REVIEW ARTICLE

PLAG1 and NCAPG-LCORL in livestock

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ABSTRACT

A recent progress on stature genetics has revealed simple genetic architecture in livestock animals in contrast to that in humans. PLAG1 and/or NCAPG-LCORL, both of which are known as a locus for adult human height, have been detected for association with body weight/height in cattle and horses, and for selective sweep in dogs and pigs. The findings indicate a significant impact of these loci on mammalian growth or body size and usefulness of the natural variants for selective breeding. However, association with an unfavorable trait, such as late puberty or risk for a neuropathic disease, was also reported for the respective loci, indicating an importance to discriminate between causality and association. Here I review the recent findings on quantitative trait loci (QTL) for stature in livestock animals, mainly focusing on the PLAG1 and NCAPG-LCORL loci. I also describe our recent efforts to identify the causative variation for the third major locus for carcass weight in Japanese Black cattle.

Key words: body weight, height, NCAPG-LCORL, PLAG1, selective sweep.

INTRODUCTION

Large-scale genome-wide association studies (GWAS) in humans have identified many novel loci for complex diseases and traits. Adult human height is a classical polygenic trait and has been analyzed as a model for quantitative genetics. Heritability of adult human height is estimated as approximately 80% (Fisher 1918; Silventoinen et al. 2003; Visscher et al. 2006), while only a small portion of the heritability was explained by the common variants identified by GWAS of tens of thousands of people (Visscher 2008; Manolio et al. 2009). To explain the “missing heritability”, a meta-analysis combining several GWAS has been conducted to increase statistical power of detecting associated loci. The most recent GWAS for adult human height used the data from >253,000 individuals and identified 697 variants in 423 loci (Wood et al. 2014). The genome-wide significant single nucleotide polymorphisms (SNPs) explained 16% of heritability and all common variants together captured 60% of heritability (Wood et al. 2014). The study also identified several genes and pathways not previously connected with human skeletal growth (Wood et al. 2014).

In livestock, quantitative trait locus (QTL) mapping and GWAS have been conducted for economically important traits. Several traits such as carcass weight, calving difficulty and puberty are known to relate with body size and have been extensively examined. We previously performed GWAS for carcass weight in Japanese Black cattle and revealed three major loci, designated as CW-1, -2 and -3, of which CW-1 and -2 corresponded to adult human height loci, PLAG1 and NCAPG-LCORL, respectively (Setoguchi et al. 2009; Nishimura et al. 2012). The three loci together explained approximately one-third of genetic variance of the GWAS population (Nishimura et al. 2012). The PLAG1 and NCAPG-LCORL regions were also detected for an association in different cattle breeds and other livestock species (Tables 1 and 2). The CW-3 QTL was detected in a specific line of Japanese Black (Nishimura et al. 2012), which hampered narrowing down the QTL region. However, recent advances in genomic technologies such as high-density SNP chips and target resequencing enabled us to easily find a marker SNP in linkage disequilibrium (LD) with the QTL and screen candidate causative variations. The next challenge is to identify the causative variation. An excellent work by Karim et al. (2011), that identified quantitative trait nucleotides (QTNs) for bovine stature at the PLAG1 locus, indicates that a comprehensive study with genetic, genomic and molecular biological approaches is required to identify the causative variation.

Here I review the studies that reported a QTL or association around the CW-1 and -2 regions, and then discuss a strategy to identify the causative variation using CW-3 as an example.

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Table 1  Quantitative trait loci (QTLs) detected around the PLAG1 locus in livestock

| Species          | Breeds                               | No. of animals | Associated traits                                                                 | Freq. of the height-increasing allele | Reference               |
|------------------|--------------------------------------|----------------|------------------------------------------------------------------------------------|--------------------------------------|-------------------------|
| Cattle           | F2 from Holstein-Friesian and Jersey | 864 cows       | Height at 18 months of age, body weight at birth and 6, 12, 18 months of age, live weight | Holstein-Friesian: 0.78; Jersey: 0.05 | Karim et al. (2011)    |
|                  | New Zealand Holstein-Friesian        | 942 female calves | Peripuberal weight                                                                 | 0.85                                 | Littlejohn et al. (2012) |
| Japanese Black   |                                       | 1156 steers     | Carcass weight                                                                     | 0.754                                | Nishimura et al. (2012) |
| Brahman, Tropical Composite | Female cattle of 843 Brahman and 866 Tropical Composite | | Age when first corpus luteum was observed, reduced blood insulin-like growth factor (IGF)-1 level at 24 months of age, hip height at 24 months of age, scanned rump fat thickness at P8 position | -                                   | Hawken et al. (2012) |
| Brahman          |                                       | 1118 bulls      | Blood IGF-1 level at 6 months of age, Scrotal circumference at 12 months of age     | -                                    | Fortes et al. (2012)   |
| Tropical Composite | 1085 bulls                           |                | Blood IGF-1 level at 6 months of age                                               | -                                    | Fortes et al. (2013b)  |
| Bos taurus, Brahman, Tropical Composite | >1000 Bos taurus cattle | | Live weight at feedlot entry, hip height at post-weaning                               | Bos taurus: 0.96; Brahman: 0.5; Tropical Composite: 0.68 | Fortes et al. (2013a)  |
| Tropical Composite | ~1000 females of Brahman            |                | Age when first corpus luteum was observed, reduced blood IGF-1 level at the first wet season, live weight when first corpus luteum was observed | -                                    | -                       |
| Tropical Composite | ~1100 males of Brahman              |                | Age at 26 cm of scrotal circumference                                                | -                                    | -                       |
| Nellore          |                                       | 654 bulls       | Estimated breeding value (EBV) for birth weight                                     | 0.18-0.27                            | Utsunomiya et al. (2013) |
| Nellore          |                                       | 861 bulls       | EBV for scrotal circumference                                                        | MAP² = 0.23                          | Utsunomiya et al. (2014) |
| German Fleckvieh |                                       | 1800 bulls      | EBV for paternal calving ease, body size and daily gain                              | 0.16                                 | Pausch et al. (2011)   |
| US beef breeds   |                                       | 18 274 animals from 10 US beef breeds | Body weights, calving ease, carcass, weaning and yearling weights                   | -                                    | Saatchi et al. (2014)  |
| Pig              | European domestic pig populations, European wild boars, Asian domestic pigs | 418 animals from European domestic pig populations, 40 European wild boars, 21 Asian domestic pigs | Candidate selective sweep region overlapped with a major QTL for body length in an intercross between Large White and wild boar | -                                    | Rubin et al. (2012)   |

*Minor allele frequency is shown in the study.
Table 2  Quantitative trait loci (QTLs) detected around the NCAPG-LCORL locus in livestock

| Species          | Breeds                                                                 | No. of animals | Associated traits                                                                 | Freq. of the height-increasing allele | Reference                   |
|------------------|-------------------------------------------------------------------------|----------------|------------------------------------------------------------------------------------|---------------------------------------|------------------------------|
| Cattle           | Japanese Black, Japanese Brown japanese Black, F2 from Charolais and German Holstein | >280 offspring × 5 half-sib families | Carcass weight, longissimus muscle area, lower subcutaneous fat thickness           | -                                     | Setoguchi et al. (2009)     |
|                  | Japanese Black, F2 from Charolais and German Holstein                    | 792 Japanese Black steers and 161 F2 bulls | Increase in body frame size at puberty                                               | Japanese Black: 0.4; F2 bulls: 0.49  | Setoguchi et al. (2011)     |
|                  | Japanese Black                                                          | 1156 steers    | Carcass weight                                                                      | 0.2                                   | Nishimura et al. (2012)     |
|                  | F2 from Charolais and German Holstein                                   | 733 animals    | Birth weight                                                                       | German Holsteins: 0.2                 | Eberlein et al. (2009)      |
|                  | F2 from Charolais and German Holstein                                   | 156 males      | Increased plasma arginine level, average daily gain, lower percentage of perirenal and subcutaneous fat in carcass weight | -                                     | Weikard et al. (2010)      |
|                  | Crossbred                                                               | ~2500 animals  | Birth weight, weaning weight, yeaing weight                                          | 0.33                                  | Snelling et al. (2010)     |
|                  | Crossbred                                                               | 400 animals (validation population) | Average feed intake, average daily gain, hot carcass weight, ribeye area, lower adjusted fat thickness | -                                     | Lindholm-Perry et al. (2011) |
|                  | Norweign Red                                                            | 2552 bulls     | Daughter yield deviation for direct effect on dystocia                              | -                                     | Olsen et al. (2009)        |
|                  | Piedmontese                                                             | 323 bulls      | Estimated breeding value for direct calving ease                                     | -                                     | Bongiorni et al. (2012)    |
|                  | US beef breeds                                                          | 18 274 animals from 10 US beef breeds | Body weights, calving ease direct, weaning weight maternal                           | -                                     | Saatchi et al. (2014)      |
| Horse            | Franches-Montagnes                                                     | 1077 animals   | Withers height, conformation of legs, ventral border of mandible, correctness of gait, expression of head | 0.0845                                | Signer-Hasler et al. (2012) |
|                  | German Warmblood                                                        | 782 stallions  | Withers height                                                                      | 0.65                                  | Tetens et al. (2013)       |
|                  | Hanoverian                                                              | 214 animals    | Height                                                                              | 0.55                                  | Metzger et al. (2013)      |
|                  | Thoroughbreds                                                           | 282 affected and 268 controls | Height, laryngeal neuropathy                                                        | Affected: 0.259; Unaffected: 0.119   | Boyko et al. (2014)        |
| Pig              | European domestic pig populations, European wild boars, Asian domestic pigs | 418 animals from European domestic pig populations, 40 European wild boars, 21 Asian domestic pigs | Candidate selective sweep region overlapped with a major QTL for body length in an intercross between Large White and wild boar Highly differentiated region across breeds | -                                     | Rubin et al. (2012)       |
| Dog              | 46 diverse breeds                                                       | 509 animals    |                                                                                     | -                                     | Vaysse et al. (2011)       |
PLAG1

*PLAG1* (pleiomorphic adenoma gene 1) is a proto-oncogene encoding a zinc-finger containing a transcription factor of which ectopic expression is crucial in the formation of pleomorphic adenomas of salivary glands (Kas et al. 1997). The region including *PLAG1* and some neighboring genes (HSA8q12.1) has been identified as one of the loci for adult human height both in European (Gudbjartsson et al. 2008; Lettre et al. 2008; Lango Allen et al. 2010; Wood et al. 2014) and Asian populations (Cho et al. 2009; Kim et al. 2010; Okada et al. 2010), although associated SNPs were different between populations.

In cattle, Karim et al. (2011) identified regulatory QTNs locating between *PLAG1* and *CHCHD7* for stature (weight and height) in the F2 population from Holstein-Friesian and Jersey cattle. The QTNs, locating at 25 Mb on BTA14 (UMD3.1), are comprised of a repeat number variation of a (CCG)n trinucleotide repeat and an adjacent SNP, both of which were shown to influence bi-directional promoter strength and affect binding of nuclear factors (Karim et al. 2011). Consistent with these results, increased gene expressions of *PLAG1*, *CHCHD7* and other neighboring genes were observed in tissues from 79 fetuses in a height-increasing allele and/or genotype-dependent manner (Karim et al. 2011). A splice-site variant of *CHCHD7* occurring in a null allele was found to have no additional effects on height or weight, resulting in elimination of *CHCHD7* as the only causative gene (Karim et al. 2011). In contrast, *PLAG1* appears the most promising causative gene for the QTL, because *Plag1* knockout mice show dwarfism in the absence of other symptoms (Hensen et al. 2004), showing consistency with the result that the bovine variant enhancing the gene expression increased withers height.

Table 1 shows the studies that detected a QTL or association around the *PLAG1* locus in livestock. In Japanese Black cattle, we detected the strongest association for carcass weight in an 830-kb interval containing *PLAG1* (Nishimura et al. 2012). Target resequencing revealed that the Q haplotype in Japanese Black shared only QTNs with the Q haplotype of the F1 sires reported by Karim et al. (2011), which eliminated other candidate causative variations in the 780-kb critical region identified by Karim et al. (2011) (Nishimura et al. 2012).

Fortes et al. (2013a) showed that the height-increasing allele of *PLAG1* constitutes a small haplotype block in *Bos taurus* cattle but a 20-Mb long haplotype in Brahman (*Bos indicus*), suggesting that the allele was recently introgressed into Brahman from *Bos taurus* cattle and strongly selected. The height-increasing allele in *Bos taurus* also increases height and weight in Brahman and Tropical Composites, and was associated with decreased serum insulin-like growth factor 1 (IGF1) level (Hawken et al. 2012; Fortes et al. 2012, 2013a,b). Since IGF1 is a growth stimulator, the direction of the association appears apparently paradoxical (Fortes et al. 2013a). The height-increasing allele was also associated with late puberty in Brahman that was defined by observation of the first corpus luteum in heifers and age at 26 cm of scrotal circumference (SC) in bulls (Fortes et al. 2013a). In Tropical Composites, the association with late puberty was detected in heifers (Fortes et al. 2013a) but not in bulls (Fortes et al. 2013b). Utsunomiya et al. (2014) also detected an association of the region containing *PLAG1* with estimated breeding value (EBV) for SC in Nellore (*Bos indicus*) bulls, although the direction of the association is not indicated. Currently there is no information about *PLAG1* for affecting timing of puberty. In a mouse model, Plag1-knockout mice show dwarfism and reduced fertility: Plag1<sup>−/−</sup> males impregnate wild-type females at a lower rate and Plag1<sup>−/−</sup> females show reduced litter size (Hensen et al. 2004). A recent human GWAS using >182,000 women identified 106 genomic loci for age at menarche, in which the *PLAG1* locus was not detected (Perry et al. 2014). It is possible that late puberty in Brahman is caused by a hitchhiking effect of the introgressed allele, that is, another variation in a nearby gene affecting puberty.

Pausch et al. (2011) identified two genomic regions (on BTA14 and BTA21) associated with EBV for paternal calving ease in German Fleckvieh bulls, one of which was a 1.58-Mb interval including *PLAG1*. Because the QTL alleles lowering EBV for calving ease were associated with increased EBV for body size and daily gain (Pausch et al. 2011), the stature QTNs at the *PLAG1* locus is likely causative for both calving ease and body weight. Utsunomiya et al. (2013) detected association of the *PLAG1* region with birth weight in Nellore (*Bos indicus*), although they did not show whether the *PLAG1* allele is segregated in the population. Recent human GWAS using tens of thousands of people identified several loci for birth weight (Horikoshi et al. 2013) and length (van der Valk et al. 2014), both of which included the adult human height loci, *HMG2* and *LCORL*. Since *HMG2* stimulates *PLAG1* gene expression (Klemke et al. 2014), *PLAG1* might be another locus for birth weight and length in humans that was not detected in the GWAS.

Table 1 shows a frequency of the height-increasing allele of the SNP with the strongest association in each study population. The frequencies are polarized in *Bos taurus* breeds: high in Holstein-Friesian and Japanese Black, and low in Jersey and Fleckvieh.

In domestic pigs, Rubin et al. (2012) detected strong signature of selection in three regions, *NR6A1*, *PLAG1* and *LCORL*. Non-synonymous amino acid substitution Pro192Leu in NR6A1 was shown to enhance binding to its co-repressors and proposed to be causative to increase the numbers of vertebrae (Mikawa et al. 2007), while any obvious candidate mutations were not found in the coding sequences of *PLAG1* and *LCORL*, suggesting...
that regulatory mutations were selected for these genes (Rubin et al. 2012).

IGF2 and other growth factors have been identified as putative targets of PLAG1 (Voz et al. 2004). Recent studies showed that PLAG1 expression is stimulated by HMGAA2 (Klemke et al. 2014) and decreased by miR-141 (Tang et al. 2013). Future studies will reveal a detailed molecular network of PLAG1 to enhance skeletal growth, which will give a cue to understand apparently paradoxical associations between an increase in height/weight and a reduced serum IGF1 level or late puberty.

**NCAPG-LCORL**

The NCAPG-LCORL region has been identified as a locus for adult human height in European (Gudbjartsson et al. 2008; Weedon et al. 2008; Soranzo et al. 2009; Lango Allen et al. 2010; Wood et al. 2014), Japanese (Okada et al. 2010) and African/African American populations (N’Diaye et al. 2011; Carty et al. 2012). The region is also associated with peak height velocity in infancy (PHV1) (Sovio et al. 2009), birth weight (Horikoshi et al. 2013) and birth length (van der Valk et al. 2014). The SNP with the strongest association locates in an intron of LCORL in European and African/African American populations but downstream of DCAF16 in a Japanese population. N’Diaye et al. (2011) indicated that an LD block in a European population contains DCAF16, NCAPG and LCORL, while only LCORL is contained in an LD block of the individuals of African ancestry. Furthermore, the best associated SNP in African-derived populations was associated with LCORL gene expression levels in lymphoblastoid cell lines derived from 56 unrelated Yoruba individuals (P = 0.0026) (N’Diaye et al. 2011). In contrast, Lango Allen et al. (2010) performed expression QTL (eQTL) analyses in lymphocyte (n = 830), osteoblast (n = 104), liver (n = 567) and omentum (n = 742) from individuals of European ancestry, in which lymphocyte and omentum eQTLs were found in this region but not correlated with the height SNP. Wood et al. (2014) also examined associations of the 697 height SNPs with eQTL in peripheral blood that was analyzed using the data from 2360 unrelated individuals of European ancestry and with non-synonymous variants detected in the 1000 Genomes Phase 1 release. Neither eQTLs nor non-synonymous variants were detected for association with the height SNP in this region.

There is no information indicating a link between height and a gene in this region. LCORL (ligand dependent nuclear receptor corepressor-like) is a transcription factor that may function during spermatogenesis in the testes (Kunieda et al. 2003). NCAPG (non-SMC condensin I complex, subunit G) is a regulatory subunit of the mammalian condensin I complex and is important during mitotic cell division (Dej et al. 2004). DCAF16 (DDB1 and CUL4 associated factor 16) may function as a substrate receptor for CUL4-DDB1 E3 ubiquitin-protein ligase complex (Wen et al. 2007). The LD analysis by N’Diaye et al. (2011) indicates that the causative variation should exist within LCORL at least in African-derived populations, while the causative gene remains unknown. A recent study showed that obesity-associated variants within introns of FTO are functionally connected, at megabase distances, with the homeobox gene IRX3 (Smemo et al. 2014). In this locus, 3 untranslated regions (UTRs) of LCORL and NCAPG are overlapped in human and mouse genomes (http://genomics.ucsc.edu/cgi-bin/hgGateway), raising the possibility of regulated alternate expression (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/). Creation of Lacr knockout mice may show direct involvement of LCORL in body length.

Table 2 shows the studies that detected a QTL or association with carcass weight (Setoguchi et al. 2009), birth weight (Eberlein et al. 2009; Snelling et al. 2010), weaning and weaning weight (Snelling et al. 2010), peri-pubertal weight gain (Weikard et al. 2010) and increase in body frame size (Setoguchi et al. 2011). The LD block in Japanese Black encompassed a 591-kb interval including FAM184B, DCAF16, NCAPG and LCORL and non-synonymous amino acid substitution Ile442Met (p.I442M) in NCAPG was found as a candidate causative variation (Setoguchi et al. 2009). The NCAPG p.I442M allele was shared in the different cattle breeds used in these studies. Gutiérrez-Gil et al. (2012) detected a bone QTL close to the NCAPG-LCORL region, but excluded NCAPG p.I442M (at 38.8 Mb on BTA6, UMD3.1) as a causal mutation and detected the strongest association with the SNP at 45.9 Mb on BTA6 (UMD3.1). In our analysis, one of the Japanese Black sires segregating the carcass weight QTL was homozygous between 39.2 and 61.0 Mb on BTA6 (UMD3.1), therefore the region was excluded (Setoguchi et al. 2009).

Interestingly, the NCAPG-LCORL locus shows pleiotropy. Association of the height/weight-increasing allele with reduced subcutaneous fat thickness has been consistently detected among the studies (Setoguchi et al. 2009; Weikard et al. 2010; Lindholm-Perry et al. 2011; Hoshiba et al. 2013).

Association with calving ease or dystocia has been also detected around this region in different cattle breeds (Olsen et al. 2009; Bongiorni et al. 2012; Saatchi et al. 2014). The association may be explained by an increase in birth weight and length due to the height-increasing allele, although the authors mentioned other candidate genes.

The region including NCAPG-LCORL has been detected for association with height in several horse breeds (Singer-Hasler et al. 2012; Tetens et al. 2013; Metzger et al. 2013; Boyko et al. 2014). Boyko et al. (2014) reported that the LCORL region was also associated with recurrent laryngeal neuropathy (RLN) explaining 6%
of the variation. The height-increasing allele was the risk allele for the disease, consistent with the clinically reported connection between height and RLN (Boyko et al. 2014).

The LCORL region was also identified as highly differentiated between dog breeds (Vaysse et al. 2011) and a selective sweep region in European domestic pigs (Rubin et al. 2012).

Association between gene expression levels of the candidate genes and genotypes or phenotypes has been examined in some studies. Eberlein et al. (2009) showed a trend that NCAPG gene expression was decreased according to numbers of the height-increasing allele in fetal placenta, using three qq, two Qq and one QQ purebred German Holsteins: the Q denotes the height-increasing allele encoding Met-442 in NCAPG. Lindholm-Perry et al. (2013) reported a negative correlation between average feed intake and LCORL transcript abundance in adipose tissue from cows (n = 81, P = 0.02) and heifers (n = 94, P = 0.045), while a positive correlation was detected between average feed intake and LCORL transcript abundance (P = 0.04) or protein level (P = 0.01) in muscle tissues from steers (n = 31). In muscle tissues from cows (n = 86), NCAPG transcript abundance was associated with average daily gain (P = 0.009) (Lindholm-Perry et al. 2013). Weikard et al. (2010) conducted metabolic profiling (n = 156 male cattle) to reveal a physiological pathway related with this locus and showed that the amino acid arginine and its metabolite symmetric dimethylarginine were associated with the height-increasing allele (NCAPG pI442M). Metzger et al. (2013) reported that a variant locating at 62 kb-upstream region of LCORL, which is in a transcription factor II D (TFIID)-binding motif, was associated with horse height and that the height-increasing allele was associated with reduced gene expression of LCORL in hair root (total 44 horses from five breeds).

GENETIC ARCHITECTURE FOR STATURE

Each locus for adult human height explains ~0.3% to ~0.5% of the phenotypic variance (Visscher 2008), corresponding to less than 0.625% of genetic variation (calculated by assuming heritability as 80%). The genome-wide significant SNPs in 423 loci together explained 16% of heritability (Wood et al. 2014). In contrast, genetic architecture for stature is simple in domestic animals. In cattle, several major loci including PLAG1 and NCAPG-LCORL explained more than a third of genetic variance in a breed (Saatchi et al. 2014). In horses, only the LCORL region explained 11% and 18% of the phenotypic variance in Franches-Montagnes and German Warmblood, respectively (Signer-Hasler et al. 2012; Tetens et al. 2013). Makvandi-Nejad et al. (2012) reported that four loci explained 83% of size variance in the 48 horses from 16 breeds and two loci explained 59% of the variance in thoroughbred size, although these estimates are likely to be upwardly biased by the small and selected sample. The two loci, PLAG1 and LCORL, together explained 18.4% of the residual variance in body length in an intercross between Large White pigs and wild boar (Rubin et al. 2012).

A locus with a large effect on a favorable trait tends to be strongly selected and fixed in domestic animals. Identification of the causal variants may reveal why the effect size is large in domestic animals. Human height loci are substantially enriched for regulatory variants (Wood et al. 2014). The bovine QTNs at the PLAG1 locus are regulatory and increased less than two-fold gene expression (average 1.2-fold), while the QTL genotype effects on live weight were +19.9 kg (QQ), 0 kg (Qq) and −23.5 kg (qq) and the QTL explained 9.9% of live weight variance of the F2 population (Karim et al. 2011). The large effect may be explained by a critical role of the PLAG1 transcription factor in up-regulating growth factors (Voz et al. 2004).

CW-3

The CW-3 QTL on BTA8 have been detected in several paternal half-sib families in Japanese Black the sires of which shared an identical-by-descendant haplotype encompassing a >14 Mb interval (Nishimura et al. 2011). Imputation of the 50K genotypes from the GWAS to high-density SNP genotypes and a haplotype-based association analysis highlighted a 3.3 Mb interval as a candidate region (Takasuga A, 2014, unpublished data). Below I describe the efforts that have been made to identify the causative variation and pitfalls of the respective analyses.

1 Target resequencing: many candidate causative variations were obtained, while GC-rich and repeat regions remained uncovered. For example, the causative variations at the PLAG1 locus were located within a GC-rich region and were not covered by target resequencing (Nishimura et al. 2012).

2 Expression analysis: it is usually difficult to obtain tissue samples of livestock animals in an appropriate growth stage and with an appropriate genotype. A database search for human (http://www.genecards.org/) and mouse gene expression (http://www.informatics.jax.org/expression.shtml) may be helpful, although some differences may exist among species. A candidate gene for CW-3 was expressed specifically in a growth plate from a Holstein femur, while the mouse ortholog was broadly expressed in a femur (Takasuga A, 2014, unpublished data).

3 Literature analysis to select functional candidates: OMIM (http://www.omim.org/) and a catalog of published genome-wide association studies (http://www.genome.gov/gwastudies/) may be helpful to select a primary candidate. In the case of CW-3, the 3.3 Mb candidate region included several genes that are involved in skeletal growth and/or associated with adult human height (Nishimura et al. 2012).
In vivo studies using a knockout mouse: International Mouse Strain Resource (http://www.findmice.org/) provides mutant mouse and ES cell lines. Taconic Biosciences (http://www.taconic.com/) is a commercial supplier that assembles more than 4000 knockout mouse lines in the repository. We found a non-synonymous variation in α-galactosidase A gene associated with adult human height, which was predicted to be a damaging mutation by SIFT (http://sift.bii.a-star.edu.sg/) and Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/). But the mice deficient for the gene showed neither abnormalities nor a change in body weight and length (Takasuga A, 2014, unpublished data). The lesson from the study is that a damaging mutation can be present in a gene that is not essential for growth.

Recently we detected a linkage between CW-3 and the skeletal abnormalities characterized by joint- and/or hip bone-enlargement (Takasuga A, 2014, unpublished data). Now we are pursuing the causative gene for the genetic disease, because the causal mutation is probably not a regulatory mutation but a non-synonymous or a splicing-junction mutation altering a coding sequence. Since disease mutations are usually infrequent, they may be distinguished from common variants using the data from whole-genome sequencing of 234 bulls and pooled libraries from 54 steers in Japanese Black (Hirano et al. 2013).

Perspectives

In livestock, genomic loci with a large effect on a favorable trait have been often accompanied with an unfavorable character. Non-synonymous variant Lys232Ala in DGAT1 increases fat content but decreases milk yield in cattle (Grisart et al. 2001, 2004). A frame-shift or Cys636Arg mutation in MRC2 increases musculature in a heterozygous carrier status but causes the recessive Crooked Tail Syndrome in Belgian Blue cattle (Faququelle et al. 2009; Sartelet et al. 2012). Therefore it is important to identify whether the unfavorable character (late puberty in Brahman cattle, RLN in horses and the skeletal abnormalities in Japanese Black cattle) is caused by the causative variation for stature or by a closely located but different mutation. Although many efforts with in vitro and in vivo studies are needed to identify the causative variation, they cannot be avoided. A large effect size may make it easier to identify the causative variation in domestic animals than in human, and in turn, identified causative variation may explain its large effect size and will give an insight to understand the underlying molecular mechanism.

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