation have been reported to date (25–30) and differences and controversies exist in the pathophysiological role of *CATSPER* in the acrosome reaction and/or the clinical impact of its deficiency. In this study, we report a new familial case of unexplained male infertility and IVF failure linked with hearing loss in twin brothers. Our work provides a deep characterization of this case, combining genetic, molecular (assessment of acrosome reaction and $[Ca]_i$), and phenotypic (clinical examination, conventional sperm parameters, and results of ART attempts) approaches.

Previous clinical investigations identified a genetic deletion of the 15q15.3 locus in Mr. S's twin brother that encompasses both the *STRC* gene (involved in hearing function) and *CATSPER 2*. Interestingly, our genetic analysis also confirmed the presence of the genetic deletion of *STRC* in Mr. S. The deletion of the 15q15.3 region is rarely reported in the literature but is often associated with male infertility which is consistent with our results (Table 1) (8, 26–36). In keeping with our observations of Mr. S, *CATSPER 2*-deficient mice exhibit normal semen parameters but are infertile with a defect in sperm's hyperactivated motility (37, 38). Deciphering the functional outcomes of *CATSPER* mutations in human clinical cases is therefore of fundamental importance.

In our study, *CATSPER 2*-deficient spermatozoa did not show significant morphological alterations. These results are consistent with murine studies that found no effect of genetic deletion of different *CATSPER* subunits on sperm production and morphology (24, 39, 40). However, because of significant morphological and functional differences between mouse and human spermatozoa, these latter studies cannot be directly transposed to humans. In accordance with our study, Luo et al. (8) identified no abnormal semen parameters in a patient with a comparable *CATSPER 2* deletion.

Achieving an acrosome reaction is a key event in any normal natural fertilization. Our experiments demonstrated that progesterone, as a *CATSPER* agonist, failed to induce

FIGURE 2



Semen from Mr. S, carrying a *CATSPER 2* deletion, exhibited no calcium entry in response to progesterone. (A) Calcium mobilization assay performed on fluo-8-loaded fertile control sperm. Injection of different agonists (arrow) was performed at 30 seconds. The fluorescence of the calcium fluo-8 probe was recorded over 230 seconds and represents the fluorescent level normalized by the baseline level (F/baseline), which is the level of fluorescence before injection. (B, C) Intracellular calcium responses to 32 μM progesterone (B) or 10 μM ionomycin (C) were compared between semen from the fertile control donor (gray curves) and the infertile patient Mr. S (black curves). The graphs represent the mean profile of 2 independent experiments.

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an acrosome reaction in Mr. S's spermatozoa. Since the *CATSPER 2* gene is deleted in Mr. S, our result is in agreement with studies reporting that *CATSPER* is required for a progesterone-induced acrosome reaction (8, 41, 42). This inability to achieve progesterone-induced acrosome reaction is likely to be one of the functional consequences of *CATSPER 2* deletion that explains Mr. S's infertility.

The calcium flux analysis performed simultaneously corroborates the results on the acrosome reaction. In our fertile control donor, we validated that progesterone-induced a calcium signaling that was, in turn, fully dependent on *CATSPER*, being abolished both by the depletion of extracellular calcium and the CatSper antagonist Mibefradil, as described by Strünker et al. (19). The inability of progesterone, but not ionomycin, to increase $[Ca^{2+}]_i$ is indicative of a loss of *CATSPER* function in Mr. S. Similar results in sperm from a patient presenting a *CATSPER 2* deletion were demonstrated by Luo et al. (8). In addition to acrosome reaction, calcium signals drive various central behaviors of spermatozoa such as chemotaxis, capacitation and hyperactivation (43). Hence, the inability of *CATSPER*-deficient sperm to induce progesterone-dependent calcium influx and subsequent signaling gives a mechanistic explanation for Mr. S's infertility.

Interestingly, spermatozoa from Mr. S showed a positive but reduced ability to undergo acrosome reaction in response to ionomycin compared with our fertile control donor. In the same way, ionomycin-induced calcium signals were slightly reduced in Mr. S compared with our fertile control. The primary mechanism described for ionomycin is the binding of extracellular calcium to promote the passage of calcium into the intracellular compartment. However, Dedkova et al. (44) described that, depending on its concentration, ionomycin may also promote calcium release from intracellular organelles into the intracellular compartment or facilitate extracellular calcium entry through activation of unknown Ca^{2+} channels. Therefore, knowing that *CATSPER* is 1 of the major Ca^{2+} channels on the surface of spermatozoa, this

last mechanism could explain a lower calcium flow and ionomycin-induced acrosome reaction in spermatozoa from Mr. S.

With regard to the outcomes of ART, the failure of extracellular calcium mobilization and acrosome reaction in Mr. S provides a plausible explanation for why conventional IVF failed where IVF–ICSI was successful for Mr. S and his wife. From a clinical point of view, our work emphasizes the usefulness of calcium flow assays in diagnosing unexplained male fertility. Moreover, the IVF–ICSI procedure is an efficient tool for obtaining fertilization for infertile men and should be proposed as the first option in the presence of these indications.

The originality of our case is to give a complete genetic, molecular and clinical description of our patient with a *CATSPER 2* mutation. Although the majority of *CATSPER* mutations are associated with abnormal conventional sperm parameters (8/11 on Table 1) (8, 26–30), greater vigilance is required in male infertility qualified as "idiopathic," as in this case. We performed genetic analysis and identification of the *CATSPER* channel abnormality in Mr. S and his brother. Both of these patients and their spouses suffered from medical care that was too long and repeatedly failed to achieve IUI. In this context, although this manuscript presents a familial case, our work once again underlines the necessity of performing a complete questionnaire for both male and female parties during infertility investigation. When infertility is coupled with partial or complete hearing loss, it is crucial to be more vigilant and to initiate genetic exploration and counseling and promptly refer the couple for IVF–ICSI treatment, even if the sperm parameters appear normal.

In addition, there is a growing need for human models to further study *CATSPER* mutations. The *CATSPER* family has relatively low sequence homologies between different species (14). For example, the homology of the different *CATSPER* subunits between mice and humans is relatively low, ranging from 50% (*CATSPER 1*) to 69% (*CATSPER 4*). This could explain the significant differences in *CATSPER* function and regulation between the different species (45).

In terms of the limitations of our study, it is important to note the absence of a complete family tree for Mr. S. Furthermore, we were not able to conduct calcium flow and acrosome reaction tests on the brother of our patient for geographical reasons. Moreover, it would have been interesting to perform a specific exploration of sperm capacitation, but we could not do so because we lacked fresh samples.

CONCLUSIONS

In conclusion, this study led to a better understanding of certain cases of idiopathic male infertility. It allowed us to show the link between the *CATSPER* 2 mutation and the functional deficit of the calcium channels, inducing a lack of acrosome reaction, infertility and numerous failure of ART attempts (IUI and conventional IVF), before turning to ICSI. The special feature of this study relates to the small number of cases described in the literature. Only one other case of *CATSPER* 2 mutation linked to idiopathic infertility has been reported to date (8). Clinical testing does not currently carry out a detection of *CATSPER* abnormalities. *CATSPER* abnormalities may therefore represent an underdiagnosed cause of male infertility (8).

As *STRC* deletions associated with *CATSPER 2* deletions make up for more than one-third of STRC abnormalities (9), the presence of slight to moderate hearing loss associated with infertility should prompt a genetic diagnosis of *STRC* mutation, particularly in the absence of any other cause of infertility in both members of the couple.

Acknowledgments

The authors thank the donor and patient for their willingness to provide information and sperm samples. This study was supported by Germethèque BioBank (France – Toulouse site), which provided samples (BB-0033-00081, CRB Germethèque, Toulouse, France [Germethèque number 20220807]). The authors thank the genetic laboratory of the University Hospital of Poitiers, France, for performing blood molecular genetic analysis.

Declaration of Interests

S.G. reports that the doctoral salary was supported by Medical Research Foundation (FRM – FDT202204014931) and the National Research Institute for agriculture, feed and environment (INRAE). C.G. has nothing to disclose. L.T. has nothing to disclose. J.M. has nothing to disclose. M.C. has nothing to disclose. F.I. has nothing to disclose. É.H. has nothing to disclose. C.R. has nothing to disclose. N.V. has nothing to disclose. C.D. has nothing to disclose. C.B. has nothing to disclose. N.G. has nothing to disclose.

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