Journal of Dairy Research (2004) **71** 182–187. © Proprietors of Journal of Dairy Research 2004
DOI: 10.1017/S0022029904000032 Printed in the United Kingdom

DGAT1 polymorphism in *Bos indicus* and *Bos taurus* cattle breeds

Bernhard Kaupe[1], Andreas Winter[2], Ruedi Fries[2] and Georg Erhardt[1]*

[1] Department of Animal Breeding and Genetics, Justus-Liebig-University Giessen, Ludwigstrasse 216, 35390 Giessen, Germany
[2] Technical University Munich, Freising-Weihenstephan, Germany

Received 2 December 2002 and accepted for publication 23 April 2003

As a result of multiple QTL-mapping projects in recent years, a quantitative trait locus for milk fat percentage and milk yield has been described on *BTA14*. Recent reports name the acyl-CoA : diacylglycerol acyltransferase (*DGAT1*) gene on *BTA14* as a potential candidate gene, with a nonconservative substitution of lysine by alanine (*K232A*) producing a major effect on milk composition and yield. $DGAT1^K$ appears to be the ancestral allele and the *K232A* substitution probably occurred after the divergence of the *Bos indicus* and *Bos taurus* lineages. These findings prompted us to genotype 1748 DNA samples of 38 different *Bos taurus* and *Bos indicus* cattle breeds from 13 countries on five continents (Europe, Africa, Asia, North America and South America), to examine the occurrence of the *DGAT1* polymorphism and characterize the *K232A* substitution in cattle breeds of different origins and selected for different purposes (e.g., beef, dairy and dual purpose). Calculating pairwise F_{ST} values for pooled subpopulations showed least divergence for *Bos indicus* breeds with high milk fat percentage. Fixation of $DGAT1^A$ was found in some *Bos taurus* breeds and fixation of $DGAT1^K$ in one *Bos indicus* breed. Breeds of no known organized breeding background from the Near East domestication centre of *Bos taurus* and taurine African N'Dama cattle were found to possess intermediate frequencies of $DGAT1^K$. While beef breeds tended to harbour higher $DGAT1^A$ levels, dairy cattle showed everything from very low levels of $DGAT1^K$ to unexpectedly high frequencies of this allele.

Keywords: *DGAT1*, allele frequencies, cattle breeds, bovine diversity.

In dairy cattle breeding programmes, milk yield, fat and protein percentage were improved in the past by applying quantitative genetic methods. Geldermann (1975) showed that individual chromosomal locations were responsible for variation in economically important traits. To reveal these chromosomal regions in more detail, linkage maps in cattle were established (Barendse et al. 1997; Kappes et al. 1997; Thomsen et al. 2000) and used for genomic scans of populations to detect quantitative trait loci (QTL).

Within the different national and international QTL-mapping projects in recent years, a QTL for milk fat percentage and milk yield on *BTA14* has been described, mainly in Holstein Friesian populations (Coppieters et al. 1998; Ron et al. 1998; Heyen et al. 1999; Riquet et al. 1999; Looft et al. 2000; Thomsen et al. 2000). Within the course of fine-mapping of this QTL, it became evident that the acyl-CoA : diacylglycerol acyltransferase (*DGAT1*) gene, which maps to the centromeric end of *BTA14*, is a potential candidate gene, as reported for a Dutch/New Zealand Holstein Friesian population (Grisart et al. 2002) and German Fleckvieh (Winter et al. 2002). In these breeds, a transition/transversion mutation resulting in the nonconservative substitution of lysine by alanine (*K232A*) produces a major effect on milk composition and yield. The $DGAT1^K$ allele exceeds the $DGAT1^A$ allele by +0·34 percentage units in fat, +0·08 percentage units in protein, and +10·46 kg in fat yield, while milk and protein yield are reduced (−316 kg, −5·64 kg, respectively) (Grisart et al. 2002). Similar effects occur in German Simmental and German Holsteins (G Thaller, personal communication).

Milk yield, together with milk fat content, were the dominant parameters in dairy cattle breeding programmes of North America and Europe in the past. But as both *DGAT1* alleles of the *K232A* substitution may occur in cattle breeds independently of their respective milk fat content, the evolutionary background of this mutation is of potential interest. The objective of the current study was to examine

*For correspondence; e-mail: Georg.Erhardt@agrar.uni-giessen.de

https://doi.org/10.1017/S0022029904000032 Published online by Cambridge University Press

and characterize the occurrence of the *K232A* substitution in the *DGAT1* gene in cattle breeds of different origins and selected for different purposes.

Materials and Methods

We genotyped 1748 DNA samples of 38 different *Bos taurus* and *Bos indicus* cattle breeds of different selection background (beef, dairy and dual purpose) together with breeds of no known systematic breeding from the Near East domestication area of *Bos taurus*. Samples originated from 13 countries on five continents (Europe, Africa, Asia, North America and South America). Genotyping was carried out by a PCR-RFLP test based on the *K232A* (*AAG→GCG*) substitution in *DGAT1*.

Sampling

Cattle breeds screened for the *K232A* substitution in *DGAT1* in this survey were contributed either as anonymous DNA samples, as anonymous EDTA-whole blood samples or as semen straws of unrelated AI bulls (no parents or grandparents in common), and were gathered from several research projects studying cattle diversity.

German Brown cattle are understood as the old-style German Brown cattle influenced originally only by Swiss and Austrian Browns. German Browns remained clear of admixture with US Brown Swiss. German Brown Swiss cattle are the result of 35 years of male-mediated upgrading of original German Brown with US Brown Swiss sires. German Black Pied cattle are understood to have common roots with other European Friesian cattle that can be traced back to old Jutland cattle (Porter, 1991). Despite an independent German herdbook, there was constant gene flow from the Netherlands and Sweden. Genetic resource populations of German Black Pied cattle (age classes 1966–1989) contributed 22 and 23 samples from sires from the former West Germany and East Germany, respectively. German Holsteins are the result of 35 years of male-mediated upgrading of original German Black-and-Whites with US and Canadian Holstein sires. British Friesian cattle have evolved equally from Jutland cattle with constant genetic inputs from Dutch Friesian cattle (Porter, 1991).

DNA preparation

Isolation of DNA included the salting-out procedure of Miller et al. (1988), the high-salt method of Montgomery & Sise (1990) and the phenol-chloroform extraction method of Lien et al. (1990).

PCR and RFLP-test

PCR was performed in a BIORAD *i*cycler, Version 1.280 (BIO-RAD Laboratories Ltd., Hemel Hempstead HP2 7TD, UK). A 411 bp fragment of the bovine *DGAT1* gene that contained the *K232A* substitution was amplified. PCR reactions were performed in a 20-µl volume using 50 ng of genomic DNA, 1 × PCR buffer containing 1·5 mM-$MgCl_2$, 0·2 mM-dNTP, 0·5 pM of each primer, 5% DMSO and 0·5 U Hotstar™ *Taq polymerase* (QUIAGEN GmbH, 40724 Hilden, Germany). Addition of 5% DMSO to the reaction allowed equal amplification of both alleles. Primer sequences were: *forward* 5′-GCACCATCCTCTTCCTCAAG-3′ and *reverse* 5′-GGAAGCGCTTTCGGATG-3′. The PCR profile included 15 min at 95 °C; 35 cycles of 60 s at 94 °C, 60 s at 60 °C, 60 s at 72 °C; and a final 10-min extension at 72 °C. To detect allelic variation at nucleotide positions 10433 and 10434 of the *DGAT1* gene (Genbank Accession no. AJ318490), 2 µl of amplified DNA was digested with 2 U of *Cfr*I restriction enzyme (MBI Fermentas GmbH, 68789 St. Leon-Rot, Germany) for 3 h at 37 °C and separated subsequently on a 2% agarose gel with 3 V/cm in 0·5 × TBE buffer. Gels were stained with ethidium bromide and visualized under UV light. Phenotypes were identified by differential migration due to fragment size.

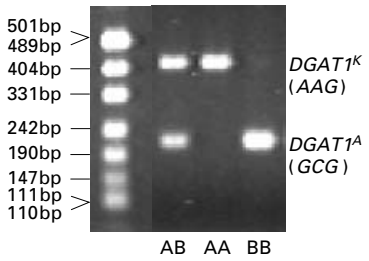


Fig. 1. Three distinct phenotypes representing heterozygous and homozygous status for both *DGAT1* alleles.

Statistical evaluation

For statistical evaluation, the GENEPOP program version 3.1d (Raymond & Rousset, 1995) was used to estimate gene and genotype frequencies of *DGAT1*. Wright's F_{ST} values were calculated to show degree of drift among populations (Nei, 1977).

Results

Typing *DGAT1* allelic variation by PCR-RFLP utilizing DNA from different sources of an extensive range of different cattle breeds showed a clear separation of three different phenotypes (Fig. 1). Differences between breeds in the occurrence and frequency of both alleles could be discerned (Fig. 2). Pooled subpopulations could be grouped into three classes according to their milk fat content (Jensen, 1995); low, medium and high fat showed allelic differentiation. Subsequently calculated pairwise F_{ST} values show the smallest F_{ST} value for the last group. If *Bos indicus* breeds are added as a fourth pool, then the high-fat group shows least divergence from these additional cattle (Table 2).

https://doi.org/10.1017/S0022029904000032 Published online by Cambridge University Press

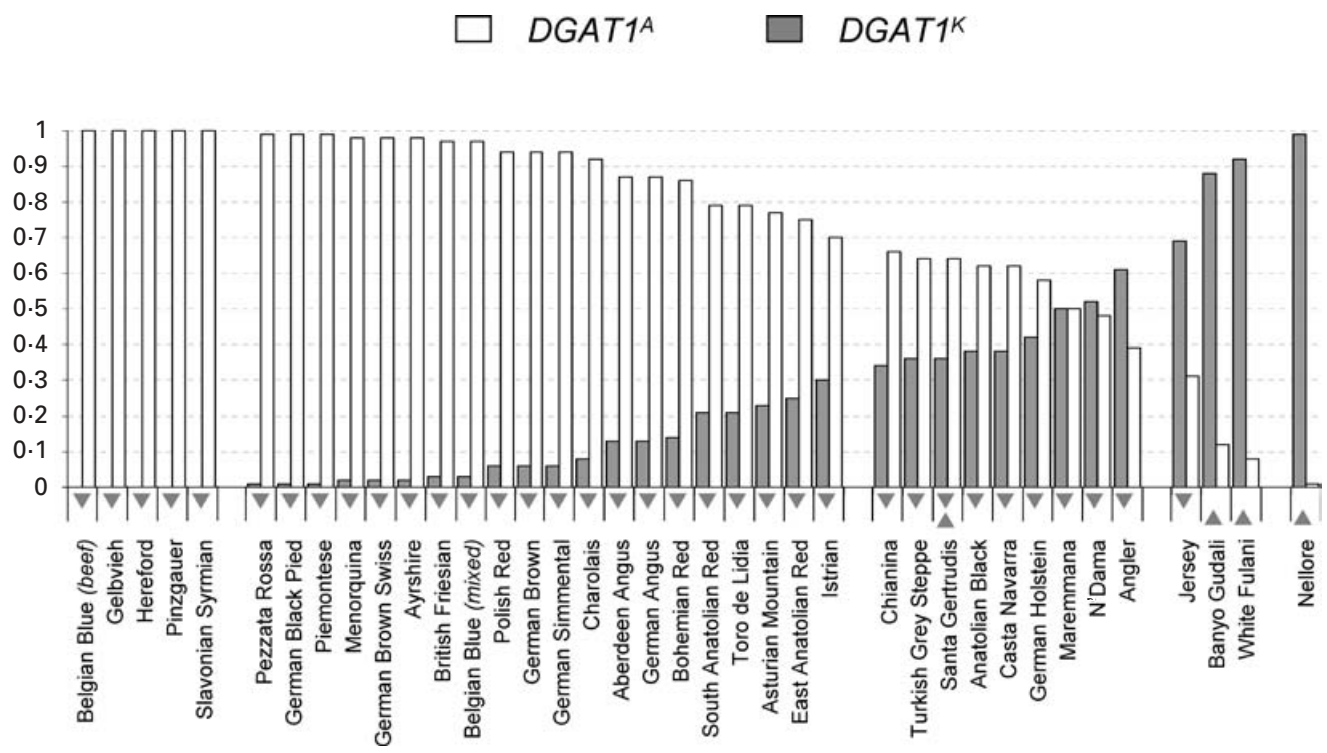


Fig. 2. Variation of the *K232A* substitution in the *DGAT1* gene in 38 cattle breeds of differing selection background. *Bos taurus* breeds (▼) and *Bos indicus* breeds (▲).

A large proportion of breeds show low to intermediate allele frequencies (1–66%) for $DGAT1^K$. These include breeds of no known systematic breeding from the geographical region of Near East domestication centre of *Bos taurus*, African N'Dama cattle as well as today's highly selected *Bos taurus* dairy and beef breeds. Breeds with fixed $DGAT1^A$ belong exclusively to *Bos taurus* type cattle, whereas the single breed with fixation of $DGAT1^K$ is of the *Bos indicus* type. Ten breeds agreed in expected and observed heterozygosity, while 17 breeds showed higher, and six breeds showed lower than predicted frequencies for the heterozygote genotype (Table 1).

$DGAT1^K$ was not found in Belgian Blue (beef), Gelbvieh, Hereford, Pinzgauer and Slavonian Syrmian. The Nellore population of 46 animals was homozygous for $DGAT1^K$ except for one heterozygous animal, which could be attributed to untypical introgression. Both $DGAT1^A$ and $DGAT1^K$ occurred in differing proportions in all other breeds genotyped. *Bos taurus* type cattle exhibited lower $DGAT1^K$ frequencies than *Bos indicus* type cattle: the lowest frequency of $DGAT1^K$ was found in Pezzata Rossa and Piemontese through German Brown Swiss and Ayrshire to German Simmental and Charolais (1–8%). The highest $DGAT1^K$ frequencies were harboured by African zebu Banyo Gudali and White Fulani (88–92%).

Dual purpose breeds had the narrowest range of $DGAT1^K$ frequencies (1–23%, from Pezzata Rossa to Asturian Mountain). Beef breeds had a wider range of $DGAT1^K$ frequencies (1–34%, from Piemontese to Chianina) than dairy breeds, with the widest range from German Brown Swiss and Ayrshire through German Holstein to Angler and Jersey (2–69%) as shown in Fig. 2.

Within the black pied dairy cattle population, differences were found between the 'Old World' Friesian and the 'New World' Holstein populations. British Friesian together with German Black Pied (Gene resource) populations are almost homozygous for $DGAT1^A$ (97–99%) compared with German Holstein with an intermediate $DGAT1^K$ frequency (58%). Unlike the aforementioned Friesian and Holstein populations, no significant deviations were detected between German Brown and German Brown Swiss populations with very low frequencies of $DGAT1^K$ (2–6%).

Special breeds like Turkish Anatolian Black, South Anatolian Red, East Anatolian Red and Grey Steppe together with African N'Dama cattle showed intermediate $DGAT1^K$ frequencies (21–52%).

Discussion

Cattle breeds analysed for *DGAT1* allele frequencies represented the complete range of variance from fixation of $DGAT1^A$ to fixation of $DGAT1^K$. According to Grisart et al. (2002) and Winter et al. (2002), $DGAT1^K$ appears to be the ancestral allele and the *K232A* substitution most likely occurred after the separation of the *Bos indicus* and *Bos taurus* lineages over 200 000 years ago (Loftus et al. 1994).

https://doi.org/10.1017/S0022029904000032 Published online by Cambridge University Press

Table 1. *DGAT1* allele frequencies including observed and expected heterozygosity in 38 cattle breeds

		Allele-frequency		Heterozygosity	
Breeds	*n*	*DGAT1*^{K}	*DGAT1*^{A}	observed	expected
1 Aberdeen Angus	43	0·13	0·87	0·20	0·21
2 Anatolian Black	73	0·38	0·62	0·48	0·47
3 Angler	48	0·61	0·39	0·60	0·48
4 Asturian Mountain	50	0·23	0·77	0·26	0·35
5 Ayrshire	41	0·02	0·98	0·05	0·04
6 Banyo Gudali	72	0·88	0·12	0·24	0·21
7 Belgian Blue (*beef*)	30	—	1·00	*DGAT1*^{A}	
8 Belgian Blue (*mixed*)	15	0·03	0·97	0·07	0·06
9 Bohemian Red	44	0·14	0·86	0·27	0·24
10 British Frisian	49	0·03	0·97	0·06	0·06
11 Casta Navarra	42	0·38	0·62	0·43	0·47
12 Charolais	31	0·08	0·92	0·16	0·15
13 Chianina	44	0·34	0·66	0·36	0·24
14 East Anatolian Red	50	0·25	0·75	0·30	0·38
15 Gelbvieh	30	—	1·00	*DGAT1*^{A}	
16 German Angus	54	0·13	0·87	0·26	0·23
17 German Black Pied (Gene resource population)	41	0·01	0·99	0·02	0·02
18 German Brown	8	0·06	0·94	0·13	0·11
19 German Brown Swiss	48	0·02	0·98	0·04	0·04
20 German Holstein	79	0·42	0·58	0·51	0·49
21 German Simmental	126	0·06	0·94	0·11	0·11
22 Hereford	50	—	1·00	*DGAT1*^{A}	
23 Istrian	49	0·30	0·70	0·42	0·42
24 Jersey	47	0·69	0·31	0·36	0·43
25 Maremmana	48	0·50	0·50	0·63	0·50
26 Menorquina	50	0·02	0·98	0·04	0·04
27 N'Dama	25	0·52	0·48	0·56	0·50
28 Nellore	46	0·99	0·01	0·02	0·02
29 Pezzata Rossa	47	0·01	0·99	0·02	0·02
30 Piemontese	40	0·01	0·99	0·03	0·02
31 Pinzgauer	42	—	1·00	*DGAT1*^{A}	
32 Polish Red	44	0·06	0·94	0·11	0·11
33 Santa Gertrudis	48	0·36	0·64	0·56	0·46
34 Slavonian Syrmian	6	—	1·00	*DGAT1*^{A}	
35 South Anatolian Red	48	0·21	0·79	0·38	0·33
36 Toro de Lidia	47	0·21	0·79	0·34	0·33
37 Turkish Grey Steppe	49	0·36	0·64	0·35	0·46
38 White Fulani	44	0·92	0·08	0·15	0·15

Table 2. Pairwise F_{ST} values indicating genetic divergence between breed groups of different milk fat content (%) according to Jensen (1995) and *Bos indicus* breeds (numeral breed denominations as in Table 1)

			F_{ST} values (pairwise)			
Breed groups			1	2	3	4
Group 1	3·4–3·9% milk fat	1	—	0·0125	0·2326	0·6793
Group 2	4·0–4·4% milk fat	2	0·0125	—	0·3312	0·7581
Group 3	4·5–6·3% milk fat	3	0·2326	0·3312	—	0·2693
Group 4	*Bos indicus*	4	0·6793	0·7581	0·2693	—
Group 1	Breeds: 3, 5, 8, 9, 11, 22, 25, 32, 34, 35, 38					
Group 2	Breeds: 1, 4, 6, 7, 10, 14, 15, 16, 17, 23, 24, 28, 30, 36, 37					
Group 3	Breeds: 2, 12, 13, 18, 19, 20, 21, 26, 27, 29, 31, 33					
Group 4	Breeds: 20, 21, 27					

https://doi.org/10.1017/S0022029904000032 Published online by Cambridge University Press

Allele frequency distribution shows the tendency towards high frequencies of *DGAT1*^{A} in *Bos taurus* breeds and the same effect for *DGAT1*^{K} in *Bos indicus*. We found fixation of *DGAT1*^{A} in five *Bos taurus* breeds and Winter et al. (2002) found fixation of *DGAT1*^{K} in three different Indian *Bos indicus* breeds. That *DGAT1*^{A} was found in the African N'Dama cattle breed as well as in European *Bos taurus* breeds renders it possible that the *K232A* substitution occurred after divergence of taurine and indicine *Bos* lineages, but before the separation of the two *Bos taurus* strains. This is because N'Dama cattle are suspected to have been domesticated independently of European *Bos taurus* (MacHugh et al. 1997; Loftus et al. 1999; Troy et al. 2001). After the domestication of wild cattle, selection for *DGAT1*^{A} presumably occurred because of early selection for milk volume. The *K232A* substitution possibly also results in a lower energy drain on the cow leading to increased fertility. As follicular dynamics are altered by negative energy balance (Lucy et al. 1992), natural selection might have already favoured a high *DGAT1*^{A} frequency in undomesticated aurochs once the substitution originated. MacHugh et al. (1999) found diverging mtDNA haplotypes in comparing European aurochs (*Bos primigenius*) and European cattle populations, which indicates the existence of genetically different subtypes of wild aurochs. Likewise, Troy et al. (2001) showed high divergence between mtDNA haplotypes of domesticated cattle breeds and ancient British aurochs. Certainly, an undomesticated and genetically distinct subtype of *Bos primigenius* existed alongside domesticated *Bos taurus* cattle for several thousand years (Medjugorac et al. 1994).

Nevertheless there are *Bos taurus* dairy breeds today that harbour relatively high frequencies of *DGAT1*^{K}. In both the Jersey Island breed and in German Angler cattle, this was probably due to constant selection for milk fat. In British Jersey, we found a frequency of 0·69 for *DGAT1*^{K}; Spelman et al. (2002) found 0·88 in New Zealand Jersey. The Jersey's ancestors are thought to have been of the Celtic type (Porter, 1991). Eastern Turkey is known to be the domestication centre of *Bos primigenius*. Considering that Near Eastern cattle may have retained some allelic variation from the wild ox (Loftus et al. 1999), Turkish cattle are of special interest. Winter et al. (2002) found common haplotypes between Anatolian Black cattle (which are autochthonous to this region) and Jersey as well as between Anatolian Black cattle and Indian *Bos indicus* Sahival. Previous investigations on blood polymorphisms found Jersey cattle to be different from European cattle. *HBB*^{B} frequency in *Bos indicus* cattle (0·4) is markedly higher than in *Bos taurus* cattle (0·0–0·2) (Baker & Manwell, 1980), but approaching equality in the Jersey breed (Hines, 1999). As the domestication centres of both *Bos taurus* and *Bos indicus* are in closer geographical proximity than distances covered by migrating Neolithic human tribes, early contact between *Bos indicus* and *Bos taurus* cattle cannot be excluded. If a *DGAT1*^{A} homozygote ancestor of today's *Bos taurus* cattle ever existed, introgression of *DGAT1*^{K} must have occurred. It is very likely that there were early cattle strains coming to Europe being heterozygous for *DGAT1*$^{K+A}$, from which breeds like Jersey were derived. On the other hand, new genotypes could evolve through repeated crossbreeding with wild aurochs and secondary domestication events (Medjugorac et al. 1994). This possibly constituted the origin of breeds with high *DGAT1*^{A} frequencies from which today's homozygous breeds developed. Any selection for milk yield thereafter must have augmented the trend for *DGAT1*^{A} homozygosity. Pairwise F_{ST} values calculated between three subpopulations grouped according to their milk fat content and an additional *Bos indicus* breed group showed that selection for milk yield with declining fat percentage could lead to growing genetic divergence from the *DGAT1* wild allele.

We concluded from frequencies for *DGAT1*^{K} of 0·01 in German black-and-white cattle and 0·03 in British Friesian cattle that the founder population of both, namely Friesian and Jutland black-and-white cattle (Porter, 1991), probably did not carry this allele. Assuming a constant appreciation of milk fat as a source of energy in human nutrition over the last 200 years, there would have existed selection for this trait along with milk yield in black-and-white North European dairy cattle. Accordingly, frequencies of *DGAT1*^{K} higher than 0·01–0·03 ought to be found in European Friesians of today had this allele been present before.

As German Holstein cattle are the result of 35 years of male-mediated upgrading of German black-and-white cattle with American and Canadian Holstein sires, we should expect comparable *DGAT1*$^{A+K}$ frequencies in both strains. However, we found intermediate frequencies of *DGAT1*$^{A+K}$ in German Holstein. This result agrees with frequencies found in New Zealand Holstein cattle of 80–90% US and Canadian Holstein influence (Spelman et al. 2002). According to Porter (1991) 95% of the black-and-white cattle imported into North America from 1852 onwards came from the Netherlands. If *DGAT1*^{K} was not present in their founder population, introgression into the Holstein breed might have occurred during its formative years through close contact with the Jersey breed in US milk production industries. Winter et al. (2002) compared *DGAT1* haplotypes and detected, among others, one common haplotype between German Holstein, Jersey and German Simmental (Fleckvieh). The occurrence of this haplotype in German Simmental can be explained through recent introgression of Red Holstein alleles into this breed. A common haplotype between Holstein and Jersey can only be attributed to cross-breeding events. Having then entered the breed, *DGAT1*^{K} could rise in frequency through selection, which from the 1950s onwards was predominantly for milk fat (Grisart et al. 2002).

With diagnostic tests for *DGAT1* it is possible to look on the classification of a wide range of cattle breeds from a new perspective, which might be helpful in future breeding plans and which could contribute to further cattle diversity studies. *DGAT1* gives the opportunity for more

https://doi.org/10.1017/S0022029904000032 Published online by Cambridge University Press

effective selection of dairy cattle for milk fat content and might harbour important implications for beef cattle as well.

We thank EM Ibeagha and OC Jann, Germany, C Özbeyaz and N Eker, Turkey, JL Williams, Scotland, P Ajmone-Marsan, Italy, M Premzl, Herzegovina, P Zaragoza, Spain, J Citek, Czech Republic, R Zieminski, Poland, K Moazami-Goudarzi, France, H Lenstra, Netherlands, L Panicke, Germany and German AI stations for the contribution of samples. C Ehling and B Adler, Germany are gratefully acknowledged for information on gene resource cattle pedigrees. The authors also thank Olaf Bininda-Emonds for comments on the manuscript.

References

Baker CA & Manwell C 1980 Chemical classification of cattle. 1. Breed groups. *Animal Blood Groups and Biochemical Genetics* **11** 127

Barendse W, Vaiman D, Kemp SJ, Sugimoto Y, Armitage SM, Williams JL, Sun HS, Eggen A, Agaba M, Aleyasin SA, Band M, Bishop MD, Buitkamp J, Byrne K, Collins F, Cooper L, Coppieters W, Denys B, Drinkwater RD, Easterday K, Elduque C, Ennis S, Erhardt G, Ferreti L, Flavin N, Gao Q, Georges M, Gurung R, Harzilius B, Hawkins G, Hetzel J, Hirano T, Hulme D, Jorgensen C, Kessler M, Kirkpatrick BW, Konfortov B, Kostia S, Kuhn C, Lenstra JA, Leveziel H, Lewin HA, Leyhe B, Lil L, Martin Burriel I, McGraw RA, Miller JR, Moody DE, Moore SS, Nakane IJ, Nijman IJ, Olsaker I, Pomp D, Rando A, Ron M, Shalom A, Teale AJ, Thieven U, Urquhart BGD, Vage DI, Van de Weghe A, Varvio S, Velmala R, Vilkki J, Weikard R, Woodside C, Womack JE, Zanotti M & Zaragoza P 1997 A medium-density genetic linkage map of the bovine genome. *Mammalian Genome* **8** 21–28

Coppieters W, Riquet J, Arranz JJ, Berzi P, Cambisano N, Grisart B, Karim L, Marcq F, Moreau L, Nezer C, Simon P, Vanmanshoven P, Wagenaar D & Georges M 1998 A QTL with major effect on milk yield and composition maps to bovine Chromosome 14. *Mammalian Genome* **9** 540–544

Geldermann H 1975 Investigation on inheritance of quantitative characters in animal by gene markers (I. Methods). *Theoretical and Applied Genetics* **46** 319–330

Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M & Snell R 2002 Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine *DGAT1* gene with major effect on milk yield and composition. *Genome Research* **12** 222–231

Heyen DW, Weller JI, Ron M, Band M, Beever JE, Feldmesser E, Da Y, Wiggans PM, Van Raden PM & Lewin HA 1999 A genome scan for QTL influencing milk production and health traits in dairy cattle. *Physiological Genomics* **1** 165–181

Hines HC 1999 Blood groups and biochemical polymorphisms. In *The Genetics of Cattle* (Eds R Fries & A Ruvinsky). Wallingford, UK: CABI Publishing

Jensen RG 1995 *Handbook of Milk Composition*. San Diego, USA: Academic Press

Kappes SM, Keele JW, Stone RT, McGraw RA, Sonstegard TS, Smith TPL, Lopez-Corrales NL & Beattie CW 1997 A second-generation linkage map of the bovine genome. *Genome Research* **7** 235–249

Lien S, Rogne S, Brovold MJ & Aleström P 1990 A method for isolation of DNA from frozen (A.I.) bulls semen. *Journal of Animal Breeding and Genetics* **107** 74

Loftus RT, MacHugh DE, Bradley DG, Sharp PM & Cunningham EP 1994 Evidence for two independent domestications of cattle. *Proceedings of the National Academy of Sciencies of the USA* **91** 2757–2761

Loftus RT, Ertugrul O, Harba AH, El-Barody MAA, MacHugh DE, Park SDE & Bradley DG 1999 A microsatellite survey of cattle from a centre of origin: the Near East. *Molecular Evolution* **8** 2015–2022

Looft C, Reinsch N, Karall-Albrecht C, Paul S, Brink M, Thomsen H, Brockmann G, Kühn C, Schwerin M & Kalm E 2001 A mammary gland EST showing linkage disequilibrium to a milk production QTL on bovine Chromosome 14. *Mammalian Genome* **12** 646–650

Lucy MC, Savio JD, Badinga L, De La Sota RL & Thatcher WW 1992 Factors that affect ovarian follicular dynamics in cattle. *Journal of Animal Science* **70** 3615–3626

MacHugh DE, Shriver MD, Loftus RT, Cunningham P & Bradley D 1997 Microsatellite DNA variation and the evolution, domestication and phylogeography of taurine and Zebu cattle (*Bos taurus* and *Bos indicus*). *Genetics* **146** 1071–1086

MacHugh DE, Troy CS, McCormick F, Olsaker I, Eythórsdóttir E & Bradley DG 1999 Early medieval cattle remains from a Scandinavian settlement in Dublin: genetic analysis and comparison with extant breeds. *Philosophical Transactions of the Royal Society of London B* **354** 99–109

Medjugorac I, Kustermann W, Lazar P, Russ I & Pirchner F 1994 Marker-derived phylogeny of European cattle supports demic expansion of agriculture. *Animal Genetics* **25** (Suppl. 1) 19–27

Miller SA, Dykes DD & Polesky HF 1988 A simple salting-out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research* **16** 1215

Montgomery GW & Sise JA 1990 Extraction of DNA from sheep white blood cells. *New Zealand Journal of Agricultural Research* **33** 437–441

Nei M 1977 F-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics, London* **41** 225–233

Porter V 1991 *Cattle – A Handbook to the Breeds of the World*. New York, USA: Facts on File, Inc

Raymond M & Rousset F 1995 GENEPOP Version 1.2: Population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86** 248–249

Riquet J, Coppieters W, Cambisano N, Arranz JJ, Berzi P, Davis SK, Grisart B, Farnir F, Karim L, Mni M, Simon P, Taylor JF, Vanmanshoven P, Wagenaar D, Womack JE & Georges M 1999 Fine-mapping of quantitative trait loci by identity by descent in outbred populations: application to milk production in dairy cattle. *Proceedings of the National Academy of Sciences of the USA* **16** 9252–9257

Ron M, Heyen DW, Weller JL, Band M, Feldmesser E, Pasternak H, Da Y, Wiggans GR, Vanraden PM, Ezra E & Lewin HA 1998 Detection and analysis of a locus affecting milk concentration in the US and Israeli dairy cattle population. *Proceedings of the 6th World Congress of Genetics and Applied Livestock Production*, Armidale, Australia **26** 422–425

Spelman RJ, Ford CA, McElhinney P, Gregory GC & Snell RG 2002 Characterization of the DGAT1 gene in the New Zealand dairy population. *Journal of Dairy Science* **85** 3514–3517

Thomsen H, Reinsch N, Xu N, Looft C, Gruppe S, Kühn C, Brockmann GA, Schwerin M, Leyhe-Horn B, Hiendleder S, Erhardt G, Medjugorac I, Russ I, Förster M, Brenig B, Reinhardt F, Reents R, Blümel J, Averdunk G & Kalm E 2000 A male linkage map for the ADR granddaughter design. *Journal of Animal Breeding and Genetics* **117** 289–306

Troy CS, MacHugh DE, Bailey JF, Magee DA, Loftus RT, Cunningham P, Chamberlain AT, Sykes BC & Bradley DG 2001 Genetic evidence for Near-Eastern origins of European cattle. *Nature* **410** 1088–1091

Winter A, Krämer W, Werner FAO, Kollers S, Kata S, Durstewitz G, Buitkamp J, Womack JE, Thaller G & Fries R 2002 Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA: diacylglycerol acyltransferase (*DGAT1*) with variation at a quantitative trait locus for milk fat content. *Proceedings of the National Academy of Sciences of the USA* **16** 9252–9257

https://doi.org/10.1017/S0022029904000032 Published online by Cambridge University Press