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► To cite this version:

Zine-eddine Kherraf, Anne-laure Barbotin, Guillaume Martinez, Aurélien Mazet, Caroline Cazin, et al.. A splice donor variant of GAS8 induces structural disorganization of the axoneme in sperm flagella and leads to nonsyndromic male infertility. *Clinical Genetics*, 2023, 105 (2), pp.220-225. 10.1111/cge.14450 . hal-04601943

HAL Id: hal-04601943

<https://hal.science/hal-04601943v1>

Submitted on 5 Jun 2024

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SHORT REPORT

A splice donor variant of *GAS8* induces structural disorganization of the axoneme in sperm flagella and leads to nonsyndromic male infertility

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Funding information

French National Research Agency, Grant/Award Number: FLAGEL-OME ANR 19-CE17-0014

Abstract

Motile cilia and flagella are closely related organelles structured around a highly conserved axoneme whose formation and maintenance involve proteins from hundreds of genes. Defects in many of these genes have been described to induce primary ciliary dyskinesia (PCD) mainly characterized by chronic respiratory infections, situs inversus and/or infertility. In men, cilia/flagella-related infertility is usually caused by asthenozoospermia due to multiple morphological abnormalities of the sperm flagella (MMAF). Here, we investigated a cohort of 196 infertile men displaying a typical MMAF phenotype without any other PCD symptoms. Analysis of WES data identified a single case carrying a deleterious homozygous *GAS8* variant altering a splice donor consensus site. This gene, also known as *DRC4*, encodes a subunit of the Nexin-Dynein Regulatory Complex (N-DRC), and has been already associated to male infertility and mild PCD. Confirming the deleterious effect of the candidate variant, *GAS8* staining by immunofluorescence did not evidence any signal from the patient's spermatozoa whereas a strong signal was present along the whole flagella length in control cells. Concordant with its role in the N-DRC, transmission electron microscopy evidenced peripheral microtubule doublets misalignments. We confirm here the importance of *GAS8* in the N-DRC and observed that its absence induces a typical MMAF phenotype not necessarily accompanied by other PCD symptoms.

KEYWORDS

asthenozoospermia, *DRC4*, *GAS8*, male infertility, MMAF, PCD

1 | INTRODUCTION

Disruption of spermatogenesis leads to a wide range of sperm defects including asthenozoospermia which is defined by an alteration of

sperm motility. One of the most severe conditions of asthenozoospermia is due to Multiple Morphological Abnormalities of the Sperm Flagella (MMAF), a phenotype characterized by a mosaic of sperm morphological defects including absent, bent, short, irregular and

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coiled flagella.¹ More than 40 MMAF-associated genes have been so far related to human infertility, thus demonstrating the high genetic heterogeneity of this phenotype.² However about half of MMAF cases remain unexplained.³

In this study, we recruited and investigated by whole exome sequencing (WES), a patient displaying a primary non-syndromic infertility due to MMAF. We identified and validated a novel loss-of-function (LoF) variant in *GAS8* (OMIM: # 605178), a gene encoding a protein localized in the axoneme of cilia and sperm flagella. Previous studies demonstrated that *GAS8* LoF variants are responsible for mild symptoms of Primary Ciliary Dyskinesia (PCD, OMIM: # 244400), a genetic multisystemic disorder characterized by impaired ciliary function, leading to a chronic sinopulmonary disease, referred to as Kartagener syndrome when associated to situs inversus totalis.⁴ It has been reported that PCD patients carrying *GAS8* variants are infertile but their phenotype was not fully explored and reported. Here, we demonstrated the involvement of a novel pathogenic *GAS8* variant in a non-syndromic case of male infertility due to MMAF.

2 | MATERIALS AND METHODS

2.1 | Participant and ethical approval statement

All samples, from 196 MMAF subjects were sent to Grenoble Alpes Hospital for genetic analysis. This study respects all ethical guidelines (Declaration of Helsinki) and was approved by local ethics committees and the French Data Protection Authority (DR-2016-392) and samples collection (Fertithèque) was declared to the French Ministry of health (DC-2015-2580). Informed consent was obtained from patients

prior to sample collection and analyses. Semen samples were prepared and analyzed as described previously.⁵ The patient gave his informed consent to donate and store spermatozoa not used for semen analysis in GERMETHEQUE Biobank (BB-0033-00081).

2.2 | WES analysis and variant verification

WES and bioinformatics analyses were performed according to our previously described protocol using the human genome assembly GRCh38 as a reference.⁶ The selected variant was validated by Sanger sequencing using ABI 3130XL and SeqScape software (Applied Biosystems; Foster City, CA). Primers are listed in Table S1.

2.3 | Transmission electron microscopy (TEM) analysis

To better characterize the impact of the reported candidate variant on sperm ultrastructure, sperm from a fertile control and from patient P0543 were subjected to transmission electron microscopy as described previously.⁵

2.4 | Immunofluorescence (IF) Analysis of Sperm Cells

Immunostaining was carried out on sperm cells from fertile control subjects and from individual P0543. All the procedure is described in.⁵ Antibodies uses for these experiments are listed in Table S2.

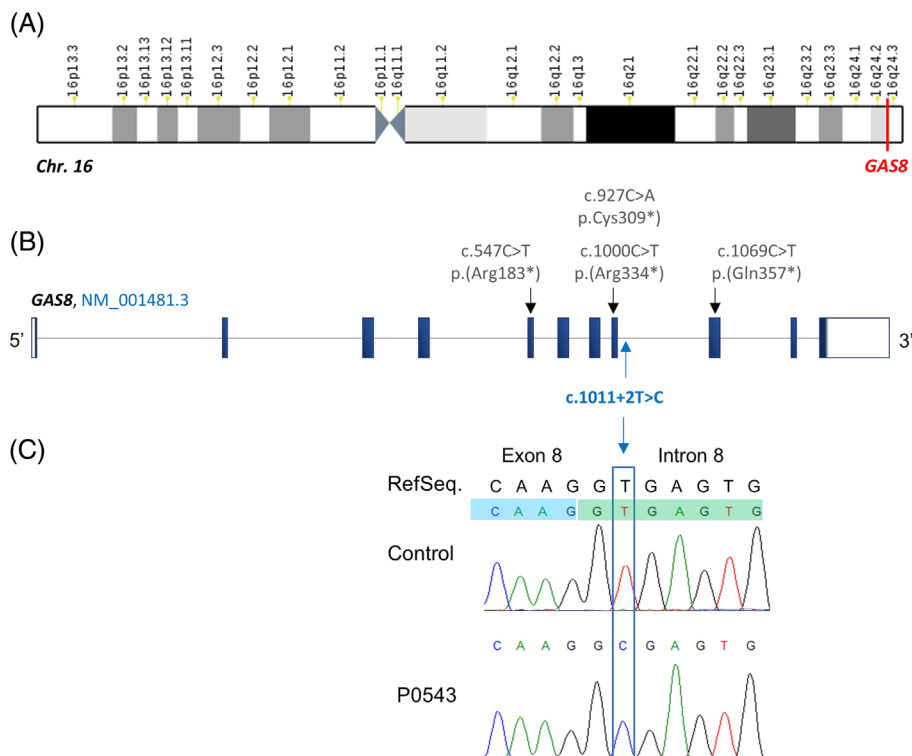


FIGURE 1 Identification of a rare homozygous splicing variant in *GAS8*. (A) *GAS8* is located at chromosome (chr.) 16q24.3, g.90019629-90 044 975. (B) *GAS8* (NM_001481.3) comprises 11 exons. The novel *GAS8* variant, marked in blue, is located in intron 8 and affects the consensus GT sequence of the splice donor site (c.1011 + 2 T > C). Variants described in previous studies are marked in gray. (C) Sanger sequencing confirmed the presence of the candidate *GAS8* variant in P0543. [Colour figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | WES identified a homozygous splicing variant in GAS8

Previous WES analyses of our MMAF cohort permitted to identify bi-allelic variants in a total of 92 men (47%) in 22 confirmed MMAF-associated genes. In the remaining undiagnosed individuals, we identified one subject (P0543) carrying a homozygous deleterious variants in the intron 8 of GAS8, NM_001481.3:c.1011 + 2 T > C (Figure 1A,B). This variant is very rare (MAF: 6.575×10^{-6} in gnomAD, v3.1.2) and was predicted to alter a consensus splice donor site (SIP score: 98.41% [91.47%–99.96%]) (Figure S1). Sanger sequencing confirmed the presence of this

variant (Figure 1C). Finally, this variant was selected as a strong candidate among the list of the other variants identified in P0543 to explain its infertility (Table S3).

3.2 | Patient phenotypic characterization

Patient P0543, a 32 years old male, The subject consulted for infertility at the Reproductive Biology laboratory-CECOS of Rouen University Hospital (France). This patient, originating from West Africa, was born from related parents. He had a brother showing primary infertility due to severe asthenozoospermia who consulted to a different ART center and was described with a MMAF phenotype (Figure 2A). Physical examination of the patient was normal. Sperm analysis

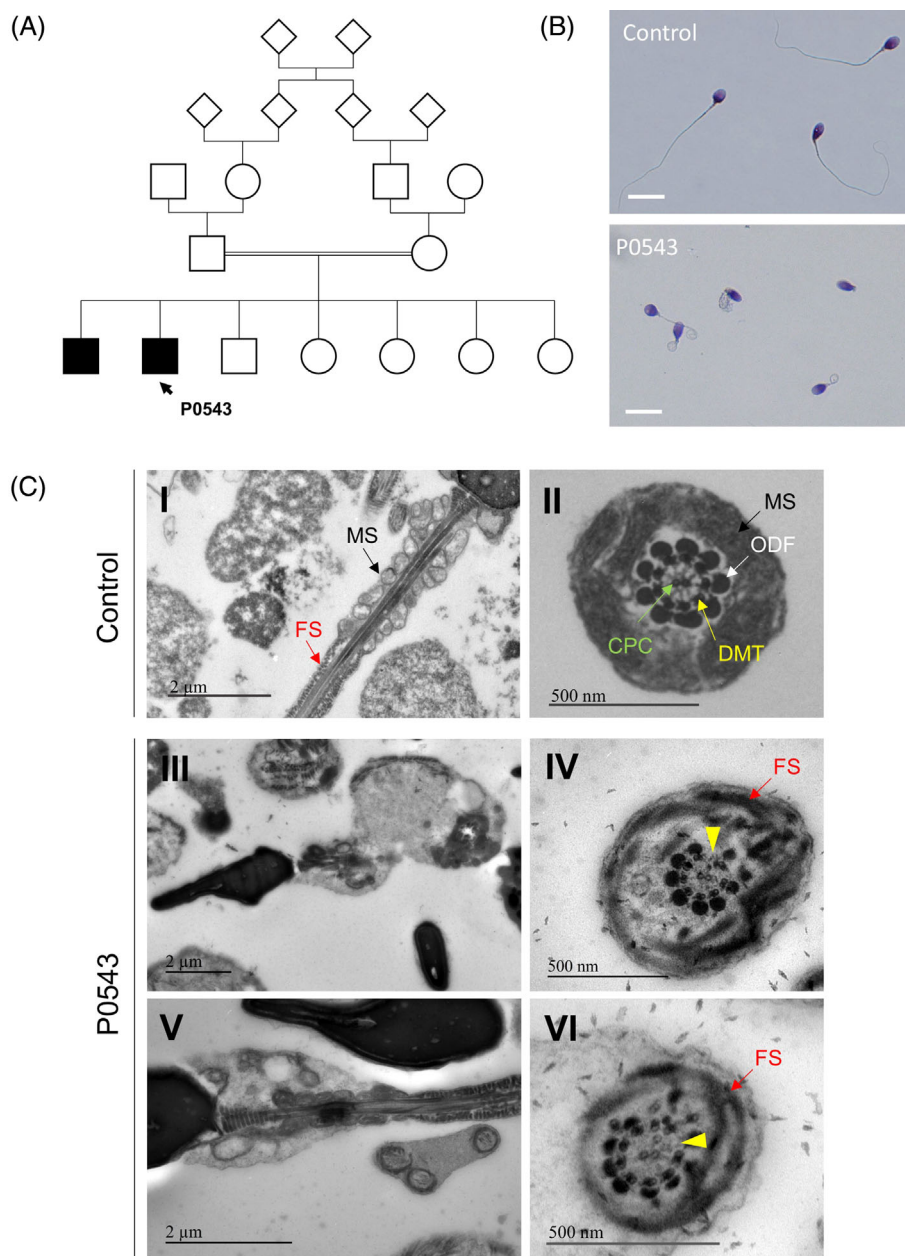


FIGURE 2 A consanguineous family with an infertile man displaying primary infertility due to multiple morphological abnormalities of the sperm flagella (MMAF). (A) Pedigree analysis. (B) Morphological analysis of sperm illustrating a typical MMAF phenotype. (C) Ultrastructure of the spermatozoa from P0543 compared to that of control sperm. Yellow head-arrows indicate abnormal DMT assemblage and cohesion. CPC, central pair complex; FS, fibrous sheath; MS, mitochondrial sheath; ODF, outer dense fibers. [Colour figure can be viewed at wileyonlinelibrary.com]

evidenced a complete astheno-teratozoospermia with 100% of immotile and morphologically abnormal spermatozoa, most of which (93%) displayed multiple morphological abnormalities of the flagellum with 53% of absent, 24% of short, 16% of curled or bent and flagella (Figure 2B). Further clinical assessment of this patient suggested a condition of nonsyndromic infertility without PCD-symptoms except for a medical past history of asthma and recurrent otitis during childhood.

3.3 | TEM revealed N-DRC defects associated with abnormal flagellum assembly and elongation

TEM analysis of patient's sperm evidenced severe and recurrent elongation defects associated with fibrous sheath dysplasia and reduced numbers and abnormal arrangement of mitochondria in the mid-piece (Figure 2C). Moreover, most observed cross sections of flagella showed a preserved "9 + 2" axonemal structure but also a frequent

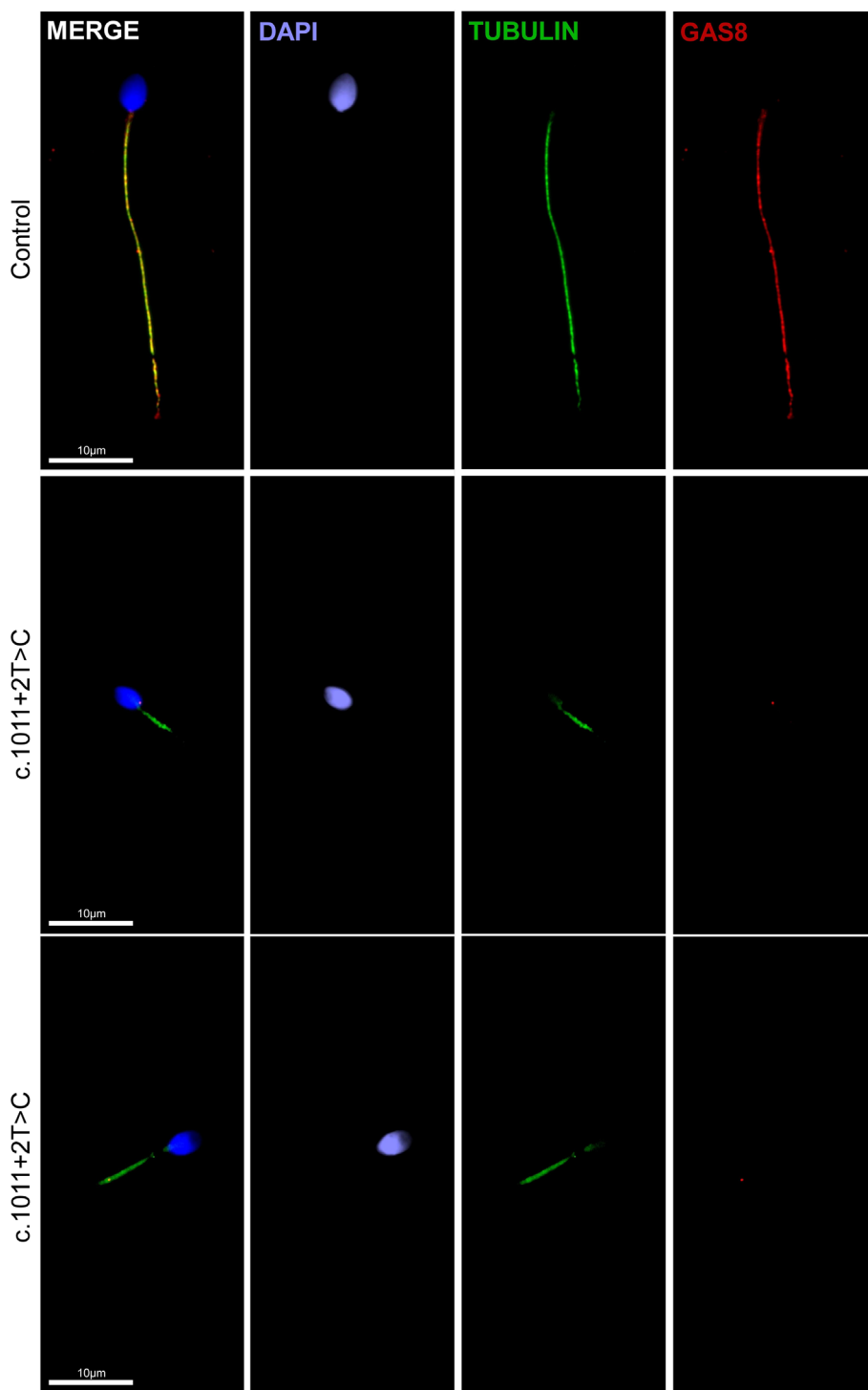


FIGURE 3 GAS8 immunostaining of sperm cells from a fertile control subject and the infertile patient carrying the candidate GAS8 variant. Sperm cells were stained with a polyclonal anti-GAS8 (red) and anti-acetylated α -tubulin (green) antibodies. DNA was counterstained with DAPI (4',6-diamidino-2-phenylindole). GAS8 signal was strongly detected in control sperm over the entire length of the flagellum. However, the GAS8 signal was not detected in the sperm flagella from P0543 who carries the GAS8 variant. [Colour figure can be viewed at wileyonlinelibrary.com]

detachment or absence of some peripheral microtubule doublets suggesting a possible disruption of the link given by the N-DRC.

3.4 | IF experiments showed the loss of GAS8 in sperm flagella from patient carrying the candidate variant

GAS8 is localized in the axoneme of the flagella and is a component of the N-DRC (nexin-dynein regulatory complex), which links adjacent peripheral doublet microtubules (DMTs). To demonstrate the impact of the candidate GAS8 variant on protein expression and localization, we performed IF staining of sperm from controls and P0543 using an antibody raised against the C-terminus domain of GAS8 (aa 379–469). In control spermatozoa, GAS8 and the axonemal marker acetylated α -tubulin co-localize within the axoneme (Figure 3). However, spermatozoa from the patient showed no GAS8 signal in the flagellar axoneme indicating that this protein was either absent or truncated (Figure 3).

4 | DISCUSSION

Previous studies identified bi-allelic LoF variants in GAS8 as genetic factors responsible for PCD and severe primary male infertility due to asthenozoospermia, but few cases have been reported and the reproductive phenotype has been seldom explored.^{7–9} Our study identified a novel homozygous LoF variant in GAS8 for a patient displaying primary infertility due to MMAF without apparent PCD symptoms. GAS8 is highly expressed in cilia of epithelial cells of pulmonary bronchi and fallopian tubes, and also predominantly expressed in the adult testes during the late stages of spermatogenesis concomitantly with the formation of flagella.¹⁰ The sperm flagellum contains a structure known as the axoneme, which runs through its entire length.¹¹

The axoneme comprises nine DMTs surrounding a central pair complex of two singlet microtubules (9 + 2 configuration). The N-DRC is a thin structure that bridges neighboring DMTs and restricts sliding motions between DMTs. As a result, the N-DRC converts dynein-generated sliding motion into flagellar bending motion.¹² To date, biochemical and genetic studies have revealed that the N-DRC is composed of 12 subunits.^{12,13} GAS8 encodes the DRC4 subunit of this complex. It was demonstrated that, during axonemal assembly, DRC4 travels within cilia and flagella as cargoes on intra-flagellar transport (IFT) particles.¹⁴ DRC4 was also described to interact with DRC1 (OMIM: # 615288) and CCDC65 (OMIM: # 611088) subunits to form the core structure of the N-DRC which serves as a scaffold for the assembly of the functional subunits. Mutations in the main DRC subunit genes cause the disassembly of the N-DRC, defects in ciliary movement and the abnormal assembly of several closely associated structures.^{7,15,16} CCDC65 pathogenic variants induce subtle ciliary beating defects and lead to PCD, whereas DRC1 deficiency was reported to cause both PCD and male infertility due to MMAF.^{16–18}

GAS8 deficiency leads to N-DRC disruption and cause PCD and asthenozoospermia.^{7,8} Unlike *Gas8* knockout mice that display severe PCD phenotypes including hydrocephalus and situs inversus, bi-allelic GAS8 pathogenic variants in humans have been associated to milder symptoms such as insufficient mucociliary clearance of the airways which may result in a chronic destructive respiratory disorder.⁹ It is therefore possible that our patient who does not present PCD symptoms is at an early stage of the respiratory disease. Indeed, it was shown that the genetic background may play a role in delaying the disease onset and progression in PCD-patients. In this issue, a recent study performed a precise genotype–phenotype correlation analysis in a series of PCD patients carrying mutations in *RSPH9*, a well-established causative PCD gene, reporting a wide phenotypic variability even among patients bearing the same variant.¹⁹ In another study, authors generated several *Drc1* mutant mouse strains and observed that the phenotypic manifestations were strain-dependent.¹⁶ Overall, these data underscore the fact that differences in genetic background may influence the penetrance of PCD-related mutations.

In conclusion, we herein identified a novel homozygous LoF GAS8 variant in a MMAF patient. We also propose the TEM analysis as a complementary approach in addition to WES for the investigation of MMAF and particularly for the detection of N-DRC related-defects.

AUTHOR CONTRIBUTIONS

Zine-Eddine Kherraf, Nicolas Thierry-Mieg, Charles Coutton, Caroline Cazin, and Pierre F Ray performed and analyzed the genetic data. Anne-Laure Barbotin performed TEM analysis. Guillaume Martinez, Caroline Cazin, and Aurélie Mazet performed the immunofluorescence assays. Aurélie Rives-Feraille and Nathalie Rives provided clinical samples and data. Pierre F Ray and Zine-Eddine Kherraf designed the study, supervised all molecular laboratory work. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

We thank all the individuals for their cooperation, as well as all the referring physicians.

FUNDING INFORMATION

This work was supported by the French National Research Agency (grant FLAGEL-OME ANR-19-CE17-0014).

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at <https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14450>.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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REFERENCES

- Ben Khelifa M, Coutton C, Zouari R, et al. Mutations in DNAH1, which encodes an inner arm heavy chain dynein, lead to male infertility from multiple morphological abnormalities of the sperm flagella. *Am J Hum Genet.* 2014;94(1):95-104.
- Wang J, Wang W, Shen L, et al. Clinical detection, diagnosis and treatment of morphological abnormalities of sperm flagella: a review of literature. *Front Genet.* 2022;13:1034951.
- Touré A, Martinez G, Kherraf ZE, et al. The genetic architecture of morphological abnormalities of the sperm tail. *Hum Genet Janv.* 2021; 140(1):21-42.
- Wei S, Xie H, Cheng Y. Progress in diagnosis of primary ciliary dyskinesia. *J Paediatr Child Health.* 2022;58(10):1736-1740.
- Martinez G, Barbotin AL, Cazin C, et al. New mutations in DNHD1 cause multiple morphological abnormalities of the sperm flagella. *Int J Mol Sci.* 2023;24(3):2559.
- Kherraf ZE, Cazin C, Bouker A, et al. Whole-exome sequencing improves the diagnosis and care of men with non-obstructive azoospermia. *Am J Hum Genet.* 2022;109(3):508-517.
- Olbrich H, Cremers C, Loges NT, et al. Loss-of-function GAS8 mutations cause primary ciliary dyskinesia and disrupt the nexin-dynein regulatory complex. *Am J Hum Genet.* 2015;97(4): 546-554.
- Jeanson L, Thomas L, Copin B, et al. Mutations in GAS8, a gene encoding a nexin-dynein regulatory complex subunit, cause primary ciliary dyskinesia with axonemal disorganization. *Hum Mutat août.* 2016;37(8):776-785.
- Lewis WR, Malarkey EB, Tritschler D, et al. Mutation of growth arrest specific 8 reveals a role in motile cilia function and human disease. *PLoS Genet.* 2016;12(7):e1006220.
- Yeh SD, Chen YJ, Chang ACY, et al. Isolation and properties of Gas8, a growth arrest-specific gene regulated during male gametogenesis to produce a protein associated with the sperm motility apparatus. *J Biol Chem.* 2002;277(8):6311-6317.
- Lindemann CB, Lesich KA. Functional anatomy of the mammalian sperm flagellum. *Cytoskeleton Hoboken NJ.* 2016;73(11):652-669.
- Ghaneaian A, Majhi S, McCaffrey CL, et al. Integrated modeling of the Nexin-dynein regulatory complex reveals its regulatory mechanism. bioRxiv. 2023.
- Bower R, Tritschler D, Vanderwaal K, et al. The N-DRC forms a conserved biochemical complex that maintains outer doublet alignment and limits microtubule sliding in motile axonemes. *Mol Biol Cell Avr.* 2013;24(8):1134-1152.
- Wren KN, Craft JM, Tritschler D, et al. A differential cargo-loading model of ciliary length regulation by IFT. *Curr Biol CB.* 2013;23(24): 2463-2471.
- Bower R, Tritschler D, Mills KV, Heuser T, Nicastro D, Porter ME. DRC2/CCDC65 is a central hub for assembly of the nexin-dynein regulatory complex and other regulators of ciliary and flagellar motility. *Mol Biol Cell.* 2018;29(2):137-153.
- Zhang J, He X, Wu H, et al. Loss of DRC1 function leads to multiple morphological abnormalities of the sperm flagella and male infertility in human and mouse. *Hum Mol Genet.* 2021;30(21):1996-2011.
- Horani A, Brody SL, Ferkol TW, et al. CCDC65 mutation causes primary ciliary dyskinesia with normal ultrastructure and hyperkinetic cilia. *PLoS One.* 2013;8(8):e72299.
- Lei C, Yang D, Wang R, et al. DRC1 deficiency caused primary ciliary dyskinesia and MMAF in a Chinese patient. *J Hum Genet Avr.* 2022; 67(4):197-201.
- Yiallourous PK, Kouis P, Pirpa P, et al. Wide phenotypic variability in RSPH9-associated primary ciliary dyskinesia: review of a case-series from Cyprus. *J Thorac Dis Mai.* 2019;11(5):2067-2075.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kherraf Z-E, Barbotin A-L, Martinez G, et al. A splice donor variant of GAS8 induces structural disorganization of the axoneme in sperm flagella and leads to nonsyndromic male infertility. *Clinical Genetics.* 2024; 105(2):220-225. doi:10.1111/cge.14450