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Detection of structural variants linked to mutton flavor and odor in two closely related black goat breeds

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Abstract

Background Mutton quality is closely related to genetic variants and gene expression alterations during growth and development, resulting in differences in nutritional values, flavor, and odor.

Results We first evaluated and compared the composition of crude protein, crude fat, cholesterol, amino acid (AA), and fatty acid (FA) in the longissimus dorsi muscle of Guizhou black goats (GZB, $n=5$) and Yunshang black goats (YBG, $n=6$). The contents of cholesterol and FA related to odor in GZB were significantly lower than that in YBG, while the concentrations of umami amino acids and intramuscular fat were significantly higher in GZB. Furthermore, structural variants (SVs) in the genomes of GZB ($n=30$) and YBG ($n=11$) were explored. It was found that some regions in Chr 10/12/18 were densely involved with a large number of SVs in the genomes of GZB and YBG. By setting $F_{ST} \geq 0.25$, we got 837 stratified SVs, of which 25 SVs (involved in 12 genes, e.g., *CORO1A*, *CLIC6*, *PCSK2*, and *TMEM9*) were limited in GZB. Functional enrichment analysis of 14 protein-coding genes (e.g., *ENPEP*, *LIPC*, *ABCA5*, and *SLC6A15*) revealed multiple terms and pathways related with metabolisms of AA, FA, and cholesterol. The SVs ($n=10$) obtained by the whole genome resequencing were confirmed in percentages of 36.67 to 86.67% ($n=96$) by PCR method. The SVa and SVd polymorphisms indicated a moderate negative correlation with HMGCS1 activity ($n=17$).

Conclusion This study is the first to comprehensively reveal potential SVs related to mutton nutritional values, flavor, and odor based on genomic compare between two black goat breeds with closely genetic relationship. The SVs generated in this study provide a data resource for deeper studies to understand the genomic characteristics and possible evolutionary outcomes with better nutritional values, flavor and extremely light odor.

Keywords Structural variant, Fixation index, Mutton, Guizhou black goat, Yunshang black goat

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Background

With the development of the economy and the improvement of living standards, human beings' requirements for all kinds of meat quality are constantly increasing, and protecting and optimizing the breeding with better meat quality have become one of the major challenges faced by the livestock and poultry industry. Domestic scholars believe that the combination effects of linear fatty acids such as caproic acid, caprylic acid, and capric acid are contributed to the unique odor of mutton [1]. Previous studies show that 4-methyloctanoic acid (MOA) and 4-methylnonanoic acid (MNA) in volatile branched-chain fatty acids play an important role in mutton odor [2, 3]. Guizhou black goat (GZB or G) is a local breed living in the wet-cold mountain area of the Yunnan-Guizhou plateau. It presents excellent characteristics such as better mutton flavor (umami amino acid content > 8.9 g/100 g), light odor (hexanoic acid, decanoic acid, and branched fatty acid content are lower), low cholesterol content (cholesterol content < 0.02 g/100 g), and good palatability (fat content: 3.5-5.0 g/100 g) [4]. Excessive cholesterol intake is an initiating risk factor for atherosclerosis. Many genes play a central role in cholesterol synthesis, including *HMGCR*, *HMGCS1*, *FDPS*, *CYP51*, *TM7SF2*, *EBP*, *SC5D*, and *DHCR7* [5]. Yunnan black goat (YBG or Y) is the first new breed of black goat for mutton in China. It possesses nice characteristics including fast growth (adult male goat), perennial oestrus, high fecundity, and good mutton production performance [6, 7]. Our previous study [8] showed that these two black goat breeds had a close genetic relationship. However, their mutton quality traits differ greatly and the related genetic basis is poorly understood.

Based on whole genome resequencing analysis, variant characteristics in genes and selection signatures associated with economic traits can be revealed [9–11]. Structural variants (SVs) have long been described as being involved in the origin, adaption, and domestication of animal species [12]. SVs may have a greater potential impact on multiple biological processes than single nucleotide variants [13–16]. Many studies have shown that structural variants are closely related to the trait difference species. Several SVs associated with yield and fiber quality improvement in allotetraploid cotton have been identified based on pan-genome and genome-wide association analyses (GWAS) [12]. Some researchers have found SVs harbored in many functionally important genes in *Drosophila* that may affect complex phenotypes [17]. A study of binary crossbred pigs identified a new pattern of SVs or tandem duplication regulating phenotypic traits, where many important SVs were embedded in or adjacent to long noncoding RNAs, suggesting their functional importance [18].

Traditional crossbreeding is time-consuming and inefficient. It is hoped that the genetic basis behind the differences in mutton flavor traits can be excavated, and the genes and structural variants related to these traits can be disclosed, which will lay a theoretical foundation for livestock breeding in future.

Results

Mutton of Guizhou black goat was featured with excellent nutritional values, flavor, and light odor

To explore the differences in mutton nutrition between the two goat breeds, we tested the contents of several mutton components. The results showed that the mutton nutrition of the Guizhou black goat (GZB or G) was significantly higher than that of the Yunshang black goat [19] (YBG or Y). There were significant differences ($P < 0.05$) in monounsaturated fatty acids (MUFA, C14:1, C15:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C22:1n9, and C24:1), polyunsaturated fatty acids (PUFA, C18:2n6t, C18:3n3, C18:3n6, C20:3n6, C20:5n3, C20:4n6, and C22:2), saturated fatty acids (SFA, C6:0, C10:0, C11:0, C13:0, C14:0, C16:0, C17:0, C18:0, C20:0, and C24:0), branched-chain fatty acids (MOA and MNA), umami amino acids (Glu, Arg, Ala, and Gly), crude fat and cholesterol (Fig. 1, Table S1). The GZB contained higher SFA, MUFA, and n-3 PUFA than YBG, while n-6 PUFA was lower. The ratios of n-6/n-3 PUFA and PUFA/SFA in GZB were 2.42 and 0.11, while those ratios in YBG were 15.61 and 0.40, respectively (Table S1).

Population-stratified SVs and candidate genes related to mutton flavor and odor

The analysis of the selection signature indicated that the GZB genome has multiple single nucleotide variants and selected genes may be involved in the formation of mutton quality traits [8]. To further explore more potential genetic basis of the mutton quality trait, we conducted SVs detection and comparative analysis based on the whole genome resequencing data of 30 GZBs (~ 15.3 ×) and 11 YBGs (~ 22.46 ×). It identified 30,732 SVs (including 19,931 deletions (DELs), 4,274 inversions (INVs), 4,049 tandem duplications (DUPs), 1,576 insertions (INSs), and 902 translocations (TRAs) in the genome of GZB and 28,703 SVs (including 18,482 DELs, 3,945 DUPs, 3,965 INVs, 1,488 INSs, and 823 TRAs in the genome of YBG (Fig. 2a). These SVs were classified into four types, including shared (identified in all samples), major (identified in ≥ 50% of samples), polymorphic (identified in > 1 sample), and singleton (identified in only one sample) SVs. GZB harbored 794 shared SVs, 5,723 major SVs, 20,136 polymorphic SVs, and 4,079 singleton SVs. Comparing the SVs of the two populations, GZB had 6,327 population-specific SVs and YBG occupied 4,298 (Fig. 2b). The population-specific variants of GZB and

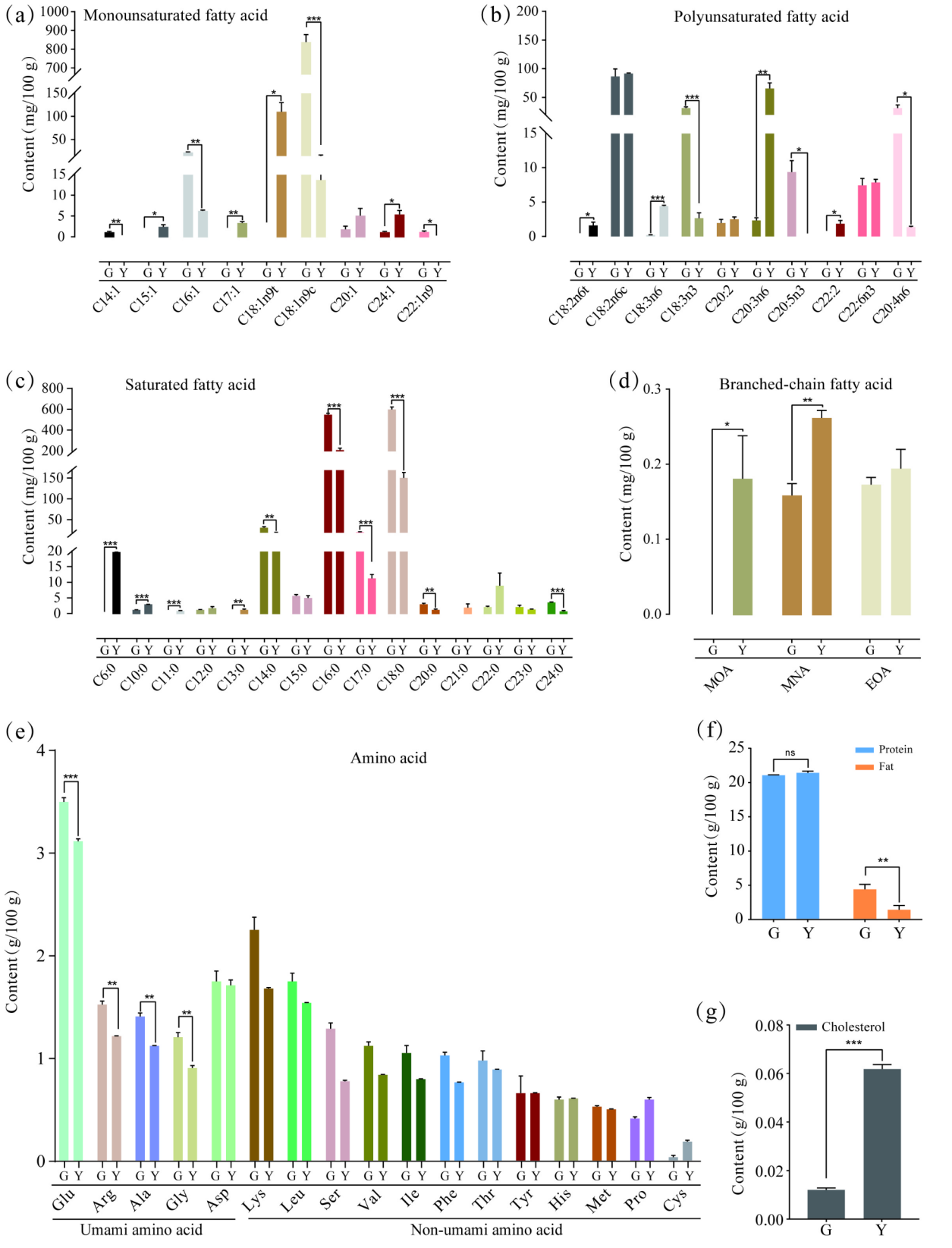

Fig. 1 Comparative analysis of nutritional components in mutton of GZB and YBG. **a**, monounsaturated fatty acid. **b**, polyunsaturated fatty acid. **c**, saturated fatty acid. **d**, branched fatty acid. **e**, amino acid. **f**, protein and intramuscular fat. **g**, cholesterol. *, **, and *** mean $P<0.05$, $P<0.01$, and $P<0.001$, respectively. **G**: Guizhou black goat. **Y**: Yunshang black goat

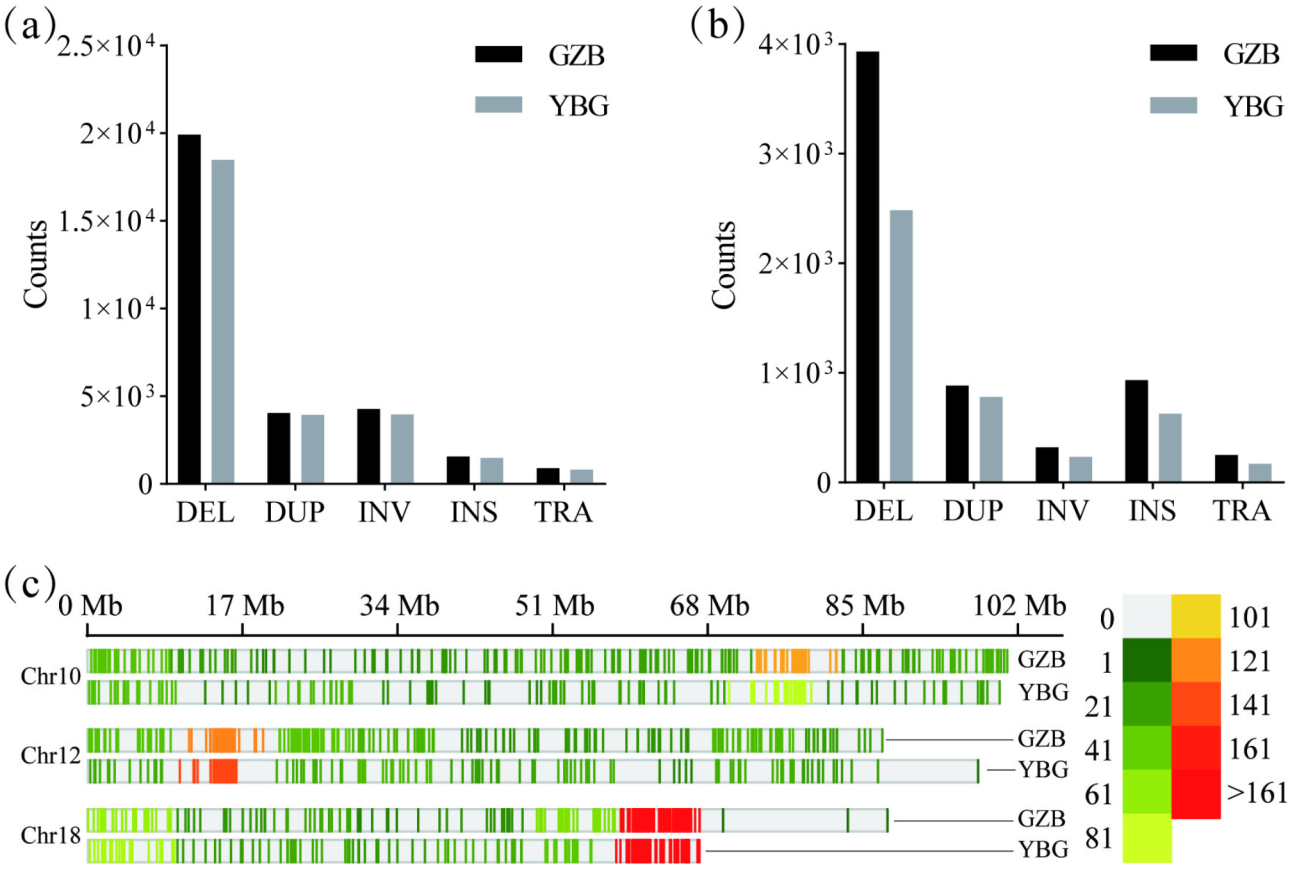

Fig. 2 The distribution of SVs in the genomes of GZB and YBG. **(a)** Quantitative distribution of different types of total SVs in the genomes of GZB and YBG. (**b)** Quantitative distribution of different types of population-specific SVs in the genomes of GZB and YBG. (**c)** Distribution of population-specific SVs on three chromosomes of GZB and YBG

YBG were distributed in all autosomes, and some regions on Chr 10/12/18 were more densely enriched, but there were some differences in density and distribution (Fig. 2c and Fig. S1). By thresholds of setting fixation index (F_{ST})≥0.25, we obtained 837 stratified SVs (corresponding to 368 stratified protein-coding genes) (Table S2), of which 25 were specific to GZB (Table 1; Figs. 3) and 367 were endemic to YBG (Table S3). Functional enrichment analysis of 14 protein-coding genes found multiple terms and pathways involving AA, FA, and cholesterol metabolisms, such as branched-chain AA: sodium symporter activity, triacylglycerol metabolism, and positive regulation of reverse cholesterol transport (Table 2, Table S4, Fig. 3).

Accuracy and polymorphism of structural variants

To verify the results of SV calling, we randomly selected 10 DEL variants on different chromosomes for accuracy and polymorphism analysis. It showed that the accuracy of SV calling ranged from 36. 67 to 86. 67% and the polymorphisms presented different consistencies with the callings (Table 3; Fig. 4, and Fig.S2-3).

The "M" in red color represented the DNA molecular marker ladder.

Correlation between the SVs polymorphisms and the rate-limiting enzyme activity of cholesterol synthesis in GZB

Cholesterol oxides may impart an unpleasant odor that degrades the flavor of mutton. The cholesterol content of mutton was very low in GZB. To explore the correlation between the SVs polymorphisms and cholesterol content, we detected the activities of three rate-limiting enzymes, including HMGCS1, HMGCR, and FDPS related to cholesterol synthesis in liver tissues of 17 GZBs. Subsequently, we analyzed the correlation coefficient r between the SVs polymorphisms (96 GZBs) validated (Table 3) and the ELISA results of enzymes (17 GZBs). As shown in Fig. 5, polymorphisms of SVa and SVd had a moderate negative correlation with HMGCS1 activity, while SVj had a weak positive correlation. SVd and SVg polymorphisms showed a weak positive correlation with FDPS activity. SVh and SVj polymorphisms presented a weak negative correlation with HMGCR activity. Although the ANOVA results between the SVs polymorphisms and enzyme activities were unsatisfactory (P>0.05), the

Table 1 SVs specific to GZB when $F_{ST} \geq 0.25$

SV_ID	Length/nt	F_{ST}	Gene name	Exon	Ratio
5_115344748_115347809_INV	3061	1.0000	*MIRLET7B*	-	30
11_104537584_104543182_INV	5598	1.0000	ENSCHIG00000018540	1/9	30
13_37237288_37237390_DEL	-102	0.7897	*PCSK2*	-	28
12_85826509_85826949_DEL	-440	0.7071	-	-	27
5_90810228_90810306_DEL	-78	0.6356	-	-	26
22_59881606_59884151_INV	2545	0.5729	*PLXNA1*	1/5	25
6_115785587_115786003_DEL	-416	0.5444	*TACC3*	-	25
7_86989312_86989559_DEL	-247	0.4246	-	-	24
10_78026196_78514725_DUP	488,529	0.4246	ENSCHIG00000013389	-	22
16_78187717_78187956_DEL	-239	0.4246	*TMEM9*	-	22
25_26042593_26042929_DEL	-336	0.4043	*CORO1A*	10/10	22
17_23412226_23412885_DEL	-659	0.3850	-	-	21
1_131020888_131021046_DEL	-158	0.3492	-	-	20
7_31759278_31759337_DEL	-59	0.3326	-	-	20
8_19328144_19328208_DEL	-64	0.3167	-	-	19
17_67465300_67465580_DEL	-280	0.3015	-	-	25
17_67493985_67495907_DEL	-1922	0.3015	-	-	25
20_71114891_71115238_DEL	-347	0.3015	ENSCHIG00000018789	-	21
21_52341637_52341992_DEL	-355	0.3015	-	-	20
5_24344629_24354339_DEL	-9710	0.2870	ENSCHIG00000001450	-	22
5_35592262_35594793_DEL	-2531	0.2870	*TMEM117*	-	24
6_38861264_38861814_DEL	-550	0.2870	-	-	25
21_66537202_66537273_DEL	-71	0.2870	-	-	22
1_41054244_41054321_DEL	-77	0.2597	*CLIC6*	6/6	22
4_119376588_119376650_DEL	-62	0.2597	-	-	24

significance test of correlation coefficient r was reliable ($P<0.05$).

Discussion

The flavor and odor of mutton are key factors for the popularity in market

Previous researchers have concluded that flavor and odor are important attributes next to color, contributing to overall meat evaluation and reflecting differences in meat quality between species [20]. The taste and flavor of mutton are also greatly influenced by amino acids types [21]. It is generally believed that there are three main types of amino acids: umami amino acids, sweet amino acids, and bitter amino acids [22]. Aspartic acid and glutamic acid, for example, are umami amino acids, and their higher contents elevate the distinctive flavor of mutton [23]. In this study, the content of four kinds of umami amino acids such as glutamate and arginine in the mutton of GZB was significantly higher than that in YBG (Fig. 1e), which could indicate its degree of flavor in mutton to some extent. Hu [24] et al. demonstrated that the supplementation of glutamic acid not only decreases backfat thickness but also enhances the muscle fatty acid composition [25]. Research has demonstrated that volatile branched-chain fatty acids (BCFAs) plays a crucial role in determining the odor of mutton. Among these, MOA, EOA, and MNA are the primary compounds that contribute to the characteristic odor in mutton [26]. Our study revealed that the contents of MOA and MNA in the mutton of GZB were notably decreased compared to those found in YBG. Collectively, these findings indicated that the mutton of GZB possessed a stronger flavor, a subtler odor, and met a higher acceptance of consumers.

Changes of n-6/n-3 PUFA and P/S ratio affecting mutton quality or nutrition value

As consumers become aware of the health benefits of higher PUFAs and lower SFAs in meat, livestock farming also tends to produce healthier products. The ratio of n-6/n-3 PUFA in the optimum nutritional standard of meat is not more than 4, but P/S is less than 0.4 [27]. The ratios of n-6/n-3 PUFA and P/S in GZB are 2.42 and 0.11, while those ratios in YBG were 15.61 and 0.40, respectively. The ratios of n-6/n-3 PUFA and P/S are 3.8 and 0.6 in Iranian indigenous Lori goat [28], 3.43 and 0.2 in Hu sheep [29], , respectively. P/S and n-6/n-3 PUFA ratios indicate the nutritional value of meat, but many kinds of meat do not meet the prescribed nutritional health standards, resulting in unbalanced fatty acid intakes by human beings. Therefore, it is necessary to provide theoretical guidance to the livestock industry to adjust fatty acid content up to more beneficial levels to meet the needs for human health.

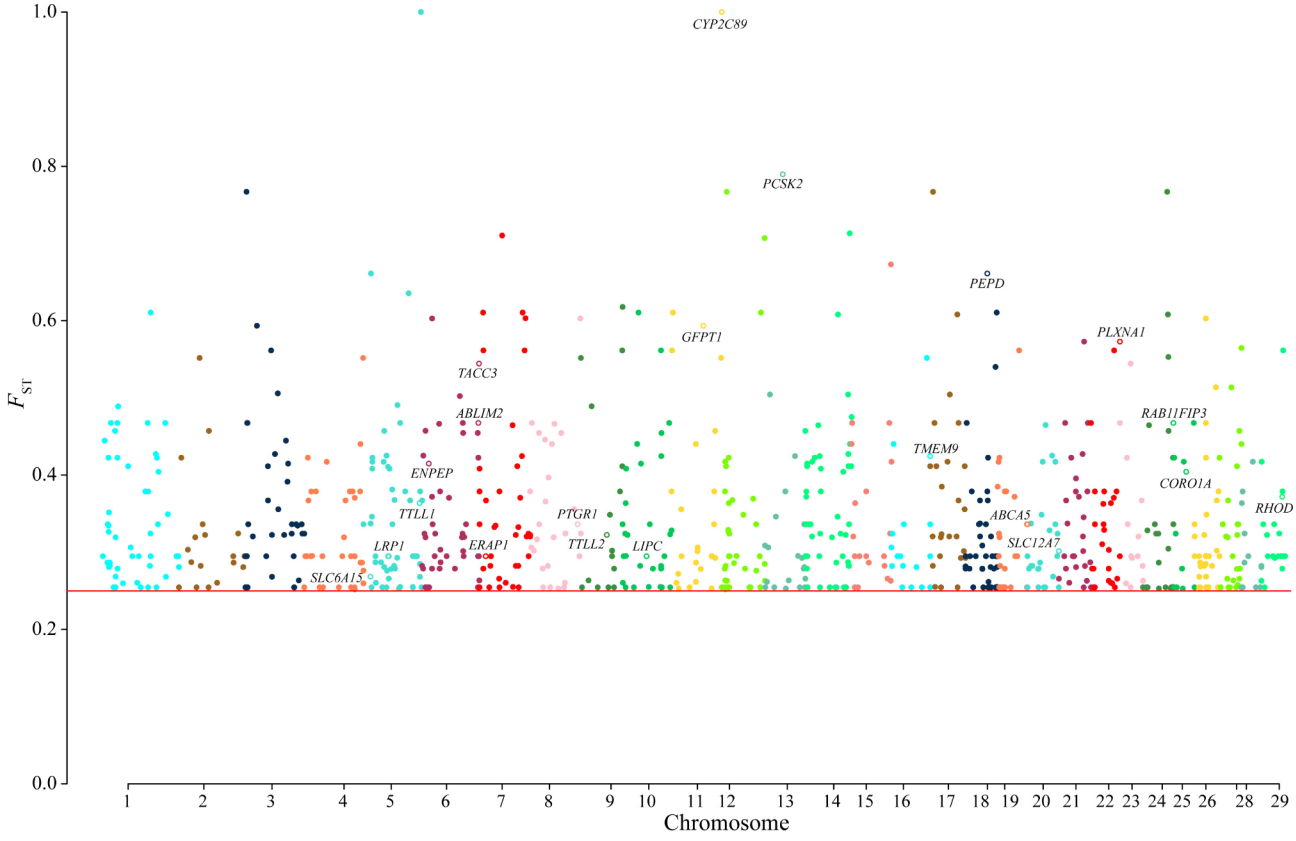

Fig. 3 Distribution of genes corresponding to stratified SVs in the genome of GZB

Accuracy of detecting SVs based on short-read whole-genome sequencing (srWGS)

By contrast, millions of genomes have been sequenced or commissioned in international projects and studies through srWGS. With the advance and rise of long-read WGS (lrWGS) technology, the sensitivity of SV detection from genomes has increased significantly. Disappointingly, the current cost of lrWGS is significantly higher than srWGS. Given the comparative advantage of srWGS in current genomic research, we conducted this study to look for potential structural variants associated with mutton quality. Initial surveys have implied highly variable outcomes and limited overall concordance in SV detection between the two technologies [30]. However, some researchers observe a very high (93.8%) concordance between srWGS and lrWGS in detecting deletion variants in 90% of the human reference genome. In addition, lrWGS performs much better in detecting SV of insertion [31]. And it is also confirmed the reliability of srWGS in detecting SV of deletion. Therefore, we pay more attention to deletion structural variants in this study. Although their validation rates was not satisfactory, it could still provide directions for our further research. In this study, we used three softwares (Delly, Manta, and Dysgu) for SV calling. The false positive rates might be reduced by introducing more SV detection softwares, such as GRIDSS [32], Lumpy [33], SVseq2 [34], or SoftSV [35].

Promising genes and SVs for improving the flavor, odor, and cholesterol content of mutton

The previous study demonstrates that Merinoland sheep exhibits a higher concentration of 4-alkyl branched-chain fatty acids, whereas Ile de French and Merinoland sheep hybrids possesses lower levels of these fatty acids [36]. The candidate genes *CYP2B6*, *ACOT12*, *THEM4*, *ACSF2*, *LPIN1*, and *ADCY4* have been recognized as potentially playing a role in regulating the synthesis of BCFAs in sheep livers [37]. Analysis of transcriptome expression profiles in liver tissues from sheep exhibiting variable mutton odor and flavor indicated that *CYP2A6*, *UGT2B18*, *SULT1C1*, and *GSTM1* could be the dominant candidate genes involved in the regulation of these distinct odor and flavor characteristics [38]. Furthermore, a comparative study [39] between house feeding and free grazing conditions in black goats revealed that numerous genes play crucial roles in the synthesis of fatty acids and unsaturated fatty acids, including *FXR*, *LDLR*, *FDFT1*, *HMGCS1*, *ACSS2*, *SCARB1*, and *SCD*. We also found that some stratified genes in this study, such as *CYP2C89*, *HMGCS2*, and *SULT1B1*, might be involved in the production of odor and flavor.

Table 2 Some stratified genes were significantly enriched in AA, FA, and cholesterol-related terms/pathways

GO term/Pathway	Gene Name	*P*-value
aminopeptidase activity	*ENPEP/PEPD/ERAP1*	0.00075
apolipoprotein binding	*LRP1/LIPC*	0.00299
high-density lipoprotein particle remodeling	*LIPC/ABCA5*	0.00575
plasma lipoprotein particle remodeling	*LIPC/ABCA5*	0.0121
protein-lipid complex remodeling	*LIPC/ABCA5*	0.0121
13-lipoxin reductase activity	*PTGR1*	0.0106
positive regulation of reverse cholesterol transport	*ABCA5*	0.0106
regulation of reverse cholesterol transport	*ABCA5*	0.0106
branched-chain amino acid: sodium symporter activity	*SLC6A15*	0.021
negative regulation of adiponectin secretion	*RAB11FIP3*	0.021
acid-amino acid ligase activity	*TTLL1/TTLL2*	0.0264
Triacylglycerol metabolism	*LIPC*	0.0314
L-glutamine aminotransferase activity	*GFPT1*	0.0314
lamellipodium assembly	*ABLIM2/RHOD*	0.0306
lipoprotein lipase activity	*LIPC*	0.0416
lipoxin A4 metabolic process	*PTGR1*	0.0416
very-low-density lipoprotein particle remodeling	*PTGR1*	0.0416
triglyceride-rich lipoprotein particle remodeling	*PTGR1*	0.0416

Several studies have shown that ABCA5 can regulate cholesterol homeostasis [39–42]. In this study, we found that there were two insertion type SVs in the *ABCA5* gene of YBG, which might lead to cholesterol instability and made cholesterol content significantly higher than GZB. The situation is similar in the *LIPC* gene, which also has one SV of insertion that may lead to dysregulation of cholesterol synthesis [43–46]. Some studies suggest that SV of *CORO1A* may lead to cholesterol efflux and lipoprotein uptake blocked in some cells [47–49].

Conclusion

Our results indicated that mutton of GZB was better than that of YBG in nutritional value, flavor, and odor. The GZB contained higher SFA, MUFA, and n-3 PUFA than YBG, while the contents of cholesterol and n-6 PUFA were lower. The stratified SVs were detected from the genomic regions that mainly related to mutton nutritional value, flavor, and odor. Functional enrichment analysis of 14 protein-coding genes corresponding to the stratified SVs revealed multiple terms and pathways involving in metabolisms of AA, FA, and cholesterol. SVa (2_1225462_1225627_DEL) and SVd (6_31713624_31713762_DEL) polymorphisms showed a moderate negative correlation with HMGCS1 enzyme activity. These results would lay the groundwork for continued protection of resources and the improvement of mutton quality in goat breeds.

Methods

Slaughter and determination of nutrient components in mutton

We sampled fresh longissimus dorsi muscle and liver tissues from adult male GZB (12-month old, $n=17$) with natural grazing in Nayong country of Guizhou province, China. The GZB were slaughtered under standardized conditions (GB/T 43562−2023). Artificial electric anesthesia was used to induce coma. After fainting, it was in coma and its heart should be kept beating. It should not be repeatedly stunned or killed. Electric hemp operators shall wear qualified insulating boots and gloves. The voltage of electric hemp is 90–110 V, and the current is 0.5–1.0 A. Dip in 5% saline before electroanesthesia. The artificial electric anesthesia sites were the roots of two ears. The duration of anesthesia was 3–5 s. Fatty acid (FA), amino acid (AA), crude protein, crude fat, and cholesterol contents were detected according to different National Food Safety Standards of China (GB5009.168-2016, GB5009.124-2016, GB5009.5-2016, GB5009.6-2016, and GB5009.128-2016, respectively). Volatile branched-chain fatty acids were detected according to

Table 3 Validation of SV calling by Sanger sequencing, PCR, and agarose gel electrophoresis

No.	Gene Name	SV_ID	Length/nt	Validation Rate/%	Polymorphism		
					II	ID	DD
SVa	*ACTL8*	2_1225462_1225627_DEL	165	53.33	7	18	71
SVb	*CORO1A*	25_26042593_26042929_DEL	336	70.00	1	0	95
SVc	*CYRIB*	14_71266335_71266493_DEL	158	83.33	23	49	24
SVd	*GRID2*	6_31713624_31713762_DEL	138	70.00	5	45	46
SVe	*HMGCS2*	3_97009494_97009595_DEL	101	43.33	75	2	19
SVf	*PLXND1*	22_56161354_56161645_DEL	291	36.67	11	39	46
SVg	*RAI14*	20_39332619_39332799_DEL	180	63.33	26	44	26
SVh	*SORL1*	15_50249177_50249329_DEL	152	86.67	22	41	33
SVi	*SULT1B1*	6_85780059_85780236_DEL	177	86.67	33	38	25
SVj	*SUPT4H1*	19_8946148_8946331_DEL	183	40.00	15	39	42

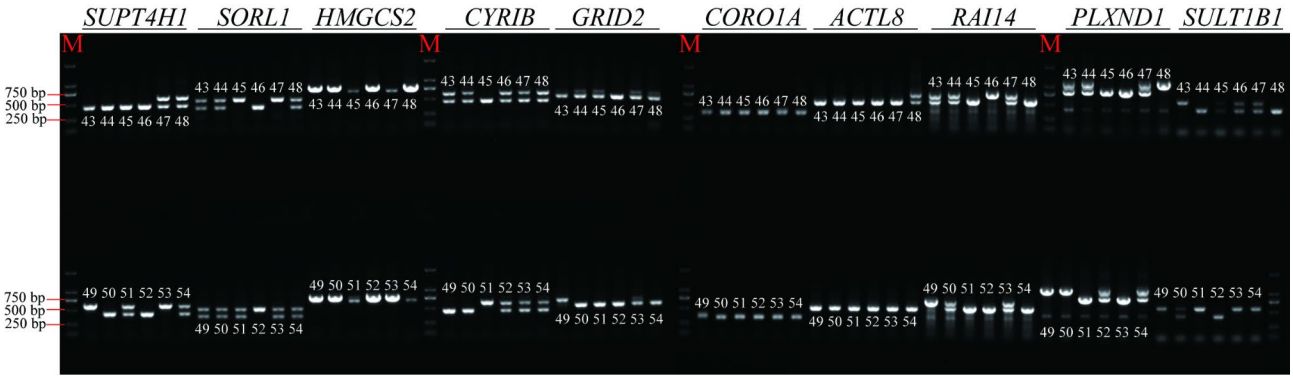

Fig. 4 Verification of SVs' polymorphisms by PCR method and agarose gel electrophoresis (#43–54, typeset by Adobe Illustrator software)

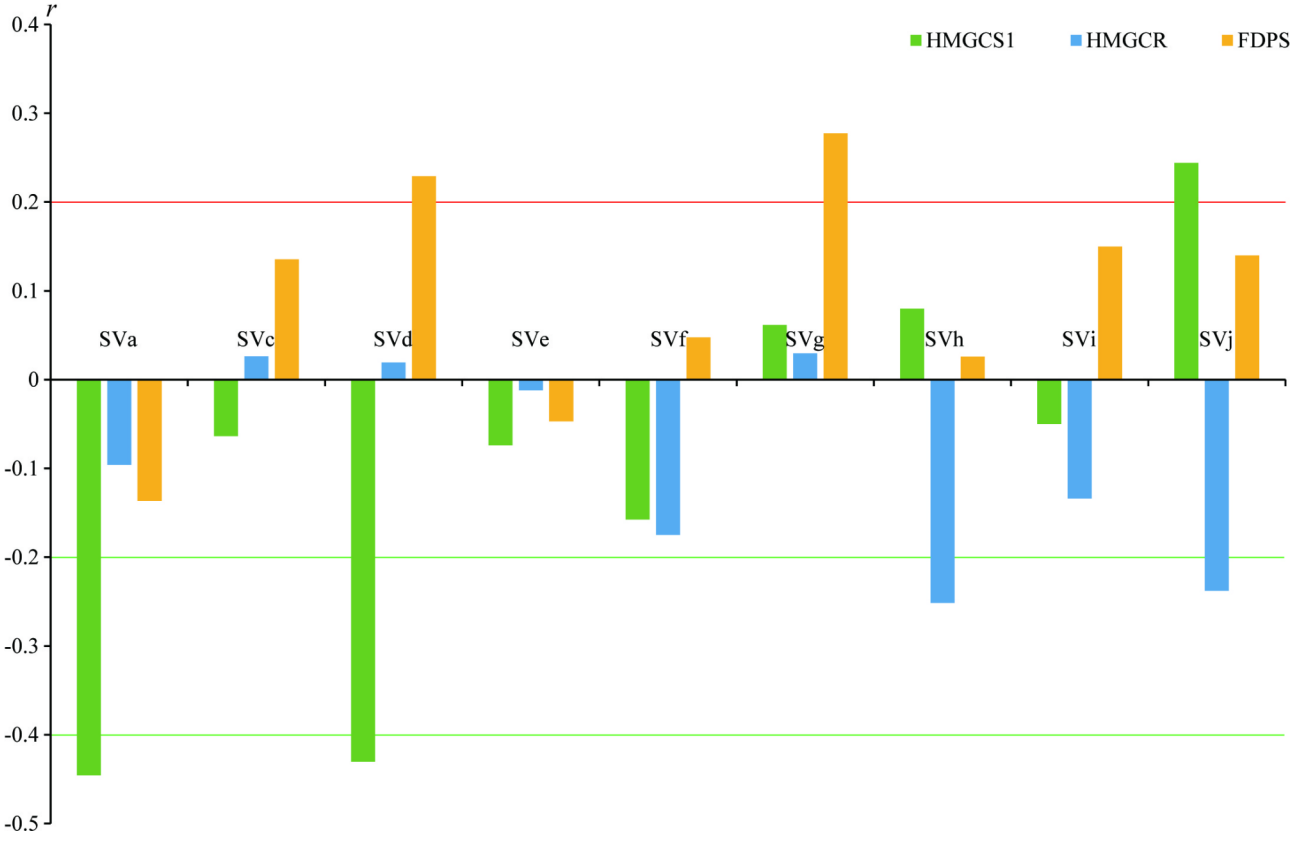

Fig. 5 Correlation coefficient *r* between the SVs polymorphisms and the rate-limiting enzymes activities related to cholesterol synthesis

Kaffarnik et al. [36], which is based on the conversion of the fatty acids in sheep fat into fattyacid methyl esters followed by the direct gas chromatography with mass spectrometry (GC/MS) analysis. The required sensitivity of the GC/MS method was obtained by switching to the selected ion monitoring (SIM) mode.

Population resequencing

We randomly sampled a total of 96 GZB (similar body length, height, and chest circumference, et al.) from different pens on five larger core breeding farms (>300 goats) to reduce affinity between samples. Genomic DNA was extracted from the ear tissue of each individual. Of which, the whole-genome resequencing of 30 GZB was performed by a DNBSEQ-T7 sequencer (BGI, Shenzhen, China) according to the manufacturer's recommendations. In addition, we downloaded the genome data of 11 Yunshang black goat individuals (PRJNA611688) from the European Bioinformatics Institute website (www.ebi.ac.uk/).

Structural variant calling

Fastp v0.23.4 [50] was used for assessing a per-base sequence quality with default parameters. The command was like 'fastp -i /data/A1_1.fastq.gz -I /data/A1_2.fastq.gz -o /data/A1_1.QC.gz -O /data/A1_2.QC.gz'. The high-quality 150 bp paired-end reads were aligned to the goat reference genome ARS1, using the Burrows-Wheeler aligner v.0.7.8 software [51] with default parameters, such as bwa mem -R ' @RG\tID: SCG\tSM: A' -t 64 / data/ARS1.fa /data/A1_1.QC.gz /data/A1_2.QC.gz>A1.sam. We then converted the mapping reads into bam files and sorted the files using SAMtools v.1.9 [52] by default parameters, such as samtools view -bS A1.sam>A1.sort.bam. Duplicates were removed by the MarkDuplicates module in GATK v.4.3.0.0 [53] with command 'gatk --java-options "-Xmx16g -Djava.io.tmpdir=./tmp" MarkDuplicates -I A1.sort, bam -M A1.metrics --CREATE_INDEX -O A1.sort.MarkDup.bam'. We used Manta [54] v1.6.0, Delly [55] v.0.8.3, and Dysgu [56] v1.5.0 to call SVs, respectively, and SURVIVOR [57] to merge the calling results. The corresponding command scripts were as follows, #Manta: (1) samtools view -T ARS1.fa -C -o A1.sort.MarkDup.cram A1.sort.MarkDup.bam (2) configManta.py --bam A1.sort.MarkDup.bam --referenceFasta ARS1.fa --runDir runDir/ (3) python runWorkflow.py -m local -j 80. #Delly: (1) samtools index -b A1.sort.MarkDup.bam (2) delly call -t 25 -g ARS1.fa -o A1.bcf A1.sort.MarkDup.bam (3) delly merge -o sites.bcf s1.bcf s2.bcf . sN.bcf (4) bcftools convert -O v -o sites.vcf sites.bcf. #Dysgu: (1). for i in A1 A2 A3…A30\ (2) do dysgu run -p30 ARS1.fa temp_dir$[i] $[i].dedup.bqsr.bam>GZB$[i]svs.vcf\ (3) Done. Finally, SURVIVOR was used to merge the structural variants obtained by the above three softwares with default parameters, such as SURVIVOR merge sample_A1 1000 2 1 1 0 50 A1_merged.vcf. "1000" indicated that the distance between SVs allowed to be merged would not exceed 1000 bp. "2" meant that SVs identified by both tools would be output. The first "1" means that SVs identified by both tools and of the same type would be output. The second "1" meant that SVs identified by both tools and in the same direction would be output. The "50" represented that SVs with a length of more than 50 bp would be considered.

Fixation index calculation

The *F*-statistics (F_{ST}) value was calculated according to the formulas in previous study [58, 59].

$$p = (2*N_{AA}+1*N_{Aa})/2N, q = 1 - p \quad (1/2)$$

$$\bar{p} = (2*N_{1AA}+1*N_{1Aa}+2*N_{2AA}+1*N_{2Aa})/2*(N_1+N_2), \bar{q} = 1 - \bar{p} \quad (3/4)$$

$$H_{exp1} = 1 - (p_1^2+q_1^2), H_{exp2} = 1 - (p_2^2+q_2^2) \quad (5/6)$$

$$H_S = 1 - (\bar{p}^2+\bar{q}^2), H_T = (H_{exp1}*N_1+H_{exp2}*N_2)/(N_1+N_2) \quad (7/8)$$

$$F_{ST} = 1 - H_S/H_T \quad (9)$$

Structural variant validation

To check the confidence of SVs calling, we randomly validated 10 SVs in specific genes from 96 individuals that were genotyped by PCR and agarose gel electrophoresis. The primers used for PCR were designed with DNAMAN v9.0.1.116 (Lynnon Biosoft, USA). The PCR reactions were carried out in 20 μL volume containing 10 μL of 2×Taq PCR Master Mix (TIANGEN Biotech, Beijing, China), 0.4 μL (10 μM) for each forward and reverse primer (Table S5), 1 μL DNA templates (30–100 ng/mL), and the remainder supplied with ddH_2O. The reactions were performed by a BIO-RAD T100 Thermal Cycler with conditions of an initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, annealing at 60/62/65°C for 30 s and extension at 72 °C for 45 s, and then a final extension at 72 °C for 5 min. Subsequently, we compared genotypes of each SV identified by whole-genome resequencing with those obtained by the PCR and agarose gel electrophoresis for the same individuals.

Enzyme-linked immunosorbent assay of HMGCS1, HMGCR, and FDPS

Samples were obtained from the livers of GZB after ethanol gavage, and over 100 μL of serum samples were separated by centrifuge and stored at -80 °C for analysis. The enzyme activities of HMGCS1, HMGCR, and FDPS were determined with ELISA kits (Meimian Biotechnology, Yancheng, Jiangsu, China) according to the manufacturer's instruction.

Statistical analysis

Means and standard deviations of the nutrient components in mutton were calculated using Microsoft Excel. Significant difference analysis of nutrient components percentage between GZB and YBG groups was performed using the t-test, and $P<0.05$ was considered significant. Correlation analysis between SV genotypes (II, ID, and DD) and enzyme activities (HMGCS1, HMGCR, and FDPS) was performed. A positive correlation meant that the presence of structural variation leads to the increased enzyme activity, while a negative correlation lead to activity decrease. SPSS v24 was used to complete the statistical analysis.

Abbreviations

GZB	Guizhou black goat
YBG	Yunshang black goat
SNP	Single nucleotide polymorphism
SV	Structural variant
WGS	Whole-genome sequencing
lrWGS	Long-read WGS
srWGS	Short-read WGS
F_{ST}	Fixation index
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
MOA	4-methyloctanoic acid
MNA	4-methylnonanoic acid
FA	Fatty acid
AA	Amino acid
HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase
FDPS	Farnesyl diphosphate synthase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-024-10874-2.

Supplementary Material 1

Author contributions

JW and XR conceived and designed the experiments. XN, SH, and DS contributed to the sample collections and determination of nutritional components in mutton. LC performed the analysis of whole-genome resequencing data, the sample DNA extraction, and the SV validation experiment. LC drafted the manuscript. All authors read and approved the final manuscript.

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Data availability

Sequences were private from ENA with the Bioproject accession numbers PRJEB67694.

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee of Guizhou University following the recommendation of the Regulations for the Administration of Affairs Concerning Experimental Animals of China. The study is also reported in accordance with the ARRIVE guidelines. The 35 goats were privately owned by some farms in Guizhou, China, and we obtained consent from the owners to use the goats in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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