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Segregation of the InDel Mutation in *IRF2BP2* Discriminating Fleece Type in North African and Iranian Sheep Breeds

Research Article

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ABSTRACT

In sheep, the woolly or hairy fleece type shows variation within and between breeds and populations. Recently, the woolly allele was shown to result from the insertion of an antisens retrogene into the 3'UTR of the ovine *IRF2BP2* gene. The purpose of this study was to examine this InDel polymorphism within the *IRF2BP2* gene using a specific PCR genotyping in 17 North African (Algeria and Tunisia) and Iranian sheep breeds showing various fleece types. The genotyping of the overall animals (n=908) showed the segregation of the two alleles insertion (I) and deletion (D), and the three genotypes (II, ID and DD) with various frequencies. Ouled Djellal, Rembi and Tâadmit known as woolly breeds had 100%, 94% and 94% of II homozygous animals, respectively. In contrast, Ifilène and Sidaou known as hairy breeds had 100% and 99% of DD homozygous animals. Other breeds showed the segregation of the I allele with a frequency from 3% to 91%. An association analysis, carried out only for the Algerian sheep breeds, revealed that this polymorphism was significantly associated with the fleece type trait ($P<0.05$). Thus, the current work meets previous results to further confirm the genetic effect of the insertion occurring into the 3′ UTR of the *IRF2BP2* gene on the fleece type trait of sheep breeds.

KEY WORDS association, gene, hairy, woolly.

INTRODUCTION

Improvement in sheep wool and hair production is of critical importance in several countries (Purvis and Franklin, 2005). As one of the most important traits, fleece weight and wool quality have received much more consideration (Holman and Malau-Aduli, 2012) particularly in North Africa where the wool industry comes second after meat or milk in Algeria and Tunisia (Ansari-Renani, 2012; Harizi *et al.* 2015; Belharfi *et al.* 2018). In these countries, the wool is sold in the local market and used in carpet industry and craftsmanship, also to produce clothes and bedding elements as mattresses, blankets and pillows (Djennadi, 2006; Harizi and Abidi, 2015). Various genetic and environmental factors can simultaneously affect wool traits having low to moderate heritability levels (Naidoo *et al.* 2004; Zhao *et al.* 2021). Indeed, the ranges of heritability estimates were 0.2 to 0.57 for the fleece weight and 0.5 to 0.68 for the fiber diameter (Snyman *et al.* 1996; Wuliji *et al.* 2001; Safari *et al.* 2005; Safari *et al.* 2007). Proteomic and biochemical studies allowed better understanding of properties and composition of ovine wool fiber. It comprises several proteins, and particularly numerous keratins (KRTs), keratin-associated proteins (KRTAPs) and trichohyalin (Yu *et al.* 2009; Gong *et al.* 2016). This latest type of protein has been defined as an interfilamenteous matrix, which also comprises the KRTAPs (Dale *et al.* 1980). Several authors revealed that the physical properties of fiber are mostly regulated by the spatial organization of those proteins and the nature of their chemical bonding in the matrix (Powell and Rogers, 1997; Li *et al.* 2009; Harel and Christiano, 2012; Dierks *et al.* 2013). Quite a lot of genetics, genomics and transcriptomics investigations, possibly focusing of these candidate genes have searched for molecular variations that can affect wool fiber and fleece quality in sheep (Allain and Renieri, 2010; Gutierrez-Gil *et al.* 2017; Zhang *et al.* 2017; Ebrahimi *et al.* 2017; Bai *et al.* 2019; Li *et al.* 2019; Li *et al.* 2020; Ghoreishifar *et al.* 2021; Shi *et al.* 2021). The molecular origin of fleece type variation (woolly vs. hairy) was resolved recently (Demars *et al.* 2017). These authors demonstrated that the “woolly” allele results from the insertion of an antisense *EIF2S2* retrogene (called *asEIF2S2*) into the 3′ UTR of the *IRF2BP2* gene. These results shed also light on the evolutionary variation of the fleece type indicating that ancestral ovine species were characterized by a long and hairy fleece. The second wave of domestication involved the improvement of wool and milk traits and was accompanied by a shift of the fibers to become less hairy leading the modern domestic sheep (Ovis aries) to have a short and woolly fleece, associated with the retrogene inserted into *IRF2BP2* (Chessa *et al.* 2009; Demars *et al.* 2017).

In North Africa, Algeria has the largest sheep population with 29 million heads in 2021. It exhibits a great phenotypic diversity, particularly for fleece type and color (Djaout *et al.* 2017), The Ouled Djellal breed with a white woolly fleece has been introduced either by the Ben-Hillal who came to Algeria in the 13th century from Hidjaz (Arabia) or earlier by the Roman from Italy in the 5th century (Trouette, 1929). Rembi is a composite woolly breed between the Djebel Amour mouflon (also called Laroui) and the Ouled Djellal breed, exibiting the conformation of Ouled Djellal and the color and horn of mouflon (Magneville, 1959). The prolific D’man breed is a Saharan breed from the oases of southwest Algeria (Chellig, 1992). The coloring of D’man sheep varies a lot and the fleece type could be either woolly or hairy. The rustic Berbère breed (also called Azoulaï wool Berbère) is the oldest sheep breed in the Maghreb. This is a small size breed with a shiny white wool (Djaout *et al.* 2017). The Sidaou hairy breed is well adapted to Saharan climate and it is believed to originate in Mali or Sudan (Chellig, 1992). The Tâadmit breed is a cross between the Ouled Djellal and the Merinos breeds started in 1922 with the double objective of wool and meat production (Trouette, 1929). The Hamra breed with brown skin and woolly white fleece (Djaout *et al.* 2017). Darâa animal has black skin (head and limbs) and brown wool, the wool of this breed is used for the manufacture of Bernousse (Djaout *et al.* 2017). The Srandi (or Sordi, Sardi) breed with its Sardi counterpart from Morocco, locates in the Algerian-Moroccan border but its origin is not really defined. It is characterized by white wool and black spots on the ears, eyes, legs and snout. The Tazegzawt or Ham breed shows a white woolly fleece and a face with dark bluish spots (Djaout *et al.* 2017). The Ifilène hairy breed is native from Mali, Niger and Nigeria (also known as Uda) (Djaout *et al.* 2017). The Barbarine breed present in East Algeria and reared for meat, milk and wool purposes holds its origin form the fat-tailed Tunisian Barbarine, but exhibits a half fat tail (Djaout *et al.* 2017).

Tunisia raises 6.5 million sheep, mostly of four breeds: Barbarine, Queue Fine de l'Ouest, Noire de Thibar, and Sicilo-Sarde (ONAGRI, 2018). The Barbarine and Queue Fine de l'Ouest are the most common breeds, accounting for more than 90% of Tunisian sheep (Iniguez, 2006). The fat-tailed Barbarine is a woolly breed with white fleece that originated in the Asiatic steppes (Khaldi, 1989). The Noire de Thibar breed, used for wool and meat production, was created through crossbreeding between the native Queue Fine de l'Ouest and the French Merinos d'Arles woolly breeds (Chalh *et al.* 2007). The Sicilo-Sarde breed has a woolly white fleece and was created in the late 1800s by a cross between the Italian Sarda and the Comisana breeds (Djemali, 2000).

According to the Iranian Ministry of Agriculture (2020), there are more than 45 million sheep in Iran, representing almost 30 different native breeds. The Mehraban sheep, which are raised in the western Iranian province of Hamedan, is one of the most interesting sheep breeds in this country. The Mehraban sheep is a fat-tailed carpet wool sheep with a light body, dark face, and brown neck that can thrive in rocky and severe conditions. Meat production is the main breeding purpose of Mehraban sheep.

Studies on fleece are absent or even minimal in the Maghreb countries (Algeria and Tunisia) and in Iran. The purpose of the present study was to improve the genetic understanding of ovine fleece type variation (woolly and hairy), and to further confirm the relation between the genetic polymorphism at the *IRF2BP2* locus and this morphological phenotype.

MATERIALS AND METHODS

Sample collection

Whole blood was collected from the jugular vein using vacutainers containing K2-EDTA during routine animal sanitary controls by an authorized veterinarian. In Algeria, blood samples were collected randomly for twelve sheep breeds between the years 1999 and 2019: Sidaou (n=72), Hamra (n=73), Sardi (n=11), Ifilène (n=9), Darâa (n=8), Ouled Djellal (n=37), Tâadmit (n=54), D'man (n=45), Tazegzawt (n=24), Barbarine (n=37), Rembi (n=95),and Berbère (n=26). In Tunisia, sampling was carried out between the years 2010 and 2014 on sheep belonging to Barbarine (n=272), Queue Fine de l'Ouest (n=25), Noire de Thibar (n=23) and Sicilo Sarde (n=22) breeds. Mehraban samples (n=75) from Iran were collected at random between 2008 and 2016 as already described (Talebi *et al.* 2018). For all breeds, data collected was recorded during a field survey based on herds of different sizes. Information on the geographic distribution of sampling in Algeria and Tunisia is shown in the supplemental Figure 1 and distribution of Mehraban sheep breed was in Western part of the Iran with the GPS Coordinates Lat: 34.79922 / N 34˚ 47' 57.192", Long: 48.51456 / 48˚ 30' 52.416").

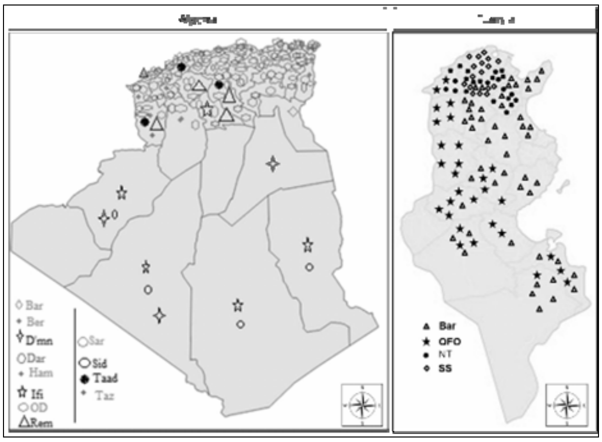

Figure 1 Distribution of sampled flocks from sixteen sheep breeds. Algeria (Bar: Barbarine, Ber: Berbère, D'mn: D'man, Dar: Daraa, Ham: Hamra, Ifi: Ifilène, OD: Ouled Djellal, Rem: Rembi, Sar: Sardi, Sid: Sidaou, Taad: Taadmit, Taz: Tazegzawt), Tunisia (Bar: Barbarine, QFO: Queue Fine de l'Ouest, NT: Noire de Thibar and SS: SiciloSarde)

Phenotypic data

We collected phenotypic information for fleece only from Algerian sheep breeds, sampled. We showed that all breeds samples had hairy (like that of a goat, is coarse and rough) or woolly fleece (Wool fibers are soft and delicate and have a circular cross-section and are very fine, with a diameter ranging from 20 to 80 microns) (Djaout *et al.* 2017; Belharfi *et al.* 2018) (Figure 2).

Figure 2 Fleece type variation observed in Algerian breeds. (a, b) Sidaou animal with a hairy fleece type. (c,d) Ouled Djellal animal with a woolly fleece type

All phenotypic data are reported in Table 1. One representative animal for each breed of each country is in the Figure 3, Figure 4 and Figure 5.

PCR amplification and genotyping

Genomic DNA from blood sample was extracted following the salting out protocol (Miller *et al.* 1988). The genotyping of the Indel polymorphism in the 3' UTR of the *IRF2BP2* gene followed the original design developed in Demars *et al.* (2017). It consists in an allele-specific PCR amplification using three primers (Table 2) in the same reaction, two outside and one inside the insertion (Figure 6). This resulted in a 225 bp fragment to attest for the insertion, and a 465 bp fragment to attest for the deletion (Figure 7). PCR reaction was performed in a final volume of 20 µL containing 20 ng of genomic DNA, 0.5U GoTaq DNA polymerase (Promega), 4 µL GoTaq PCR buffer (5X), 0.2 mM dNTPs, 0.6 µM of the forward primer and 0.3 µM of each reverse primer (Table 2). PCR amplification was carried out on a 2720 thermal cycler (Life Technologies) with the following condition: 5 min denaturation at 95 ˚C, 40 amplification cycles of 30 s at 95 ˚C, 30 s at 56 ˚C, 1 min at 72 ˚C and 7min final elongation at 72 ˚C. Genotype analysis of the PCR products was resolved on 1% agarose gel electrophoresis. -

Data analysis

Genotypic and allelic frequencies were calculated within and across breed, based on counting the respective genotypes of individual animals. Cervus v. 3.0.3 (Marshall *et al.* 1998) software was used to analyze the number of alleles, observed (Ho) and expected heterozygosity (He: corrected for sampling bias) and polymorphic information content (PIC).

Table 1 Features and information of the studied sheep breeds

Country	Breed	Geographic distribution	Production	Tail type	Observed fleece type	According to FAO
Algeria	Barbarine_Algeria	Steppe and Northeast of desert	Meat	Fat	Wool	Wool
	Berbère	Mountain	Meat	Thin	Wool	Wool
	D'man	Oasis	Meat	Thin	Wool / Hair	Wool
	Darâa	Steppe	Meat	Thin	Wool	
	Hamra	Steppe	Meat	Thin	Wool	Wool
	Ifilène	desert	Meat	Thin	Hair	
	Ouled Djellal	North Steppe and Northern Sahara	Meat	Thin	Wool	Wool
	Rembi	Steppe	Meat	Thin	Wool	Wool
	Sardi	Mountain	Meat	Thin	Wool	
	Sidaou	Desert	Meat	Thin	Hair	Hair
	Tâadmit	Steppe	Meat	Thin	Wool	Wool
	Tazegzawt	Mountain	Meat	Thin	Wool	
Tunisia	Barbarine_Tunisia	Over the country	Meat	Fat		
	Noire de Thibar	North	Meat	Thin		
	Queue Fine de l'Ouest	Over the country except the desert	Meat	Thin		Wool
	Sicilo Sarde	North Ouest	Milk	Thin		Wool
Iran	Mehraban	Hamedan province	Meat	Fat		Wool

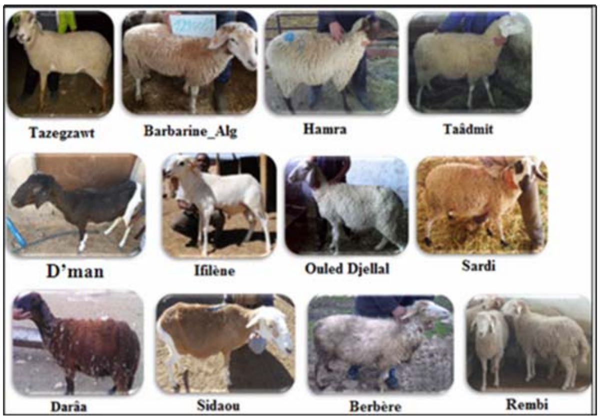

Figure 3 Photographs of the Algerian studied sheep breeds

Figure 4 Photographs of the Tunisian studied sheep breeds

Genepop v.4 software (Raymond and Rousset, 1995) was utilized to calculate the exact test for Hardy–Weinberg equilibrium. Analyses of molecular variance (AMOVA) were carried out on two datasets by using the Arlequin program (Excoffier *et al.* 2005). The first analysis included data for all seventeen breeds; the second dataset consisted in performing the analysis by grouping breeds according to their original country (Algeria, Tunisia and Iran).

Figure 5 Photographs of the Iranian studied sheep breed

The association of InDel in *IRF2BP2* gene with the fleece type (woolly/hairy) in the ovine Algerian breeds was tested using SPSS 26.0. Three packages of R program (vcd, ggstatsplot and ggplot2) were used to draw a specific plot. It combines a bar plot (to visualize a contingency table) and the result of the Chi-square test of independence.

RESULTS AND DISCUSSION

A previous report identified an approximately 1400 bp Indel into the 3′ UTR of the ovine *IRF2BP2* gene associated with fleece type mainly in French sheep breeds (Demars *et al.* 2017). We have specifically search for this polymorphism segregation in North African and Iranian local sheep breeds. After resolution on 1% agarose gel, the PCR genotyping of this Indel realized on 908 DNA samples from 17 North African and Iranian ovine breeds (12 Algerian breeds: Sidaou, Hamra, Sardi, Ifilène, Darâa, Ouled Djellal, Tâadmit, D'man, Tazegzawt, Barbarine, Rembi and Berbère; 4 Tunisian breeds: Queue Fine de l'Ouest, Barbarine, Noire de Thibar and Sicilo-Sarde; and 1 Iranian breed: Mehraban).

Table 2 The sequence and position of IRF2BP2 PCR primers for InDel genotyping

Sequence	Strand	Genomic coordinates (Oar_v3.1)
Forward: TGGTTCACAACCCAGCTTTT	Plus	Chr25:7452383-7452402
Reverse 1: CCGGTAACATGAATTTGCAC	Minus	Chr25:7450817-7450836
Reverse 2: TGGCTGGACAGGTTTACCAT	Minus	Chr25:7452025-7452044

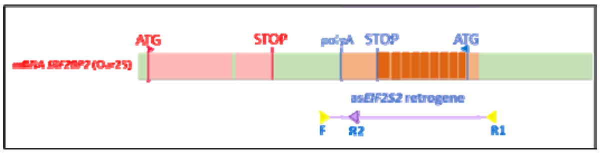

Figure 6 The position of the primers in the target sequence of the *IRF2BP2* gene (adapted from Demars *et al.* 2017). F (Forward), R1 (Reverse 1), R2 (Reverse 2)

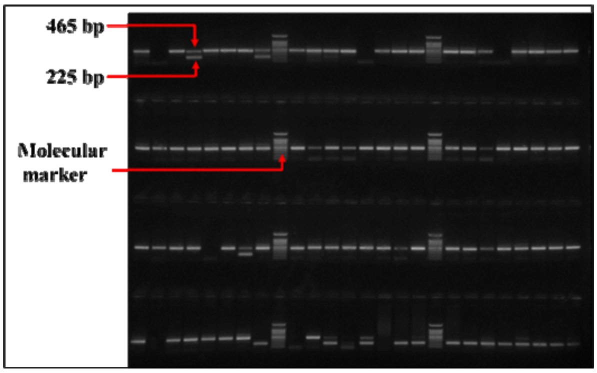

Figure 7 Electrophoresis pattern of the different genotypes observed: II (465 bp), ID (465 bp, 225 bp) and DD (225 bp, 225 bp) resulting of the target sequence of the *IRF2BP2* gene amplification in studied sheep breeds

We have detected the 3 expected genotypes, insertion at homozygous state (II), deletion at homozygous state (DD) and heterozygous (ID) (Figure 7). A contingency table of the genotyping results per breed is given in Table 3. The genotypic and allelic frequencies of each allele I and D of *IRF2BP2* were calculated.

Table 3 shows that the I "woolly" allele did not segregate or at very low frequency (between 1% and 3%) in Ifilène, Sidaou and Mehraban breeds, with no II homozygous animals observed. In contrast, the ancestral D "hairy" allele did not segregate in Ouled Djellal or at low frequency (between 3% and 9%) in Tâadmit, Rembi and Sardi breeds, with no DD homozygous animals observed, excepted one in Rembi. The other breeds exhibited the three genotypes but often by not respecting the Hardy-Weinberg equilibrium, with an excess of both homozygous forms and a deficit in heterozygous compared to the expected distribution.

Population genetic analysis revealed that the Ho were lower than 0.5, and for most of the breeds, the values of Ho were lower than He. Only three breeds (Barbarine of Tunisia, Sardi and Darâa) showed Ho values higher than He.

Except breeds with only one observed genotype (II or DD), polymorphism information content (PIC) values ranged from 0.014 to 0.366. The InDel polymorphism has moderate PIC in all Tunisian breeds (from 0.282 to 0.354), whereas it was more variable in Algerian breeds from 0.014 to 0.366, Iranian Mehraban being low (0.051).

The phenotyping of fleece type, hairy or woolly, was performed only for Algerian sheep breeds for which wool samples were collected. The InDel polymorphism of *IRF2BP2* showed significant association with the fleece type ($P<0.05$). As shown in Figure 8, the woolly fleece type was determined by the segregation of the I allele, 100% of II animals and 98% of ID animals being woolly. In contrast, most of the hairy animals were DD, homozygous carriers of the hairy ancestral allele. However, 31% of DD animals showed woolly fleece type. In the last years, some studies found that variations in this gene could affect sheep domestication and breed improvement also reported that *IRF2BP2* gene might affect fleece trait (Kalds *et al.* 2022; Li *et al.* 2022; Lv *et al.* 2022; Guo *et al.* 2023).

Algeria and Tunisia witnessed a substantial population surge during the latter half of the 20th century (Kateb and Ouadah-Bedidi, 2001). This growth coincided with a shift in demand for animal-based food sources, prompting significant alterations in sheep breeding objectives. However, no breeding programs have been implemented to enhance fleece quality in these nations. While studies focused on fleece and wool quality are absent in Tunisia, a few have been conducted in Algeria, the most recent being those carried out by Belharfi *et al.* (2018). Approximately 9000-11000 years ago, sheep were domesticated in the Fertile Crescent region (Zeder, 2008). The emergence of the long and hairy fleece phenotype preceded the development of the short and woolly fleece phenotype during the second wave of domestication, suggesting that the hairy fleece type is ancestral This variation in fleece type from hairy to wooly is genetically determined by the insertion of an antisense *EIF2S2* retrogene (called asEIF2S2) into the 3′ UTR of the IRF2BP2 gene (Demars *et al.* 2017).

This study has revealed that the *IRF2BP2* InDel alleles (I and D) segregate in numerous sheep breeds across Algeria, Tunisia, and one breed in Iran. Notably, the rustic Ifilène and Sidaou breeds, both originating from the Saharan countries and traditionally raised by Touareg populations (Lahlou-Kassi *et al.* 1989; Chellig, 1992; Djaout *et al.* 2017), are known as hairy breeds.

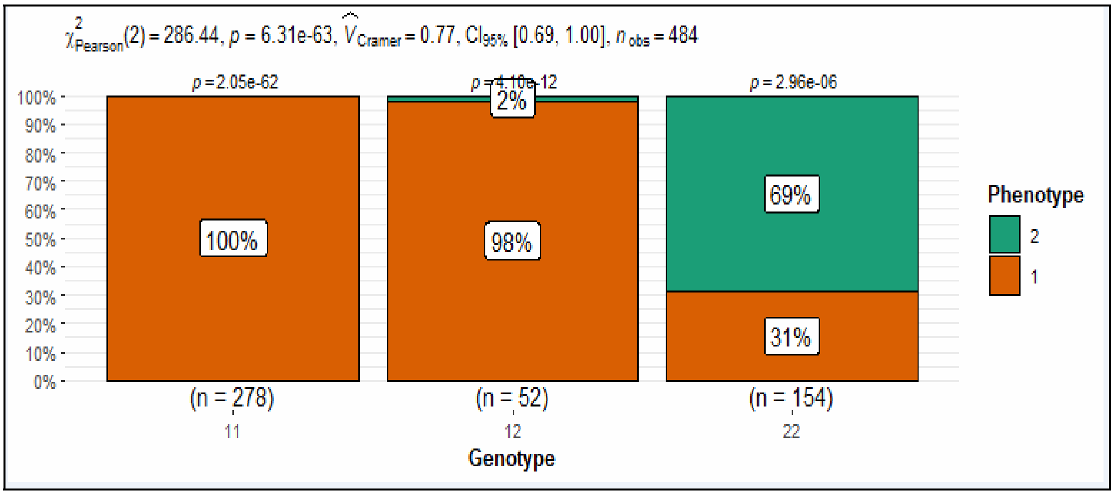

Figure 8 A barplot drawn using R program. It displays the result of the Chi-square test of independence. (1: woolly, 2: hairy)

Table 3 Genotypic and allelic frequencies and population indexes for InDel polymorphism in *IRF2BP2* gene

Breeds	Size	Genotypic Frequencies (%)			Allelic Frequencies (%)		HWE	Population parameters		
	N	II	ID	DD	I	D	P-values	Ho	He	PIC
Ifilène	9	0	0	100	0	100	ND	0.000	0.000	0.000
Sidaou	72	0	1	99	1	99	NS	0.014	0.014	0.014
Mehraban	75	0	5	95	3	97	NS	0.053	0.052	0.051
D'man	45	9	9	82	13	87	***	0.089	0.234	0.204
Sicilo Sarde	22	18	9	73	23	77	***	0.091	0.359	0.290
Barbarine_Tunisia	272	12	47	41	36	64	NS	0.467	0.461	0.354
Berbère	26	46	27	27	60	40	*	0.269	0.491	0.366
Hamra	73	48	26	26	61	39	***	0.260	0.479	0.363
Barbarine__Algeria	37	51	22	27	62	38	**	0.216	0.477	0.360
Tazegzawt	24	71	4	25	73	27	***	0.042	0.403	0.317
Noire de Thibar	23	74	9	17	78	22	***	0.087	0.348	0.282
Queue Fine de l'Ouest	25	72	12	16	78	22	**	0.120	0.350	0.284
Darâa	8	63	37	0	81	19	NS	0.375	0.325	0.258
Sardi	11	82	18	0	91	9	NS	0.182	0.173	0.152
Rembi	95	94	5	1	96	4	*	0.053	0.071	0.068
Tâadmit	54	94	6	0	97	3	NS	0.056	0.055	0.053
Ouled Djellal	37	100	0	0	100	0	ND	0.000	0.000	0.000

[1] I: insertion; D: deletion; HWE: Hardy–Weinberg equilibrium; Ho: homozygosity; He: heterozygosity; PIC: polymorphism information content.
ND: not determined and NS: non significant.
* (P<0.05); ** (P<0.01) and *** (P<0.001).

Interestingly, almost all animals from these breeds were genotyped as DD, homozygous for the ancestral hairy allele. In the Iranian Mehraban breed, the D allele frequency (97%) was also exceptionally high, with only four ID heterozygous animals observed among the 75 tested. This suggests a hairy fleece type for this breed. However, the FAO domestic animal diversity information system (Table 1) classified this breed as wooly, despite its production of coarse fiber used for carpet production. But is also possible seen the high frequency of the DD genotype in several woolly phenotype sheep breeds suggests that the phenotype is polygenic with the *IRF2BP2* gene as the dominant gene.

In light of these findings, this classification as wooly should be revised. In contrast, the Ouled Djellal sheep breed, the most developed for meat production in Algeria, exhibits a modern woolly fleece with fixation of the I allele, with all animals being homozygous for this allele. Similarly, the Sardi, Darâa, Tâadmit, and Rembi breeds displayed a high frequency of the I allele (>80%), with minimal or no DD animals. It is noteworthy that most of these breeds trace their ancestry to crosses with Ouled Djellal (Trouette, 1929; Magneville, 1959). Most of the other breeds exhibited the segregation of all three genotypes, indicating the potential coexistence of both hairy and wooly animals within these populations as observed in D'man. However, the distribution of the II, ID, and DD genotypes did not conform to the Hardy-Weinberg equilibrium of genotypes. This deviation could be attributed to the history of uncontrolled mating and crossbreeding within these breeds (Kdidi *et al.* 2015; Ameur Ameur *et al.* 2018). For all breeds considered, the average expected heterozygosity (He) exceeded the observed heterozygosity (Table 3). This result could be attributed to an isolate-breaking effect, potentially characteristic of certain Tunisian and Algerian sheep breeds. In Tunisia, crossbreeding between the Barbarine and Queue fine de l'Ouest breeds and between the Barbarine and Noire de Thibar breed remain the most common practices. This shift towards thin-tailed breeds (Queue fine de l'Ouest and Noire de Thibar) and their crosses has been driven by butchers and farmers who have encountered difficulties in selling the excess fat from the Barbarine tail (accounting for up to 15% of the carcass weight) (Bedhiaf-Romdhani *et al.* 2008).

This work confirms the original results of Demars *et al.* (2017), showing the relationship between the I allele with the woolly phenotype and the D allele with the hairy phenotype. Despite a significant association between the IRF2BP2 InDel polymorphism and the fleece type (woolly/hairy) evaluated in Algerian sheep breeds, a substantial number of DD animals were observed and classified as wooly, even though their genotype would predict a hairy phenotype. This discrepancy could be attributed to either phenotypic misclassification or the influence of other genetic mutations affecting fleece type or quality. Keratins and Keratin Associated Proteins (KAPs) have been implicated in most hair abnormalities reported in human disorders (Shimomura *et al.* 2010) and sheep fiber anomalies (Li *et al.* 2009). Moreover, SNP variants in the ovine Follistatin (FST) gene, involved in hair follicle morphogenesis, were also associated with wool fiber characteristics (Ma *et al.* 2017).

CONCLUSION

The present work studied the InDel polymorphism of the ovine *IRF2BP2* gene in relation to fleece type across major North African and Iranian sheep breeds. It reaffirms the pivotal role of the I insertion allele in the modern wooly fleece phenotype compared to the ancestral hairy D allele. The co-occurrence of both I and D alleles at relatively high frequencies in most populations examined raises the prospect of genetically enhancing wool quality from coarse hairy fibers to finer woolly fibers within each population without resorting to crossbreeding. Systematic genotyping of this Indel-type mutation could assist in establishing effective breeding programs aimed at managing fleece quality in Algerian and Tunisian sheep breeds.

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REFERENCES

Allain D. and Renieri C. (2010). Genetics of fiber production and fleece characteristics in small ruminants. Angora rabbit and South American camelids. *Animal*. **4(9),** 1472-1481.

Ameur Ameur A., Ata N., Benyoucef M.T., Djaout A., Azzi N., Yilmaz O. and Gaouar S.B.S. (2018). New genetic identification and characterisation of 12 Algerian sheep breeds by microsatellite markers. *Italian J. Anim. Sci.* **17(1),** 38-48.

Ansari-Renani H.R. (2012). Fiber quality of Iranian carpet-wool sheep breeds. *Media Peternakan*. **35(3),** 179-179.

Bai L., Zhou H., Gong H., Tao J., Ma Q., Ding W. and Hickford J.G. (2019). Variation in the ovine KAP8-1 gene affects wool fibre uniformity in Chinese Tan sheep. *Small Rumin. Res.* **178,** 18-21.

Bedhiaf-Romdhani S., Djemali M., Zaklouta M. and Iniguez L. (2008). Monitoring crossbreeding trends in Aitken native Tunisian sheep breeds. *Small Rumin. Res.* **74(1),** 274-278.

Belharfi F.Z., Djaout A., Ameur Ameur A., Sahraoui H. and Gaouar S.B.S. (2018). A comparative study of wool quality in sheep breeds in western Algeria. *Gen. Biodiv. J.* **2(1),** 19-25.

Chalh A., El Gazzah M., Djemali M. and Chalbi N. (2007). Genetic and phenotypic characterization of the Tunisian Noire De Thibar lambs on their growth traits. *J. Biol. Sci.* **7,** 1347-1353.

Chellig R. (1992). Les «Races» Ovines Algériennes. Office des Publications Universitaires, Alger.

Chessa B., Pereira F., Arnaud F., Amorim A., Goyache F., Mainland I., Kao R.R., Pemberton J.M., Beraldi D., Stear M.J., Alberti A., Pittau M., Iannuzzi L., Banabazi M.H., Kazwala R.R., Zhang Y.P., Arranz J.J., Ali. B.A., Wang Z. and Palmarini M. (2009). Revealing the history of sheep domestication using retrovirus integrations. *Science*. **324(5926),** 532-536.

Dale B.A., Lonsdale-Eccles J.D. and Holbrook K.A. (1980). Stratum corneum basic protein: an interfilamentous matrix protein of epidermal keratin. *Curr. Probl. Dermatol.* **10,** 311-325.

Demars J., Cano M., Drouilhet L., Plisson-Petit F., Bardou P., Fabre S., Servin B., Sarry J., Woloszyn F., Mulsant P., Foulquier D., Carrière F., Aletru M., Rodde N., Cauet S., Bouchez O., Pirson M., Tosser-Klopp G. and Allain D. (2017). Genome-wide identification of the mutation underlying fleece variation and discriminating ancestral hairy species from modern woolly sheep. *Mol. Biol. Evol.* **34(7),** 1722-1729.

Dierks C., Lehner S., Philipp U. and Distl O. (2013). Elimination of keratin 71 as candidate for hairlessness in Don Sphynx cats. *Anim. Genet.* **44,** 607-612.

Djaout A., Afri-Bouzebda F., Chekal F., El-Bouyahiaoui R., Rabhi A., Boubekeur A. and Gaouar S.B.S. (2017). Etat de la biodiversité des «races» ovines algériennes. *Gen. Biodiv. J.* **1(1),** 1-17.

Djemali M. (2000). Genetic improvement objectives of sheep and goats in Tunisia. Lessons learned. Pp. 121-127 in Proc. 43rd Sémin. Méditerran., Zaragoza, Spain.

Djennadi S. (2006). Entrepreneurship among the Berber people in Algeria. *Int. J. Entrepren. Small Bus.* **3(6),** 691-695.

Ebrahimi F., Gholizadeh M., Rahimi-Mianji G. and Farhadi A. (2017). Detection of QTL for greasy fleece weight in sheep using a 50 K single nucleotide polymorphism chip. *Trop. Anim. Health Prod.* **49(8),** 1657-1662.

Excoffier L., Laval G. and Schneider S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evol. Bioinform*. **1,** 47-50.

Ghoreishifar S.M., Rochus C.M., Moghaddaszadeh-Ahrabi S., Davoudi P., Ardestani S.S., Zinovieva N.A. and Johansson A.M. (2021). Shared ancestry and signatures of recent selection in goat and sheep. *Genes*. **12(3),** 433-441.

Gong H., Zhou H., Forrest R.H., Li S., Wang J., Dyer J.M. and Hickford J.G. (2016). Wool keratin-associated protein genes in sheep-a review. *Genes*. **7(6),** 24-32.

Guo Y., Bai F., Wang J., Fu S., Zhang Y., Liu X., Zhang Z., Shao J., Li R., Wang F., Zhang L., Zheng H., Wang X., Liu Y. and Jiang Y. (2023). Design and characterization of a high-resolution multiple-SNP capture array by target sequencing for sheep. *J. Anim. Sci.* **101,** 33-41.

Gutierrez-Gil B., Esteban-Blanco C., Wiener P., Chitneedi P.K., Suarez-Vega A. and Arranz J.J. (2017). High-resolution analysis of selection sweeps identified between fine-wool Merino and coarse-wool Churra sheep breeds. *Genet. Sel.* **49(1),** 1-24.

Harel S. and Christiano A.M. (2012). Keratin 71 mutations: from water dogs to woolly hair. *J. Invest. Dermatol*. **132,** 2315-2317.

Harizi T. and Abidi F. (2015). Characteristics of virgin and pulled wool fibres used in Tunisian handmade carpets. *Int. J. Sci. Technol. Res*. **4(10),** 368-372.

Harizi W., Chaki S., Bourse G. and Ourak M. (2015). Mechanical damage characterization of glass fiber-reinforced polymer laminates by ultrasonic maps. *Composites B Eng*. **70,** 131-137.

Holman B.W.B. and Malau-Aduli A.E.O. (2012). A review of sheep wool quality traits. *Annu. Res. Rev. Biol*. **2(1),** 1-14.

Iniguez L. (2006). Characterization of Small Breeds in West Asia and North Africa. International Center for Agriculture Research in the Dry Areas (ICARDA), West Asia. Aleppo, Syria.

Kalds P., Zhou S., Gao Y., Cai B., Huang S., Chen Y. and Wang X. (2022). Genetics of the phenotypic evolution in sheep: A molecular look at diversity-driving genes. *Genet. Sel.* **54(1),** 1-27.

Kateb K. and Ouadah-Bedidi Z. (2001). L'actualitédémographique du Maghreb. Actes de la DESCO Université d'été, Montréal, Canada.

Kdidi S., Calvo J.H., González-Calvo L., Ben-Sassi M., Khorchani T. and Yahyaoui M.H. (2015). Genetic relationship and admixture in four Tunisian sheep breeds revealed by microsatellite markers. *Small Rumin. Res*. **131,** 64-69.

Khaldi G. (1989). Barbarine sheep. In: Small ruminant in the Near East, vol. III, North Africa FAO. *Anim. Prod. Health.* **74,** 96-135.

Lahlou-Kassi A., Berger Y.M., Bradford G.E., Boukhliq G.R., Tibary A., Derqaoui L. and Boujenane I. (1989). Performance of D'Man and Sardi Sheep on Accelerated Lambing: Fertility, Litter Size, Postpartum Anoestrus and Puberty. *Small Rumin. Res*. **2(3),** 225-239.

Li S., Chen W., Zheng X., Liu Z., Yang G., Hu X. and Mou C. (2020).Comparative investigation of coarse and fine wool sheep skin indicates the early regulators for skin and wool diversity. *Gene*. **758,** 1-9.

Li W., Gong H., Zhou H., Wang J., Li S., Liu X. and Hickford J.G. (2019). Variation in KRTAP6-1 affects wool fibre diameter in New Zealand Romney ewes. *Arch. Anim. Breed.* **62(2),** 509-515.

Li S.W., Ouyang H.S., Rogers G.E. and Bawden C.S. (2009). Characterization of the structural and molecular defects in fibres and follicles of the Merino felting lustre mutant. *Exp. Dermatol*. **18,** 134-142.

Li X., Yuan L., Zhang X., Zhang D., Zhao Y., Chen J. and Zhang X. (2022). Whole genome re-sequencing reveals artificial and natural selection for milk traits in East Friesian sheep. *Front. Vet. Sci.* **9,** 1-10.

Lv F.H., Cao Y.H., Liu G.J., Luo L.Y., Lu R., Liu M.J. and Li M.H. (2022). Whole-genome resequencing of worldwide wild and domestic sheep elucidates genetic diversity, introgression, and agronomically important loci. *Mol. Biol. Evol.* **39(2),** 353-359.

Ma G.W., Chu Y.K., Zhang W.J., Qin F.Y., Xu S.S., Yang H., Rong E.G, Du Z.Q., Wang S.Z., Li H. and Wang N. (2017). Polymorphisms of FST gene and their association with wool quality traits in Chinese Merino sheep. *PLoS One*. **12(4),** e0174868.

Magneville D. (1959). Observation sur le mouton Algérien, ses qualités et ses défauts. *Elevage et Cultures.* **126,** 12-17.

Marshall T.C., Slate J.B.K.E., Kruuk L.E.B. and Pemberton J.M. (1998). Statistical confidence for likelihood based paternity inference in natural populations. *Mol. Ecol.* **7(5),** 639-655.

Miller S.A., Dykes D.D. and Polesky H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16(3),** 1215-1221.

Naidoo P., Cloete S. and Olivier J. (2004). Heritability estimates and correlations between subjectively assessed and objectively measured fleece traits in Merino sheep. *South African J. Anim. Sci*. **34(6),** 13-17.

ONAGRI. (2018). Annuaire de production animale. http://www.onagri.tn/statistiques. Accessed Jun. 2021.

Powell B.C. and Rogers G.E. (1997). The role of keratin proteins and their genes in the growth, structure and properties of hair. Pp. 59-148 in Formation and Structure of Human Hair. P. Jolles, H. Zahn and E. Hocker, Eds., Birkhauser Verlag, Basel.

Purvis I.W. and Franklin I.R. (2005). Major genes and QTL influencing wool production and quality: A review. *Genet. Sel.* **37(1),** 1-11.

Raymond M. and Rousset F. (1995). GENEPOP (version 4): Population genetics software for exact tests and ecumenicism. *J. Hered.* **86(3),** 248-249.

Safari E., Fogarty N.M. and Gilmour A.R. (2005). A review of genetic parameter estimates for wool, growth, meat and reproduction traits in sheep. *Livest. Prod. Sci.* **92(3),** 271-289.

Safari E., Fogarty N.M., Gilmour A.R., Atkins K.D., Mortimer S.I., Swan A.A. and Van der Werf J.H.J. (2007). Across population genetic parameters for wool, growth, and reproduction traits in Australian Merino sheep. 2. Estimates of heritability and variance components. *Australian J. Agric. Res.* **58(2),** 177-184.

Shi X., Wu J., Lang X., Wang C., Bai Y., Riley D.G. and Ma X. (2021). Comparative transcriptome and histological analyses provide insights into the skin pigmentation in Minxian black fur sheep (Ovisaries). *Peer J.* **9,** e11122.

Shimomura Y., Wajid M., Petukhova L., Kurban M. and Christiano A.M. (2010). Autosomal-dominant woolly hair resulting from disruption of keratin 74 (KRT74), a potential determinant of human hair texture. *Am. J. Hum. Genet*. **86,** 632-638.

Snyman M.A., Olivier W.J. and Olivier J.J. (1996). Variance components and genetic parameters for body weight and fleece traits of Merino sheep in an arid environment. *South African J. Anim. Sci*. **26(1),** 11-14.

Talebi R., Ahmadi A., Afraz F., Sarry J., Woloszyn F. and Fabre S. (2018). Detection of single nucleotide polymorphisms at major prolificacy genes in the Mehraban sheep and association with litter size. *Ann. Anim. Sci.* **18(3),** 685-698.

Trouette M. (1929). Les «races» d'Algérie. Pp. 299-302 in Proc. Congr. Mouton, Paris, France.

Wuliji T., Dodds K.G., Land J.T.J., Andrews R.N. and Turner P.R. (2001). Selection for ultrafine Merino sheep in New Zealand: heritability, phenotypic and genetic correlations of live weight, fleece weight and wool characteristics in yearlings. *Anim. Sci.* **72(2),** 241-250.

Yu Z., Gordan S.W., Nixon A.J., Bawden C.S., Rogers M.A., Wildermoth J.E. and Pearson A.J. (2009). Expression patterns of keratin intermediate filament and keratin associated protein genes in wool follicles. *Differentiation.* **77(3),** 307-316.

Zeder M.A. (2008). Domestication and early agriculture in the Mediterranean Basin: Origins, diffusion, and impact. Proc. Natl. Acad. Sci. USA**. 105(33),** 11597-11604.

Zhang L., Sun F., Jin H., Dalrymple B.P., Cao Y., Wei T., Vuocolo T., Zhang M., Piao Q. and Ingham A.B. (2017). Comparison of transcriptomic patterns measured in the skin of Chinese fine and coarse wool sheep breeds. *Sci. Rep.* **7(1),** 14301-14311.

Zhao H., Guo T., Lu Z., Liu J., Zhu S., Quia G., Han M., Yuan C., Wang T., Li F., Zhang Y., Hou F., Yue Y. and Yang B. (2021). Genome-wide association studies detects candidate genes for wool traits by re-sequencing in Chinese fine-wool sheep. *BMC Genom*. **22,** 127-135.