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Complex Vertebral Malformation: a Recessive Disorder in Holstein Friesian Cattle – a Review

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ABSTRACT

The present review is a concise summary of findings on one of the most important recessive hereditary disorders, the Complex Vertebral Malformation (CVM) in cattle. It is a disease of Holstein calves characterized by complex anomalies of the vertebral column and limbs in aborted fetuses and in prematurely born, stillborn and neonates. The recessive homozygous form is lethal and since carrier animals have viability, CVM frequency increases by use of carrier bulls in Artificial insemination (AI). The gene SLC35A3 has been identified as the culprit and a point mutation was eventually identified within the alleles encoding bovine SLC35A3 in a Holstein calf affected with CVM. This mutation causes valine to phenylalanine substitution at the amino acid level. PCR-PIRA, PCR-SSCP, AS-PCR and Tetra-primer ARMS-PCR are certain PCR based techniques which could be used to screen CVM in cattle. The use of these molecular technologies promises quick detection of carriers, enables their culling and consequently controls and prevents the spread of CVM in the population.

Keywords: CVM, genetic disorder, Holstein Friesian, SLC35A3 gene, PCR-RFLP

INTRODUCTION

Complex vertebral malformation (CVM) is a recessively inherited disorder leading to frequent abortion of fetuses or vertebral anomalies and prenatal death (Agerholm *et al.*, 2001, 2004; Nielsen *et al.*, 2003). The syndrome was discovered in the Danish Holstein population in 1999, but shortly thereafter, its occurrence was reported between 12 to 20% in Netherlands (Wouda *et al.*, 2000), United States (Duncan *et al.*, 2001), United Kingdom (Revell, 2001) *and* Japan (Nagahata *et al.*, 2002). Genealogical records revealed that the calves suffering from CVM were genetically related to the US Holstein sire Penstate Ivanhoe Star (US1441440, born in 1963) and his son Carlin-M Ivanhoe Bell (US1667366) which had been used in dairy cattle breeding worldwide for two decades due to the superior lactation performance of their daughters. Consequently, the number of animals genetically related to the carrier bulls was very high and the disease-causing mutation was widespread among Holstein cattle throughout the world (Thomsen *et al.*, 2006). In stillborn, aborted and pre-term calves, CVM has been characterized by shortened cervical and thoracic regions of the vertebral column and symmetric arthrogryphosis (Agerholm *et al.*, 2001; Duncan *et al.*, 2001). Multiple hemivertebrae, scoliosis and synostosis, fused and mis-shaped vertebral column has also been described.

Molecular basis of CVM: CVM is caused by a point mutation (missense mutation) from G to T at nucleotide position 559 of the bovine solute carrier family 35 member 3 (SLC35A3) gene, which causes valine to phenylalanine substitution at the amino acid level (Kanae *et al.*, 2005) depicted in Figure 1. The *SLC35A3* gene encodes a Golgi-resident UDP-*N*-acetyl glucosamine transporter (Thomsen *et al.*, 2006). UDP-*N*-acetyl glucosamine transporter transports nucleotide sugars from cytosol to Golgi body to use as a substrate for glycosylation of protein, lipid and proteoglycans (Guillen *et al.*, 1998). Bovine SLC35A3 plays a great role in the development of the axial skeleton, demonstrating that some of the molecular mechanisms that operate during formation of vertebrae and ribs depend on carbohydrate modification in the Golgi apparatus (Thomsen *et al.*, 2006).

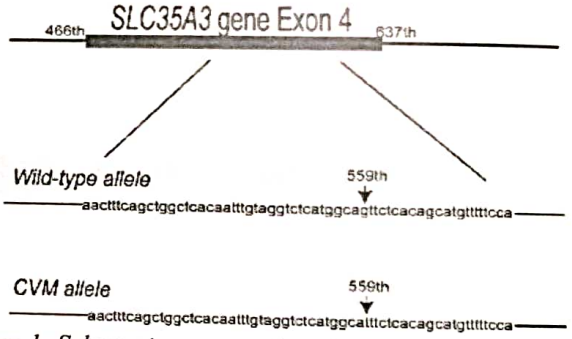

Figure 1: Schematic presentation of exon 4, depicted by a solid box, partial exon 4 sequences of wild-type and CVM SLC35A3 gene (Kanae et al., 2005).

The data demonstrates that the gene responsible for CVM encodes a member of the solute carrier family SLC35, which are enzymes transporting nucleotide-sugars from the cytosol into the lumen of the endoplasmic reticulum and/or the Golgi apparatus. In these organelles, nucleotide sugars are utilized by glycosyltranferases to synthesize the sugar chains of glycoproteins, glycolipids *and* carbohydrate polymers. Several studies lend support for an essential role of nucleotide sugar transportation in development and disease. Observations on human glycosylation diseases further attest to the importance of nucleotide-sugar transportation. Thus, the human disease called congenital disorder of glycosylation (CDG) type IIc (formerly leukocyte adhesion deficiency type II) occurs when GDP-fucose uptake into the Golgi is defective, resulting in clinical manifestations such as facial dysmorphology, growth and mental retardation *and* lack of cell-surface expression of fucosylated glyco-conjugates like the ABH and Lewis-related blood group antigens. Also, fucose-containing carbohydrate ligands required for selectin interactions are absent, leading to leukocyte adhesion

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Luhn *et al.*, 2001). Recently, a related glycosylation disorder CDG type IIf was shown to result from inactivation of the Golgi CMP-sialic acid transporter (Martinez-Duncker *et al.*, 2005). Bovine SLC35A3 is the first nucleotide- sugar transporter shown to play a role in the development of the axial skeleton, demonstrating that some of the molecular mechanisms that operate during formation of vertebrae and ribs depend on carbohydrate modification in the Golgi apparatus. The present disease model may be exploited to predict the human clinical phenotype as well as to provide further insight into the disease mechanism.

Clinical, pathological and radiographic features of CVM: In some studies protrusion of the tongue was often found and in some cases the ears were symmetrically displaced caudo-ventrally. In stillborn, aborted and pre-term calves, CVM has been characterized by shortened cervical and thoracic regions of the vertebral column, bilateral symmetric contraction of the metacarpo-phalangeal and metatarso-phalangeal joints *and* symmetric arthrogryphosis (Agerholm *et al.* 2001; Duncan *et al.* 2001). Multiple hemivertebrae, scoliosis *and* synostosis, fused and mis-shaped vertebral column (Nagahata *et al.*, 2002) have also been described (Figure 2). The heads of affected calves were of normal size except when dystocia-related subcutaneous edema was present (Agerholm *et al.*, 2001). Secondary palatoschisis, unilateral dermoid attached to the conjunctiva and slight brachygnathia superior has been reported (Agerholm *et al.*, 2001; Thomsen *et al.*, 2006).

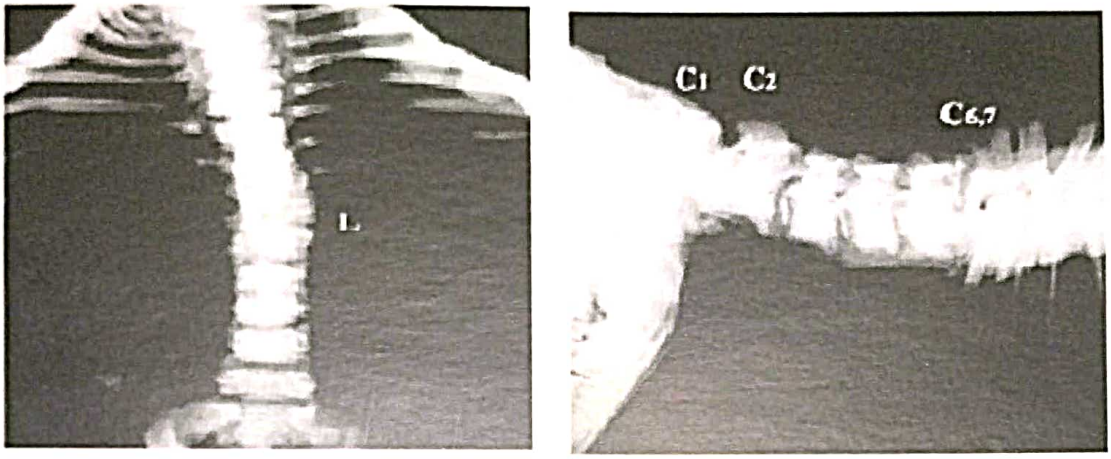

Figure 2: A: Radiograph of ventral thoracic and lumbar vertebrae of the affected Calf. Scoliosis of vertebral column. L: lumbar vertebrae (Nagahata et al., 2002) ; B : Radiograph of lateral cervical and thoracic vertebrae of the affected calf. Fused and malshaped vertebrae. C1: First cervical vertebrae, Atlas. C2: Second cervical vertebrae, Axis. Hemivertebrae. C6, 7: Sixth and seventh cervical vertebra (Nagahata et al., 2002).

The proximal and occasionally middle parts of the ribs were fused, as were spinous processes in areas with vertebral malformations. The intercostal spaces were nonparallel, with the ribs radiating from the malformed vertebral region. The abdomen was protruding in some cases and herniation of the abomasum is also reported with symmetrical arthrogryposis of the anterior limbs (Nagahata *et al.*, 2002). Generally vertebral malformation occurred from the first cervical vertebra to the second lumbar vertebra, in lumbar vertebra No. 5 and in caudal vertebra Nos. 13, 18, 19 and 20 (Agerholm *et al.*, 2001).

CVM and its relationship with other traits: Studies of Danish Holstein showed that the extent of foetal mortality was approximately 77% prior to gestation day 260 (Nielsen *et al.*, 2003). This is reflected in a significantly reduced ratio of CVM affected calves in breeding studies. The symptoms of the defect have not been observed in carriers of CVM (Agreholm *et al.*, 2001). No productive and reproductive difference between carrier and non- carrier animals has been reported. The only difference, which is very important, was increase in the rate of intra-uterine mortality. The risk of return to service was higher in carrier animals (Berglund *et al.*, 2004).

Prevalence of CVM: The syndrome was first discovered in the Danish Holstein population in 1999, but shortly thereafter reports documented the presence of CVM in United States (Duncan *et al.*, 2001), United Kingdom (Revell, 2001), Netherlands (Wouda *et al*, 2000 & 2001), Japan (Nagahata *et al.* 2002), Sweden (Berglund *et al.* 2004), Czech Republic (Citek *et al.* 2006) China and Belgium (Mateusen *et al.* 2004). Berglund *et al.* (2004) estimated that 2,200 affected fetuses were produced annually between 1995 and 1999 in Holstein population in Sweden, while the annual loss in Germany was estimated to be more than 8,000 fetuses between 1997 and 2000 (Konersmann *et al.*, 2003). The defective allele for CVM had spread in Holstein populations worldwide though extensive exploitation of sires that later turned out to be carriers of the defect. Konersmann *et al.* (2003) reported that 13.2% of 957 sires used for insemination in Germany were diagnosed as carriers of CVM, while a prevalence of 31% and 32.5% was found in Denmark and Japan, respectively (Thomsen *et al*, 2006, Nagahata *et al*, 2002). Out of the 605 bulls examined, 150 T/G heterozygotes were diagnosed, including 118 that were sons of known CVM carriers (Rusc and Kaminski 2007). Qin Chu *et al.* (2008) using the polymerase chain reaction–single-stranded conformational polymorphism (PCR-SSCP) technique, found 10 CVM carriers among 68 at-risk Chinese Holstein bulls and 282 carriers were found among 602 cows at-risk. Ghanem *et al.* (2008) examined 200 Holstein crossbred cows and found 26 (13%) cows were CVM carrier. Mahdi (2008) examined 52 HF crossbred bulls and found that frequency of carriers was 20.35% in India.

Table: Frequency of CVM carriers among Holstein bulls in different countries

Country	No. of Bulls tested	CVM Carrier %	Reference
Japan	40	32.20	Nagahata *et al.* (2002)
Germany	957	13.20	Konersmann *et al.* (2003)
Sweden	228	23.00	Berglund *et al.* (2004)
USA	11868	17.76	Holstein Association USA (2006)
Poland	605	24.79	Rusc and Kaminski (2007)
Iran	144	0	Rezaee *et al*, (2008)
China	68	14.71	Qin Chu *et al.* (2008)
India	52	20.35	Mahdi (2008)

DNA testing for identifying different genotypes of CVM: Different methods have been used for identification of single nucleotide polymorphism in SLC35A3 gene. Rusc and Kaminski (2007) used a new PCR-SSCP method (polymerase chain reaction-single stranded conformation polymorphism) and concluded that their method was highly reliable and could be applied for widespread screening program aimed at reducing the incidence of the CVM defect.

Kanae *et al.* (2005) introduced "Polymerase chain reaction-primer introduced restriction analysis" (PCR-PIRA) for detecting a single nucleotide mutation in any gene without a restriction site around the mutation site. In this study, primers were designed to introduce *PstI* or *EcoT22* sites into PCR products from the wild type and CVM alleles, respectively. The wild-type allele, heterozygotes and homozygotes of the CVM allele could be discriminated by restriction fragment length polymorphism analysis. Specific introduction of restriction sites into PCR products depending on the change in a single nucleotide of template was shown using a variety of DNA polymerases and PCR machines.

Agreholm *et al.* (2004) performed genotyping of the CVM locus in a template-directed single-base extension assay, using the AcycloPrime-FP SNP detection kit (Perkin-Elmer Life Sciences). This genotyping method is based on an initial PCR amplification of the locus containing the single-base mutation followed by a specific template-directed single-base extension at the mutation site. Fluorescent signals revealing the nature of the base at the mutation site was detected by fluorescence polarization in a Victor.

Ghanem *et al.* (2008) introduced DNA typing of CVM with allele specific PCR reaction (AS-PCR) and they found this as a reliable method for detection of Single nucleotide polymorphism. Tetra-primer ARMS-PCR (amplification refractory mutation system- Polymerase Chain Reaction) is a relatively new method. It employs two primer pairs to amplify, respectively, the two different alleles of a SNP in a single PCR reaction. A computer program for designing primers was developed. Tetra-primer ARMS-PCR was combined with micro-plate array diagonal gel electrophoresis, gaining the advantage of high throughput for gel-based resolution of tetra-primer ARMS-PCR products. The technique was applied to analyze a number of SNPs and the results were completely consistent with those from an independent method, restriction fragment length polymorphism analysis. It is a rapid, simple, low cost and high throughput methodology for SNP genotyping (Shu *et al.*, 2001).

Checklist for managing CVM in a herd

1) A system should be set up for accurate recording of sire and maternal grandsire ID for all cows in the herd.

2) List or spreadsheet file should be made showing the CVM status of the sire and maternal grandsire of each cow in the herd.

3) Selection index should be used such as Lifetime Net Merit, to identify the group of AI sires likely to be used in the herd during the next three months.

4) CVM carrier bulls should be avoided for using on any cow whose sire or maternal grandsire is a carrier.

5) Modern genetic tools help us to identify undesirable genes like CVM and to eliminate them in a rapid and efficient manner and should be utilized.

CONCLUSION

The massive spread of genetic disorder in cattle like CVM in recent years is caused by the extensive use of elite sires and latent heterozygous carriers. Artificial insemination accelerates the spread of undesirable recessives worldwide. However, the new methods of molecular genetics enable us to find the cause at gene level. They make it possible to detect heterozygous animals, to control the genetic health of the population and in a way, to anticipate the accumulation of recessive alleles. The first step in controlling genetic defects like CVM is to establish pedigree records for the animals in a herd. Once this is done, it's relatively easy to avoid mating known carrier bulls to cows whose sire or maternal grandsire is also a carrier, because virtually all AI sires will be tested. One can do this by visually inspecting pedigrees, by developing a simple spreadsheet program, or by using a computerized mating program. It's probably unwise to "panic" and exclude all CVM carrier bulls from a breeding program. Many bulls that carry the undesirable CVM gene may also carry numerous other genes with positive effects on milk production, milk component percentage, udder shape and size, somatic cell count and other key traits. If one discards all of these bulls, one may end up using a somewhat mediocre group of bulls instead, just to avoid CVM. Avoid using CVM carrier bulls on cows whose sire or maternal grandsire are CVM carriers.

REFERENCES

Agerholm JS, Bendixen Candersen O. and Ambjerg J. 2001. Complex vertebral malformation in Holstein calves. *J. Vet. Diagn. Invest.* 13: 283-289.

Agerholm JS, Andersen O, Almskou MB, Bendixen C, Arnbjerg J.

Aamand G, Nielsen US, Panitz F and Petersen AH. 2004. Evaluation of the inheritance of complex vertebral malformation syndrome by breeding studies. *Acta. Vet. Scand.* 45:133–137.

Berglund B, Persson A and Stalhammer H. 2004.Effects of complex vertebral malformation on fertility in Swedish Holstein cattle. *Acta. Vet. Scand.* 45: 161-165.

Citek J, Rehout V, Hradecka E and Vecerek L. 2006. Molecular genetic analyses of the locus of complex vertebral malformation. *Anim. Sci.* 23: 39-42.

Duncan RB, Carrig CB, Agerholm JS and Bendixen C 2001. Complex vertebral malformation in a Holstein calf: report of a case in the USA. *J. Vet. Diagn. Invest.* 13: 333-336.

Ghanem ME, Akita M, Suzuki T, Kasuga A and Nishibori M. 2008. Complex vertebral malformation in Holstein cows in Japan and its inheritance to crossbred F1generation. *Am. Rep. Sci.* 103: 348-354.

Guillen E, Claudia A and Carlos BH. 1998. Mammalian Golgi apparatus UDP-N-acetyl glucosamine transporter: Molecular cloning by phenotypic correction of a yeast mutant. *Proc. Natl. Acad. Sci. U.S.* 95: 7888-7892.

Kanae Y, Endoh D, Nagahata H and Hayashi MA. 2005. Method for detecting complex vertebral malformation in Holstein calves using polymerase chain reaction-primer introduced restriction analysis. *J. Vet. Diagn. Invest.* 17: 258-262.

Konersmann Y, Wemheuer W and Brenig B. 2003. Origin, distribution and relevance of the CVM defect within the Holstein-Friesian population. *Zuchtungskunde* 75: 9-15.

Lubke, T, Marquardt, Etzioni A, Hartmann E, von Figura K. and Korner C 2001.Complementation cloning identifies CDG-IIc, a new types of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. *Nat. Genet.* 28: 73-76.

Luhn K, Wild K, Eckhardt M, Gerardy-Schahn R*and* Vestweber D. 2001. The gene defective in leukocyte adhesion deficiency II encode a putative GDP-fucose transporter. *Nat. Genet.* 28: 69-72.

Mahdi M. 2008. Genetic screening of Karan fries Bulls for Bovine Leukocyte Adhesion Deficiency and Complex Vertebral Malformation. M.V.Sc. Thesis, NDRI, Karnal. pp 1-48

Malher X, Beaudeau F and Philipot JM. 2006. Effects of sire and dam genotype for complex vertebral malformation (CVM) on risk of return-to-service in Holstein dairy cows and heifers. *Theriogenology* 65: 1215-1225.

Martinez-Duncker I, Dupre T, Piller V, Piller, Candelier J, Trichet Tchernia G, Oriol R and Mollicone R. 2005. Genetic complementation reveals a novel human congenital disorder glycosylation of type II, due to inactivation of the Golgi CMP-siac acid transporter. *Blood.* 105: 2671-2676.

Mateusen B, Last T, Vercauteren G, Mommens G and Kruif A. 2004. Complex vertebral malformation in a Belgian Holstein Friesian calf. *Vlaams-Diergeneeskundig-Tijdschrift* 73: 341-343.

Nagahata H, Oota H, Nitanai A, Oikawa S, Higuchi, Nakade T, Kurosawa T, Morita M and Ogawa H. 2002. Complex vertebral malformation in a stillborn Holstein calf in *Japan. J. Vet. Med. Sci.* 64: 1107-1112.

Nielsen US, Aamand GP, Andersen O, Bendixen C, Nielsen VH and Agerholm JS. 2003. Effects of complex vertebral malformation on fertility traits in Holstein cattle. *Livest. Prod. Sci.* 79: 233-238.

Qin Chu, Dongxiao Sun, Ying Yu, Yi Zhang and Yua Zhang. 2008. Identification of complex vertebral malformation carriers in Chinese Holstein. *J. Vet. Diagn. Invest.* 20: 228-230.

Revell S. 2001. Complex vertebral malformation in a Holstein calf in the UK. *Vet. Res.* 149: 659- 660.

Rusc A. and Kaminski, S. 2007 Prevalence of complex vertebral malformation carriers among Polish Holstein-Friesian bulls. *J Appl Genet* 48:247-252.

Shu Ye, Sahar Dhillon, Xiayi Keandrew R, Collins*and* Ian NM. 2001. An efficient procedure for genotyping single nucleotide polymorphisms. *Nucleic Acids Res* 29: e88

Thomsen B, Horn P, Panitz F, Bendixen E, Petersen AH, Holm LE, Nielsen VH, Agerholm JS, Arnbjerg J and Bendixen C. 2006. A missense mutation in the bovine SLC35A3 gene, encoding an UDP-N-acetylglucosamine transporter, causes complex vertebral malformation. *Genome Res* 16: 97-105

Wouda W, Visser IJ, Borst GH, Vos JH, Zeeuwen AA and Peperkamp NH. 2000. Developmental anomalies in aborted and stillborn calves in the Netherlands, *Veterinary Record* 147: 612.

Wouda W, Borst GH, Vos JH, Peperkamp NH, Visser IJ and Zeeuven AA. 2001. A newly recognized hereditary syndrome in Holstein- Friesian calves characterized by vertebral malformations, cervicotruncal dwarfism, arthrogryphosis and cardiac defects. Proc. 19th Meeting Eur. Soc. Vet. Path. Thessaloniki, 168.