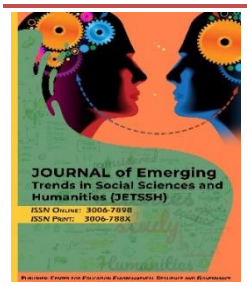

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Genome-wide Association Study For Coat Colour and Other Qualitative Morphological Traits in Indigenous Goat Breeds of Sindh-Pakistan Using 50k SNP Bead Chip

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ABSTRACT

Sindh province harbours the largest number of indigenous goat breeds in Pakistan, and these breeds are distinguished from one another largely on the basis of qualitative morphological traits such as coat colour and pattern, eye colour, hair length, nose shape and horn orientation. The present study was designed to identify genomic regions and candidate genes associated with seven qualitative morphological traits in nine indigenous goat breeds of Sindh, viz. Barri, Bugi-Tori, Chappar, Jattan, Kachan, Kamori, Pateri, Tapri and Thari. Phenotypes were recorded on 395 goats (49 males and 346 females) through standardised photography and a structured proforma, and genomic DNA extracted from jugular blood was genotyped with the Illumina Goat SNP50 BeadChip. Association analysis was performed in PLINK 1.9 using a case-control model for six binary traits and a quantitative model for nose length, fitting breed, sex and correlated traits as covariates, with Benjamini-Hochberg control of the false discovery rate. A total of 38 SNPs reached significance: five for black body coat, four for flowery colour pattern, one for brown eye colour, seven for short hair, nine for Roman nose shape and twelve for upward horn orientation, whereas no SNP was significant for nose length. Two SNPs on chromosomes 1 (IL1RAP) and 9 (PRKN) were shared between black coat and flowery pattern. These markers provide the first genome-wide evidence on the architecture of qualitative morphological traits in Pakistani goats and offer a starting point for marker-assisted selection of breed-defining phenotypes.

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Introduction

Goats are the most numerous livestock species in Pakistan and the country ranks among the largest goat-holding nations of the world, with a national herd of 89.4 million head, of which 20.89 million (23.4%) are kept in Sindh (GOP, 2024). The species is central to the livelihood of resource-poor rural households because of its low feed cost, short generation interval, high fertility and ability to convert poor-quality browse into meat and milk. Thirty-six goat breeds are documented in the country, distributed across agro-ecological zones ranging from irrigated plains to high mountain plateaus and deserts (Khan et al., 2008; Moaeen-ud-Din, 2020).

Sindh, the third largest province of Pakistan, is the richest single province in terms of goat diversity, being home to nine of these documented breeds viz. Barri, Bugi-Tori, Chappar, Jattan, Kachan, Kamori, Pateri, Tapri and Thari (Moaeen-ud-Din, 2020). Bugi-Tori and Chappar are reared for milk, meat and hair, while the remainder are essentially dual purpose (Khan et al., 2008). These breeds are traditionally identified and traded on the basis of appearance rather than performance records. Pateri goats carry characteristic dark brown spotting on the neck, legs and head over a white base colour, with spirally twisted horns directed upward and hair of medium to long length (Mari et al., 2022). Barri shows a flowery pattern of white splashing over a solid white base, Bugi-Tori a white base with black spotting, Tapri a predominantly white coat, and Kamori a dark brown base coat bearing moon spots.

Coat colour is more than an aesthetic attribute. It contributes to thermoregulation in hot arid environments, it forms the basis on which farmers classify and price animals, and it shapes human perception of the animal, as has been demonstrated for coat colour and ear shape in dogs (Fratkin and Baker, 2013). Eye colour, a polygenic character determined by the quantity of melanosomes in the iris and the pigment they contain, varies between blue, brown and intermediate types in Pakistani goats (Negro et al., 2017). Hair length has direct adaptive significance for heat dissipation, while horn orientation determines handling safety and injury risk within the flock. Despite the economic and practical relevance of these traits, essentially nothing is known of the genomic regions controlling them in Pakistani goat populations.

The release of the goat reference genome and of the Illumina Goat SNP50 BeadChip by the International Goat Genome Consortium made genome-wide association study (GWAS) feasible in this species (Tosser-Klopp et al., 2014). Elsewhere, the chip has been used to map coat colour and mohair traits in the Iranian Markhoz goat (Nazari-Ghadikolaei et al., 2018), wattles in Swiss breeds (Reber et al., 2015), and coat colour, beard, black dorsal line and hind leg hair in Chinese native breeds (Sun et al., 2022). In Pakistan, genome-wide marker panels have so far been applied to characterise population structure, genetic diversity and selection signatures in seven indigenous populations (Kumar et al., 2018) and in the seven documented breeds of Punjab (Muner et al., 2021), and to map growth and body conformation traits in Punjab breeds (Moaeen-ud-Din et al., 2022). No association study has yet been reported for qualitative morphological traits in any Pakistani goat breed.

The present study was therefore designed to identify SNPs and candidate genes associated with black body coat, flowery colour pattern, brown eye colour, short hair, Roman nose shape, nose length and upward horn orientation in the indigenous goat breeds of Sindh province using the Goat SNP50 BeadChip.

Materials and Methods

Study area and experimental animals: The study was carried out in Sindh province, which extends about 579 km from north to south and averages 281 km from east to west, covering a territorial area of 140,915 sq. km. Its ecosystems range from the Indus delta and associated wetlands to desert, rangeland and irrigated landscapes, and the province supports a large indigenous small-ruminant population. Animals were sampled from the breeding tracts of the nine breeds in the districts of Hyderabad, Matiyari, Hala, Methi, Umer Kot, Tharparkar, Thatta, Badin, Dadu and Mirpur Khas. A total of 395 goats (49 males and 346 females) of the breeds Barri, Bugi-Tori, Chappar, Jattan, Kachan, Kamori, Pateri, Tapri and Thari were recorded. Approximately 25 unrelated animals per breed were sampled at random across several flocks so that the within-breed diversity of each population

was represented. Sampling of a smaller number of males than females is not expected to bias genome-wide estimates appreciably.

Phenotypic recording: Each animal was assigned a unique identity and photographed from all angles under a uniform camera setting (ISO 300, f/16, no filter) so that colour phenotypes could be scored consistently and re-verified. Data were entered on a structured proforma. Coat colour was recorded in four base categories (white, black, grey and brown), each further subdivided where necessary according to the presence of patches or spots of a second colour; the flowery pattern was recorded as splashing of white over a base colour. Eye colour was scored as black, blue, grey or brown. Body hair length was measured in a bunch from the backline of the animal and classified as short (up to 2 cm), medium (2.1-4 cm) or long (more than 4 cm). Horn shape was recorded as straight, curled or twisted, horn orientation as upward, backward or outward, and horn length as small, medium or large; animals were additionally classified as horned or polled. Nose profile was recorded as straight, concave or convex, the convex (Roman) profile being taken as the case class in the association analysis, and nose length was measured. Head size, ear length, ear shape and tail length were recorded on the same three-level scale but were not carried forward to association analysis. Every level of every trait was assigned a numerical code before analysis. Seven traits were carried forward to association analysis: black body coat, flowery colour pattern, brown eye colour, short hair, Roman nose shape, nose length and upward horn orientation.

Blood sampling and DNA extraction: Blood was drawn from the jugular vein into EDTA vacutainers by qualified veterinarians. Genomic DNA was extracted by the organic phenol-chloroform procedure (Ciulla et al., 1988). Concentration and purity were checked on a NanoDrop Q5000 microvolume spectrophotometer, samples with a 260/280 ratio between 1.8 and 2.0 being accepted as pure (Desjardins and Conklin, 2010).

Genotyping and quality control: Genotyping was performed at Iowa State University, USA, under the Pak-USAID project "Collaborative Research for Genetic Conservation and Improvement of Pakistani Goat Breeds", using the Illumina Goat SNP50 BeadChip, which carries 52,295 SNPs distributed across the caprine genome (Tosser-Klopp et al., 2014). Raw intensities were processed in GenomeStudio (Illumina) with a GenCall score threshold of 0.15 and an average call rate of 99%. Quality control was completed in PLINK 1.9 (Purcell et al., 2007) applying animal and SNP call rate of 99%, minor allele frequency of 5% and Hardy-Weinberg equilibrium at $P < 0.01$. Six animals and 1,431 SNPs were removed, leaving 360 animals and 42,490 SNPs for association analysis.

Genome-wide association analysis: Association analysis was carried out in PLINK 1.9 (Purcell et al., 2007) using the model

$$y = G\beta G + X\beta X + e$$

where y is the phenotype of the individual, G is the matrix of genotypes for the variant under test, βG is the effect of the genotype, X is the matrix of covariates, βX the effects of the covariates and e the residual error. A case-control model was applied to the six binary traits and a quantitative model to nose length. Breed and sex were fitted as covariates for every trait, together with correlated traits as detailed in Table 1, so that associations reflect within-breed rather than between-breed variation. Raw probabilities were corrected for multiple testing by the Benjamini-Hochberg false discovery rate procedure. Manhattan and quantile-quantile plots were drawn in R 4.2.1 (R Core Team, 2022). The genomic inflation factor (λ) was obtained for each trait from the median chi-square statistic using the --adjust option of PLINK 1.9, in order to assess whether residual population structure had inflated the test statistics.

Gene annotation: SNPs surviving correction were positioned on the ARS1 assembly of the Capra hircus genome in the NCBI Genome Data Viewer, and genes lying within or in the immediate flanking region of each associated SNP were retrieved. Gene function was annotated using the GeneCards database (www.genecards.org).

Results

The distribution of cases and controls, the missing phenotypes and the covariates fitted for each of the seven traits are given in Table 1. Association analysis identified 38 SNPs that remained

significant after false discovery rate correction. Five SNPs were associated with black body coat, four with flowery colour pattern, one with brown eye colour, seven with short hair, nine with Roman nose shape and twelve with upward horn orientation. No SNP reached significance for nose length. The complete list of significant markers, their chromosomal positions, the nearest annotated gene and the corresponding raw and corrected probabilities is presented in Table 2, and the function of each associated gene in Table 3. The genome-wide distribution of the association signals and the corresponding quantile-quantile plots are shown in Figures 1 and 2.

For black body coat the strongest signal was snp40497-scaffold519-4753 on chromosome 1, close to IL1RAP (raw P = 5.03E-21), followed by markers on chromosomes 13, 14, 9 and 30. For the flowery colour pattern the strongest signal was snp39216-scaffold5-2083487 on chromosome 9, lying within PRKN (raw P = 6.19E-36), followed by SNPs near LIX1 on chromosome 7, ANTXR2 on chromosome 6 and IL1RAP on chromosome 1. Two markers, snp40497-scaffold519-4753 on chromosome 1 and snp39216-scaffold5-2083487 on chromosome 9, were common to both coat colour traits.

A single marker, snp52777-scaffold791-743883 on chromosome 14, was associated with brown eye colour and lies approximately 415 kb from MIR124A. For short hair, seven markers were significant, two of them on chromosome 15 close to ARNTL/BMAL1 and P4HA3, and the remainder within or near ATP10D, SPIDR, LOC102180841, RSL24D1 and INHBA. Nine markers were associated with Roman nose shape, including two on chromosome 3 adjacent to S100A10 and S100A13 and one close to KIAA0586 on chromosome 10, which carried the lowest probability for this trait. Twelve markers were associated with upward horn orientation, the strongest being snp48394-scaffold687-2154148 within CDC14A on chromosome 3.

Table 1: *Model, sample size and covariates used for the genome-wide association analysis of seven morphological traits in Sindh goat breeds*

Trait	Model	Number of individuals	Missing phenotypes	Covariates
Black body coat	Case-control	53 cases, 213 controls	94	Breed, sex, flowery colour pattern, brown eye colour
Flowery colour pattern	Case-control	30 cases, 236 controls	94	Breed, sex, black body coat, brown eye colour
Brown eye colour	Case-control	260 cases, 40 controls	94	Breed, sex, black body coat, flowery colour pattern
Short hair	Case-control	225 cases, 42 controls	93	Breed, sex, spiral horns, upward horns
Nose length	Quantitative	311	49	Breed, sex, age, Roman nose
Roman nose shape	Case-control	178 cases, 99 controls	83	Breed, sex, nose length
Upward horn orientation	Case-control	146 cases, 121 controls	93	Breed, sex, short hair, spiral horns

Cases and controls refer to the presence and absence of the trait, respectively.

Table 2: *SNPs significantly associated with six qualitative morphological traits in Sindh goat breeds, with the nearest annotated gene on the ARS1 assembly*

Trait	SNP	Chr	ARS1 position (bp)	Nearest gene	Distance (bp)	Genes in the region	Raw P	FDR-BH
Black body coat	snp40497-scaffold519-4753	1	76,279,644	*IL1RAP*	+68,781	1081	5.03E-21	1.54E-16
	snp58679-scaffold956-1096397	13	69,126,159	*TRNAW-CCA*	+68,784	907	2.04E-18	3.12E-14
	snp23162-scaffold231-977044	14	61,987,175	*C14H8orf22/PPDPFL*	+72,349	641	9.72E-18	9.94E-14
	snp39216-scaffold5-2083487	9	85,488,881	*PARK2/PRKN*	Within	626	6.34E-17	3.96E-13
	snp7219-scaffold1267-268453	30	10,419,742	Assembly not available	–	–	6.46E-17	3.96E-13
Flowery colour pattern	snp39216-scaffold5-2083487	9	85,488,881	*PARK2/PRKN*	Within	626	6.19E-36	1.90E-31
	snp494-scaffold1013-33666	7	14,058,559	*LIX1*	Within	1557	1.81E-29	2.78E-25
	snp927-scaffold1025-601285	6	94,998,169	*ANTXR2*	+58,574	787	6.35E-28	6.48E-24
	snp40497-scaffold519-4753	1	76,279,644	*IL1RAP*	+68,781	1081	1.87E-27	1.43E-23
Brown eye colour	snp52777-scaffold791-743883	14	53,393,590	*MIR124A*	–415,101	641	4.24E-08	1.30E-03
Short hair	snp45805-scaffold628-2398413	15	42,790,540	*ARNTL/BMAL1*	+142,249	1234	6.65E-16	2.04E-11
	snp58057-scaffold94-3857854	6	66,755,919	*ATP10D*	Within	787	5.16E-15	7.91E-11
	snp23178-scaffold231-1651912	14	62,662,043	*SPIDR*	Within	641	9.28E-14	9.48E-10
	snp24137-scaffold246-1419289	12	15,575,387	*LOC102180841*	Within	453	1.39E-13	1.07E-09
	snp48089-scaffold68-3609220	10	48,002,865	*RSL24D1*	+68,361	1174	1.67E-12	7.88E-09
	snp42014-scaffold548-3173570	15	29,354,057	*P4HA3*	+4,116	1234	1.67E-12	7.88E-09
	snp58495-scaffold950-3034288	4	40,537,236	*INHBA*	+324,886	975	1.80E-12	7.88E-09

Trait	SNP	Chr	ARS1 position (bp)	Nearest gene	Distance (bp)	Genes in the region	Raw P	FDR-BH
Roman nose shape	snp58295-scaffold947-455430	10	32,389,320	*KIAA0586*	+2,635	1174	7.96E-20	2.02E-15
	snp16204-scaffold1704-179798	3	101,806,921	*S100A10*	–9,688	1639	1.32E-19	2.02E-15
	snp44073-scaffold597-759138	16	42,763,032	*LOC108637776*	–20,042	798	1.54E-17	1.57E-13
	snp19833-scaffold199-5760351	17	29,529,591	*GRIA2*	+403,530	624	5.21E-17	3.26E-13
	snp34450-scaffold405-775613	5	12,855,027	*TMTC2*	+843,172	1498	5.62E-17	3.26E-13
	snp41725-scaffold543-606045	3	103,396,911	*S100A13*	Within	1639	6.38E-17	3.26E-13
	snp41966-scaffold548-970378	15	27,150,865	*THAP12*	Within	1234	1.39E-16	6.08E-13
	snp55216-scaffold85-1273613	7	17,381,410	*FAM172A*	Within	1557	2.30E-16	8.29E-13
	snp33679-scaffold395-2440453	11	16,003,596	*FAM98A*	–5,016	1156	2.43E-16	8.29E-13
Upward horn orientation	snp48394-scaffold687-2154148	3	77,707,105	*CDC14A*	Within	1639	4.20E-14	1.29E-09
	snp39094-scaffold498-544295	12	11,055,898	*FARP1*	Within	453	4.15E-11	4.60E-07
	snp24974-scaffold257-186470	3	61,862,222	*SSX2IP*	–53,732	1639	4.50E-11	4.60E-07
	snp56292-scaffold881-1210039	13	44,791,734	*PFKP*	–218,962	907	8.65E-11	6.63E-07
	snp51518-scaffold755-722030	14	59,766,841	*RP1*	–33,859	641	1.40E-10	8.55E-07
	snp47017-scaffold658-128998	11	101,527,546	*MED27*	Within	1156	1.92E-10	8.89E-07
	snp29058-scaffold312-4935525	2	61,096,167	*LOC102180354*	+11,188	1087	2.34E-10	8.89E-07
	snp33966-scaffold40-1056358	20	52,472,439	*LOC102188712*	–57,687	419	2.39E-10	8.89E-07

Trait	SNP	Chr	ARS1 position (bp)	Nearest gene	Distance (bp)	Genes in the region	Raw P	FDR-BH
	snp36216-scaffold433-1192931	6	80,926,961	*LOC108636228*	–79,234	787	2.92E-10	8.89E-07
	snp42413-scaffold560-375211	30	84,966,861	Assembly not available	–	–	3.19E-10	8.89E-07
	snp17709-scaffold184-112045	10	24,287,998	*GPHN*	Within	1174	3.19E-10	8.89E-07
	snp36336-scaffold435-3490645	1	72,784,147	*ATP13A3*	+2,465	1081	3.62E-10	9.25E-07

"Within" indicates that the SNP lies inside the annotated gene; negative distances indicate that the gene lies upstream of the SNP. No SNP was significant for nose length.

Table 3: *Annotation of genes associated with morphological traits in Sindh goat breeds (source: www.genecards.org)*

Associated gene	Human homologue	Gene function
IL1RAP	—	Interleukin 1 receptor accessory protein
TRNAW-CCA	—	Transfer RNA tryptophan (anticodon CCA)
C14H8orf22/PPDPFL	—	Pancreatic progenitor cell differentiation and proliferation factor like
PARK2/PRKN	—	Parkin RBR E3 ubiquitin protein ligase
LIX1	—	Limb and CNS expressed 1
ANTXR2	—	ANTXR cell adhesion molecule 2
MIR124A (MIR124-1)	—	MicroRNA 124-1
ARNTL/BMAL1	—	Basic helix-loop-helix ARNT like 1
ATP10D	—	ATPase phospholipid transporting 10D
SPIDR	—	Scaffold protein involved in DNA repair
LOC102180841	*ABCC4*	ATP binding cassette subfamily C member 4
RSL24D1	—	Ribosomal L24 domain containing 1
P4HA3	—	Prolyl 4-hydroxylase subunit alpha 3
INHBA	—	Inhibin subunit beta A
KIAA0586	—	Talpid3, ciliogenesis and hedgehog signalling
S100A10	—	S100 calcium binding protein A10
LOC108637776	*LOC127267332*	H3K27ac-H3K4me1 hESC enhancer region
GRIA2	—	Glutamate ionotropic receptor AMPA type subunit 2
TMTC2	—	Transmembrane O-mannosyltransferase targeting cadherins 2
S100A13	—	S100 calcium binding protein A13
THAP12	—	THAP domain containing 12

Associated gene	**Human homologue**	**Gene function**
FAM172A	—	Family with sequence similarity 172 member A
FAM98A	—	Family with sequence similarity 98 member A
CDC14A	—	Cell division cycle 14A
FARP1	—	FERM, ARH/RhoGEF and pleckstrin domain protein 1
SSX2IP	—	SSX family member 2 interacting protein
PFKP	—	Phosphofructokinase, platelet
RP1	—	RP1 axonemal microtubule associated
MED27	—	Mediator complex subunit 27
LOC102180354	*LINC01826*	Long intergenic non-protein coding RNA 1826
LOC102188712	*NDUFV2*	NADH:ubiquinone oxidoreductase core subunit V2
LOC108636228	*RPLP1*	Ribosomal protein lateral stalk subunit P1
GPHN	—	Gephyrin
ATP13A3	—	ATPase 13A3

A dash indicates that the caprine gene symbol is identical to the human symbol.

The quantile-quantile plots (Figure 2) followed the null expectation closely across the bulk of the distribution for every trait, departing from it only in the extreme upper tail where the associated markers lie, which indicates that the fitting of breed and sex as covariates controlled residual population structure adequately. The corresponding genomic inflation factors were λ = [INSERT VALUE] for black body coat, [INSERT] for flowery colour pattern, [INSERT] for brown eye colour, [INSERT] for short hair, [INSERT] for Roman nose shape, [INSERT] for nose length and [INSERT] for upward horn orientation.

Figure 1: *Manhattan plots showing genome-wide significant SNPs for seven morphological traits in Sindh goat breeds. Horizontal red and blue lines indicate the genome-wide and chromosome-wide significance thresholds respectively.*

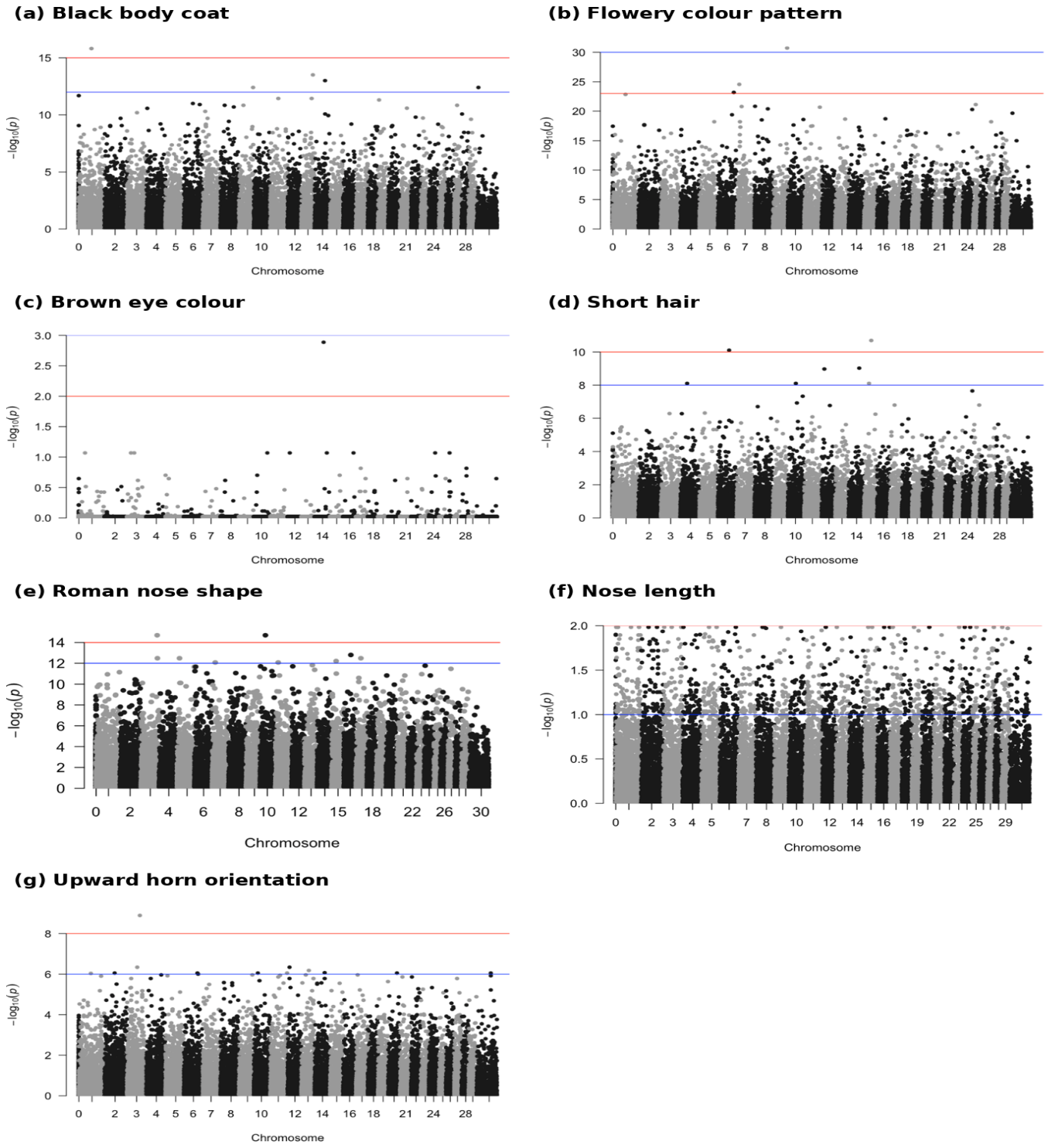
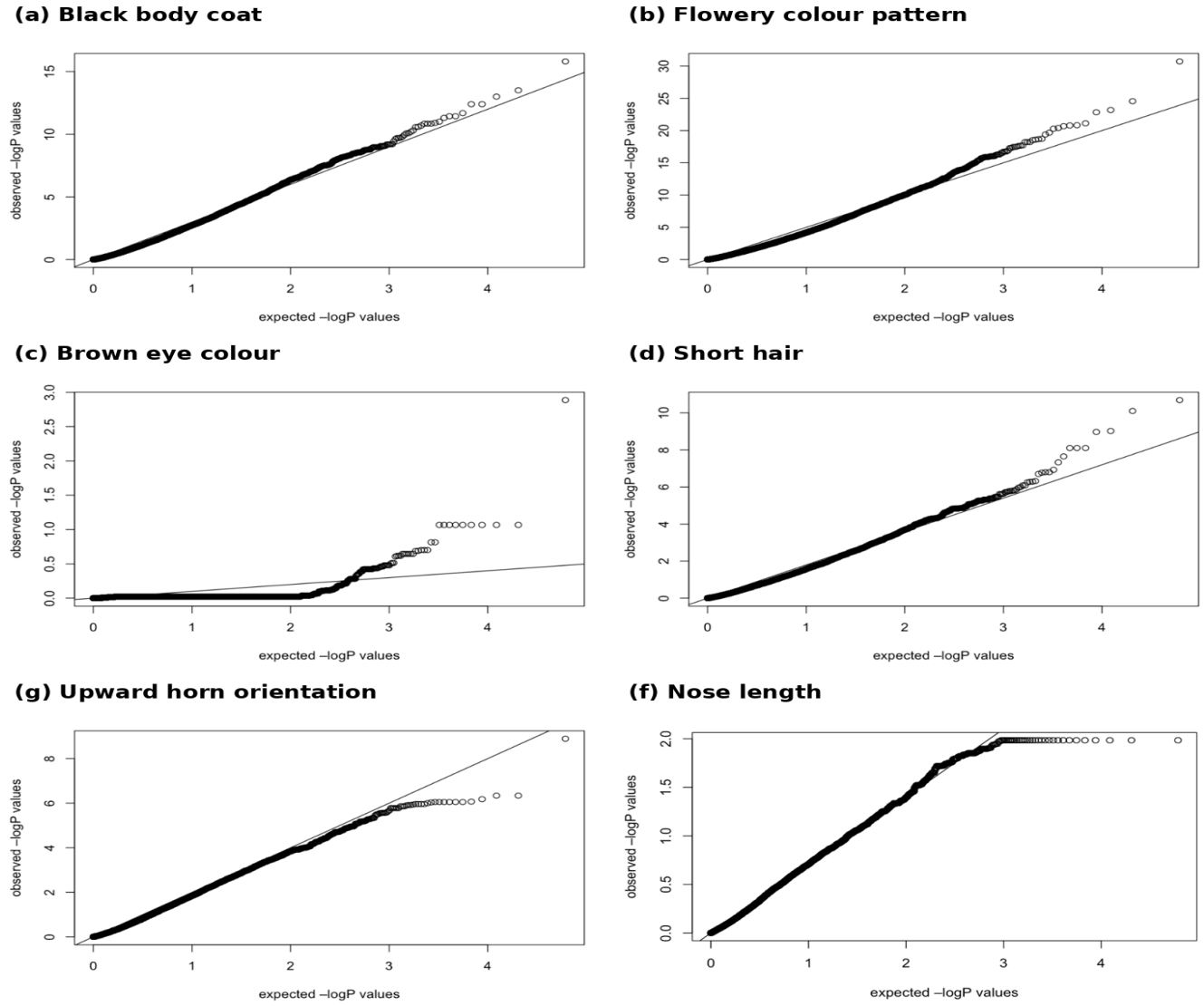


Figure 2: *Quantile-quantile plots of observed against expected –log10 P values for six of the seven traits analysed in Sindh goat breeds. The panel for Roman nose shape (e) is to be added.*



Discussion

Black body coat and flowery colour pattern: Coat colour has been a criterion of selection in goats for centuries, both because of its physiological role in hot climates and because farmers pay a premium for particular colours and patterns. Seven markers were significant for the two colour traits examined here, two of which were shared. The marker on chromosome 9 lies within PRKN, which encodes the E3 ubiquitin protein ligase parkin, responsible for the disposal of damaged and surplus proteins and better known for its involvement in Parkinson disease in humans (Ferreira and Massano, 2017). The marker on chromosome 1 lies close to IL1RAP and that on chromosome 6 near ANTXR2, both of which participate in cellular interaction and adhesion. Sun et al. (2022) similarly reported SORCS3, DYM and PDE4D rather than the classical pigmentation loci for coat colour in three Chinese native breeds, and attributed this to the polygenic nature of the trait.

It is notable that the canonical pigmentation genes ASIP, KIT, TYRP1 and MC1R, which explain colour variation in Valais goats (Henkel et al., 2021) and in the Markhoz goat, where ASIP, ITCH, AHCY and RALY on chromosome 13 were associated with black and brown coat and KIT and PDGFRA on chromosome 6 with white coat (Nazari-Ghadikolaei et al., 2018), did not emerge in the present analysis. Three explanations are plausible. First, coat colour in the Sindh populations is largely confounded with breed, and fitting breed as a covariate removes precisely that component of the variance in which the major pigmentation loci segregate. Second, the number

of cases available for the two colour traits was modest, which limits power at the stringent thresholds applied. Third, the 50K panel provides on average one marker per 50 kb and may not tag causal variants at these loci in the populations studied. Resequencing of the ASIP and KIT regions in a larger sample of coloured and white animals would resolve the question directly.

Brown eye colour: Eye colour reflects the quantity of melanosomes in the iris and the type of pigment they contain, and it is polygenic in mammals (Negro et al., 2017; Lui and Stokkermans, 2021). Pakistani goats display blue, brown and mixed iris colours. The single significant marker detected here lies near MIR124A, which encodes a microRNA with a documented role in ocular development: over-expression of miR-124 in the nervous system produces abnormal eye development in Drosophila (Wang et al., 2014), and miR-124a suppresses Lhx2 and thereby prevents apoptosis of retinal cells during development (Sanuki et al., 2011). Since iris colour deepens during development in mammals, a regulatory role for MIR124A in brown iris formation is biologically plausible, although the very small control group for this trait means that the association requires confirmation.

Short hair: Hair length is directly relevant to heat tolerance in the hot arid tracts of lower Sindh and also carries economic value where goat hair is used for rugs. Two of the seven significant markers lie on chromosome 15 near ARNTL/BMAL1 and P4HA3. BMAL1 is a core circadian gene (Lavtar et al., 2018) that heterodimerises with CLOCK and regulates the hair growth cycle; in its absence REV-ERBα is down-regulated and P21 up-regulated, arresting hair follicles in the G1 phase and delaying anagen (Geyfman and Andersen, 2010). P4HA3 catalyses post-translational hydroxylation of collagen (Atkinson et al., 2019), and collagen is a principal component of the extracellular matrix that governs cell proliferation and differentiation, including that of hair follicle cells (Bonnans et al., 2014). SPIDR participates in double-strand break repair during replication and may therefore influence the rapidly dividing follicular tissue, ATP10D transports glucosylceramide across the plasma membrane (Sigruener et al., 2017), and RSL24D1 is required for ribosome biogenesis and hence for protein synthesis (McCool et al., 2023). Sun et al. (2022) reported GLRB, GRIA2 and PGBD5 for hind leg hair in Chinese breeds, so the genes implicated in hair phenotypes differ appreciably between populations.

Roman nose shape: A pronounced Roman nose is a recognised feature of several Pakistani goat breeds. Nine markers were significant, two of them on chromosome 3 close to the calcium-binding proteins S100A10 and S100A13, and one close to KIAA0586 on chromosome 10. KIAA0586 is required for ciliogenesis and sonic hedgehog signalling, a pathway central to craniofacial and forebrain development, and its mutation causes Joubert syndrome with a characteristic mid-brain malformation in humans (Bachmann-Gagescu et al., 2015). Markers close to TMTC2, FAM172A, FAM98A, THAP12 and GRIA2 were also detected. That no marker reached significance for nose length, a quantitative measure of the same anatomical region, suggests that the shape and the size of the muzzle are under distinct genetic control, and that the sample was underpowered for the continuous trait.

Upward horn orientation: Horns serve in defence and thermoregulation and determine the risk of injury during handling. Twelve markers were associated with upward orientation, the strongest lying within CDC14A on chromosome 3. CDC14A encodes a dual-specificity phosphatase involved in double-strand break repair, microtubule integrity, chromosome segregation and mitotic exit, and two of its recessive variants cause deafness in humans (Imtiaz et al., 2017). Other implicated genes included FARP1, previously associated with milk urea nitrogen in Holstein cattle (Ma et al., 2023) and with neuronal signalling and synapse number (Kuo et al., 2018); RPLP1, required for embryonic development of the nervous system (Perucho et al., 2014); PFKP and NDUFV2, which act in glycolysis and oxidative energy generation (Ghosh et al., 2018); MED27, a transcriptional mediator (Rienzo et al., 2014); and the structural proteins RP1 and GPHN. It should be emphasised that the trait analysed here is the direction in which horns grow and not the presence or absence of horns, which in goats is controlled by the polled intersex syndrome locus on chromosome 1; none of the markers reported here falls in that region.

Limitations: The findings should be interpreted with the design in mind. The number of animals per breed was modest, several traits had strongly unbalanced case and control groups, and the phenotypes are partly confounded with breed, so that a proportion of the signals may reflect residual population structure rather than direct association. Replication in an independent and larger sample, ideally with a principal-component correction for stratification in addition to the breed covariate, is required before any of these markers is used in selection. Functional confirmation through expression analysis of the candidate genes in skin, iris, follicle and horn bud tissue would establish causality.

In conclusion, this study provides the first genome-wide association analysis of qualitative morphological traits in Pakistani goats and identifies 38 SNPs and a set of candidate genes associated with coat colour, eye colour, hair length, nose shape and horn orientation in the nine indigenous breeds of Sindh. The markers have prospective utility for selecting animals of a preferred colour, and short hair may prove a useful selection criterion for heat tolerance in the hot arid tracts of the province. Validation in larger independent populations and functional characterisation of the identified genes are recommended before the markers are incorporated into breeding tools.

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Conflict of Interest

The authors showed no conflict of interest.

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