Copyright © 2003 by the Genetics Society of America

Note

Mosaicism of Solid Gold Supports the Causality of a Noncoding A-to-G Transition in the Determinism of the Callipyge Phenotype

Maria Smit,*[,1] Karin Segers,†[,1] Laura Garcia Carrascosa,† Tracy Shay,* Francesca Baraldi,† Gabor Gyapay,‡ Gary Snowder,§ Michel Georges,†[,2] Noelle Cockett* and Carole Charlier†

**Department of Animal, Dairy and Veterinary Sciences, College of Agriculture, Utah State University, Logan, Utah 84322-4700, †Department of Genetics, Faculty of Veterinary Medicine, University of Liège (B43), 4000-Liège, Belgium, ‡Genoscope, Centre National de Séquençage, CP 5706, 91057 EVRY Cedex, France and §United States Department of Agriculture, Agricultural Research Station, U.S. Meat Animal Research Center, Clay Center, Nebraska 68933*

Manuscript received September 17, 2002
Accepted for publication October 23, 2002

ABSTRACT

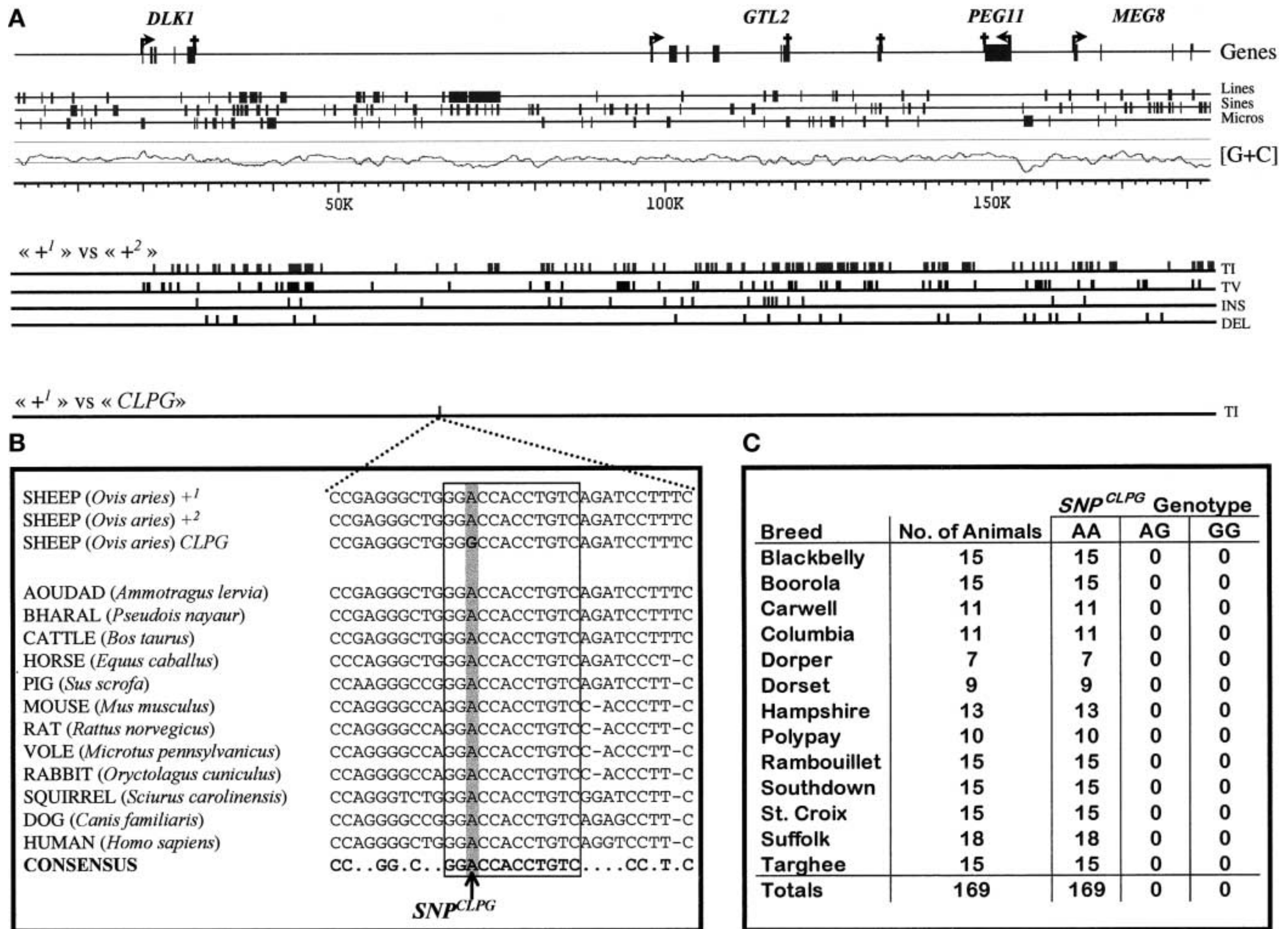
To identify the callipyge mutation, we have resequenced 184 kb spanning the *DLK1*-, *GTL2*-, *PEG11*-, and *MEG8*-imprinted domain and have identified an A-to-G transition in a highly conserved dodecamer motif between *DLK1* and *GTL2*. This was the only difference found between the callipyge (*CLPG*) allele and a phylogenetically closely related wild-type allele. We report that this SNP is in perfect association with the callipyge genotype. The demonstration that Solid Gold—the alleged founder ram of the callipyge flock—is mosaic for this SNP virtually proves the causality of this SNP in the determinism of the callipyge phenotype.

THE callipyge phenotype is an inherited muscular hypertrophy that was first reported in 1983 in a Dorset ram (Solid Gold), which transmitted this remarkable phenotype to some of its descendants. The trait reflects an increase in the proportion and diameter of fast twitch myofibers and manifests itself at ~4 weeks of age. Expression of the callipyge phenotype was shown to be fully determined by a single locus on distal OAR18q and to be subject to an unusual mode of inheritance called "polar overdominance," in which only heterozygous individuals inheriting the callipyge (*CLPG*) allele from their sire ($+^{Mat}/CLPG^{Pat}$ genotype) exhibit the muscular hypertrophy (Cockett *et al.* 1996). We recently demonstrated that the *CLPG* mutation enhances the expression of a cluster of four imprinted genes (*DLK1*, *GTL2*, *PEG11*, and *MEG8*) *in cis* without affecting their imprinting status (Charlier *et al.* 2001a). This leads to a unique expression profile for $+^{Mat}/CLPG^{Pat}$ individuals, which is thought to cause the callipyge phenotype. On the basis of these results, we hypothesized that the *CLPG* mutation affects a shared locus control element. Recently, a single nucleotide polymorphism (SNP) has been reported in an inbred ram of callipyge phenotype postulated to have inherited chromosome segments identical by descent with the exception of the mutated position (Freking *et al.* 2002). We herein report the finding of the same mutation in an independent effort and provide additional evidence supporting its causality.

To identify the *CLPG* mutation, we sequenced 184 kb spanning the *DLK1*, *GTL2*, *PEG11*, and *MEG8* genes from a *CLPG* allele as well as from a phylogenetically closely related wild-type ("$+^1$") allele. The sequence of the *CLPG* allele was obtained by cycle sequencing 209 partially overlapping PCR products averaging 1018 bp amplified from genomic DNA of a *CLPG*/*CLPG* individual. The "$+^1$" allele was likewise obtained from genomic DNA of a $+^{Mat}/CLPG^{Pat}$ individual (with callipyge phenotype) that was homozygous for eight SNPs previously reported in the *DLK1*, *GTL2*, *PEG11*, and *MEG8* genes (Charlier *et al.* 2001b), as well as for five flanking microsatellite markers (*OY15*, *OY3*, *BMS1561*, *IDVGA30*, and *CSSM18*; Fahrenkrug *et al.* 2000; Shay *et al.* 2001). The resulting *CLPG* and "$+^1$" sequences were compared with a previously reported wild-type sequence ("$+^2$") obtained from bacterial artificial chromosome clones covering the region (Charlier *et al.* 2001b; Figure 1A). A comparison of the "$+^2$" allele with the "$+^1$" and *CLPG* alleles revealed 320 and 321 differences, respectively (reported in updated GenBank accession no. AF354168). The *CLPG* and "$+^1$" sequences, however, were identical over their entire length with the exception of a single A-to-G substitution located 32,775 bp upstream of *GTL2*.

[1]Both authors contributed equally to this work.

[2]*Corresponding author:* Department of Genetics, Faculty of Veterinary Medicine, University of Liège (B43), 20 Bd. de Colonster, 4000-Liège, Belgium. E-mail: michel.georges@ulg.ac.be



Breed	No. of Animals	SNP^{CLPG} Genotype AA	AG	GG
Blackbelly	15	15	0	0
Boorola	15	15	0	0
Carwell	11	11	0	0
Columbia	11	11	0	0
Dorper	7	7	0	0
Dorset	9	9	0	0
Hampshire	13	13	0	0
Polypay	10	10	0	0
Rambouillet	15	15	0	0
Southdown	15	15	0	0
St. Croix	15	15	0	0
Suffolk	18	18	0	0
Targhee	15	15	0	0
Totals	169	169	0	0

FIGURE 1.—(A) ViewGene (KASHUK *et al.* 2002) representation of 184 kb spanning the *DLK1-GTL2*-imprinted domain showing the location of the *DLK1*, *GTL2*, *PEG11*, and *MEG8* genes; LINE, SINE, and simple sequence repeats; moving average [G + C] content; as well as the DNA sequence polymorphisms [sorted in transitions (TI), transversions (TV), insertions (INS), and deletions (DEL)] observed when (i) comparing the "$+^1$" and "$+^2$" alleles and (ii) comparing the "$+^1$" and "*CLPG*" alleles. (B) Comparison of the sequence spanning the SNP^{CLPG} among 13 mammalian species, showing the highly conserved dodecamer motif. (C) SNP^{CLPG} genotype frequencies in 169 control individuals from 13 breeds, showing the absence of the G allele.

This SNP, which has also been reported by FREKING *et al.* (2002), is referred to hereafter as SNP^{CLPG}.

Using primer sequences derived from the alignment of the orthologous human, mouse, and sheep sequences (GenBank accession nos. AL117190, AJ320506, and AF354168, respectively), we amplified and sequenced ~200 bp spanning the SNP position for 10 additional mammalian species (GenBank accession nos. AY167893–AY167902). This demonstrated that the SNP^{CLPG} affects the third position of a perfectly conserved, supposedly functional dodecamer motif (Figure 1B).

We developed a PCR-restriction fragment length polymorphism test (using *Ava*II) for the SNP^{CLPG} and genotyped a cohort of 169 individuals representing 13 distinct breeds as well as >200 individuals from our callipyge flock (SHAY *et al.* 2001). The G allele was shown to be a "private" allele, encountered exclusively in the callipyge flock. In this flock, the SNP^{CLPG} genotype was in perfect agreement with the callipyge genotype as deduced from phenotype and/or flanking marker data. Individuals with the callipyge muscular hypertrophy ($+^{Mat}/CLPG^{Pat}$) were all heterozygous "A/G," while phenotypically normal individuals were "A/A" when predicted to be of $+^{Mat}/+^{Pat}$ genotype (on the basis of flanking marker data), "A/G" when predicted to be $CLPG^{Mat}/+^{Pat}$, and "G/G" when predicted to be $CLPG^{Mat}/CLPG^{Pat}$ (data not shown). Note that we genotyped 11 Carwell sheep, a breed known for an inherited muscular hypertrophy of the rib eye that also maps to OAR18q. Although 4 of these animals exhibited the Carwell muscular hypertrophy phenotype, none of them carried the G allele.

To further test the possible causality of the SNP^{CLPG} in the determinism of the callipyge phenotype, we genotyped Solid Gold—the alleged callipyge founder ram—using genomic DNA extracted from leucocytes. Solid

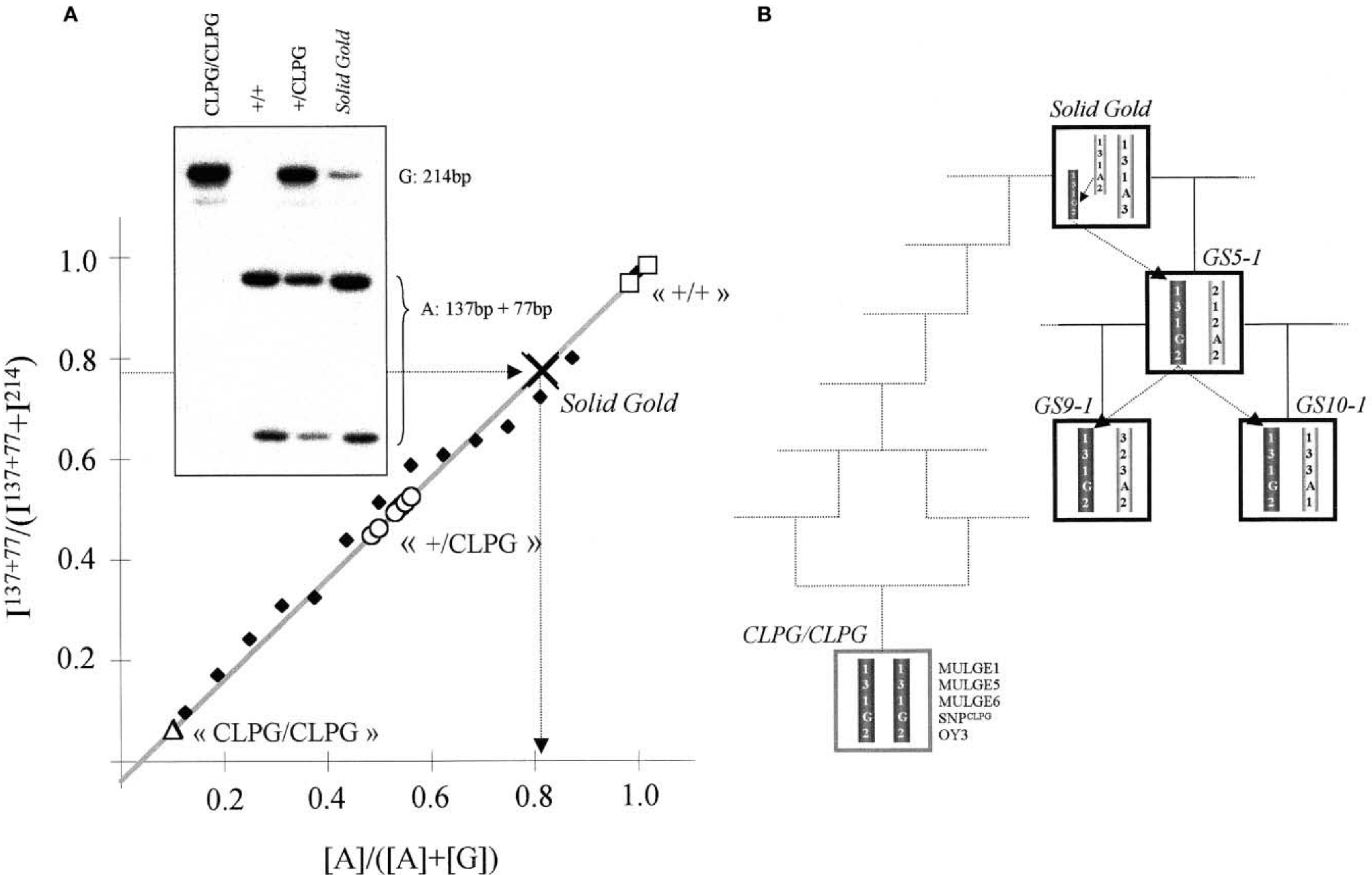


FIGURE 2.—(A) Estimating the proportion of A *vs.* G alleles in a DNA sample (*x*-axis) from the relative intensities of the 214 bp *vs.* the (137 bp + 77 bp) hot-stop PCR products (*y*-axis). The gray line is the standard curve obtained by linear regression using data points obtained from DNA samples with known A-to-G ratios (diamonds). The △, ○, and □ symbols correspond, respectively, to individuals with *CLPG/CLPG*, +/*CLPG*, and +/+ genotypes, while the × symbol corresponds to Solid Gold. (Inset) Hot-stop PCR (UEJIMA *et al.* 2000) products showing the undigested 214-bp PCR fragment (*CLPG* G allele), as well as the 137- and 77-bp *Ava*II fragments (wild-type A allele), as obtained from genomic DNA from a *CLPG/CLPG*, +/+, +/*CLPG* individual, as well as from Solid Gold. (B) Identification of the marker haplotype (solid bar) transmitted in association with the SNP^{CLPG} G allele by Solid Gold to one of his callipyge sons (GS5-1) and to two of his callipyge grandsons (GS9-1 and GS10-1) and demonstration that it is identical to the haplotype for which all *CLPG/CLPG* individuals of the Utah State University (USU) callipyge flock (SHAY *et al.* 2001) are homozygous. Genotypes are given for four microsatellite markers (*MULGE-1*, *MULGE-5*, *MULGE-6*, and *OY3*) for which the paternal allele could be identified unambiguously in the GS5-1, GS9-1, and GS10-1 individuals. Callipyge individuals are boxed in black; normal individuals are in shaded boxes.

Gold proved to carry the G allele as expected, but to our surprise he exhibited an allelic ratio of the A-to-G alleles that clearly departed from the expected 1:1 ratio. The proportion of G allele in Solid Gold's DNA was estimated more precisely by hot-stop PCR (Uejima *et al.* 2000) at ~20% (Figure 2A). None of the heterozygous animals tested in our callipyge flock demonstrated any evidence for such an allelic imbalance. This indicated that Solid Gold was either chimeric or mosaic. As leucochimerism resulting from placental anastomoses between dizygotic twins is common in ruminants, we genotyped Solid Gold for a battery of 10 highly polymorphic nonsyntenic microsatellite markers. We did not find any evidence for the presence of three or four alleles, as expected in the case of leucochimerism, allowing us to exclude this hypothesis.

We then genotyped Solid Gold, one of his callipyge sons, and two of his callipyge grandsons for microsatellite markers flanking the *DLK1-GTL2* domain. Solid Gold was shown to transmit the G allele in association with the marker haplotype known from previous studies to be associated with the *CLPG* allele (Figure 2B). No evidence could be found, however, for an allelic imbalance of any of the flanking microsatellites.

Taken together, these results indicate that Solid Gold is (A/A + G) mosaic for the SNP^{CLPG}, suggesting that the A-to-G transition occurred during its early embryonic development. This hypothesis is corroborated by the report that only 10% of the 150 offspring produced by Solid Gold were callipyge, suggesting that he was a germline mosaic as well (A. Moffat, personal communication).

The demonstration that the only mutation that differentiates the *CLPG* allele from a phylogenetically related wild-type allele occurred during the early embryonic development of the founder ram of the callipyge flock virtually proves the causality of the SNP^{CLPG} in the determinism of this fascinating phenotype.

This project was supported by grants from the FRFC (no. 2.4525.96), Crédit aux Chercheurs (no. 1.5.134.00) from the FNRS, Crédit à la Recherche from the ULg, the SSTC (no. 0135), the Utah Center of Excellence Program, the USDA/NRICGP (grant nos. 94-04358, 96-35205, and 98-03455), and the Utah Agricultural Experiment Station, USU. Carole Charlier is a Chercheur Qualifié from the FNRS.

LITERATURE CITED

Charlier, C., K. Segers, L. Karim, T. Shay, G. Gyapay *et al.*, 2001a The callipyge (CLPG) mutation enhances the expression of the coregulated *DLK1*, *GTL2*, *PEG11* and *MEG8* genes in *cis* without affecting their imprinting status. Nat. Genet. **27:** 367–369.

Charlier, C., K. Segers, D. Wagenaar, L. Karim, S. Berghmans *et al.*, 2001b Human-ovine comparative sequencing of a 250 kilobase imprinted domain encompassing the callipyge (*clpg*) gene and identification of six imprinted transcripts: *DLK1*, *DAT*, *GTL2*, *PEG11*, *antiPEG11* and *MEG8*. Genome Res. **11:** 850–862.

Cockett, N., S. Jackson, T. Shay, F. Farnir, G. Snowder *et al.*, 1996 Polar overdominance at the ovine callipyge locus. Science **273:** 236–238.

Fahrenkrug, S. C., B. A. Freking, C. E. Rexroad, III, K. A. Leymaster, S. M. Kappes *et al.*, 2000 Comparative mapping of the ovine CLPG locus. Mamm. Genome **11:** 871–876.

Freking, B. A., S. K. Murphy, A. A. Wylie, S. J. Rhodes, J. W. Keele *et al.*, 2002 Identification of the single base change causing the callipyge muscle hypertrophy phenotype, the only known example of polar overdominance in mammals. Genome Res. **12:** 1496–1506.

Kashuk, C., S. Sengupta, E. Eichler and A. Chakravarti, 2002 ViewGene: a graphical tool for polymorphism visualization and characterization. Genome Res. **12:** 333–338.

Shay, T., S. Berghmans, K. Segers, S. Meyers, J. Womack, *et al.*, 2001 Fine-mapping and construction of a bovine contig spanning a 4.6 centimorgan interval containing the CLPG locus. Mamm. Genome **12:** 141–149.

Uejima, H., M. P. Lee, H. Cui and A. P. Feinberg, 2000 Hot-stop PCR: a simple and general assay for linear quantitation of allele ratios. Nat. Genet. **25:** 375–376.

Communicating editor: C. Haley