Detection of genetic variations of five MMAF-related genes and their associations with litter size in goat

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Abstract

Background: Multiple morphological abnormalities of the sperm flagella (MMAF) makes an assignable contribution to male infertility, including QRICH2 , CFAP43 , CFAP44 , CFAP69 , CCDC39 , AKAP4 and DNAH1 gene. This work studied 28 putative indel mutations of MMAF related genes including QRICH2 , CFAP69 , CFAP43 , CCDC39 and DNAH1 gene and their correlation with the first-born litter sizes of 769 Shaanbei white cashmere (SBWC) goats. Results: Electrophoresis and DNA sequencing analysis showed the 11-bp indel within QRICH2 (QRICH2 -P4), the three indel variations in CFAP69 (CFAP69 -P4, CFAP69 -P6 and CFAP69 -P7) and the 27-bp indel of DNAH1 (DNAH1 -P1) were found to be polymorphic. The 27-bp indel variation within DNAH1 was not in consistent with HWE and the other four indel of QRICH2 and CFAP69 were in consistent with HWE. The linkage disequilibrium (LD) analysis showed the 8-bp indel (CFAP69 -P4) and the 6-bp indel (CFAP69- P6) within CFAP69 were in complete LD with each other ($D'=0.99$, $r^2=1.00$). The 27-bp indel mutation within DNAH1 was strongly significantly associated with first-born litter sizes of SBWC goats ($P \leq 0.01$) and the average litter size of II genotype was significantly greater than ID and DD genotypes ($P = 0.003$). In single-lamb and multi-lamb of goat groups, the genotype distributions of the 27-bp indel was significantly different ($P = 0.002$). While the 11-bp indel variation of QRICH2 and three indel mutations (P4, P6 and P7) of CFAP69 identified were not ($P \geq 0.05$). Conclusions: These findings suggest the 27-bp indels in the goat DNAH1 can be used as an effective molecular marker for marker-assisted selection of goats reproduction breeding in the future.

Background

In a lot of countries, especially in China, poor reproduction performance of native breed still impedes the escalation of goat industry (Cui et al., 2018; Kang et al., 2019a). Hence, finding effective and practical measures to improve goat reproduction traits is extremely urgent. Because most traits associated with reproduction are quantitative traits with low heritability, it is difficult to improve these traits by traditional breeding selection methods (Shaath et al., 2009). On the contrary, marker-assisted selection (MAS) can breed high yields and superior quality breeding quickly to enhance economic traits (Collard et al., 2008; Kang et al., 2019b; Knorst et al., 2019). Now, natural genetic variations were divided into insertion/deletion (indel), single nucleotide polymorphism (SNP) and larger structural variants (SV) (Cui et al., 2018). Among them, indels can be directly and easily detected by PCR technology making it convenient and practical. So indel detecting technique for reproduction related candidate genes has been developed and widely used in many studies (Ren et al., 2017; Wang et al., 2018; Zhang et al., 2018).

Multiple morphological abnormalities of the sperm flagella (MMAF) makes an assignable contribution to male infertility, characterized by a mosaic of morphological abnormalities of the flagellum including coiled, bent, irregular, short or/and absent flagella (Amiri-Yekta et al., 2016; Shen et al., 2019). According to Shen's report (Shen et al., 2019), to date, mutations in only seven genes have been determined in humans related to MMAF: *QRICH2* (*Glutamine Rich 2*), *CFAP43* (*Cilia And Flagella Associated Protein 43*), *CFAP44* (*Cilia And Flagella Associated Protein 44*) , *CFAP69* (*Cilia And Flagella Associated Protein 69*), *CCDC39* (*Coiled-Coil Domain Containing 39*) *AKAP4* (*A-Kinase Anchoring Protein 4*) and *DNAH1* (*Dynein*

Axonemal Heavy Chain 1) (Baccetti et al., 2005; Merveille et al., 2011; Ben Khelifa et al., 2014; Coutton et al., 2015; Tang et al., 2017; Coutton et al., 2018; Dong et al., 2018; Shen et al., 2019). Recent studies have also certified some male related genes also expressed in female reproductive structure and might were able to affect female reproduction (Zariwala et al., 2011; Kang et al., 2019a; Kang et al., 2019b). Meanwhile, there were studies confirmed *QRICH2*, *CFAP44*, *CFAP69*, *CCDC39* and *DNAH1* were broad spectrum expression including in testis, ovaries and placentas (Fagerberg et al., 2014). These findings suggest that MMAF related genes might play a crucial role in female reproduction, but there was no paper reported these genes were related to goat reproduction performance yet.

Based on the above-mentioned studies, we hypothesized that MMAF-related genes were linked to goat reproduction traits. Therefore, the objectives of this study were to identify genetic polymorphisms in the MMAF related genes in 769 SBWC goats by high-efficiency and simple indel method, to determine the relationship between allele variation of MMAF related genes and goat reproduction traits, as well as provide some scientific basis for the efficient and rapid development of goat industry.

Methods

Ear tissue samples and DNA isolation

A total of 769 adult female SBWC goats were physical maturity and were selected randomly from a large population. They all were raised in SBWC goat farm of Shaanxi Provincial Engineering and Technology Research Center of Cashmere Goats of Yulin University. These goats received the same diets and kept under same environment. Their first-born litter sizes were recorded by husbandry station recorders. These goat individuals were randomly selected from goat farm, in order to make sure that these goats were unrelated with each other. All ear tissue samples were collected before slaughter and were frozen so as to prevent sample from degradation. Genomic DNA was isolated using High salt-extraction method from ear tissues and was assayed by College of Animal Science and Technology of Northwest A&F University (Aljanabi et al., 1997). The goat DNA samples were diluted for further experiment (Lan et al., 2013).

Primer design and PCR amplification

By Ensembl online database (<http://asia.ensembl.org/>), putative indel mutations of seven MMAF related genes were studied but only *QRICH2*, *CFAP69*, *CFAP43*, *CCDC39* and *DNAH1* gene exist indel mutations which could be used as indel typing detection. According to these putative indel variations, a total of 28 pairs of primers were designed for amplifying indel loci of *QRICH2*, *CFAP69*, *CFAP43*, *CCDC39* and *DNAH1* based on the goat sequence (NC_030829.1, NC_030826.1, NC_030811.1, NC_030833.1 and NC_030808.1) using Primer software 5.0 (Canada, Premier). The traditional PCR program was performed in 13 µL of reaction mixture containing 24 µg genomic DNA; the multiplex PCR program were performed in 20 µL of reaction mixture containing 48 µg genomic DNA. PCR products were detected by electrophoresis in 3.5% agarose gel stained with ethidium bromide.

Detection of five indel mutations of MMAF-related gene

Among MMAF-related genes, for *QRICH2* and *CCDC39* gene, four putative indel mutations were verified by traditional PCR program respectively; for *CFAP43* and *CFAP69* gene, seven pairs of primers were designed to amplify product fragments severally; for *DNAH1* gene, six pairs of primers were designed for amplifying indel fragments. All primers designed were shown in **Table S1**. These indels were all amplified by PCR program in order to identify polymorphisms of MMAF-related genes in SBWC goats. For *CFAP69*-P4 and *CFAP69*-P6 loci, because fragment lengths of them were different and these two indels both were located in *CFAP69* gene, these two indel loci were amplified using multiplex PCR method and identified genotypes (Henegariu et al., 1997; de Cássia-Pires et al., 2017).

Statistical Analysis

Correlation between different genotypes and goat litter sizes were all performed by Statistical program for social sciences 23.0 software by single-factor analysis of variance procedure. Associations of the these indel loci with goat litter sizes of 769 individuals were determined using a mixed linear model. A least-squares mean test was used to determine the correlation of litter sizes with different indel genotypes according to the formula parameters:

$$Y_{ij} = \mu + S_i + HYS_j + G_i + e_{ij}$$

Here, Y_{ij} stands for the phenotypic value of each litter sizes; μ represents the overall population mean; S_i stands for the kidding year; HYS_j represents the population' mean; G_i stands for the fixed effect of genotype; and e_{ij} stands for random error (Zhao et al., 2013). All data were expressed as mean $\pm$ standard error and $P < 0.05$ was considered to be significant. Population indexes (H_o , homozygosity; H_e , heterozygosity; PIC, polymorphism information content) were calculated following Nei's methods (Nei, 1973). The chi-square (χ^2) test and hardy-Weinberg equilibrium (HWE) were performed using SHEsis (<http://analysis.bio-x.cn>) and Linkage disequilibrium (LD) structure was evaluated across these five indel mutations (Wang et al., 2019). The r^2 value was known as a pairwise measure of LD. The case of $r^2 \leq 0.33$ is known as not strong LD, $r^2 > 0.33$ regarded as sufficiently strong LD, and $r^2 = 1$ known as complete LD (Reich et al., 2001).

Results

Identification of indel polymorphisms within MMAF-related genes

According to Ensembl online database, only *QRICH2*, *CFAP43*, *CFAP69*, *CCDC39* and *DNAH1* genes exist putative indel mutations in goat and this Results identified only five indel loci within *DNAH1* (P1), *QRICH2* (P4), *CFAP69* (P4, P6, P7) were polymorphic. The information of five indel mutations are as follows: the 11-bp indel (NC_030826: rs65182110;g.54660931_54660942delCCCCACCGCACC) within *QRICH2* was confirmed by primer 4; the 8-bp indel (NC_030811: rs646423399;g.45735111_45735118delCTTAGATC), the 6-bp indel (NC_030811:rs654691768;g.45748633_45748638delGTGAAA) and the 8-bp indel (NC_030811:rs658907780;g.45775640_45775647delTCCCATTA) within *CFAP69* was authenticated by

primer 4, 6 and 7 respectively; the 27-bp indel (NC_030829:rs636295440;g.48594103_48594129delAAGAGAGCAATGTCCAGGGCGCGGGGT) within *DNAH1* was identified by primer 1. The results of PCR products of these five indels by agarose gel electrophoresis were all verified by sequence diagrams **Fig.S1**.

Genotyping and genetic parameters of genetic variations of MMAF-related genes

The results of agarose gel electrophoresis showed these five indel mutations including *QRICH2* (P4), *CFAP69* (P4, P6, P7) and *DNAH1* (P1) all generated three genotypes: homozygotic insertion type (II, insertion/insertion), heterozygote type (ID, insertion/deletion) and homozygotic deletion type (DD, deletion/deletion); for these five indels, the “D” allele presented a lower frequency compared with “I” allele, suggesting that the “I” allele occupied the dominant hierarchy in 769 individuals. For *QRICH2*-P4, *CFAP69*-4, *CFAP69*-6 and *CFAP69*-7 indel mutations, the χ^2 test indicated genotype distributions were in consistent with HWE ($P \geq 0.05$), Meanwhile, for the 27-bp indel variation within *DNAH1* gene, in 632 SBWC goat samples, the genotype distribution was not in consistent with HWE ($P = 2.37E-05$) (**Table 1**). This study analyzed the haplotypic frequencies of these five indel mutations in 769 SBWC goats. In the order of *QRICH2*-P4, *CFAP69*-P4, *CFAP69*-P6, *CFAP69*-P7 and *DNAH1*-P1, the analyzed results showed the haplotype (Hap1, I₁₁I₈I₆I₈I₂₇) had the highest haplotypic frequency (0.432) (**Table 2**).

Result of linkage disequilibrium analysis of indels within *CFAP69* gene

According to the result of genotypes of *CFAP69*-P4 and *CFAP69*-P6 indel variations (**Fig.S1**) and linkage disequilibrium (LD) analysis (**Fig.1**), this study found *CFAP69*-P7 this indel locus was not strong LD with *CFAP69*-P4 or *CFAP69*-P6 indel loci ($r^2 \leq 0.33$), but *CFAP69*-P4, *CFAP69*-P6 indels were in complete LD with each other ($D' = 0.99$, $r^2 = 1.00$), suggesting that there was certain combination between these two loci. Because the other indel loci were not located in the same Chromosome with *CFAP69* gene, this study did not analyze the LD between them with *CFAP69* gene.

Correlation analysis between indels of MMAF-related gene and goat litter size

Correlations between different genotypes of *QRICH2*, *CFAP69* and *DNAH1* indel loci with goat litter size were analyzed. For the *QRICH2*-P4, *CFAP69*-P4 and *CFAP69*-P6 and *CFAP69*-P7 indel mutations, the relationship between different genotypes of them were all not significant associated with goat litter size ($P \geq 0.05$) (**Table 3**). According to χ^2 test, the relationship between different genotypes with goat litter size was also not significant as well ($P \geq 0.05$) (**Table 4**). But for the *DNAH1*-P1 locus, mean values of goat litter size with II, ID and DD genotypes in litter sizes were significantly different. The II carriers had the highest values of litter sizes compared with ID or DD carries ($P = 0.003$) (**Table 3**). Meanwhile, χ^2 test result showed that different genotypes of the *DNAH1*-P1 indel locus in single-lamb and multi-lamb of goat samples were also significantly different ($P = 0.002$) (**Table 4**).

Discussion

MMAF, characterized by a mosaic of morphological abnormalities of the flagellum, was an important factor leading to male infertility and has been widely reported and there were some genes have been reported associating with this symptom (Amiri-Yekta et al., 2016; Shen et al., 2019). What does the MMAF-related genes have to do with female goat fertility, which has not been studied. As an MMAF related gene, *DNAH1*, also known as *HL11*, *HDHC7*, *CILD37*, *DNAHC1*, *HSRF-1*, *SPGF18* and *XLHSRF-1*, encodes an inner arm heavy chain of axonemal dynein. *DNAH1* appears as a reproduction candidate gene and belongs to broad spectrum expression including in testis, trachea, ovaries and placentas (Maiti et al., 2000; Fagerberg et al., 2014). *DNAH1* has been reported to be associated with MMAF and Primary ciliary dyskinesia (PCD) et al (Zariwala et al., 2011; Ben Khelifa et al., 2014; Imtiaz et al., 2015). MMAF makes an assignable contribution to male infertility and recent studies have also certified some male related genes also expressed in female reproductive structure and might were able to affect female reproduction (Zariwala et al., 2011; Kang et al., 2019a; Kang et al., 2019b). Similarly, PCD is a clinically and genetically heterogeneous disorder of motile cilia dysfunction typically caused by an autosomal recessive mode of inheritance. Clinically, males can be infertile due to immotile sperm flagella and female patients can also present with sub-fertility due to defective oviduct cilia (Zariwala et al., 2011; Imtiaz et al., 2015). Taken together, *DNAH1* gene could affect both male and female reproduction performance and whether affect goat reproductive trait deserved to be studied.

In this study, results showed the 27-bp indel polymorphism within *DNAH1* was significantly correlated with goat litter sizes ($P = 0.003$) and different genotypes of this indel locus in single-lamb and multi-lamb of goat samples were also significantly different ($P = 0.002$). For the other indel variations within *QRICH2* and *CFAP69*, there were no significant correlation with goat first litter size ($P \geq 0.05$) (**Table 3**). Genetic parameters of these indel mutations of *QRICH2* and *CFAP69* gene were in consistent with HWE ($P \geq 0.05$), only the 27-bp indel genotype distribution in *DNAH1* was not in consistent with HWE ($P = 2.37E-05$) (**Table 4**). Those showed with long period of artificial traditional selection, as a reproduction related site, this indel of *DNAH1* probably has been selected and the 27-bp indel within *DNAH1* gene played an important role in first-born litter sizes of SBWC goats.

Compared with exon mutations within candidate genes, variations in introns tend to more common and easier appear and deserved to be studied. Some studies have reported that polymorphisms within intron region had positive effects on gene expression and affected many economic traits of livestock by various ways (Kalkan et al., 2013; Kanzi et al., 2016; Vaz-Drago et al., 2017). The representative example is a nucleotide substitution in intron 3 of porcine *IGF2* significantly affecting skeletal muscle by transcription factor called ZBED6 (Van Laere et al., 2003; Xiang et al., 2018). Meanwhile, some reports showed transcription factors might gene expression (Cartharius et al., 2005; Kang et al., 2019a). By online website (<http://www.genomatix.de/>), we found there was no any differential transcription factor appearing in the 27-bp indel region of *DNAH1* gene, so how the 27-bp indel mutation within *DNAH1* gene significantly affects goat reproduction traits still deserves to be further studied.

Many reports have proved different gene mutation sites could regulate phenotypes of organism through LD (Gibson et al., 2013; Lynch et al., 2014; Wang et al., 2019). Based on multiplex PCR method and LD

analysis, this study first discovered *CFAP69-P4* and *CFAP69-P6* were in complete LD ($D' = 0.999$, $r^2 = 1.000$) (**Fig.1**), suggesting there was certain combination of these two loci in the process of SBWC goat evolutionary selection. These two indel mutations probably played an important role in regulating goat other phenotypes.

Conclusions

This study found the 27-bp indel within *DNAH1* intron was strongly significantly affecting goat litter sizes and could be provided as a useful DNA marker in order to selecting superior individual for goat reproduction breeding.

Declarations

Ethics approval and consent to participate

All experiments involving animals in this study were approved by the animal policy and welfare committee of Northwest Agriculture and Forestry University (Protocol Number NWAFA1008). In addition, the use and care of laboratory animals fully complied with local animal welfare guidelines, laws and policies.

Consent for publication

Not applicable.

Availability of data and materials

Relevant data are available from the corresponding author.

Competing interest

There was no conflict of interest between the authors.

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Authors' contributions

XL and XS raised thought and designed the project. HC, CP and LQ all contributed by collecting samples and recorded reproduction data. ZW, YP and LH conducted this experiment. ZW wrote the manuscript and all authors agreed with the final manuscript.

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Abbreviations

MMAF, Multiple Morphological Abnormalities of the Sperm Flagella; QRIH2, Glutamine Rich 2; CFAP69, Cilia And Flagella Associated Protein 69; CFAP44, Cilia And Flagella Associated Protein 44; CFAP43, Cilia And Flagella Associated Protein 43; CCDC39, Coiled-Coil Domain Containing 39; AKAP4, A-Kinase Anchoring Protein 4; DNAH1, Dynein Axonemal Heavy Chain 1; SBWC, Shaanbei white cashmere goat; MAS, marker assisted selection; indel, insertion/deletion; PCR, polymerase chain reaction; bp, base pair; IL, insertion/deletion; DD, deletion/deletion; ID, insertion/deletion; LD, linkage disequilibrium; Ho, homozygosity; He, heterozygosity; PIC, polymorphism information content; HWE, Hardy-Weinberg equilibrium.

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Tables

Table 1 Genotypic and allelic frequencies and population indexes for five indel mutations of MMAF-related genes

Gene	Primers	Loci	Genotypes	Frequency		Ho	He	PIC	χ^2 (p-value)
				Genotypes	Alleles				
<i>QRICH2</i>	P4	11 bp (n=300)	II (n=130)	0.433	0.650 (I)	0.545	0.355	0.351	0.680 (<i>P</i> = 0.409)
			ID (n=130)	0.433	0.350 (D)				
			DD (n=40)	0.133					
<i>CFAP69</i>	P4	8 bp (n=274)	II (n=208)	0.759	0.874 (I)	0.780	0.220	0.196	0.544 (<i>P</i> = 0.461)
			ID (n=63)	0.230	0.126 (D)				
			DD (n=3)	0.011					
	P6	6 bp (n=274)	II (n=208)	0.759	0.874 (I)	0.780	0.220	0.196	0.544 (<i>P</i> = 0.461)
			ID (n=63)	0.230	0.126 (D)				
			DD (n=3)	0.011					
	P7	8 bp (n=300)	II (n=212)	0.707	0.843 (I)	0.736	0.264	0.229	0.355 (<i>P</i> = 0.551)
			ID (n=82)	0.273	0.157 (D)				
			DD (n=6)	0.020					
<i>DNAH1</i>	P1	27 bp (n=300)	II (n=288)	0.960	0.978 (I)	0.958	0.042	0.041	5.476 (<i>P</i> = 0.020)
			ID (n=11)	0.037	0.022 (D)				
			DD (n=1)	0.003					
		27 bp (n=632)	II (n=595)	0.941	0.968 (I)	0.937	0.063	0.061	17.867 (<i>P</i> = 2.37E-05)
			ID (n=33)	0.052	0.032 (D)				
			DD (n=4)	0.007					

Note: indel mutations of *CFAP43* and *CCDC39* gene not exist polymorphisms; n represents individual number. The same below.

Table 2 Haplotypic frequencies within these five indels of MMAF-related gene in Shaanbei white cashmere goat

Haplotypes	arrangements	frequencies
Hap1	I ₁₁ I ₈ I ₆ I ₈ I ₂₇	0.432
Hap2	I ₂₇ D ₁₁ I ₈ I ₆ I ₈ I ₂₇	0.233
Hap3	I ₁₁ I ₈ I ₆ D ₈ I ₂₇	0.103
Hap4	I ₁₁ D ₈ D ₆ I ₈ I ₂₇	0.076
Hap5	D ₁₁ I ₈ I ₆ D ₈ I ₂₇	0.056
Hap6	D ₁₁ D ₈ D ₆ I ₈ I ₂₇	0.041
Hap7	I ₁₁ D ₈ D ₆ D ₈ I ₂₇	0.018
Hap8	I ₁₁ I ₈ I ₆ I ₈ D ₂₇	0.014
Hap9	D ₁₁ D ₈ D ₆ D ₈ I ₂₇	0.010
Hap10	D ₁₁ I ₈ I ₆ I ₈ D ₂₇	0.007
Hap11	I ₁₁ I ₈ I ₆ D ₈ D ₂₇	0.003
Hap12	I ₁₁ D ₈ D ₆ I ₈ D ₂₇	0.002
Hap13	D ₁₁ I ₈ I ₆ D ₈ D ₂₇	0.002
Hap14	D ₁₁ D ₈ D ₆ I ₈ D ₂₇	0.001
Hap15	I ₁₁ D ₈ D ₆ D ₈ D ₂₇	0.001
Hap16	D ₁₁ D ₈ D ₆ D ₈ D ₂₇	0.001

Note: the order of haplotype of different loci was as follow: the 11-bp indel of *QRICH2* gene; the 8-bp indel (P4), the 6-bp indel (P6) and the 8-bp indel (P7) within *CFAP69*; the 27-bp indel in *DNAH1* gene.

Table 3 Associations of indel mutations of MMAF-related genes and litter size of Shaanbei white cashmere goat (mean $\pm$ standard error)

Gene	Primers	Sizes	II	ID	DD	p-values
<i>QRICH2</i>	P4	300	1.52 $\pm$ 0.05 (n=130)	1.55 $\pm$ 0.05 (n=130)	1.60 $\pm$ 0.09 (n=40)	0.436
<i>CFAP69</i>	P4	274	1.45 $\pm$ 0.04 (n=208)	1.54 $\pm$ 0.06 (n=63)	1.67 $\pm$ 0.33 (n=3)	0.206
	P6	274	1.45 $\pm$ 0.04 (n=208)	1.54 $\pm$ 0.06 (n=63)	1.67 $\pm$ 0.33 (n=3)	0.206
	P7	300	1.56 $\pm$ 0.04 (n=212)	1.67 $\pm$ 0.06 (n=82)	1.83 $\pm$ 0.31 (n=6)	0.098
<i>DNAH1</i>	P1	300	1.95 $\pm$ 0.02 ^a (n=288)	1.55 $\pm$ 0.16 ^b (n=11)	--	0.029
		632	1.53 $\pm$ 0.02 ^A (n=595)	1.24 $\pm$ 0.08 ^B (n=33)	1.00 $\pm$ 0.00 ^C (n=4)	0.003

Note: '--' stands for n $\leq$ 3.

Table 4 Genotype distribution of MMAF-related gene indel mutations between mothers of single-lamb and multi-lamb in Shaanbei white cashmere goat

Gene	Primers	Sizes	MSL genotypes (frequencies)			MML genotypes (frequencies)			<i>p</i> -values
			II	ID	DD	II	ID	DD	
<i>QRICH2</i>	P4	300	65	62	17	65	68	23	0.705
<i>CFAP69</i>	P4	274	116	29	1	92	34	2	0.312
	P6	274	116	29	1	92	34	2	0.312
	P7	300	97	29	2	115	53	4	0.239
<i>DNAH1</i>	P1	632	298	25	4	297	8	0	0.002

Note: MSL, mothers of single lamb; MML, mothers of multi-lamb (≥2).

Figures

Figure 1

Linkage disequilibrium plot of three indel mutations within CFAP69 (CFAP69-P4, CFAP69-P6 and CFAP69-P7) in Shaanbei white cashmere goats

Supplementary Files

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