REPRODUCTION FUNCTION IN MALE PATIENTS WITH BARDET BIEDL SYNDROME

Koscinski Isabelle\textsuperscript{1,2,*}, Mark Marc\textsuperscript{3,4}, Messaddeq Nadia\textsuperscript{3}, Braun Jean-Jacques\textsuperscript{5}, Celebi Catherine\textsuperscript{4}, Muller Jean\textsuperscript{6,7}, Zinnetti-Bertschy Anna\textsuperscript{8,9}, Goetz Nathalie\textsuperscript{10}, Dollfus Hélène\textsuperscript{6,10}, Rossignol Sylvie\textsuperscript{6,11}

1 Laboratoire de Biologie de la Reproduction/CECOS Lorraine, Hôpitaux universitaires de Nancy, France

2 Université de Lorraine, Inserm, NGERE, F-54000 Nancy, France

3 Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch-Graffenstaden, France

4 Laboratoire de Biologie de la Reproduction, Hôpitaux universitaires de Strasbourg (HUS), Strasbourg, France.

5 Service ORL et CCF, Hôpital de Hautepierre, Hôpitaux universitaires de Strasbourg (HUS), Strasbourg, France.

6 Laboratoire de Génétique Médicale, Institut de Génétique Médicale d’Alsace (IGMA), INSERM U1112, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France

7 Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

8 Pôle de Psychiatrie, Santé Mentale et Addictologie, Hôpitaux Universitaires de Strasbourg, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France

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9 Neuropsychologie cognitive et physiopathologie de la schizophrénie, Unité de recherche INSERM U1114, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France

10 Service de Génétique Médicale, centre de référence pour les maladies ophtalmiques rares (CARGO), Hôpitaux Universitaires de Strasbourg, Strasbourg, France

11 Service de Pédiatrie, Hôpital de Hautepierre, Hôpitaux Universitaires de Strasbourg, Strasbourg, France

*corresponding author, person to whom reprint requests should be addressed.
Reproduction function in male BBS patients

Disclosure summary:

This study explores each step of reproduction in a cohort of 11 Bardet-Biedl male patients, from embryology of genitalia to hormonal status, sperm analysis and psychological profile, discussing the hypothetical impact of the ciliopathy.

Disclosure Statement: The authors have nothing to disclose.

Trial registration number: PHRC 2007 – ref HUS No. 4056
Abstract

Purpose: Bardet-Biedl syndrome (BBS) is a ciliopathy with a wide spectrum of symptoms due to primary cilia dysfunction including genitourinary developmental anomalies as well as impaired reproduction particularly in males. Primary cilia are known to be required at the following steps of reproduction function: (1) genitourinary organogenesis, (2) in the fetal firing of hypothalamo-pituitary axe, (3) sperm flagellum structure and (4) the first zygotic mitosis conducted by proximal sperm centriole. BBS phenotype is not fully understood.

Methods: This study explored all steps of reproduction in 11 French male patients with identified BBS mutations.

Results: BBS patients presented frequently genitourinary malformations as cryptorchidism (5/11), short scrotum (5/8), micropenis (5/8) but unexpectedly, normal testis size (7/8). Ultrasonography highlighted epididymal cysts or agenesis of one seminal vesicle in some cases. Sexual hormones levels were normal in all patients except one. Sperm numeration was normal in 8 out of the 10 obtained samples. Five to 45% of sperm presented a progressive motility. Electronic microscopy did not reveal any homogeneous abnormality. Moreover, a psychological approach pointed a decreased self-confidence linked to blindness and obesity explaining why so few BBS patients express a child wish.

Conclusions: PC dysfunction in BBS impacts embryology of the male genital tract, especially epididymis, penis and scrotum through an insufficient fetal androgen production. However, in adults, sperm structure does not seem to be impacted. These results should be confirmed in a greater BBS patient cohort, focusing on fertility.
No competing interests.

Trial registration number: PHRC 2007 – ref HUS No. 4056

Precis:

Bardet-Biedl ciliopathy impacts morphogenesis of male genital tract, but also penis and scrotum through a low fetal production of androgens. Nevertheless, sperm structure does not seem to be impacted.
Introduction

The multisystem involvement of the Bardet-Biedl syndrome (BBS, OMIM 209900) is related to the pathogenesis well recognized as a ciliopathy. Its estimated prevalence in North America and Europe is ranging from 1:140,000 to 1:160,000 live births. In the last 20 years, 22 genes have been implicated in the BBS. All of them participate in primary cilia function by coding for proteins involved in the formation of the BBSome, the chaperonin complex or the basal body with an essential role in the intraflagellar traffic.

BBS phenotype is characterized by retinal dystrophy, obesity, renal dysfunction, learning difficulties, genital anomalies and postaxial polydactyly (a pathognomonic sign in this context). Hypogonadism is part of the major diagnosis criteria in males. The generic term of hypogenitalism is often used in clinical series and micropenis, cryptorchidism, and delayed puberty are frequently reported. However, hormonal and histological data are scarce and the origin of this hypogonadism (primary or hypogonadotropic) remains unclear. Large BBS series stipulate that males are almost invariably infertile and only a few males with descendant are reported. By way of a confused extrapolation to the Kartagener syndrome, another ciliopathy (affecting motile cilia), BBS patients are supposed to be infertile by producing immotile spermatozoa.

Moreover, the potential impact of PC on fertility is considered to result from the role of cilia structures at different steps of human reproduction function and from a partial common ultrastructural architecture of all cilia.

The present study explores the reproduction function in a cohort of BBS male patients with identified mutations within different BBS genes. The results are interpreted in the
context of organogenesis of male genital organs, hormonal secretions of hypothalamic-pituitary-gonadal axis, spermatogenesis, and sexual/reproductive behavior. To our knowledge, this is the first specific study of BBS male patients’ reproduction function, contrasting with the presumption that BBS patients are infertile.
Subjects and methods

Subjects were recruited by the reference center for rare eye diseases at the Strasbourg University Hospital, France (CARGO) and were explored for fertility by the Centre of Medicine and Biology of Reproduction at the Strasbourg University Hospital, France. In total, 11 adult male BBS patients underwent a complete exploration of the gonadotropic axis including clinical examination, comprehensive male hormonal panel testing, including a GnRH stimulation test (0.1 mg Relefact; Sanofi-Aventis, Frankfurt am Main, Germany), urinary-genital ultrasonography, standard sperm analysis with a modified David classification of morphological anomalies, and Electron Transmission Microscopy whenever sperm count was high enough. The clinical examination comprised (1) the palpation of testes to evaluate scrotum length and testes volume, (2) an examination of the penis to evaluate the presence of micropenis (when the length of flaccid penis was less than 2.5 SD - measurement taken from the pubic ramus to the distal tip of the gland), (3) the evaluation of the secondary sexual characteristics, (4) the presence of gynecomastia. This exploration was a part of the French National Research Protocol ethically approved by CPP ‘EST IV’ (Strasbourg, France) (PHRC National Bardet-Biedl 2007 IDRCB 2007-A00868-45); it included also ophthalmic, olfactive, endocrine and psychological explorations. Furthermore, one patient consulted with his wife for infertility and underwent an Assisted reproductive technology (ART) program comprising 3 intra-uterine Artificial Inseminations and one In Vitro Fertilization (IVF) assisted by Intra Cytoplasmic Sperm Injection (ICSI).
Mutation analysis

Genomic DNA was isolated and explored as previously described either by direct Sanger sequencing or by high-throughput sequencing\textsuperscript{16}.

Sperm analysis:

All sperm analyses were performed in accordance with the World Health Organization recommendations\textsuperscript{17}; the morphology was analyzed using David modified criteria\textsuperscript{18}.

Electronic microscopy:

Spermatozoa were fixed at 4°C in 2.5% glutaraldehyde for 2 hours, then centrifuged. The pellet was washed in sodium cacodylate buffer 0.1M, then post-fixed in 1% osmium tetroxide in cacodylate buffer and washed again. After a progressive dehydration in ethanol (50°-70°-95°-absolute (3times) – propylene oxide (3times), each step of 10 minutes at room temperature, the pellet was embedded in epoxy (Epon) resin and ultrathin sections were obtained.

Olfactory evaluation:

A senior Ear Nose Throat (ENT) specialist evaluated all BBS patients according to the same protocol as described in Braun \textit{et al.}, 2014 \textsuperscript{19} (1) clinical evaluation of olfaction, (2) ENT examination with nasal endoscopy and (3) olfactometry using two different psychophysical methods, namely a suprathreshold evaluation of the olfaction and the UPSIT (Sensonics Inc., Haddon Heights, NJ) \textsuperscript{20-22}. 


Verbal intelligence quotient evaluation:

As described in Braun et al.\textsuperscript{19}, the estimated verbal intelligence quotient (VIQ) has been calculated on the basis of the administration of subscales from the Wechsler Adult Intelligence Scale (WAIS-III)\textsuperscript{23}.
Results

The cohort analyzed in this study is composed of 11 male patients with a clear clinical and molecular diagnosis of Bardet-Biedl syndrome. Patients were between 18 and 39 years old. The main elements of their medical history, clinical examination as well as genotype are summarized in Table 1. According to the recognized features of the BBS, all patients of this cohort presented primary and secondary features of BBS: all presented with rod-cone dystrophy. Polydactyly or brachydactyly was observed for 9 out of the 11. Similarly, 8 out of the 11 patients were obese and 3 of them had morbid obesity. Moreover, two patients had hyperinsulinism with glucose intolerance and one was diabetic. Interestingly, 7 out of 10 patients evaluated for olfaction presented an anosmia or severe hyposmia.

Genital examination and hormonal status assessment were part of the protocol, however, 3 patients refused external genitals clinical examination. Andrological data are summarized in Table 2 and Hormonal status in Table 3: BBS patients presented frequently a non-descended testis history (5 out of 11, 3 unilateral and 2 bilateral) and/or a short scrotum at adult age (5 out of 8). The length of penis was much decreased (<-2.5 SD) in 5 out of the 8 examined patients, 3 had a normal length, and 1 presented an abnormally curved penis. Unexpectedly, the size of the testes was normal (7 out of 8). They all reported a normal spontaneous puberty. The systemic androgenic effects, evaluated by the general body hair and sexual androgen dependent body hair, was normal in 7 out of 8 examined patients.

Genito-urinary ultrasonography was performed in 5 patients and highlighted abnormalities such as a cyst of prostatic utricle (one case) or epididymal cysts (one case) and or unilateral agenesis of seminal vesicle (3 out of 5 cases) that could be...
related to extreme hypospermia (<0.5 mL in two cases). Kidney cysts or ecstasies were found in 7 out of 11 patients (2 with normal semen volume, 4 with decreased semen volume and one patient with failure of semen collection).

Testosterone and gonadotropin levels (Table 3) were not interpretable in patient 8 since he was under testosterone therapy: he was then considered as having a hypogonadism (previous hormonal status not available). For the other patients, testosterone was low only in 2 cases out of 8 (2 missing data) and basal gonadotrophins were all in normal range (1 missing data). For all tested patients, the pituitary response to a GnRH stimulation test was normal (9/9). The Testosterone/ LH ratio was calculated for 8 out of 11 patients and ranged from 1.58 to 5.51.

Sperm analysis was performed in 10 out of the 11 patients (Table 4) as one failed to collect his semen (only 0.12mL of glandular secretion without epididymal fraction of ejaculate). A decreased semen volume was observed in 6 out of 10 other patients (median of 1.2mL [0.1; 12.8], and a very low semen volume (<0.5 mL) was observed in 3 out of 10 patients performing a semen collection. Sperm numeration was normal in 8 out of 10 patients (median of 72 x10^6[0; 485 x10^6], and sperm progressive motility was comprised between 5 and 45% (median of 23%). The teratozoospermia was moderately increased (median of 8% of typical forms [2; 28]), and abnormalities were heterogeneous (, as confirmed by TEM : more than 80% of the spermatozoa displayed severe morphological alterations of the head (e.g., large nuclear vacuoles) and/or the midpiece (e.g., disorganized mitochondrial sheath) which were apparently combined at random. TEM analysis also revealed that the 9+2 microtubules pattern of the axoneme was preserved in most of transverse sections through the midpiece or principal piece. Therefore, these spermatozoa were without any specific anomaly of the axoneme, having notably a
central pair of microtubules as well as para-microtubular proteins like dynein arms (Figure 1).

The \textit{BBS1} gene was implicated in 6 out of the 11 cases. Other cases comprised homozygous mutation in \textit{BBS3/ARL6, BBS5, BBS9, BBS12} or compound heterozygous mutations in \textit{BBS10}. Regarding the reproductive function no genotype to phenotype association could be observed in our cohort. Even between siblings, variations of the phenotype were observed: only one of the siblings, with the same homozygote mutation of \textit{BBS1} (c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg]), reported a cryptorchidism history. Another patient bearing the same mutation presented a severe decrease of semen volume (0.3mL).

The psychological evaluation of patients (table 1 and 5) uncovered a moderate mental retardation with a Verbal Intellectual Quotient varying between 60.5 and 102 (median 78.5) associated to a frequent lack of self-confidence mainly due to their obesity. Two patients lived with a partner; only one consulted for infertility and the couple underwent ART.
Discussion

Among the main phenotypic features of BBS hypogenitalism/hypogonadism is often used as a generic term, encompassing cryptorchidism, short scrotum, micropenis and low testicular volume that also has suggested associated infertility. If the literature reports frequently genital anomalies, in contrast, hormonal assessment is rarely performed. In some cases of BBS, the presence of hypogonadotropic hypogonadism was concluded, while in others the presence of hypogonadism of testicular origin, or both mechanisms were reported. Of note, in these reports, the diagnosis of BBS was only clinically assessed, and some patients were prepubertal or young adolescents.

In order to document this part of the syndrome, we have systematically analyzed the reproductive function of 11 males BBS patients. As illustrated in tables 2-3-4, BBS patients present an important variability of features (clinical and biological) linked to fertility.

Five patients had a history of bilateral (n=2) or unilateral cryptorchidism and 4 of them exhibited a micropenis and short scrotum. This association strongly suggests a congenital hypogonadotropic hypogonadism (CHH) with insufficient hypothalamic-pituitary induced androgen secretion after midgestation that is normally responsible for inguino-scrotal testes descent and penile growth. The neuro-endocrine regulation of GnRH hypothalamic release is quite complex and results from the interplay of activating and inhibitory inputs onto GnRH neuron during fetal life. The recent description of the fundamental role of hypothalamic kisspeptin and its receptor Kiss1r (previously named GPR54) on the GnRH secretion brought a major advance in the understanding in this field (for an extensive review, see). The regulation of GnRH release by kisspeptin signaling appears crucial for the functional integrity of the gonadotropic axis during
fetal life, for pubertal onset, as well as to maintain fertility in adults. Interestingly, in the mouse brain, primary cilia of GnRH neurons are enriched in Kiss1r and Kiss1r activity, in response to kisspeptin binding, is enhanced by the presence of cilia on GnRH neurons. This signal amplification appears determinant in the firing rate of fetal GnRH neurons, especially in males. Koemeter-Cox et al. also showed that the percentage of GnRH neurons displaying at least one Kiss1r-positive cilium was 75% in both sexes at birth and was stable during the life. However, quantifying the percentage of GnRH neurons possessing more than one Kiss1r positive cilium revealed that the frequency of multiciliate GnRH neurons significantly increases during postnatal development (from 10% at birth to 42% at P60) in parallel with sexual maturation. In Human as well as in rodents, signaling in the cilium results of clustering of receptors in the ciliary membrane. Any dysfunction of primitive cilia in BBS patient could explain a decreased activity of KISS1R signaling pathway during fetal and early post-natal life leading to micropenis and undescended testis.

This CHH seems reversible in most cases since all patients presented a spontaneous puberty, normal secondary sexual characteristics, and normal testicular volume. Testosterone levels at adult age were indeed normal in all except 2 patients with relatively low testosterone and one with testosterone replacement for years. Reversal of CHH occurs in 10 to 20% of patients, which challenges the dogma that the condition is lifelong. Desai et al. already described a case of reversible hypogonadotropic hypogonadism in a BBS man. We propose the hypothesis that in BBS patients, the increase of cilia number on GnRH neurons during puberty, as observed in mice, leading to an increase in KISS1Rs number might overcome the individual signal reduction and allow a normal puberty and normal adult gonadotropic axis in most of cases. Many case reports focused on pubertal delay. Unfortunately, exact timing and tempo of
puberty could not be assessed in our patients since it was a retrospective declarative data. Following along the lifespan testicular function in theses patient could be interesting, since cryptorchidism and obesity can both impact on gonadal function, as suggested by the lowest ratio of testosterone/LH observed in the oldest patient of our series.

The observation of frequent cystic formation in male genital tract (epididymis, seminal vesicle and prostate) can be explained by the fact that epithelial cells of genital tract, accessory gland and kidney cells share a similar meso/meta-nephrotic embryonic origin and expression of primitive cilia. The high rate of kidney cyst observed in BBS patients is in favor of this hypothesis (7 out of 11 in our series, according with the literature 16). We hypothesize that cyst formation in the male genital tract of BBS patients results from a physio-pathological mechanism similar to that described in Polycystic Kidney Disease (PKD): cysts formation would result from a dysfunction of the dimer polycystin 1-polycystin 2 (PC1-PC2) 11,41,42, inducing an excessive cell growth/proliferation and secretion. According with a recent discussion about the role of BBS proteins in the cystogenesis 43, we presume that, in ciliated epididymal cells like in kidney cells, BBSome interacts with PC1 to stabilize the complex (Figure 2). Expression of a pathogenic BBS3/Arl6 mutant (T31R) locks Arl6 in the GDP form leading to stunted cilia and inhibition of PC1 on primary cilia 44.

The diminution of semen volume appears to be due more to a partial obstruction of the genital duct by cysts of genital tract, rather than to hypogonadotropic hypogonadism not observed in our series. In patient 8, ultrasonography revealed the presence of a cyst of prostatic utricle, which is an embryologic Müllerian remnant and should not be confused with other ciliopathy related cysts of the genital tract, which could also affect
the prostate. Another cause of hypovolemia is the unilateral agenesis of a seminal vesicle, potentially related to the prenatal embryogenesis of this gland \(^{45,46}\), which begins by GW 14 to 16 from the Wolffian duct, and is dependent on testosterone. The prenatal defect of fetal testosterone production linked to the prenatal hypogonadotropic hypogonadism in BBS patients could explain this rather frequent abnormality (2 patients out of 5 patients explored by ultrasonography).

Furthermore, this study discloses the conserved motility of the spermatozoa in a great proportion of the BBS patients studied as the integrity of the 9 peripheral + 1 central microtubules doublet is conserved. The variable asthenozoospermia observed (5 to 45% of progressive motility) in the series could rather be due to the poor spermatogenesis conditions due to the short scrotum and the obesity disturbing scrotal thermoregulation \(^{47}\). The notion of infertility in BBS patients linked to a suspected major asthenozoospermia results from a confusion between motile cilia and primary cilia. Conserved sperm motility as well as results of ART performed with spermatozoa of one patient suggest a normal functionality of sperm flagellum and proximal centriole, even if all cilia structure share common some structural elements and common mechanism of genesis \(^{11,33}\).

The functionality of the sperm centriole has been checked in patient 3 through the IVF attempt which succeeded in the birth of a healthy child.

The absence of obvious correlation between genotype and phenotype of BBS patients has been already mentioned in a more general context, not focused on reproduction \(^{16}\). The most frequent mutation (single missense mutation of exon 12 of \(BBS1\) (p.M390R)), known to induce sometimes a mild phenotype seems to lead, in our series, to moderate
abnormalities of genitalia, suggesting a moderate fetal hypothalamo-pituitary impairment and finally a moderate fetal androgen deficit.

Abnormal tail morphologies of the spermatozoa were observed often in our series (patient 1, patient 3, patient 6 and patient 10) without significant impact on their motility. In addition, the structure of the axoneme in our patients was not specifically disturbed in Electron Transmission Microscopy. These observations contrast with those in mice carrying similar mutations: spermatozoa of Bbs1 (M390R/M390R) knockout mice presented no flagellum even if the cilia of these mice presented a normal axonemal structure, including 9 peripheral microtubules doublets + 1 central doublet arrangement of axonemal microtubules, and elongated cilia with abnormally swollen distal ends suggesting the mutation may impair the completion of flagella assembly. Similarly, Bbs4-null mice develop normally their somatic motile and primary cilia, suggesting that Bbs4 is dispensable for global cilia genesis, but interestingly, male Bbs4-null mice do not form spermatozoa flagella, suggesting a difference in the formation of the different types of tails. Other Bbs null mice (Bbs2, Bbs7) present also disturbed sperm flagellum synthesis. Although BBS6 belongs to another family of BBS proteins, characterized as chaperonins, Bbs6-null mice present also impaired spermatozoa tail synthesis. In 2008, Walsh et al. highlighted an important role of chaperonin proteins in the fusion mechanism of acrosomal function (acrosomal building by proacrosomal vesicles fusion and acrosomal reaction by acrosome membrane fusion to plasma membrane of sperm head). Since several BBS proteins interact with other chaperonin proteins, it is possible that mutations of BBS proteins of the chaperonin-like family have an impact on sperm acrosomal function and thus on spontaneous fertility. In
this case, the ART solution would consist in carrying out an ICSI-IVF (which is what was performed for the patient consulting for infertility in our series).

**Psychology and implications in the sexual behaviour of BBS**

Intellectual disability is a variable feature of BBS and like in earlier reports\textsuperscript{54,55}, our patients have their VIQ close to 80 (60.5-102, mean = median = 78.5). Nevertheless, all of them except one had a professional activity (from groom to telephone advisor). Similarly to the observation made by Kerr et al.\textsuperscript{54}, we observed emotional immaturity in some BBS patients, with frequent inappropriate emotional outbursts but, in contrary, no disinhibited behavior or inability to recognize social cues.

Handicap linked to blindness, frequent lack of self-confidence mainly due to obesity, associated to these psychological traits could explain the low proportion of BBS patients living with a partner (2 out of 11 in our series) and consulting for child wish (only one out of 11).

**Conclusion**

BBS is a pleiotropic syndrome affecting reproductive prognostic through: (1) fetal hypogonadotropic hypogonadism resulting in micropenis, short scrotum and cryptorchidism, (2) variable impairment in spermatogenesis due to the proximity of testes to abdomen (3) susceptibility to cystic formation of genital tract leading to partial obstruction of genital ducts, and (4) sexual/reproductive behaviour linked to psychological traits of BBS patients.

However, in contrast with the literature we suggest that: (1) the hypothalamic-pituitary-gonadal axis can function normally in adults despite a frequent severe obesity,
(2) the sperm motility can be normal and (3) sperm is able to fertilize mature oocyte and the centriole is able to conduct the first zygote divisions.

Very few cases of spontaneous fatherhood are reported, maybe more linked to an impaired sexual behaviour. Our study is reassuring about the possibility for male BBS patients to benefit of reproductive medicine care.

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Legend of Figures

**Figure 1:** Sperm morphology in BBS patients

1-4: Harris Shorr staining, from different patients: 1: Patient 2; 2: patient 8; 3: patient 6; 4: patient 9. NS: Normal Spermatozoa; IF: Isolated Flagellum; IH: Isolated Head; CF: coiled Flagellum; MF: MultiFlagellar sperm; ST: Short Tail; RS: Round Spermatid; EMP: Enlarged middle piece. The proportion of Isolated Flagella (IF) is increased. Coiled flagellum, short tail, enlarged middle piece suggest an impaired spermiogenesis.

A-F. Electron microscopy analysis of spermatozoa. (A,B) and (C-F) correspond to two different patients. (A and C-F) Commonly observed defects include: acrosomes detached from the nucleus (Ac in A,C,E) and malformed (Ac in E), excess of cytoplasm in the head (Cy in A,C,E) and flagellum (Cy in D,F), disorganized mitochondrial sheaths (Mi, in C,D) and large nuclear vacuoles (Va, in C,E). (B,D,F) The majority of the cross sections of axonemes show a normal 9 plus 2 microtubules pattern and well-preserved dynein arms (arrowheads in B). Note that abnormalities of axonemes in the patients spermatozoa (e.g., absence of the central doublet of microtubules; not illustrated here) are almost systematically associated with poor preservation of the adjacent mitochondria, therefore strongly suggesting that they reflect a state of cellular necrosis.

**Figure 2:** Main mechanisms involved in the cystogenesis

A: In epithelial kidney or epididymal cells, primary cilia constitute a reserve of polycystin 1 (PC1) and polycystin 2 (PC2). PC1 assembly with PC2 to form a dimere
inducing from the endoplasmic reticulum an exit of calcium. A high concentration of cytoplasmic Ca2+ represses Adenylate Cyclase VI (AC VI). The AC VI produces cAMP stimulating AMPk pathway and PKA pathway which are resulting in a stimulation of cell proliferation and CFTR-linked chlore secretion respectively. BBSome of the primary cilia stabilizes the PC1 complex and allows a functional dimerisation with PC2.

B: An anomaly of BBSome decreases the stability of PC1-PC2 dimere, resulting further in an insufficient Ca2+ intracytoplasmic concentration, an insufficient repression of AC VI and finally to an excessive cell proliferation and excretion of chlore and fluid through the CFTR. Excessive cell proliferation and excessive fluid secretion lead to cyst formation.
Table 1: Clinical and genetic data for our cohort. Mutations in patient 1 and 10 affect the splicing of their respective gene.²⁴,²⁵ VIQ: Verbal Intelligence Quotient, NA: not available

| Patient | Age (years) | Mutation | Rod-cone dystrophy | Hands/feet anomalies | BMIs | Micropenises | Cryptorchidism | Kidney cysts or ectasia | Diabetes | Glucose intolerance | Olfaction defect | VIQ |
|---------|-------------|----------|--------------------|---------------------|------|--------------|---------------|----------------------|----------|---------------------|----------------|-----|
| 1       | 22          | BBS1: c.[479G>A];[479G>A], p.[Arg160Gln];[Arg160Gln] | yes | brachydactyly | >3 | 0 | NA | no | no | no | mild hyposmia | 60.5 |
| 2       | 25          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg] | yes | no | <3 | 0 | no | no | no | no | normal | 64 |
| 3       | 24          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg] | yes | no | <3 | 0 | no | right | no | no | severe hyposmia | 62 |
| 4       | 36          | BBS1: c.[382C>T];[382C>T], p.[Gln128*];[Gln128*] | yes | polydactyly | >3 | 0 | yes | bilateral | no | no | NA | NA |
| 5       | 24          | BBS5: c.[413G>C];[413G>C], p.[Arg138Pro];[Arg138Pro] | yes | polydactyly | >3 | 0 | yes | moderate | right | yes | no | severe hyposmia | 93 |
| 6       | 35          | BBS10: c.[271dup];[963T>G], p.[Cys911efuls*5];[Tyr321*] | yes | polydactyly | >4 | 0 | yes | bilateral | yes | no | anosmia | 90 |
| 7       | 38          | BBS1: c.[1169T>G];[1214_1215i]ns[MT113356];[Met390Arg];[Ala406Glnfs*47] | yes | polydactyly | >2 | 5 | NA | no | yes | no | severe hyposmia | 62 |
| 8       | 22          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg] | yes | polydactyly | >4 | 0 | yes | no | yes | no | moderate hyposmia | 94.5 |
| 9       | 26          | BBS9: c. [703_707del];[832C>T], p.[Val235Phefs*6];[Arg278*] | yes | polydactyly | >4 | 0 | no but angulation | no | yes | mild for | yes | anosmia | 102 |
| 10      | 18          | BBS3: c.[535G>A];[535G>A], p.[Asp179Asn];[Asp179Asn] | yes | polydactyly | >3 | 0 | NA | no | yes | yes | anosmia | 88.5 |
| 11      | 39          | BBS12: c.[1037T>C];[1037T>C], p.[Ile346Thr];[Ile346Thr] | yes | polydactyly | >3 | 5 | yes | left | yes | yes | anosmia | 68.5 |

Total patients Mean 25

11/11 9/11 8/11 5/8 5/11 7/1 3/11 9/10 Median 78.5
Table 2: Andrological data

| Patient | Age (years) | Mutation                                                                 | Cryptorchidism | Micropenis | Testis size | Scrotum length | Genito-urinary ultrasonography | Kidney ultrasonography (kidney cysts or ectasia) |
|---------|-------------|---------------------------------------------------------------------------|----------------|------------|-------------|----------------|-------------------------------|---------------------------------------------|
| 1       | 22          | BBS1: c.[479G>A];[479G>A], p.[Arg160Gln];[Arg160Gln]                       | no             | Examination not performed | Examination not performed | normal                       | no                            |
| 2       | 25          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg]                    | no             | no         | normal      | normal         | not performed                | no                            |
| 3       | 24          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg]                    | right          | no         | normal      | normal         | not performed                | no                            |
| 4       | 36          | BBS1: c.[382C>T];[382C>T], p.[Gln128*];[Gln128*]                           | bilateral      | yes        | normal      | short          | right seminal vesicle non observed; right epididymal cyst | no                            |
| 5       | 24          | BBS5: c.[413G>C];[413G>C], p.[Arg138Pro];[Arg138Pro]                      | right          | yes moderate | normal      | short          | right epididymal cyst      | yes                            |
| 6       | 35          | BBS10: c.[271dup];[963T>G], p.[Cys91Leufs*5];[Tyr321*]                     | bilateral      | yes        | normal      | normal         | right seminal vesicle non observed; left varicocele; bilateral epididymal cysts | yes                            |
| 7       | 38          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg]                    | no             | not performed | not performed | not performed | not performed                | yes                            |
| 8       | 22          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg]                    | no             | yes        | normal      | very short     | enlargement of prostatic utricle | yes                            |
| 9       | 26          | BBS9: c.[1703-?_886+?del];[832C>T], p.[Val235Phets*6];[Arg278*]           | no             | no but angulation | normal      | short          | not performed                | yes mild form                  |
| 10      | 18          | BBS3: c.[535G>A];[535G>A], p.[Asp179Asn];[Asp179Asn]                      | no             | not performed | not performed | not performed | not performed                | yes                            |
| 11      | 39          | BBS12: c.[1037T>C];[1037T>C], p.[Ile346Thr];[Ile346Thr]                   | left           | yes        | bilateral    | moderate       | not performed                | yes                            |
Table 3: Hormonal status. * Patient 8 under testosterone therapy

| Patient | Age (years) | Mutation | Basal FSH IU/l [1.5-12] | FSH at the peak (after GnRH stimulation) [1.5-2 x basal] | Basal LH IU/l [1.5-8.5] | LH at the peak (after GnRH stimulation) [3-4 x basal] | Basal total testosterone nmol/l [10.4-41.6] | Testosterone/LH (nMol / IU) | PRL mIU/l [86-324] | Leptin (µg/l) |
|---------|-------------|----------|------------------------|----------------------------------------------------------|-------------------------|-----------------------------------------------|--------------------------------|---------------------------|------------------------|------------------|
| 1       | 22          | BBS1: c. [479G>A] [479G>A], p.[Arg160Gln][Arg160Gln] | 2.42 | 4.65 | 4.71 | 29.3 | 16.3 | 3.46 | 239 | 21.2 |
| 2       | 25          | BBS1: c. [1169T>G];[1169T>G], p.[Met390Arg][Met390Arg] | 2.67 | 5.02 | 2.94 | 20.8 | not performed | not performed | not performed | not performed | not performed |
| 3       | 24          | BBS1: c. [1169T>G];[1169T>G], p.[Met390Arg][Met390Arg] | 5.37 | 7.15 | 3.21 | 15.7 | 17.7 | 5.51 | 176 | not performed |
| 4       | 36          | BBS1: c. [382C>T];[382C>T], p.[Gln128*][Gln128*] | not performed | not performed | not performed | not performed | not performed | not performed | not performed | not performed |
| 5       | 24          | BBS5: c. [413G>C];[413G>C], p.[Arg138Pro][Arg138Pro] | 3.26 | 5.94 | 3.64 | 20.6 | 13.8 | 3.79 | 134 | 12.6 |
| 6       | 35          | BBS10: c. [271dup];[963T>G], p.[Cys91Leufs*5][Tyr321*] | 2.08 | 3.69 | 3.38 | 12.5 | 12.8 | 3.77 | 106 | not performed |
| 7       | 38          | BBS1: c. [1169T>G];[1214_1215insMT113356], p.[Met390Arg][Ala406Glnfs*47] | 3.51 | 6.37 | 4.11 | 26.9 | 11.1 | 2.70 | 94.7 | 4.78 |
| 8       | 22          | BBS1: c. [1169T>G];[1169T>G], p.[Met390Arg][Met390Arg] | 0.85 | not performed | 0.71 | not performed | 18.7 | Not applicable | 295 | 19.8 |
| 9       | 26          | BBS9: c. [703-7_886-8del];[832C>T], p.[Val235Phefs*6][Arg278*] | 1.56 | 3.16 | 1.66 | 12 | 6.9 | 4.15 | normal | 50.4 |
| 10      | 18          | BBS3: c. [535G>A];[535G>A], p.[Asp179Asn][Asp179Asn] | 2.84 | 8.46 | 3.57 | 51.8 | 8.6 | 2.4 | 72.1 | not performed |
| 11      | 39          | BBS12: c. [1037T>C];[1037T>C], p.[Ile345Thr][Ile345Thr] | 9.41 | 20.5 | 7.02 | 43 | 11.1 | 1.58 | 209 | not performed |
| Patient | Age (years) | Semen volume (mL) | pH | Viscosity | Numeration spz/Ejaculate (N >39 x 10^6) | Progressive motility (%) | Total motility (%) | Vitality (%) | Morphology |
|---------|-------------|------------------|----|-----------|--------------------------------------|--------------------------|---------------------|-------------|------------|
|         |             |                  |    |           |                                      |                          |                     |             |            |
| 1       | 22          | 12,8             | 6  | normal    | 0,09 x10^6                          | 0                        | 0                   | Not performed | 10/19 enrolled tail, 18/19 abnormal acrosome, 1/20 TF |
| 2       | 25          | 1,2 (da=2j)      | 8  | Normal    | 324 x 10^6                          | 45                       | 50                  | 69           | 6% enrolled tail, 68% abnormal acrosome; 11% TF; isolated tails: 17% of observed spermatozoa |
| 3       | 24          | 2,3              | 7,8| Normal    | 138 x10^6                           | 25                       | 30                  | 61           | 32% enrolled tail, 86% abnormal acrosome; 2% TF; isolated tails: 85% of observed spermatozoa |
| 4       | 36          | 6,5              | 6,8| Normal    | 15                                   | 0                        | 0                   | 0            | not interpretable |
| 5       | 24          | 0,12            | 6,3| Normal    | 0                                    | Not applicable           | Not applicable      | Not applicable | Not applicable |
| 6       | 35          | 0,3              | 7,8| Normal    | 79,2 x10^6                          | 37                       | 52                  | >60          | 50% abnormal tail, 11 à 20% TF |
| 7       | 38          | 0,3              | 8,1| Normal    | 217 x10^6                           | 20                       | 30                  | >30          | 28% TF |
| 8       | 22          | 2,7              | 6,6| Normal    | 32,1 x10^6                          | 9                        | 11                  | 33           | 13% multitailed sperm; 14% enrolled tail; 15% isolated tails; 4% TF |
| 9       | 26          | 0,9 (DA=2j)      | 7,5| Normal    | 62,3 x 10^6                         | 5                        | 10                  | 66           | 8% TF; 6% isolated tails |
Table 4: Sperm analysis. * Patient 5 failed to produce ejaculate.

| Patient | Sperm Analysis | Motility | Count | Abnormalities |
|---------|----------------|----------|-------|---------------|
| 10      | 18             | Normal   | 481 x 10^6 | 39% abnormal tail; 8% TF; <5% isolated tails |
| 11      | 39             | Normal   | 72 x 10^6  | 7% TF 82% abnormal acrosome; 12% isolated tails |
### Table 5: Psychological concerns

| Patient | Age (years) | Mutation | BMI | blindness | VIQ | profession | living with a partner | child wishing |
|---------|-------------|----------|-----|-----------|-----|------------|-----------------------|---------------|
| 1       | 22          | BBS1: c.[479G>A];[479G>A], p.[Arg160Gln];[Arg160Gln] | >3 0 | yes       | 60,5 | baker      | no                    | no            |
| 2       | 25          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg] | <3 0 | yes       | 64   | telephon advisor | no                    | no            |
| 3       | 24          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg] | <3 0 | No | 62 | telephon advisor | yes | yes          |
| 4       | 36          | BBS1: c.[382C>T];[382C>T], p.[Gln128*];[Gln128*] | >3 0 | yes | not perform | metallurg y worker | yes | no            |
| 5       | 24          | BBSS: c.[413G>C];[413G>C], p.[Arg138Pro];[Arg138Pro] | >3 0 | yes | 93 | groom | no | no            |
| 6       | 35          | BBS10: c.[271dup];[963T>G], p.[Cys91Leufs*5];[Tyr321*] | >4 0 | yes | 90 | office worker | no | no            |
| 7       | 38          | BBS1: c.[1169T>G];[1214_1215ins[MT113356]], p.[Met390Arg];[[Ala406Glnfs*47]] | <2 5 | yes | 62 | profession | no | no            |
| 8       | 22          | BBS1: c.[1169T>G];[1169T>G], p.[Met390Arg];[Met390Arg] | >4 0 | yes | 94,5 | economy student | no | no            |
| 9       | 26          | BBSS: c. [703-7_886+del];[832C>T], p.[Val235Phefs*6];[Arg278*] | >4 0 | yes | 102 | telephone advisor | no | no            |
| 10      | 18          | BBS3: c.[535G>A];[535G>A], p.[Asp179Asn];[Asp179Asn] | >3 0 | yes | 88,5 | musician (guitarist) | no | no            |
| 11      | 39          | BBS12: c.[1037T>C];[1037T>C], p.[Ile346Thr];[Ile346Thr] | >3 5 | yes | 68,5 | wood sawmill worker | no | no            |
