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TITLE: Novel *ADGRG2* truncating variants in patients with X-linked Congenital Absence of Vas Deferens

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SHORT TITLE: Epidemiology of *ADGRG2* mutations in CAVD patients

KEYWORDS: Male infertility*, ADGRG2, CFTR*, Congenital Absence of Vas Deferens, Obstructive Azoospermia

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ANDR.12744

ABSTRACT:

Background: Congenital Absence of Vas Deferens (CAVD) represents a major cause of obstructive azoospermia and is mainly related to biallelic alteration of the *CFTR* gene, also involved in Cystic Fibrosis. Using whole exome sequencing, we recently identified hemizygous loss-of-function mutations in the Adhesion G Protein-coupled Receptor G2 gene (*ADGRG2*) as responsible of isolated CAVD in the absence of associated unilateral renal agenesis.

Objectives: The objective of this study is to retrospectively perform *ADGRG2* sequencing on a large cohort of patients with CAVD and 0 or only 1 *CFTR* defective allele identified after comprehensive testing in order to (i) define more precisely the spectrum and the frequency of *ADGRG2* mutations within Caucasian population (ii) explore the possibility of co-occurrence of *CFTR* and *ADGRG2* mutations.

Materials and methods: We collected 53 DNA samples from CAVD patients with 0 (n=23) or 1 (n=30) alteration identified after comprehensive *CFTR* testing in order to perform *ADGRG2* sequencing. Twenty patients had normal ultrasonographic renal examination and renal status was not documented for 33 patients.

Results: We identified 6 new truncating *ADGRG2* mutations in 8 patients including two twin brothers: c.251C>G (p.Ser84*), c.1013delC (p.Pro338Hisfs*4), c.1460delG (p.Gly487Alafs*9), c.2096dupT (p.Phe700Ilefs*29), c.2473C>T (p.Arg825*) and c.1731_1839+373del (p.Asn578Thrfs*12) which is a 596 base pair deletion affecting the last 5 bases of exon 21 and the whole exon 22. Five of the 8 patients also harbored an heterozygous *CFTR* mutation which we consider as incidental regarding the high penetrance expected for *ADGRG2* truncating variants. The frequency of *ADGRG2* truncating mutation was 26% (5/19 unrelated patients) when presence of both kidneys was attested by ultrasonography and 6.1% (=2/33) among patients with unknown renal status.

Discussion & Conclusion: Our results confirm the interest of *ADGRG2* sequencing in patients with CAVD not formerly related to CFTR dysfunction, especially in absence of associated unilateral renal agenesis.

INTRODUCTION:

Congenital Bilateral Absence of Vas Deferens (CBAVD [MIM : 277180]) is found in approximately 25% of men with obstructive azoospermia and globally accounts for 1-2% of the cases of male infertility (Wosnitzer & Goldstein, 2014; Yu et al., 2012) with a prevalence estimated between 1/1,000 and 1/10,000 (Stuhrmann & Dörk, 2000). CAVD is related to a complete or partial defect of the mesonephric duct derivatives that may include the renal portion in a subset of patients (McCallum et al., 2001). In medical practice, CAVD can be identified in three subgroups of patients: (i) men suffering from Cystic Fibrosis (CF [MIM: 219700]), (ii) apparently healthy men with solitary kidney and (iii) apparently healthy men without kidney abnormalities (referred as "isolated CAVD" in the text).

CF is an autosomal recessive disease caused by biallelic mutations in the Cystic Fibrosis Transmembrane Regulator (*CFTR* [MIM: 602421]) gene, involved in transmembrane chloride and bicarbonate transport in epithelial cells, mainly affecting lungs, digestive tract, sweat glands and vas deferens in men. CF is the most frequent monogenic lethal disease in the Caucasian population with incidence generally comprised between 1/1800 and 1/6000 in European countries (Farrell, 2008). More than 95% of males with CF are infertile due to CAVD (Claustres, 2000) presumably due to a progressive atrophy of the vas deferens, resulting from electrolytes and fluid transport defects, rather than an agenesis (Gaillard et al., 1997).

In apparently healthy men, solitary kidney occurs in approximately 20% of CAVD cases (Augarten et al., 1994; Daudin et al., 2000; Llabador et al., 2015). Contrary to CAVD due to *CFTR* dysfunction, the association of CAVD with solitary kidney is considered as a developmental defect of the early mesonephric or Wolffian duct (McCallum et al., 2001). Currently, the role played by genetic determination in the development of CAVD when associated with solitary kidney remains poorly understood whereas the molecular basis of isolated CAVD was discovered early in the nineties (Dumur et al, 1990) and has been studied extensively. Most of the time isolated CAVD occurs as an autosomic recessive condition with biallelic alterations identified on the *CFTR* gene (the association of two CF-causing mutations, resulting in total or almost total loss of the CFTR protein function, is associated with an early-onset multivisceral phenotype of CF whereas isolated CAVD or others delayed "*CFTR*-related disorders" are caused by combination of a mild mutation with a CF-causing mutation or with a second mild mutation) (Claustres, 2005; Castellani et al., 2008; Bombieri et al., 2011). The proportion of isolated CAVD patients harboring 2 *CFTR* pathogenic variants varies largely among studies and ethnicities, from 22% in India to more than 80% in French population (Sharma et al., 2009; Bareil et al., 2007; Ratbi et al., 2007). Until recently, *CFTR* was the only gene known to cause isolated CAVD and etiology remained uncertain for patients with 0 or only 1 *CFTR* alteration identified after comprehensive *CFTR* screening. In 2016, we reported the identification of loss-of-function mutations in the Adhesion G Protein-coupled Receptor G2 gene (*ADGRG2*, [MIM: 300572]) in 4 patients with isolated CAVD and no *CFTR* mutation identified after complete gene screening (Patat et al., 2016). The *ADGRG2* gene is located on the chromosome X and encodes for an orphan G protein-coupled receptor highly expressed within the efferent ducts. The exact function and ligand

of ADGRG2 remain unknown but strong evidence supports a role in testicular fluid reabsorption and knock-out male mice exhibit an obstructive infertility phenotype (Davies et al., 2004; Zhang et al., 2018). CAVD patients with *ADGRG2* pathogenic mutations were phenotypically indistinguishable of those with *CFTR* mutations suggesting a common physiopathological mechanism (Patat et al., 2016).

In the present study, we aimed to delineate more precisely the mutational spectrum and the frequency of *ADGRG2* mutations in patients with isolated CAVD not formerly related to CFTR dysfunction, including patients without any *CFTR* alteration as well as patients who carry just one heterozygous *CFTR* alteration after comprehensive screening. Indeed, for those heterozygous patients the implication of *CFTR* remains uncertain especially considering the high frequency of *CFTR* disease-causing variants in the healthy population. By including patients with 1 *CFTR* alteration we also aimed to explore the possibility of co-occurrence of one *CFTR* pathogenic mutation with an *ADGRG2* variant. For those purposes, we collected DNA samples from CAVD patients with 0 or 1 *CFTR* potentially pathogenic mutation and no evidence of unilateral renal agenesis among the GenMucoFrance network of laboratories (French laboratories specialized in *CFTR* molecular analysis) in order to perform *ADGRG2* sequencing.

Materials and Methods

Patients

A total of 53 genomic DNA samples collected between 1992 and 2017 were selected by four French laboratories specialized in *CFTR* molecular testing (Bordeaux, Lille, Montpellier and Paris-Cochin) corresponding to 51 apparently healthy infertile men and 2 newborn twin brothers with bilateral absence of the vas deferens discovered during inguinal hernia surgery. A standardized description form was filled out for each patient to collect data regarding *CFTR* analysis, renal ultrasonography, clinical and ultrasound examination of the genital tract, semen analysis and geographic origins. Inclusion criteria were (i) CBAVD confirmed by clinical and/or ultrasound examination or unilateral absence of a vas deferens with azoospermia documented by semen analysis (ii) 0 or 1 potentially pathogenic *CFTR* allele after comprehensive testing including screening of the 27 exons and flanking regions, large rearrangement analysis, promoter analysis and target sequencing of seven deep-intronic mutations (c.870-1113_870-1110delGAAT, c.1585-9412A>G, c.1680-886A>G, c.2989-313A>T, c.3469-1304C>G, c.3718-2477C>T and c.3874-4522A>G). DNA sequencing was performed by Sanger sequencing or Next-Generation Sequencing and large rearrangement analysis by MLPA or QMPSF. Among the 53 patients who fulfilled the inclusion criteria, 23 had no *CFTR* mutations and 30 had one *CFTR* mutation identified. Ten patients were heterozygous for a CF-causing mutation (c.1521_1523del (p.Phe508del) (n=7), c.1657C>T (p.Arg553*), c.870-1113_870-1110delGAAT, c.617T>G (p.Leu206Trp)), 15 were heterozygous for a mild mutation associated with *CFTR*-RD (c.1210-34_1210-6TG[12]T[5] (n=6), c.960A>T (p.Leu320Val), c.[1210-34_1210-6TG[11]T[5](;)1684G>A] (p.Val562Ile on a $(TG)_{11}T_5$ background) (n=2), c.2991G>C (p.Leu997Phe) (n=3), c.4056G>C (p.Gln1352His) (n=2), c.4097T>C (p.Ile1366Thr)) and 5 were heterozygous for a variant of unclear significance (c.[221G>A(;)890G>A] (p.[Arg74Gln(;)Arg297Gln] (n=2), c.571T>G (p.Phe191Val), c.2876C>T (p.Ala959Val) and c.3935A>G (p.Asp1312Gly)).

Patients included in the previous study (Patat et al., 2016) or patients with known unilateral renal agenesis were not included in the present study. The presence of both kidneys was confirmed by ultrasonography for 20 patients but remains undocumented for 33 patients (Figure 1). Data from ultrasonographic exploration of the genital tract were available for 15 patients and semen analysis results were available for 24 patients.
The study was approved by ethics committee “Comité de Protection des Personnes Nord-Ouest IV”.

ADGRG2 sequencing

ADGRG2 coding exons (reference transcript NM_001079858.2) and flanking sequences (at least 20 nucleotides upstream and downstream except for exon 29 only 8 nucleotides upstream) were amplified using in-house primers with the Access Array system (Fluidigm) and sequenced on a MiSeq sequencer (Illumina) with a Nano flow cell and 2x250 v.2 chemistry. Results were analyzed with the bioinformatic pipelines MiSeq Reporter v.2.5.1 and SeqNext v.4.1.2. Potentially pathogenic sequence variations were

confirmed by Sanger sequencing. We also sequenced with the Sanger method every amplicon not covered by at least 40 reads after MiSeq sequencing.

RESULTS

Sequencing of the full *ADGRG2* coding sequence and flanking regions was performed for the 53 DNA samples and identified 6 novel hemizygous truncating *ADGRG2* mutations predicted to result in a frameshift and/or a premature stop codon in 8 patients: c.251C>G (p.Ser84*) in P2, c.1013delC (p.Pro338Hisfs*4) in P1 & P6, c.1460delG (p.Gly487Alafs*9) in P3, c.2096dupT (p.Phe700Ilefs*29) in P5, c.2473C>T (p.Arg825*) in P4 and c.1731_1839+373del (p.Asn578Thrfs*12) in P7 & P8. Five of the 8 patients also harboring an heterozygous *CFTR* mutation (P1 & P3 with p.Phe508del, 2 twin brothers P7 & P8 with p.[Arg74Gln(;)Arg297Gln] and P2 with p.Val562Ile on a $(TG)_{11}T_5$ background (Figure 1 & 2, Table 1). The c.1731_1839+373del mutation corresponds to the deletion of 596 base pairs affecting the last 5 bases of exon 21 and the whole exon 22 in *ADGRG2* gene. This rearrangement was predicted to result in a frameshift leading to the creation of a premature stop codon in transcripts. The presence of a large deletion was suspected after repeated failure to amplify exons 21 and 22 by Next Generation Sequencing and Sanger sequencing for the two related patients. We then performed long range PCR using exons 20 and 23 primers coupled with nested primers to identify the breakpoints and to confirm the presence of this large deletion.

A truncating *ADGRG2* mutation was found in 6/20 (30.0%) or 5/19 unrelated (26%) patients with documented absence of renal malformation, while only in 2/33 (6.1%) patients with unknown renal status. Additionally we found the rare missense *ADGRG2* variant c.56T>C (p.Leu19Pro) in a CAVD patient with unknown renal status also heterozygous for *CFTR* p.Val562Ile variant on a $(TG)_{11}T_5$ background. All 6 truncating mutations and p.Leu19Pro variant were confirmed by Sanger sequencing.

Seven of the eight patients with a truncating *ADGRG2* mutation had CBAVD at clinical examination and 1 had unilateral absence of the right VD (Table 1). Semen analysis results were available for 5 patients showing constant azoospermia (5/5), low semen volume (3/3) and pH<7.2 (3/3). Ultrasonographic exploration of the reproductive tract was available for 4 patients with 3/4 showing abnormalities of the seminal vesicles (1 with bilateral absence, 1 with unilateral absence and 1 with absence of the right seminal vesicle and hypotrophy on the left side).

DISCUSSION

Isolated CAVD is a rare cause of male infertility and most of the time due to biallelic alteration of the *CFTR* gene, leading to defective electrolyte and fluid transport across epithelial membrane of the excurrent ducts and causing progressive atrophy. In 2016, loss of function mutations of the *ADGRG2* gene have been reported as responsible for a similar phenotype of isolated CAVD in 4 French patients with no *CFTR* mutation (Patat et al., 2016). Subsequently, two others truncating *ADGRG2* mutations have been reported in association with CAVD in one large Pakistani family (Khan et al., 2018) and one Chinese patient (Yuan et al., 2019). The present work is a collaborative retrospective study involving 4 laboratories of the GenMucoFrance network with the objective to investigate more precisely the frequency and the nature of *ADGRG2* mutations in CAVD cases which were not formally related to *CFTR*.

Among the 53 patients included in this study 8 were hemizygous for 6 different truncating mutations of the *ADGRG2* gene predicted to result in a complete loss of function of the ADGRG2 protein. The two patients harboring the c.1013del mutation had no known family relationship but originated from the same region (North of France) and the two patients carrying the c.1731_1839+373del were twin brothers. The 6 truncating mutations identified in this study are described for the first time in patients and are not reported in the gnomAD database (regrouping whole exome or genome data from more than 140 000 individuals). These results further reinforce the role of *ADGRG2* loss-of-function mutations in isolated CAVD and document an important allelic heterogeneity. When clinical data were available, CAVD patients with *ADGRG2* truncating mutation showed constant azoospermia (5/5), low semen volume (3/3), pH<7.2 (3/3) and frequent seminal vesicles abnormalities (3/4). These observations confirm the phenotypic uniformity with CAVD caused by *CFTR* mutations.

In the subgroup with documented presence of both kidneys we found 30% (n=6/20) of patients with an *ADGRG2* truncating mutation or 26% (n=5/19) when considering only unrelated patients. This is higher than the data of 15% (n=4/26) in the original study (Patat et al., 2016) despite the inclusion of patients with one *CFTR* defective allele. Taken together, our two studies performed on French patients suggest that *ADGRG2* truncating mutations may explain approximately 20% (n=9/45) of isolated CAVD, in the absence of two *CFTR* defective alleles identified. This frequency of truncating variant is in contrast with data reported on Chinese population in which two studies involving respectively 18 and 30 CAVD patients carrying 0 or 1 *CFTR* alteration identified only one *ADGRG2* truncating mutation (Yang et al., 2018; Yuan et al., 2019).

In our subgroup with unknown renal status only 6.1% (n=2/33) of the patients had an *ADGRG2* truncating mutation. This lower rate could be attributed to a high proportion of renal agenesis in those patients, in line with the results of the original study (Patat et al., 2016) where no *ADGRG2* mutation were identified among 28 CAVD patient with renal agenesis.

Patients with only one *CFTR* defective allele after comprehensive screening were included in this study because heterozygosity for a *CFTR* alteration is relatively frequent and is theoretically not sufficient to produce a CAVD phenotype. Indeed, the frequency of CF-causing variants is approximately 1/30 individuals in European and U.S. populations (Farrell, 2008) but the frequency of variant potentially involved in CAVD is much more higher. When looking simultaneously at CF-causing, mild and rare unknown variants the frequency of heterozygous carriers increases to approximately 20% of healthy French individuals (Raynal et al., 2019). Even if it remains possible that a comprehensive *CFTR* molecular study can miss the second event, like a deep-intronic mutation (Bonini et al., 2015; Bergougnoux et al., 2018) or a complex chromosomal rearrangement, we hypothesized that for at least a portion of those patients the presence of a *CFTR* mutation may be incidental and that *ADGRG2* may be responsible for the development of CAVD. A second hypothesis is the possibility of digenic inheritance involving the interaction of two alterations on two different genes as described earlier for several conditions (Schäffer 2013). Recent data obtained on mice identified interactions between ADGRG2 and CFTR in the efferent ductules with ADGRG2 constitutive activity maintaining basic CFTR current and pH homeostasis (Zhang et al., 2018). If ADGRG2 and CFTR are parts of the same pathway which regulates ion and fluid reabsorption in the efferent ducts, it is tempting to hypothesize that the combination of two minor dysfunctions, that would have remained asymptomatic if taken separately, may trigger the development of the CAVD phenotype. In our study 5 patients harboring a *CFTR* pathogenic allele (P1 & P3 with p.Phe508del, P2 with p.Val562Ile variant on a $(TG)_{11}T_5$ background and 2 twin brothers P7 & P8 with p.[Arg74Gln(;)Arg297Gln]) were also hemizygous for an *ADGRG2* truncating mutation. Considering that a high penetrance is expected for *ADGRG2* truncating mutations, we conclude that the presence of the *CFTR* mutation may be incidental in these patients and that ADGRG2 loss of function is sufficient to produce the CAVD phenotype. We found only one patient (P9) with a genotype compatible with digenic inheritance involving hemizygous p.Leu19Pro missense variant on *ADGRG2* associated with heterozygous *CFTR* variants p.Val562Ile and $(TG)_{11}T_5$ which are most probably located in *cis* based on epidemiologic data and result in a complex allele associated with mild CFTR dysfunction (Claustres et al., 2017). The p.Leu19 position is located on the signal sequence (amino acids 1 to 37) involved in ADGRG2 protein integration into the endoplasmic reticulum membrane during early biogenesis and enabling correct transport to the plasma membrane (Oberman et al., 2003). The impact of the p.Leu19Pro variant on protein function is predominantly predicted as neutral by *in silico* tools such as CADD-v1.4 (13.5 score is below the recommended cut-off of 15) and Varsome (8 benign predictions from DANN, FATHMM, FATHMM-MKL, GERP, LRT, MutationAssessor, MutationTaster and PROVEAN versus 1 pathogenic prediction from SIFT) (Adzhubei et al., 2010; Kopanos et al., 2019; Kumar et al., 2009; Quang et al., 2015; Schwarz et al., 2014; Tavtigian et al., 2008). According to the 2015 American College of Medical Genetics and Genomics Standards and Guidelines (Richards et al., 2015) the p.Leu19Pro variant should be considered as likely benign. Its frequency of 0.057% in European populations in the gnomAD v2.1.1 database (Lek et al., 2016) does not support a fully penetrant pathogenic effect but could be compatible with a digenic inheritance involving *CFTR*. Nevertheless, considering mostly benign *in silico* predictions and unknown renal status for this patient, we considered the implication of the genotype in

the CAVD phenotype as unlikely. If we cannot formally exclude that a mechanism involving *CFTR* heterozygosity and *ADGRG2* mild dysfunction may lead to CAVD, our results on 30 CAVD patients who were simple heterozygous for a *CFTR* mutation do not support this hypothesis, at least as a frequent mechanism.

In this study, we also report the case of two premature newborn twin brothers (32 weeks and four days gestation) presenting bilateral absence of the vas deferens discovered during inguinal hernia surgery at 6 weeks of life and harboring a 596 base pair deletion of *ADGRG2* encompassing the last 5 nucleotides of exon 21 and the totality of exon 22. This is simultaneously the first description of a large rearrangement of the *ADGRG2* gene and the first observation of CAVD due to *ADGRG2* mutation in neonates, indicating that for these two twin brothers the atresia of the vas deferens occurred *in utero*.

CONCLUSION

In conclusion, our study describes six new *ADGRG2* truncating mutations and confirms the implication of ADGRG2 in approximately 20% of French patients with isolated CAVD not related to CFTR dysfunction. Our results underline the importance of *ADGRG2* sequencing in CAVD patients with no *CFTR* mutation identified after comprehensive testing but also in patients with only one *CFTR* defective allele, ideally after renal agenesis exclusion.

ACKNOWLEDGMENTS

The authors thank all clinicians who referred the patients included in this study and the technical teams of the laboratories.

DISCLOSURES

The authors have no conflicts of interest to declare.

AUTHORS CONTRIBUTION

Conceived and designed the experiments: AP, EB. Selected DNA samples and performed *CFTR* analysis: AP, AB, EG, MR, CR, TB, PF, MH, GL. Performed the experiments: CW, MK. Analyzed the data: AP. Wrote the paper: AP. Revised the paper: AB, EG, MR, CR, TB, GL, EB.

REFERENCE LIST

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS & Sunyaev SR. (2010) A method and server for predicting damaging missense mutations. Nat Methods, 7, 248-249.

Augarten A, Yahav Y, Kerem BS, Halle D, Laufer J, Szeinberg A, Dor J, Mashiach S & Madgar I. (1994) Congenital absence of vas deferens in the absence of cystic fibrosis. Lancet, 344, 1473-1474.

Bareil C, Guittard C, Altieri JP, Templin C, Claustres M & des Georges M. (2007) Comprehensive and rapid genotyping of mutations and haplotypes in Congenital absence of the vas deferens and other cystic fibrosis transmembrane conductance regulator-related disorders. J Mol Diagn, 9, 582-8.

Bergougnoux A, Délétang K, Pommier A, Varilh J, Houriez F, Altieri JP, Koenig M, Férec C, Claustres M, Lalau G, Bienvenu T, Audrézet MP, Pagin A, Girodon E, Raynal C & Taulan-Cadars M. (2018) Functional characterization and phenotypic spectrum of three recurrent disease-causing deep intronic variants of the CFTR gene. J Cyst Fibros, doi: 10.1016/j.jcf.2018.10.012.

Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, et *al*.et Ferec C. (2011) Recommendations for the classification of diseases as CFTR-related disorders. J Cyst Fibros. 2011 Jun;10 Suppl 2:S86-102.

Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P *et al. et* Elborn JS. (2008) Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros, 7, 179-96.

Claustres M, Guittard C, Bozon D, Chevalier F, Verlingue C, Ferec C *et al. et* Des Georges M. (2000) Spectrum of CFTR mutations in cystic fibrosis and in congenital absence of the vas deferens in France. Hum Mutat, 16, 143-156.

Claustres M. (2005) Molecular pathology of the CFTR locus in male infertility. Reprod Biomed Online, 10, 14-41.

Claustres M, Thèze C, des Georges M, Baux D, Girodon E, Bienvenu T et *al*., et Bareil C. (2017) CFTR-France, a national relational patient database for sharing genetic and phenotypic data associated with rare CFTR variants. Hum Mut, 38, 1297-1315.

Daudin M, Bieth E, Bujan L, Massat G, Pontonnier F & Mieusset R. (2000) Congenital absence of the vas deferens: clinical characteristics, biological parameters, cystic fibrosis transmembrane conductance regulator gene mutations, and implications for genetic counseling. Fertil Steril, 74, 1164-1174.

Davies B, Baumann C, Kirchhoff C, Ivell R, Nubbemeyer R, Habenicht UF, Theuring F & Gottwald U. (2004) Targeted deletion of the epididymal receptor HE6 results in fluid dysregulation and male infertility. Mol Cell Biol, 24, 8642-8648.

Dumur V, Gervais R, Rigot JM, Lafitte JJ, Manouvrier S, Biserte J, Mazeman E & Roussel P. (1990) Abnormal distribution of CF delta F508 allele in azoospermic men with congenital aplasia of epididymis and vas deferens. Lancet, 336, 512.

Farrell PM. (2008) The prevalence of cystic fibrosis in the European Union. J Cyst Fibros, 7, 450-453.

Gaillard DA, Carré-Pigeon F & Lallemand A. (1997) Normal vas deferens in fetuses with cystic fibrosis. J Urol, 158, 1549-1552.

Khan MJ, Pollock N, Jiang H, Castro C, Nazli R, Ahmed J, Basit S, Rajkovic A & Yatsenko AN. (2018) X-linked ADGRG2 mutation and obstructive azoospermia in a large Pakistani family. Sci Rep, 8, 16280.

Kopanos C, Tsiolkas V, Kouris A, Chapple CE, Albarca Aguilera M, Meyer R, Massouras A. (2019) VarSome: the human genomic variant search engine. Bioinformatics, 35, 1978-1980.

Kumar P, Henikoff S & Ng PC. (2009) Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc, 4, 1073-1081.

Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T *et al. et* Exome Aggregation Consortium. Analysis of protein-coding genetic variation in 60,706 humans. Nature, 536, 285-291.

Llabador MA, Pagin A, Lefebvre-Maunoury C, Marcelli F, Leroy-Martin B, Rigot JM & Mitchell V. (2015) Congenital absence of the vas deferens: the impact of spermatogenesis quality on intracytoplasmic sperm injection outcomes in 108 men. Andrology, 3, 473-480.

McCallum T, Milunsky J, Munarriz R, Carson R, Sadeghi-Nejad H & Oates R. (2001) Unilateral renal agenesis associated with Congenital absence of the vas deferens: phenotypic findings and genetic considerations. Hum Reprod, 16, 282-288.

Obermann H, Samalecos A, Osterhoff C, Schröder B, Heller R & Kirchhoff C. (2003) HE6, a two-subunit heptahelical receptor associated with apical membranes of efferent and epididymal duct epithelia. Mol Reprod Dev, 64, 13-26.

Patat O, Pagin A, Siegfried A, Mitchell V, Chassaing N, Faguer S, Monteil L, Gaston V, Bujan L, Courtade-Saïdi M, Marcelli F, Lalau G, Rigot JM, Mieusset R & Bieth E. (2016) Truncating Mutations in the Adhesion G Protein-Coupled Receptor G2 Gene ADGRG2 Cause an X-Linked Congenital Absence of Vas Deferens. Am J Hum Genet, 99, 437-442.

Quang D, Chen Y & Xie X. (2015) DANN: a deep learning approach for annotating the pathogenicity of genetic variants. Bioinformatics, 31, 761–763.

Ratbi I, Legendre M, Niel F, Martin J, Soufir JC, Izard V, Costes B, Costa C, Goossens M & Girodon E. (2007) Detection of cystic fibrosis transmembrane conductance regulator (CFTR) gene rearrangements

enriches the mutation spectrum in Congenital absence of the vas deferens and impacts on genetic counselling. Hum Reprod, 22, 1285-1291.

Raynal C, Girodon E, Pagin A, Audrezet MP, Reboul MP, Dufernez F, Cabet F, Bergougnoux F, Fanen P, Ferec C & Bienvenu. CFTR gene variants: A predisposition factor to aquagenic palmoplantar keratoderma. (2019) Br J Dermatol, DOI: 10.1111/bjd.18162.

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL & ACMG Laboratory Quality Assurance Committee. (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med, 17, 405-424.

Schäffer AA. (2013) Digenic inheritance in medical genetics. J Med Genet, 50, 641-652.

Schwarz JM, Rödelsperger C, Schuelke M & Seelow D. (2010) MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods, 7, 575-576.

Sharma N, Acharya N, Singh SK, Singh M, Sharma U & Prasad R. (2009) Heterogenous spectrum of CFTR gene mutations in Indian patients with congenital absence of vas deferens. Hum Reprod, 24, 1229-1236.

Stuhrmann M & Dork T. (2000) CFTR gene mutations and male infertility. Andrologia, 32:71-83.

Tavtigian SV, Greenblatt MS, Lesueur F & Byrnes GB. (2008) In silico analysis of missense substitutions using sequence-alignment based methods. Hum Mutat, 29, 1327-1336.

Wosnitzer MS & Goldstein M. (2014) Obstructive azoospermia. Urol Clin North Am, 41, 83–95.

Yu J, Chen Z, Ni Y, & Li Z. (2012) CFTR mutations in men with Congenital absence of the vas deferens (CAVD): a systemic review and meta-analysis. Hum Reprod, 27, 25–35.

Yuan P, Liang ZK, Liang H, Zheng LY, Li D, Li J, Zhang J, Tian J, Lai LH, Zhang K, He ZY, Zhang QX & Wang WJ. (2019) Expanding the phenotypic and genetic spectrum of Chinese patients with congenital absence of vas deferens bearing CFTR and ADGRG2 alleles. Andrology, doi: 10.1111/andr.12592.

Zhang DL, Sun YJ, Ma ML, Wang YJ, Lin H, Li RR *et al. et* Sun JP. (2018) Gq activity- and β-arrestin-1 scaffolding-mediated ADGRG2/CFTR coupling are required for male fertility. Elife, doi: 10.7554/eLife.33432.

Figure & Tables

Patient ID	**Age at CAVD diagnosis**	**ADGRG2 (hemizygous)**	***CFTR (heterozygous)***	**Geographic origins**	**Andrological clinical examination**	**Renal status**	**Semen analysis**	**Reproductive tract ultrasonography**	**Additional information**
P1	30 y.	c.1013delC (p.Pro338Hisfs*4)	c.1521_1523del (p.Phe508del)	France (North)	Bilateral absence of VD	Normal	Azoospermia Hypovolemia < 2 mL pH < 7.2	Bilateral absence of VD Bilateral EP enlargement Unilateral absence of VS	Normal clinical respiratory examination, pulmonary function and chest scan
P2	33 y.	c.251C>G (p.Ser84*)	c.1684G>A (p.Val562Ile) and $(TG)_{11}T_5$	NA	Bilateral absence of VD + Bilateral EP enlargement	Normal	Azoospermia Hypovolemia < 2 mL pH < 7.2	Bilateral absence of VD Bilateral EP enlargement	
P3	41 y.	c.1460delG (p.Gly487Alafs*9)	c.1521_1523del (p.Phe508del)	NA	Bilateral absence of VD	Normal	Azoospermia (Volemia and pH NA)	Bilateral absence of SV	
P4	34 y.	c.2473C>T (p.Arg825*)	-	NA	Bilateral absence of VD + Bilateral EP enlargement	NA	Azoospermia (Volemia and pH NA)	Absence of right SV and hypotrophy of left SV Bilateral absence of VD	Normal clinical respiratory examination, pulmonary function and chest scan
P5	41 y.	c.2096dupT	-	Armenia	Bilateral	NA	NA	NA	

		p.Phe700Ilefs*29)			absence of VD				
P6	26 y.	c.1013delC p.Pro338Hisfs*4)	-	France (North)	Unilateral absence of VD (right)	Normal	Azoospermia Hypovolemia < 2 mL pH < 7.2	NA	One child obtained by medically assisted procreation after testicular sperm retrieval
P7	6 w.	c.1731_1839+373del (p.Asn578Thrfs*12)	c.[221G>A;890G>A] (p.[Arg74Gln;Arg297Gln]	France (North)	Bilateral absence of VD (discovered during inguinal hernia surgery at 6 weeks of life)	Normal	NA	NA	Twin brother of P8
P8	6 w.	c.1731_1839+373del (p.Asn578Thrfs*12)	c.[221G>A;890G>A] p.[Arg74Gln;Arg297Gln]	France (North)	Bilateral absence of VD (discovered during inguinal hernia surgery at 6 weeks of life)	Normal	NA	NA	Twin brother of P7
P9	44 y.	c.56T>C (p.Leu19Pro)	c.1684G>A (p.Val562Ile) and $(TG)_{11}T_5$	NA	Bilateral absence of	NA	Azoospermia	NA	c.56T>C is classified as likely

					VD		Hypovolemia < 2 mL pH < 7.2		benign by ACMG Guidelines

Table 1 : Clinical, biological and morphological findings in patients with *ADGRG2* mutations. Variant nomenclatures are given at nucleotidic (c.) and proteic (p.) levels (y. : years; w. : weeks; VD : Vas Deferens; SV : Seminal Vesicles; EP : Epididymis; NA : Not Available).

Figure legends

Figure 1: Study overview with *ADGRG2* truncating mutations (in bold) and *CFTR* associated variants (in grey).

Figure 2: Schematic representation of ADGRG2 structure with the position of the truncating mutations (adapted from Patat et al., 2016). The variants identified in this study are in bold. GPS: GPCR proteolysis site. STP region: Serine-Threonine-Proline-rich region.