
LOCATION OF GENES ASSOCIATED WITH HAIR LENGTH OF RABBIT.

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ABSTRACT

Hair length is not only a crucial evaluation index of wool quality but also of great importance in annual wool production in Angora rabbit. To reveal the genetics of hair length in rabbit, Wan strain angora rabbit and Rex rabbit were used to construct backcross populations segregated for hair length in the present study. Then gene location of hair length was conducted by integration of BSA method and SLAF-seq technology. There were remarkable differences in hair length between angora and short populations of BC1 (104.4±8.00 vs 36.6±1.46 mm for coarse wool, and 74.1±4.53 vs 26.6±1.05 mm for fine wool, \(P<0.01\)). SLAF-seq generated 2,810M of data containing 18.33M pair-end reads on 181,531 SLAF tags. Among them, there were 30,010 SLAF tags with polymorphisms and 175,744 SNPs and Indels were used for testing genetic differentiation between two segregated populations with different hair length. Ultimately, rabbit hair length was located at 46.28M region on chromosome 15, which comprised of 232 genes e.g. FGF5. The results will greatly facilitate the further determination of major genes for hair length in rabbit and accelerate the genetic progress on this trait using molecular marker-assisted selection.

Key words: Rabbit, Hair length, Gene location, SLAF-seq, Backcross.

INTRODUCTION

Wool fiber length is an important trait for fur animal, which decides the wool quality as the crucial index of evaluation. Angora rabbit and Rex are important livestock reared for wool and fur usage. Angora rabbit are characterized with its long hair owing to continued long-term anagen of hair follicle, while Rex rabbit with the marked shortening hair of same length. The two natural mutant breeds provide a facility for genetic analysis of hair length as animal models. Chantry-Darmon et al. (2006) constructed the first-generation microsatellite-based integrated genetic and cytogenetic map for the European rabbit and the angora character was mapped 3 cM from INRACCDVD028 on chromosome 15 based on the three-generation reference families builded by 3 rabbit INRA strains carries wild-type and recessive alleles at angora locus. In addition, Ma (2011) performed QTL mapping of hair length using 10 microsatellites markers from chromosomes 1, 2 and 15 in two resource families of self-cross F2 and backcross generation 1 from parental meat rabbit and rex rabbit and wool length at 90d and 120d was mapped on chromosome 1 and 2, respectively.

Up to now, however, it needs more precise genomic location for hair length in rabbits mainly because of insufficient intensity and polymorphism of microsatellites on the genome used in reported literatures. Recently, the specific-length amplified fragment sequencing (SLAF-seq) was developed based on high-throughput sequencing technology, also known as reduced-representation sequencing (RRS) (Sun et al., 2013; Chen et al., 2015). The SLAF-seq technology has several advantages, such as genome-wide, high throughput, time-saving and relative low cost (Chen et al., 2013). It has been successfully applied for genetic mapping (Zhang et al., 2013), linkage analysis (Zhang et al., 2015) and association study (Wang et al., 2015). Probably, it can facilitate precise location of genes related to rabbit hair length.

The objectives of this study were firstly to construct a backcross generation segregated in hair length with Wan strain angora rabbit (WSAR) and white Rex rabbit, and secondely to locate the genomic regions associated with rabbit hair length by combination of SLAF-seq with bulked segregant analysis (BSA). The present study will be good for revealing molecular bases of rabbit hair length.
MATERIALS AND METHODS

Construction of a BC population and hair length measurement

Two breeds were used to construct a backcross population (BC) in the present study. One parental line (P) was Wan strain angora rabbit with recessive long hair trait attributed to its mutant long anagen of hair follicle. The other parental line was Rex with short white hair. There were 10 angora rabbits and 2 Rex rabbits for each kind of parents. Thirty-two (19♂ and 13♀) F1 rabbits have been generated. After interbreeding, F1 population and then BC1 with short hair were used to backcross with parental WSAR (Figure 1). Parents and all offspring were reared with identical production conditions on the experimental rabbitry of Anhui Academy of Agricultural Sciences. The hybridization commenced on autumn 2013 and terminated on spring 2015 with one generation in each year.

Hairs were clipped from three points of hip cross represented as the samples of every rabbit. Then length measurement were conducted using ruler (minimum scale=1mm) for coarse hair and fine wool sorted by normal magnifier and SZ660 stereomicroscope (Chongqing Optec Instrument Co., Ltd. China). Every type of wool fiber were measured at least 30 in number.

SLAF library construction and sequencing

Twenty seven Angora rabbits and 30 rabbits with short hair were selected from BC1 and BC2 populations. Total genomic DNA was isolated from these 57 rabbits, respectively, using the TIANamp Genomic DNA Kit (Tiangen, Beijing,China) and was quantified using the NanoDro p 2000 spectrophoto-meter (Thermo Scientific, USA). Angora pool (A-pool) and short pool (S-pool) formed through mixing DNA of equal amount from each rabbit for each group. The reference genome of rabbit was used in the present study, with a size of 2,734.47Mb (ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCA_000003625.1_OryCun2.0). Genomic DNA of two pools were digested with RsaI restriction enzyme using Oryza sativa as control after a simulated enzyme digestion with potential 129,112 SLAF tags (more than 100,000 the expected tags). The SLAF libraries were constructed according to the procedures described by Sun et al. (2013).

DNA fragments of 314-344 bp were selected as SLAF tags and prepared for pair-end sequencing on an Illumina High-seq 2500 sequencing platform (Illumina, USA). Two samples were sequenced in one lane to avoid errors and ensure the comparability between them. After filtering and trimming, paired-end reads were mapped onto the reference genome using SOAP2 (Li et al.,2009). The reads comprising of overlap and mapped to the same position in the reference genome were conducted local realignment by GATK (https://www.broadinstitute.org/gatk/guide/best-practices?bpm=DNASEq#variant-discovery-ovw). Common SNPs and InDels of outcome from GATK and samtools (http://samtools.sourceforge.net/) were used for further association analysis.

Statistical Analysis

Two-sided t-test was used to reveal the difference of hair length between two divergent groups with angora or short wool fiber. The significant differences of genotype frequency between the pools of two rabbit groups were found by calculating \( \Delta \)SNP-index method indicated by Abe et al. (2012). The loess regression fitting was carried out to determine the association-threshold also according to the method of Abe et al. (2012). The reference genome regions over the threshold were considered as potential regions related to rabbit hair length.

RESULTS AND DISCUSSION

Phenotypic and Genetic analysis of rabbit hair length

All rabbits of F1 generation had short hair (Figure 2a) like homozygous parent Rex, which indicated short hair phenotype was a dominant character, and vice-versa angora is recessive mutation (Mulsant et al., 2004). For BC generation 1, there were 34 angora rabbits and 49 short hair rabbits (Figure 2b), which fitted the 1:1 segregation ratio (\( \chi^2=2.71, P>0.05 \)). Therefore, the rabbit hair length was probably controlled by a few major genes or genomic regions which closely linked to FGF5 (Mulsant et al., 2004). For BC generation 2, there were 15 angora rabbits and 24 short hair rabbits (Figure 2c), also fitted the 1:1 segregation (\( \chi^2=2.07, P>0.05 \)).
To investigate the precise difference between angora and short populations, we attained the natural length data of each type of wool fiber gathered from BC1 generation. The average lengths of coarse wool and fine fiber were 104.4±8.00 mm and 74.1±4.53 mm, respectively, for angora population. On the contrary, those for short hair population were 36.6±1.46 mm and 26.6±1.05 mm, respectively, which similar to the hair length of Rex and/or Fujian Huang rabbit—a local variety in South China (Xu, 2011). There were significant differences between angora and short populations as for the natural length of coarse and fine wool fibers (both \( P<0.01 \)) by t-test.

SLAF-seq analysis and location of regions related to hair length

DNA samples of the two separate bulks from the BC1 population were subjected to SLAF sequencing. After SLAF library construction and high-throughput sequencing, a total of 2,810M of data containing 18.33M pair-end reads were obtained with high quality (Q30 percentage=87.73%, GC percentage=40.41%) fitting the requirements of further analysis. The numbers of SLAF tags was 181,531, and the average coverage for each tag was 93.17-fold. Out of them, 30,010 polymorphic SLAF tags were attained to account for 16.53%. According to the positions of SLAF on the rabbit genome, we calculated the polymorphic SLAF tag numbers on each chromosome, which shown that the polymorphic SLAF tags were distributed evenly on each chromosome and the rabbit genome has been simplified successfully (Figure 3a). After reads realignment and mutations determining, 365,662 SNPs (containing Indels) generated. Among them, 223,476 high quality SNPs were attained through filtering the reads whose depth below 4-fold and there were 175,744 SNPs used for differentiating two groups of DNA bulks.

Euclidean distance (ED) was calculated for every locus of SNPs based on the difference of allelic frequency followed by loess regression analysis (Hill et al., 2013). The threshold value was determined to be 0.1204. The regression value over 0.1204 indicated that the corresponding region was associated with rabbit hair length. In the present study, there was only one significantly related region spanning 46.28M (62,736,538-109,014,797) on Chromosome 15 (Figure 3b). Chantry-Darmon et al. (2006) also verify the location of angora on chromosome 15q using 109 microsatellites to scan rabbit genome but excluding chromosome 20, 21 and X. The related region composed of 232 genes which then have been done annotation through GO, COG and KEGG analyses. FGF5 gene located in this region, which had been shown to tightly linked with rabbit angora wool (Mulsant et al., 2004).

However, the difference of hair length between normal rabbit and Rex probably be attributed to an adenine Indel (named 1362IndelA) in LIPH exon 9 on chromosome 14 through twice genomic locations of Rex phenotype and candidate gene analysis (Diribarne et al., 2011). Homologous 1362delA (orylag®: a kind of selected Rex with good quality) can lead to less expression and lipase activity of LIPH than heterozygous (and/or wide type) rabbit in anagen of hair follicle, which should give rise to the shorter hair type of Rex (Diribarne et al., 2012). In
addition, hair length difference between meat rabbit and Rex may be associated with genetic variations on chromosomes 1 and 2, but it needs further researches to validate owing to less chromosomes involved (only OCU1, OCU2 and OCU15) and just three polymorphic markers used for each chromosome (Ma, 2011).

CONCLUSIONS

A small scale of backcross population was constructed in the present study and the length of each type of wool fibers were determined to be remarkably different between the angora and short groups of segregated BC1. Additionally, this is the first study on gene location in mammals using relatively moderate cost SLAF-seq successfully according to the reported literatures, which indicated that this reduced representation sequencing technology can be applied to related researches in mammals. And the hair length locus was verified to locate in 46.28M region on rabbit Chromosome 15 through genome-wide association analysis based on single nucleotide polymorphisms. Given the numerous genes in this region, it needs further studies for determination of causative major genes. Nevertheless, our study can greatly facilitate the precise location of genes associated with hair length in rabbit.

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REFERENCES


