Bi-allelic Mutations in ARMC2 Lead to Severe Astheno-Teratozoospermia Due to Sperm Flagellum Malformations in Humans and Mice

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Male infertility is a major health concern. Among its different causes, multiple morphological abnormalities of the flagella (MMAF) induces asthenozoospermia and is one of the most severe forms of qualitative sperm defects. Sperm of affected men display short, coiled, absent, and/or irregular flagella. To date, six genes (DNAH1, CFAP43, CFAP44, CFAP69, FSIP2, and WDR66) have been found to be recurrently associated with MMAF, but more than half of the cases analyzed remain unresolved, suggesting that many yet-uncharacterized gene defects account for this phenotype. Here, whole-exome sequencing (WES) was performed on 168 infertile men who had a typical MMAF phenotype. Five unrelated affected individuals carried a homozygous deleterious mutation in ARMC2, a gene not previously linked to the MMAF phenotype. Using the CRISPR-Cas9 technique, we generated homozygous Armc2 mutant mice, which also presented an MMAF phenotype, thus confirming the involvement of ARMC2 in human MMAF. Immunostaining experiments in ARMC2-mutated individuals and mutant mice evidenced the absence of the axonemal central pair complex (CPC) proteins SPAG6 and SPEF2, whereas the other tested axonemal and peri-axonemal components were present, suggesting that ARMC2 is involved in CPC assembly and/or stability. Overall, we showed that bi-allelic mutations in ARMC2 cause male infertility in humans and mice by inducing a typical MMAF phenotype, indicating that this gene is necessary for sperm flagellum structure and assembly.

The characterization of the genetic basis of male infertility represents an important challenge; more than 4,000 genes are thought to be needed for sperm production,1 and therefore defects in any of these genes can hamper spermatogenesis and induce one of many established sperm phenotypes.2 Of late, high-throughput sequencing technologies have allowed researchers to identify an increasing number of genes required for sperm production and have thus greatly facilitated efforts to explain the genetic basis of different forms of male infertility.3 This is especially true for teratozoospermia; qualitative spermatogenesis defects that lead to morphological sperm abnormalities.4,5 One of the most severe forms of qualitative sperm defects, the MMAF phenotype is responsible for astheno-teratozoospermia,6 which is characterized by the presence of immotile spermatozoa presenting with a mosaic of sperm flagellum malformations, including short, coiled, and/or absent flagella and/or flagella of irregular caliber.6 Whole-exome sequencing (WES) analysis revealed that mutations in DNAH1 (MIM: 603332), CFAP43 (MIM: 617558), CFAP44 (MIM: 617559), CFAP69 (MIM: 617949), FSIP2 (MIM: 615796), and WDR66 (MIM: 612573) account for

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the main genetic causes of MMAF.\cite{13} Rarer recessive mutations in AK7 (MIM: 615364), CEP135 (MIM: 611423), and CFAP65 (MIM: 614270) were also recently identified in different familial cases of MMAF.\cite{10,14,15} Despite these recent findings, more than half of the studied MMAF cases remain without a diagnosis, demonstrating the high genetic heterogeneity of this phenotype and the need for further genetic explorations.\cite{12}

Here, we analyzed by WES a total of 168 MMAF-affected individuals, including 78 who were previously reported\cite{9} and an additional 90 unrelated and unpublished individuals with MMAF. All but one individual were analyzed together with the same analytical pipeline, constituting a 167-strong cohort. In this main cohort, 83 individuals were of North African origin and sought consultation for primary infertility at the Clinique des Jasmins in Tunis, 52 individuals originated from the Middle East (Iran) and were treated in Tehran at the Royan Institute (Reproductive Biomedicine Research Center) for primary infertility, and 32 subjects were recruited in France: 25 at the Cochin Institute in Paris, three in Rouen, two in Grenoble, one in Lille, and one in Caen. All individuals presented with a typical MMAF phenotype, which is characterized by severe asthenozoospermia (total sperm motility below 10%) with at least three of the following flagellar abnormalities present in >5% of the spermatozoa: short, absent, coiled, bent, or irregular flagella (Table 1, Figures 1A–1D). All individuals had a normal somatic karyotype (46, XY) with normal bilateral testicular size, hormone levels, and secondary sexual characteristics. Informed consent was obtained from all the individuals participating in the study according to local protocols and the principles of the Declaration of Helsinki. The study was approved by local ethics committees, and samples were then stored in the CRB Germethèque (certification under ISO-9001 and NF-S 96-900) according to a standardized protocol or were part of the Fertinthèque collection declared to the French Ministry of health (DC-2015-2580) and the French Data Protection Authority (DR-2016-392).

WES and bioinformatics analysis were performed according to an improved version of our previously described protocol\cite{10} as described in the Supplemental Material and Methods. Data analysis of the whole cohort of 167 MMAF individuals identified 54 individuals (32.3%) with harmful mutations in known MMAF-related genes (Table S1). In 15 individuals, previously unreported variants were identified in CFAP43 (n = 2), CFAP44 (n = 1), DNAH1 (n = 6), WDR66 (n = 4), and FSIP2 (n = 2), thus confirming the importance of these genes in the etiology of the MMAF syndrome (Table S1). In addition, we identified four individuals (ARMC2-1,4) with a homozygous variant in ARMC2, a gene not previously associated with any pathology; these individuals accounted for 2.4% of our cohort. ARMC2 (GenBank: NM_032131.5) is located on chromosome 6 and contains 18 exons encoding a predicted 867-amino-acid protein (NCBI: NP8115507.4; UniProtKB: Q8NEN0). Three individuals (ARMC2-1,3,4) had a loss-of-function variant, and one (ARMC2-2) had a likely deleterious missense variant (Table 1, Table S1). In addition, we identified a fifth individual (ARMC2-3) with an ARMC2 homozygous loss-of-function variant (Table S2). This individual was of Chinese origin and sought consultation for primary infertility at the First Affiliated Hospital of Anhui Medical University (Hefei City, Anhui Province) in 2012. He was born to first-cousin parents and presented a typical MMAF phenotype, and WES analysis for this individual was performed as described in the previous report.\cite{10} Informed consent was obtained from all tested individuals, and the study was approved by the local institutional board.

The five ARMC2 variants were found in five unrelated individuals, and all were absent from control sequence databases (dbSNP, 1000 Genomes Project, NHLBI Exome Variant Server, gnomAD, and our in-house control database). The variant identified in individual ARMC2-1 is a splice variant, c.1023+1G>A, altering a consensus splice donor site of ARMC2 exon 8 (Figure 1I). To evaluate the deleterious effect of this splicing variant, we performed

### Table 1. Detailed Semen Parameters for the Five MMAF Individuals Harboring a ARMC2 Mutation

<table>
<thead>
<tr>
<th>Individuals</th>
<th>ARMC2 Mutation</th>
<th>Sperm Volume (ml)</th>
<th>Sperm Conc. (10⁶/mL)</th>
<th>Total Motility 1h</th>
<th>Vitality</th>
<th>Normal Spermatozoa</th>
<th>Absent Flagella</th>
<th>Short Flagella</th>
<th>Coiled Flagella</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARMC2-1</td>
<td>c.1023+1G&gt;A</td>
<td>4</td>
<td>35</td>
<td>6</td>
<td>NA</td>
<td>0</td>
<td>10</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td>ARMC2-2</td>
<td>c.2279T&gt;A</td>
<td>4.5</td>
<td>4.6</td>
<td>5</td>
<td>64</td>
<td>0</td>
<td>14</td>
<td>30</td>
<td>6</td>
</tr>
<tr>
<td>ARMC2-3</td>
<td>c.2353_2354delTT</td>
<td>1</td>
<td>58.5</td>
<td>0</td>
<td>16</td>
<td>3</td>
<td>31</td>
<td>37</td>
<td>23</td>
</tr>
<tr>
<td>ARMC2-4</td>
<td>c.1284_1288delACAA</td>
<td>4.2</td>
<td>2</td>
<td>0</td>
<td>35</td>
<td>2</td>
<td>0</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>ARMC2-5</td>
<td>c.421C&gt;T</td>
<td>9.2</td>
<td>10.1</td>
<td>1.1</td>
<td>NA</td>
<td>0</td>
<td>20</td>
<td>38</td>
<td>36</td>
</tr>
</tbody>
</table>

Reference limits\cite{10} = 1.5 (1.4–1.7) (12–16) (38–42) 55 (55–63) (20–26) 5 (4–6) (1–2) (15–19).

\*Reference limits (5th centiles and their 95% confidence intervals) according to the World Health Organization.
RT-PCR with RNA extracted from sperm cells from individual ARMC2_1. Amplification of a sequence ranging from cDNA exon 7–10 from a control individual yielded a normal band of 608 bp, whereas a single smaller band was obtained from cDNA of individual ARMC2_1 (Figure S1A). Sequence analysis of the amplified products demonstrated that the c.1023+1G>A variant results in exon 8 skipping (Figure S1B, Figure S1C), causing a shift in the translational reading frame and introducing a premature termination codon (p.Glu283AlafsTer2). Primer sequences and RT-PCR conditions are indicated in Table S3. The variant identified in individual ARMC2_2 is a missense variation, c.2279T>A (p.Ile760Asn), located in exon 16 (Figure 1I). No mRNA analysis or immunostaining could be performed on sperm cells from this individual because of the lack of biological samples. However, using prediction software for non-synonymous SNPs, we found that this missense change is predicted to be deleterious by SIFT (score of 0) and probably damaging by PolyPhen (score of 0.987). Moreover, the concerned amino acid (Ile760) was found to be conserved in ARMC2 orthologs (Figure S2). The two other variants identified in ARMC2_3 and ARMC2_4 were small frameshift indels, c.2353_2354delTT (p.Leu785MetfsTer5) and c.1284_1288delACAAA (p.Lys428AsnfsTer3), located in exon 17 and exon 10, respectively (Figure 1I), inducing a premature translation termination. The last identified variant (ARMC2_5) is a stop-gain variant, c.421C>T (p.Gln141Ter), located in exon 4 (Figure 1I). Familial study confirmed the presence of the homozygous loss-of-function variant in ARMC2_5 and indicated that his parents were both heterozygous and that his non-affected brother was homozygous wild-type (Figure S3A, B). The last three variants (in individuals ARMC2_3–5) introduce a premature stop codon and are therefore expected to induce nonsense-mediated mRNA decay that is likely to prevent protein synthesis. All ARMC2 variants are deposited in ClinVar under reference SUB4929442.

Overall, a total of 168 exomes from MMAF-affected individuals were analyzed, and five unrelated affected individuals were shown to carry a homozygous deleterious mutation in ARMC2. No other candidate variants reported to be associated with cilia, flagella, or male fertility were present in any of the five individuals with ARMC2 mutations. We also note that none of the individuals analyzed here carried a homozygous deleterious variant in any two (or more) of the pathological MMAF-associated genes (DNAH1, CFAP43, CFAP44, CFAP69, FSIP2, WDR66, and ARMC2), i.e., the 54 individuals with an established causal mutation did not carry another candidate variant. All mutations identified by WES were validated by Sanger sequencing, as previously described9,10 and as illustrated in Figure 1H. PCR primers and protocols used for each individual are listed in Table S4.

ARMC2 is preferentially expressed in the testis according to data from ENCODE, FANTOM, and GTEx16–18 and is described as being associated with cilia and flagella.19 We confirmed these data by RT-qPCR experiments in a panel of human tissues; these experiments indicated that expression of ARMC2 mRNA in testis is significantly higher than in other tested tissues (Figure S4). Primer sequences and RT-qPCR conditions are indicated in Table S5. According to the Uniprot server, ARMC2 is an armadillo protein composed of 12 armadillo repeats (ARM-repeat) (Figure 1I).

Sperm analysis was carried out in the source laboratories during routine biological examination of the individuals according to World Health Organization (WHO) guidelines.21 The morphology of the sperm cells was assessed with Papanicolaou staining (Figures 1A–1D). Detailed semen parameters of the five individuals with ARMC2 mutations are presented in Table 1, and the average semen parameters of the studied MMAF cohort, separated by genotypes, are described in the Table S6. Among the different parameters studied, only viability, total motility, and “lack of flagellum” presented a statistical difference.
Figure 1. Morphology of Normal and ARMC2-Mutant Spermatozoa, and the Mutations Identified in Individuals with ARMC2 Mutations

Light-microscopy analysis of spermatozoa from a fertile control individual (A) and individual ARMC2_1 (B–D). Most spermatozoa from individuals with ARMC2 mutations have flagella that are coiled (B), short (C), and/or of irregular caliber (D).

Transmission-electron-microscopy analyses of sperm cells from a control individual (E) and individual ARMC2_1 (F–G).

(E) Cross-sections of the principal piece from a fertile control individual. The axoneme is composed of nine doublets of microtubules (DMTs) circularly arranged around a central-pair complex (CPC) of microtubules (9＋2 organization). The axoneme is surrounded by

(legend continued on next page)
between the different groups, according to their genotype (one-way ANOVA test). For parameters with a positive ANOVA test, a pairwise statistical Fisher’s LSD test was employed so that significant differences between individuals with different genotypes could be identified (Figure S5). Concerning vitality, sperm from individuals with WDR66 mutations and non-characterized individuals (unknown) presented a significant increase in comparison with individuals harboring CEAP69, CFAP44, FSIP2, and ARMC2 mutations. With regard to motility, CFAP43 and CFAP44 showed the most pronounced alteration, as a result of a significant increase in sperm without a flagellum, and individuals harboring CFAP44 mutations displayed a significant increase in “no tail” sperm (Figure S5).

Sperm samples for additional phenotypic characterization could only be obtained from individual ARMC2_1. We studied the ultrastructure of sperm cells from individual ARMC2_1 by transmission electron microscopy (TEM) (Figures 1E–1G) according to the protocol previously described.9 For details, see Supplemental Material and Methods. Because of the low number of sperm cells available, only a few cross-sections (<10) were of sufficient quality for analysis. Among these sections, all were abnormal, and the main defect observed was the absence of the CPC (9 + 0 conformation) (Figure 1F). In some sections we observed a dramatic axonemal disorganization associated with peri-axonemal structural defects such as unassembled outer dense fibers (ODFs) (Figure 1G), a defect already observed in sperm from MMAF-affected individuals carrying mutations in other genes. Observation of rare longitudinal sections showed severe abnormalities such as truncated flagella or the presence of cytoplasmic structures encompassing unassembled axonemal components (not shown).

To assess the impact the absence of ARMC2 has on mouse spermatogenesis, we used the CRISPR-Cas9 technology (as previously described) to generate Armc2 mutant animals.9,22 For all experiments involving mice, animals were handled and euthanized in accordance with methods approved by the animal ethics committees of Grenoble and Geneva. All mice were adult (6 weeks or older) mice. We generated a strain with a one-nucleotide duplication in exon 4 (DupT), inducing a translational frameshift expected to lead to the complete absence of the protein or the production of a truncated protein. mRNA was extracted from Armc2 homozygous-mutant mice (Armc2mutant) and amplified by RT-PCR. The level of Armc2mutant mRNA amplification was much lower in mutant animals than in controls (Figure S6). Sanger sequencing of mRNA from Armc2 homozygous-mutant mice confirmed the production of abnormal transcripts with a premature stop codon 12 nucleotides after the first modified codon at position 135 (GenBank: NM_001034858.3 [c.403dupT]; NCBI: NP_001030030.2 [p.Tyr135LeufsTer12]) (Figure S6). The guide RNA sequence for CRISPR-Cas9 mice generation and the list of primers used for mice genotyping and RT-PCR are indicated in Table S7 and Table S8. We studied sperm morphology and observed that in contrast to what is observed in WT animals (Figure 2A), epididymal sperm from Armc2mutant males displayed a phenotype identical to the typical human MMAF phenotype: 100% of spermatozoa had short, thick, and/or coiled flagella, whereas sperm heads conserved an overall typical hooked shape (Figures 2B–2D). As could be expected, homozygous Armc2mutant males exhibited complete infertility when mated with WT females (Figure 2E), whereas homozygous Armc2mutant females were fully fertile and gave litters of normal size compared to those of WT females (8.33 ± 1.11 versus 9.67 ± 1.01 pups/litter [mean ± SE, n = 6 versus n = 3]). There was no obvious testicular anomaly; there was no difference in weight between Armc2mutant and WT testes (94 ± 20 and 92 ± 10.67 mg per testis; mean ± SE, n = 6 and n = 3, respectively) (Figure 2F). Structural defects were observed in close to 100% of spermatozoa from Armc2mutant males (Figure 2G) and were associated with a complete motility deficiency (Figure 2H). Sperm production was also affected, as shown by Armc2mutant sperm concentrations of 4.47 ± 1.29 × 10^6 sperm/mL versus concentrations of 20.75 ± 8.33 × 10^6 sperm/mL (mean ± SE, n = 6 and n = 3) in WT littermates (Figure 2I).

To define the ultrastructural defects evidenced by TEM and to characterize the molecular defects induced by ARMC2 mutation in human sperm, we studied by immunofluorescence the presence and localization of several proteins belonging to different sub-structures of the axoneme. We investigated the presence of the following proteins: SPAG6 as a marker of the CPC; DNAI2 and DNAL11 as markers of outer and inner dynein arms (ODAs and IDAs), respectively; RSPH1 as a marker of the radial spokes (RS), GAS8 as a marker of the nixin-dynein regulatory complex (N-DRC), and AKAP4 as marker of the fibrous sheath (FS). We observed that in sperm from individual ARMC2_1, staining of SPAG6, an axoneme central-pair complex protein,24 was totally absent from the flagellum (Figure 3A). In contrast, immunostaining for AKAP4, DNAL11, DNAH5, RSPH1, and GAS8 was similar to that of controls, suggesting that FS, IDAs, ODAs, RS,
and the N-DRC, respectively, were not directly affected by mutations in ARMC2 (Figure S7). Because of limited sample availability, these analyses could not be repeated on sperm from other individuals with ARMC2 mutations. Also, we regret that we could not obtain any specific ARMC2 antibodies allowing the localization of the protein in human and mouse sperm. In addition, IF experiments performed in Armc2 mutant animals showed that SPEF2 staining, another marker of the CPC, was totally absent, supporting the CPC defects observed in sperm samples from the MMAF-affected individual ARMC2-1 (Figure 3B).

We showed that the presence of bi-allelic ARMC2 mutations induces a typical MMAF phenotype in both humans and mice, indicating that this gene is necessary for spermatogenesis and, in particular, for sperm flagellum structure and motility. Bioinformatic analysis suggests that ARMC2 encodes a protein belonging to the ARM-repeat-containing protein family (Figure 1I).

ARM repeats are typically characterized by a 42-amino-acid motif composed of three a helices. Tandem ARM-repeat units fold together as “superhelixes” serving as platforms for protein-protein interactions. ARM-repeat proteins are involved in a wide range of cellular functions, including intracellular signaling, cytoskeletal regulation, and protein degradation or folding. ARM-repeat proteins are also involved in different functions in cilia and flagella; such functions include intraflagellar transport and assembly and/or stability of different axonemal components. Moreover, several ARM-repeat proteins have been described to be involved in ciliopathies or male infertility. In particular, in cattle and mice, mutations in ARMC3 and SPAG6 (a protein with eight contiguous armadillo repeats) are involved in male infertility due to sperm flagellum malformations. More recently, we also demonstrated that the absence of CFAP69, another ARM-repeat flagellum-associated protein, leads to an MMAF phenotype in humans and mice. These data clearly demonstrate that armadillo-domain repeat proteins are critical for correct spermatogenesis and flagellum formation, but the precise function of ARMC2 and of the other ARM-repeat flagellum-associated protein remains to be elucidated.
Immunostaining experiments in sperm cells from individual ARMC2_1, who harbors a splicing mutation, evidenced an absence of the SPAG6 protein, which normally locates to the C1 singlet, strongly suggesting defects in the CPC structure (Figure 3A). These findings are in concordance with TEM analysis, which revealed the absence of the central pair in the few cross-sections observed in sperm from individual ARMC2_1 (Figure 1F). Similar observations were described in the Armc2 mutant mouse, which showed abnormal staining of the SPEF2 protein (Figure 3B). IF experiments showed that other axonemal or peri-axonemal structures did not appear to be affected by the ARMC2 mutation (Figure S7), suggesting that in the absence of ARMC2, these proteins were correctly addressed and were able to maintain their normal localization within the axoneme. These observations suggest that ARMC2 might be specifically involved in the CPC assembly or stability. Interestingly, the two other armadillo-repeat proteins, SPAG6 and CFAP69, associated with MMAF phenotype are also linked to the CPC. The CPC complex consists of two singlets of microtubules, named C1 and C2, which are structurally and biochemically distinct and are surrounded by complex protein structures, known as projections, that are unique for each microtubule. It is worth noting that cilia without the CPC exist in different tissues (9 + 0 organization), indicating that the CPC is not necessary for axoneme growth and integrity; interestingly those 9 + 0 cilia are most often non-motile cilia. In contrast to cilia, the CPC seems to play a key role in maintaining the global organization of the sperm flagellum throughout spermiogenesis. Moreover, inactivation of different genes encoding proteins directly or indirectly associated with the CPC was described to lead to the MMAF phenotype in humans and in several animal models, confirming the importance of the CPC in sperm flagellum assembly and/or structure. Further experiments, including the development of functional antibodies, should now be performed so that the axonemal localization can be precisely determined and the putative role of ARMC2 in the CPC assembly or stability can be confirmed.

We present here a cohort of 167 individuals. Overall, we identified 50 individuals (29.9%) with a mutation in known MMAF-related genes; these individuals included the 35 who were previously reported (Table S1). We identified 10 different mutations in DNAH1 in 10 individuals (6%), 12 mutations in CFAP43 in 12 individuals (7.2%), two mutations in WDR66 in 11 individuals (6.6%), six mutations in CFAP44 in eight individuals (4.8%), six mutations in FSIP2 in seven individuals (4.2%), and two mutations in CFAP69 in two individuals (1.2%) (Table S1). When adding the four ARMC2-mutated individuals of the cohort, we obtained a diagnostic efficiency of 32.3% (54/167). We note that, for previously published genes, the newly described variants only include the homozygous loss-of-function variants, and we did not report the unpublished variants of questionable significance (missense variants, in-frame deletions, or variants located in splice regions other than the consensus splice sites) because we feel these need additional confirmatory work. From these results, CFAP43, WDR66, and DNAH1 appear to be the most frequently mutated genes in MMAF-affected individuals. Ten of the 11 WDR66

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**Figure 3. Immunostaining of SPAG6 and SPEF2 Revealed that the Central-Pair Complex Is Affected by Mutations in ARMC2 in Humans and Mice**

(A) Sperm cells from a fertile control individual and from individual ARMC2_1 were stained with anti-SPAG6 (rabbit polyclonal, HPA38440, Sigma-Aldrich, 1:500, green), which detects a protein located in the C1 microtubule, and anti-acetylated tubulin (monoclonal mouse T7451, Sigma-Aldrich, 1:2000, red) antibodies. SPAG6 staining uniformly decorates the full-length flagellum in the fertile control individual, whereas it is absent from the flagellum of sperm from individual ARMC2_1.

(B) Mouse sperm cells from a WT male and Armc2/−− males stained with anti-SPEF2 (rabbit polyclonal, HPA040343, Sigma-Aldrich, 1:1000, green), a marker of the projection 1b of singlet C1, and anti-acetylated tubulin (monoclonal mouse, T7451, Sigma-Aldrich, 1:500, red) antibodies. DNA was counterstained with DAPI. Contrary to the WT, the SPEF2 immunostaining is not detectable in the sperm flagellum from the Armc2/−− male. Scale bars: 10 μm.
subjects, however, harbored the same founder deletion, found only in Tunisian subjects (Table S1). The contribution of this gene in other populations could therefore be limited, although WDR66 has been reported by others to induce MMAF. WES sequencing in MMAF continues to permit the identification of new genes and pathological variants, demonstrating the efficiency of WES for investigating the genetic causes of MMAF syndrome. However, we observe here that despite regular new gene identification, the genetic causes of MMAF remain unknown for about two thirds of affected individuals. This highlights the high genetic heterogeneity of the phenotype and is consistent with the large number of genes involved in spermatogenesis. As discussed above, some variants of unknown significance in identified MMAF-associated genes could also be responsible for the infertility of some of the investigated individuals. Techniques that reliably validate the pathogenicity of these variations are currently cruelly lacking. These results also suggest that the WES approach cannot be expected to provide 100% positive diagnoses. This could also be explained in part by the fact that some variants are not detected by the technique used (e.g., such variants might include non-sequenced deep intronic variants or some exonic variants because only approximately 90% of coding nucleotides were covered) or by the current bio-informatic pipeline used for the analysis (e.g., such variants might include small duplications, structural variations, and rearrangements). To improve this diagnosis rate and to detect new variants in as-yet-uncharacterized genes, one can now envisage more powerful techniques, such as whole-genome sequencing (WGS), for MMAF-affected individuals. Several studies previously demonstrated that MMAF-affected individuals had a good prognosis after ICSI. However, it would be interesting to take all MMAF genotypes into account and compare the success rates after ICSI. Altogether, these data demonstrate that ARMC2 is essential for sperm-tail biogenesis in humans and mice and that mutations in this gene lead to drastic flagellum malformations that result in severe asthenoteratozoospermia and primary male infertility.

Accession Numbers
All ARMC2 variants reported in this manuscript are accessible in ClinVar under the accession number SUB4929442.

Supplemental Data
Supplemental Data include Supplemental Material and Methods, seven figures, and eight tables and can be found with this article online at https://doi.org/10.1016/j.ajhg.2018.12.013.

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Declaration of Interests
The authors declare no competing interests.

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Web Resources
1000 Genomes, http://www.internationalgenome.org
ENCODE, https://www.encodeproject.org
ExAC Browser, http://exac.broadinstitute.org/
FANTOM, http://fantom.gsc.riken.jp
gnomAD Browser, http://gnomad.broadinstitute.org
GTex, http://www.gtexportal.org
Online Mendelian Inheritance in Man, https://www.omim.org
SIFT, http://sift.jcvi.org


