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

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ABSTRACT

To identify the callipyge mutation, we have resequenced 184 kb spanning the DLKI-, GTL2-, PEG11-, and MEG8-imprinted domain and have identified an A-to-G transition in a highly conserved dodecamer motif between DLKI 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 spanning the muscular hypertrophy (Cockett et al. 1996). We recently demonstrated that the CLPG mutation enhances the expression of a cluster of four imprinted genes (DLKI, GTL2, PEG11, and MEG8) in cis without affecting their imprinting status (Charlier et al. 2001a). This leads to a unique expression profile for +/CLPG* 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 DLKI, 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 +/CLPG* individual (with callipyge phenotype) that was homozygous for eight SNPs previously reported in the DLKI, 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.
This SNP, which has also been reported by Freking et al. (2002), is referred to hereafter as SNPCIpg.

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 a 200 bp region of the SNP position for 10 additional mammalian species (GenBank accession nos. AY167893–AY167902). This demonstrated that the SNPCIpg affects the third position of a perfectly conserved dodecamer motif (Figure 1B).

We developed a PCR-restriction fragment length polymorphism test (using AvaI) for the SNPCIpg and genotyped a cohort of 109 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 SNPCIpg 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/CLPGPat) 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 CLPGMat/+Pat, and “G/G” when predicted to be CLPGLs/CLPGPat (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 SNPCIpg 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 AvaII 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 SNP355G 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 determination of this fascinating phenotype.

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LITERATURE CITED


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