HHS Public Access

Author manuscript

J Wildl Dis. Author manuscript; available in PMC 2018 April 01.

Published in final edited form as:

J Wildl Dis. 2017 April ; 53(2): 248–257. doi:10.7589/2016-05-113.

Hereditary Factor Vii Deficiency In The Asian Elephant (*Elephas Maximus*) Caused By A *F7* Missense Mutation

Michael Lynch[1,6], **Ken McGrath**[2], **Karthik Raj**[3], **Philippa McLaren**[4], **Karen Payne**[5], **Richard McCoy**[4], and **Urs Giger**[3]

[1]Melbourne Zoo, PO Box 74, Parkville VIC 3052, Australia

[2]Australian Genome Research Facility, Research Rd, University of Queensland, St Lucia, Qld 4072, Australia

[3]Section of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey St, Philadelphia, PA 19104-6010, USA

[4]Gribbles Veterinary Pathology, 1868 Dandenong Rd, Clayton VIC 3168, Australia

[5]Perth Zoo, 20 Labouchere Rd, South Perth WA 6951, Australia

Abstract

Hereditary disorders and genetic predispositions to disease are rarely reported in captive and free-ranging wildlife and none have been definitively identified and characterized in elephants. A wild-caught, 41-yr-old male Asian elephant (*Elephas maximus*) without an apparent increased bleeding tendency was consistently found to have prolonged prothrombin times (mean PT=55±35 s) compared to 17 other elephants (PT=10±2 s). This elephant's partial thromboplastin times (PTT) fell within the normal range of the other elephants (12–30 s). A prolonged PT in the presence of a normal PTT suggests disruption of the extrinsic pathway via deficiency of coagulation factor VII (FVII). This elephant's plasma FVII activity was very low (2%) compared to that of 15 other elephants (57–80%), but other coagulation factors activities did not differ from the control elephants. Sequencing of genomic DNA from EDTA blood revealed a single homozygous point mutation (c.202A>G) in the *F7* gene of the FVII deficient elephant which was not present in unrelated elephants. This mutation causes an amino acid substitution (p.Arg68Gly) which is predicted to be deleterious. Two living offspring of the affected elephant were heterozygous for the mutation and had normal plasma FVII activities and coagulation profiles. Tissue from a third offspring, a deceased calf, were utilized to show that it was also a heterozygote. A DNA test has been developed to enable the screening of additional elephants for this mutation. Consistent with FVII deficiency investigations in other species, the condition did not cause a serious bleeding tendency in this individual elephant.

Keywords

Blood; bleeding coagulopathy; elephant; genetic disease

[6]Corresponding author (mlynch@zoo.org.au).

Introduction

Hemostasis in mammals involves the vasculature, platelets, von Willebrand factor, and plasma coagulation factors. The process of in vivo hemostasis is currently best described by the cell based model (Furie and Furie 1992). Tissue factor (TF) from damaged endothelial cells at the site of injury activates Factor VII (FVII) to FVIIa which then activates factors in the common pathway. The small amount of thrombin generated by this pathway leads to activation of FXI and FIX of the intrinsic cascade in order to sustain fibrin formation through the common pathway. The coagulation cascades can be assessed in vitro for acquired and hereditary coagulopathies by measurement of prothrombin time (PT) and activated partial thromboplastin time (PTT). Prothrombin time measures time of fibrin formation through the extrinsic and common pathways, and the PTT determines fibrin formation through the intrinsic and common pathways (Brooks and Catalfamo 2013).

Hereditary coagulopathies are well recognized in humans and domestic dogs (*Canis familiaris*). The most serious hereditary human coagulopathies are hemophilia A and B due to a deficiency of factors VIII and IX, respectively (Triplett 2000, Brooks and Catalfamo 2013). Other hereditary coagulopathies, such as Factor VII deficiency, are generally milder and far less commonly reported in humans but may occur more frequently in several dog breeds (Triplett et al. 1985, Callan et al. 2006, Donner et al. 2016). Hereditary FVII deficiency is an autosomal recessive trait, and causes a mild bleeding tendency mostly after trauma and surgery (Triplett et al. 1985, Callan et al. 2006, Daly and Giger 2007). As this factor resides in the extrinsic cascade, it affects the PT and not the PTT. In FVII deficient human patients several mutations in the *F7* gene have been described, while the very same point missense mutation has been found in all affected domestic dog breeds including Beagles, Scottish deerhounds, Alaskan Klee Kai and others (Callan et al. 2006, Daly and Giger 2007, Kaae et al. 2007).

Hereditary diseases and genetic predispositions to disease are rarely reported in wildlife species compared to humans and domestic animals. We describe here the clinical features, routine laboratory diagnostics, and molecular genetic analyses of FVII deficiency in the Asian elephant (*Elephas maximus*).

Materials and Methods

Animals

The index case, a wild-born (~1974) Asian elephant from Malaysia, was exported to the Melbourne Zoo in Australia in 1977. The elephant had been in good general health except for chronic pododermatitis and degenerative joint disease. For the latter condition we administered intramuscular injections of sodium pentosan polysulfate (Cartrophen Vet, Biopharm, Bondi Junction, Australia) at 3 mg/kg every 3 mo. In 1996, he underwent foot surgery to remove an infected third phalanx from the digit of a forelimb, and adequate hemostasis was noted. In 2007, we incidentally found that he had a prolonged PT but normal PTT, when we collected blood to compare his coagulation profile with a female being investigated for an unrelated medical condition. During initial evaluation of his coagulopathy, we gave him 375 mg vitamin K_1 (Equisci International, Brooklyn, Australia)

daily for 6 wk without improvement in the PT. We used a higher dose of vitamin K_1 (5000 mg daily for 4 wk) for an 8 wk treatment trial in 2015 but again, we saw no improvement in the PT.

The pedigree chart for all Asian elephants born in Australian zoos from 2009 to 2015 is presented in Fig. 1. The case elephant was bred to four Asian elephant females, all born in Thailand and imported to Australia in 2006. One of the five calves (calf 3) that were sired by the case elephant had an accidental death, not associated with any potential coagulopathy. A second male (male 2) was bred with two additional females (all three adults were also part of the 2006 importation) producing a single calf from each. The 2006 import documents list different sires and dams for each of the Thai-origin elephants but more detailed pedigree information is not available.

Clinical Hemostatic Evaluations

For the PT, PTT, and coagulation factor analyses, we used standard human reference plasmas and plasma from other Asian elephants from the Australian zoo population for comparison. This approach was taken due to the lack of available elephant-specific reagents and because human reagents have been successfully used for coagulation investigations in many wild mammal species (Gentry et al. 1996, Travis and Eby 2006, Ekser et al. 2012). We collected blood samples from marginal ear veins into 3.8% sodium citrate tubes. Citrated plasma harvested from centrifuged blood samples were then either shipped chilled for immediate analysis or immediately frozen and held at -20 C. Chilled samples were analysed within 5-10 h of collection by Gribbles Veterinary Pathology, Clayton, Victoria, Australia. We transported frozen samples to the Complex Haematology Laboratory (CHL), Pathology Department, Austin Health, Heidelberg, Australia to be analyzed within 4 wk of collection. We tested data for normality and then calculated mean values and standard deviations for repeated measures of these parameters on the index case and the control group. We compared the index case with the control group using a two-tailed t test and investigated the relationship between age and FVII measures by calculation of a Pearson's correlation coefficient.

For determination of the PT and PTT, we measured the time until the formation of a fibrin clot in plasma after adding recombinant thromboplastin (HemosIL RecombiPlasTin 2G, Instrumentation Laboratory, Lexington, USA) or contact activating agent (Trinclot HS, Trinity Biotech, Jamestown, USA) and phospholipids, respectively. We used $CaCl_2$ (Fronine Laboratory Supplies, Rivertone, Australia) to initiate the coagulation reaction. Samples from elephants were tested simultaneously with human controls (Normal Control Assayed, Instrumentation Laboratory, Lexington, USA).

We measured the plasma activity of coagulation factors II, VI, VII and X using an automated coagulation analyzer (ACL TOP 500, Instrumentation Laboratory, Lexington, USA) at the CHL. The analyzer utilized a mixture of patient plasma and specific factor deficient human plasma. The principle of the test is a one stage PT assay where the degree of correction of the prolonged PT of the known deficient human plasma (HemosIL Factor Deficient Plasmas FII, FVI, FVII and FX, Instrumentation Laboratory, Lexington, USA), when test plasma is added, is proportional to the plasma factor level in the patient. The results are expressed as a

percentage for each factor activity. The reference ranges of 50-120% for human plasma factors were established at that laboratory.

Genomic DNA and cDNA Preparation

Genomic DNA was extracted from 200 μL of EDTA whole blood using the QIAamp DNA blood extraction kit (Qiagen, Hilden, Germany) and stored at -20 C. Total nucleic extractions from liver tissue (stored at -80 C) were performed using the ISOLATE II RNA/DNA extraction kit (Bioline, London, United Kingdom). Extracts were treated with DN'ase I as supplied with the kit and the resulting suspension stored at -20 C. Complementary DNA (cDNA) was made using the High Capacity cDNA Reverse Transcriptase kit (Life Technologies, Carlsbad, California, USA) and stored at -20 C prior to analysis. Samples were shipped from Australia to the School of Veterinary Medicine, University of Pennsylvania, USA for further molecular characterization of the *F7* gene. Samples were transported in compliance with all permitting required by the Australian and United States governments including that required by CITES.

Amplification and Sequencing of cDNA

An assembled and annotated reference genome of the Asian elephant is unavailable and hence we relied on the reference genome of African elephant (*Loxodonta africana*) to design primers. However, when reviewing the homology of interspecies (human, mouse, etc.) protein sequences, it became evident that the NCBI and Ensembl databases had some errors in the prediction of the African elephant *F7* gene (NCBI RefSeq ID: XM_010594038.1, Ensembl Transcript ID: ENSLAFT00000005968.3). Specifically, the 5′ end of the *F7* gene was incomplete in NCBI's prediction XM_010594038.1 and incorrect in Ensembl's prediction ENSLAFT00000005968.3. Due to gaps in the genome data the beginning of exon 4 was missing in NCBI and Ensemble referred to an incorrect region. Therefore, cDNA primers (Forward: 5′-ACAGTTTCCATCTGGAATGTCA-3′, Reverse: 5′-TCCGACCCTCAAAATCATCAAG-3′) were designed spanning from the 5′ UTR to exon 4 of the *F7* gene based on our prediction of the actual 5′ UTR location in the reference genome using interspecies homology. The primers were then used to amplify the cDNA extracted from a frozen liver sample from an Asian elephant using TaKaRa Ex Taq HotStart kit (Takara, Shiga, Japan). The obtained PCR amplicons were separated by agarose gel electrophoresis, and the bands were gel purified using QIAquick gel extraction kit (Qiagen, Hilden, Germany) and directly sequenced from both ends of the amplicons at University of Pennsylvania's DNA sequencing core facility.

Genomic DNA Sequencing and Genotyping Assay

Primers to amplify all exons and untranslated regions of *F7* gene were designed by the Australian Genome Research Facility, St Lucia, Queensland, Australia using Primer 3 software (Broad Institute, Cambridge, Massachusetts, USA; Table 1). For each exon target, PCR amplification was performed with 20 ng of gDNA and 5 pmol of primer, using AmpliTaq Gold 2× MasterMix (Life Technologies), and cycled at the following temperatures: 95 C for 7 min; 12 cycles consisting of 95 C for 30 s, 68 C (-1 C per cycle) for 30 s and 72 C for 1 min. This was followed by 29 cycles of 95 C for 30 s, 57 C for 15 s and 72 C for 1 min prior to 72 C for 10 min. The PCR products were purified using AMPure

beads (Beckman-Coulter, Lane Cove, NSW, Australia). For sequencing, purified PCR products and primers were combined with BDTv3.1 master mix and thermocycled (Life Technologies). The BDT-labelled product was purified using the CleanSEQ magnetic-bead clean-up method (Beckman-Coulter), and the labelled products were resolved on a capillary electrophoresis analyzer (Model 3730 XL, Life Technologies).

A TaqMan genotyping assay was developed to screen for the putative mutation (c.202A>G). The assay was used to test genomic DNA samples from 12 Asian elephants including the case elephant and three of his offspring.

Results

Repeated testing (n=25) over an 8 yr period of the case elephant showed persistently prolonged PT results. His mean, standard deviation, and 95% confidence interval for PT was 55±35 s (95% confidence interval: 40.4–59.7 s). This differed significantly to values obtained from 17 other Asian elephants held by Australian zoos who had a mean PT value of 10±2 s (t_{25}=5.2, μ_{case}=55, $\mu_{controls}$=9.9; P<0.01). In contrast, the PTT values from the index case were within the range of values from the control elephants (Table 2). His mean PTT of 17.0±3.4 s (95% confidence interval: 15.6–18.4 s) did not differ significantly (P=0.27) from the population mean of the control elephants (μPTT 14.9±4.2 s; n=15).

Plasma FVII activity of the case animal measured on three occasions over two years was ~2% of the human reference value and severely reduced compared to healthy control elephants (Table 2). The FVII activity mean for other elephants housed in Australia was 68±21% (n=17). For elephants of age > 6 yr age, mean FVII activity was 76±19% (n=12). However, for elephants of age ≤ 6 yr FVII activity was 47±7% (n=5) which was significantly lower than the adult animals (t=3.32. P=0.005). A positive correlation was found between age and FVII activity for elephants that were not FVII deficient (r=0.76, n=17; P<0.01). However, the other plasma coagulation factor activities measured were in the reference range of the control group without discernible age variations. There was no apparent difference in the plasma FII, FV and FX activities of the index case compared to other elephants (Table 2).

Serum biochemical parameters were measured on three occasions over 2 yr in the case animal and included indicators of hepatic disease: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), and glutamate dehydrogenase (GLDH) activities and serum bilirubin and bile acid concentrations. All were within normal ranges expected for elephants. During the study period of 9 yr the case elephant has remained healthy and also maintained a stable body weight.

Analysis of FVII cDNA Sequences

Gel electrophoresis of the amplified *F7* cDNA products showed the expected sizes. The *F7* cDNA sequences aligned perfectly with our predicted sequence, and the alignments with the reference genome showed that exon 1 is >10 kb upstream of exon 2. It also allowed correction of the errors in *F7* gene predictions shown on NCBI and Ensemble databases.

Based on this data, a partial FVII mRNA sequence of Asian elephants was submitted to NCBI Genbank database (NCBI's GenBank Accession #KX228910). Following this submission, NCBI has now made a new and more accurate version of the African elephant FVII mRNA sequence available on their database (NCBI RefSeq ID: NM_001330481.1).

Analysis of *F7* gDNA Sequences

Aligning the sequenced exonic *F7* DNA from the affected Asian elephant to the reference genome sequence (*Loxodonta africana*) revealed several polymorphisms. In the *F7* coding region of the affected elephant there were three silent and two missense polymorphisms (Table 3). One of the missense variants (c.386T>A) was also seen in samples from all the other healthy elephants ($n = 11$) that were sequenced. According to SIFT (http://sift.bii.a-star.edu.sg) and PROVEAN (http://provean.jcvi.org/index.php) software analyses this missense was predicted to be tolerated and neutral, respectively.

The other SNP (c.202A>G) was found to be homozygous only in the affected elephant (Fig. 2). This missense mutation results in an arginine to glycine substitution (p.Arg68Gly) affecting a highly conserved region (Fig. 3) of the GLA domain among vertebrates. According to SIFT and PROVEAN software analyses this missense mutation is predicted to be deleterious.

Genotyping Results

The established TaqMan genotyping assay for the SNP c.202A>G accurately identified only the affected elephant as homozygous for the mutant allele. In addition, two of the affected elephant's offspring were tested using this method and found to be heterozygous for the mutant allele. An additional offspring from the case was demonstrated as a heterozygote for the mutation by cDNA sequencing. All other Asian elephants tested using the TaqMan assay were found to be homozygous for the wild-type allele.

Discussion

We showed that an Asian elephant had a persistently prolonged PT compared to other elephants held in Australian zoos and values previously published for Asian elephants (Lewis 1974, Gentry et al. 1996). The cause of the case's prolonged PT was FVII deficiency, evidenced by both his normal PTT and extremely low FVII plasma activity (2%) compared to other Asian elephants in this study and previous reports (Lewis 1974, Gentry et al. 1996). A diagnosis of hereditary FVII deficiency was supported by finding a single disease-causing mutation in the *F7* gene of the affected elephant. We judged that it was unlikely this animal had acquired causes of FVII deficiency. Blood biochemistry and maintenance of good appetite and body weight did not support a diagnosis of hepatic disease. A toxic etiology for his prolonged PT also appeared unlikely. While the use of sodium pentosan polysulfate is known to be associated with slight prolongations of PT and PTT in humans due to interference with the binding of FXa to thrombin (Vinazzer et al. 1980, Kumagai et al. 2010), the case elephant's PTT remained normal over the 8 yr course of this study. Furthermore, his PT values did not improve with vitamin K therapy.

Comparing the *F7* DNA sequences among elephants identified one homozygous point missense mutation (c.202A>G) in the case elephant which substitutes an arginine with a glycine (p.Arg68Gly) in the highly conserved GLA domain thereby rendering the protein dysfunctional. An identical missense mutation has been identified in three humans (Giansily-Blaizot et al. 2001, Herrmann et al. 2009) but hereditary FVII deficiency in humans is also caused by mutations at many other locations on the *F7* genome (Giansily-Blaizot et al. 2001). In domestic dogs with FVII deficiency, another single missense mutation in the EGF-like domain has been found in all affected individuals thus far studied (Callan et al. 2006, Kaae et al. 2007).

Previous studies on FVII activity in elephants reported on a total of nine individual adults with values ranging from 75–140% of human control plasma (Lewis 1974, Gentry et al. 1996). Variability in FVII activity between individual elephants was also observed in the present study and is seen in humans and dogs (Marder and Shulman 1964, Kaae et al. 2007). In humans, plasma FVII activity is also known to be affected by a range of non-genetic factors including age, sleep deprivation, plasma triglycerides/cholesterol concentrations and components of the inflammatory response (Mariani et al. 1999, Passaro et al. 2008, Pinotti et al. 2010). Of these factors, perhaps the most likely reason for variance noted in FVII levels in the present study is age. As in humans (Mariani et al. 1999), a significant positive correlation between age and plasma FVII activity was demonstrated in the elephants tested here.

The case elephant's severely reduced plasma FVII activity (2%) is consistent with him being homozygous for the missense *F7* mutation. In dogs, plasma FVII activity of <10% is associated with prolonged PT times, while individuals with >10% FVII activity are unlikely to have extended PT or bleeding tendencies (Mustard et al. 1962, Marder and Shulman 1964). Moreover, dogs with FVII activity of <10% are frequently asymptomatic and may only bleed excessively after major trauma and surgery (Spurling et al. 1972, Callan et al. 2006). Similarly, in FVII deficient humans, bleeding tendencies are not expected unless plasma FVII activities are below 10% (Perry 2002), but even at these values many patients do not exhibit clinical signs of bleeding (Triplett et al. 1985). This is because the role of the extrinsic pathway is primarily involved in the initiation of the coagulation cascade by producing a rapid surge of thrombin that activates FXI, which leads to sustained activity of the intrinsic pathway. In contrast, hereditary deficiencies of factors involved with the intrinsic pathway (Factor VIII and Factor IX deficiencies) more commonly result in clinical coagulopathies (Triplett 2000). For both humans and dogs, when bleeding problems become evident their clinical expression is variable (Spurling et al. 1972, Mills et al. 1997, Peyvandi et al. 1997, Perry 2002). Manifestations of this condition include bleeding from the gums, epistaxis, post-surgical hemorrhage and hemarthroses. The case had not been noted to exhibit any of these signs and had no apparent extended hemorrhage during an invasive foot surgery procedure. Our assessment was that the case animal, while having a prolonged PT due to FVII deficiency, has likely been asymptomatic in terms of the clinical impact of this coagulopathy.

Depending on the polymorphism involved, humans who are FVII deficiency homozygotes have >50% reduction in FVII activity while heterozygotes have variably reduced activity,

typically up to 25% (Perry 2002). In dogs, as in humans, heterozygotes for the *F7* mutation have reduced plasma FVII activity (Spurling et al. 1972, Callan et al. 2006). In neither humans nor dogs are the heterozygote states associated with prolonged PT and bleeding tendencies. In our study, of the five tested elephant calves, three were the offspring of the case elephant and all were found to be heterozygous for the missense mutation. These calves had PT values and FVII activities falling within the range of values for elephants without the mutant allele. However, given we demonstrated a correlation between age and FVII activity in this study, a longitudinal study in these and unaffected calves is warranted to elucidate further the relationship between age and FVII activity in elephants and whether the heterozygote state impacts FVII activity in this species.

The case elephant was wild-born in Malaysia. The prevalence of this mutation in that and other wild elephant populations is unknown. The present study has only surveyed the current population of elephants held captive in Australian zoos. The development of a DNA mutation test associated with this study now allows ready screening of elephants for FVII deficiency. This could assist with the clinical assessment and management of PT prolongation in elephants and if desired, be taken into account in captive breeding programs.

Acknowledgments

The authors thank the staff at the Perth and Taronga Zoos for their support of this investigation. In particular we acknowledge Paul Thompson from Taronga Zoo for his coordination of sample collection and processing from their elephant group. We also thank Bron Oke for her collation of data in the early stages of this investigation. Work performed at the University of Pennsylvania was partially supported by a grant from the National Institutes of Health (# OD 010939).

Literature Cited

Brooks MB, Catalfamo JL. Current diagnostic trends in coagulation disorders among dogs and cats. Vet Clin N Am–Small. 2013; 43:1349–1372.

Callan MB, Aljamali MN, Margaritis P, Griot-Wenk ME, Pollak ES, Werner P, Giger U, High KA. A novel missense mutation responsible for factor VII deficiency in research beagle colonies. J Thromb Haemost. 2006; 4:2616–2622. [PubMed: 16961583]

Daly ML, Giger U. A rodenticide exposed and bleeding beagle dog with hereditary factor VII deficiency. J Vet Emerg Crit Care. 2007; 17:170–174.

Donner J, Kaukonen M, Anderson H, Möller F, Kyöstilä K, Sankari S, Hytönen M, Giger U, Lohi H. Genetic panel screening of nearly 100 mutations reveals new insights into the breed distribution of risk variants for canine hereditary disorders. PLoS One. 2016; 11:e0161005. [PubMed: 27525650]

Ekser B, Bianchi J, Ball S, Iwase H, Walters A, Ezzelarab M, Veroux M, Gridelli B, Wagner R, Ayares D, Cooper DKC. Comparison of hematologic, biochemical, and coagulation parameters in α1,3-galactosyltransferase gene-knockout pigs, wild-type pigs, and four primate species. Xenotransplantation. 2012; 19:342–354. [PubMed: 23145497]

Furie B, Furie BC. Molecular and cellular biology of blood coagulation. New Eng J Med. 1992; 326:800–806. [PubMed: 1538724]

Gentry PA, Ross ML, Yamada M. Blood coagulation profile of the Asian elephant (*Elephas maximus*). Zoo boil. 1996; 15:413–423.

Giansily-Blaizot M, Aguilar-Martinez P, Biron-Andreani C, Jeanjean P, Igual H, Schved JF. Analysis of the genotypes and phenotypes of 37 unrelated patients with inherited factor VII deficiency. Euro J Hum Genet. 2001; 9:105–112.

Herrmann FH, Wulff K, Auerswald G, Schulman S, Astermark J, Batorova A, Kreuz W, Pollmann H, Ruiz-Saez A, De Bosch N, Salazar-Sanchez L. Factor VII deficiency: Clinical manifestation of 717

subjects from Europe and Latin America with mutations in the factor 7 gene. Haemophilia. 2009; 15:267–280. [PubMed: 18976247]

[Accessed July 2016] PROVEAN software prediction tool. http://provean.jcvi.org/index.php

Kaae JA, Callan MB, Brooks MB. Hereditary factor VII deficiency in the Alaskan Klee Kai dog. J Vet Int Med. 2007; 21:976–981.

Kumagai K, Shirabe S, Miyata N, Murata M, Yamauchi A, Kataoka Y, Niwa M. Sodium pentosan polysulfate resulted in cartilage improvement in knee osteoarthritis–an open clinical trial. BMC Clin Pharmacol. 2010; 10:7. [PubMed: 20346179]

Lewis JH. Comparative hematology: Studies on elephants, *Elephas maximus*. Comp Biochem Phys A. 1974; 49:175–181.

Marder VJ, Shulman NR. Clinical aspects of congenital factor VII deficiency. Amer J Med. 1964; 37:182–194. [PubMed: 14206752]

Mariani G, Bernardi F, Bertina R, Vicente VV, Prydz H, Samama M, Sandset PM, Di Nucci GD, Testa MG, Bendz B, Chiarotti F, Ciarla MV, Strom R. Serum phospholipids are the main environmental determinants of activated factor VII in the most common factor VII genotype. European union concerted action “Clotart”. Haematologica. 1999; 84:620–626. [PubMed: 10406904]

Mills JN, Labuc RH, Lawley MJ. Factor VII deficiency in an Alaskan malamute. Aust Vet J. 1997; 75:320–322.

Mustard JF, Secord D, Hoeksema TD, Downie HG, Rowsell HC. Canine factor VII deficiency. Brit J Haem. 1962; 8:43–47.

Passaro A, Calzavarini S, Volpato S, Caruso P, Poli A, Fellin R, Bernardi F. Reduced factor VII and factor VIII levels and prolonged thrombin-generation times during a healthy diet in middle-aged women with mild to moderate cardiovascular disease risk. J Thromb Haem. 2008; 6:2088–2094.

Perry DJ. Factor VII deficiency. Brit J Haem. 2002; 118:689–700.

Peyvandi F, Mannucci PM, Asti D, Abdoullahi M, Di Rocco N, Sharifian R. Clinical manifestations in 28 Italian and Iranian patients with severe factor VII deficiency. Haemophilia. 1997; 3:242–246. [PubMed: 27214858]

Pinotti M, Bertolucci C, Frigato E, Branchini A, Cavallari N, Baba K, Contreras-Alcantara S, Ehlen JC, Bernardi F, Paul KN, Tosini G. Chronic sleep deprivation markedly reduces coagulation factor VII expression. Haematologica. 2010; 95:1429–1432. [PubMed: 20418241]

[Accessed July 2016] PROVEAN software prediction tool. http://provean.jcvi.org/index.php

[Accessed July 2016] SIFT software prediction tool. http://sift.bii.a-star.edu.sg

Spurling NW, Burton LK, Peacock R, Pilling T. Hereditary factor–VII deficiency in the beagle. Brit J Haem. 1972; 23:59–67.

Travis EK, Eby C. Clotting profiles and selected hematology of captive Speke's gazelles (*Gazella spekei*). J Zoo Wild Med. 1996; 37:64–67.

Triplett DA, Brandt JT, Batard MA, Dixon JL, Fair DS. Hereditary factor VII deficiency: heterogeneity defined by combined functional and immunochemical analysis. Blood. 1985; 66:1284–1287. [PubMed: 4063521]

Triplett DA. Coagulation and bleeding disorders: Review and update. Clin Chem. 2000; 46:1260–1269. [PubMed: 10926920]

Vinazzer H, Haas S, Stemberger A. Influence on the clotting mechanism of sodium pentosan polysulfate (SP54) in comparison to commercial beef lung sodium heparin. Thromb Res. 1980; 20:57–68. [PubMed: 6163222]

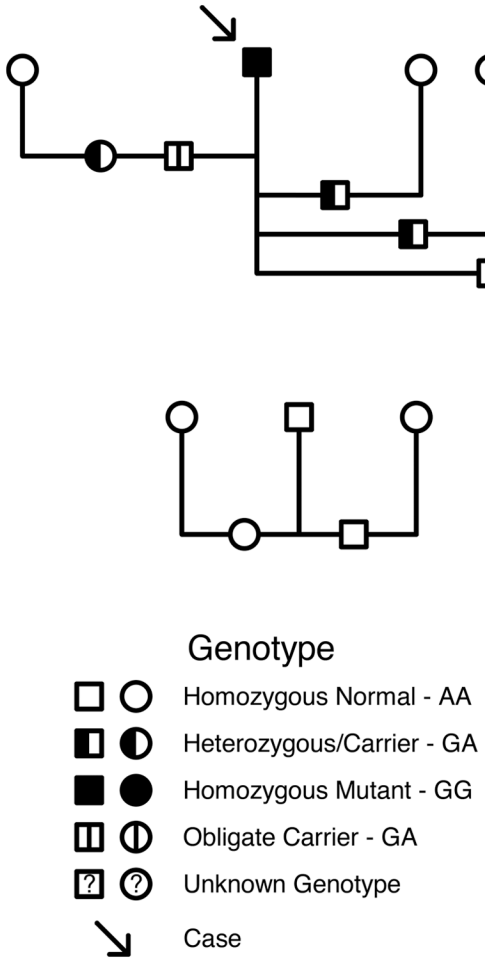

Figure 1.

Pedigree chart for all Asian elephant (*Elephas maximus*) calves born in Australian zoos between 2009 and 2015. Squares represent male individuals and circles, females.

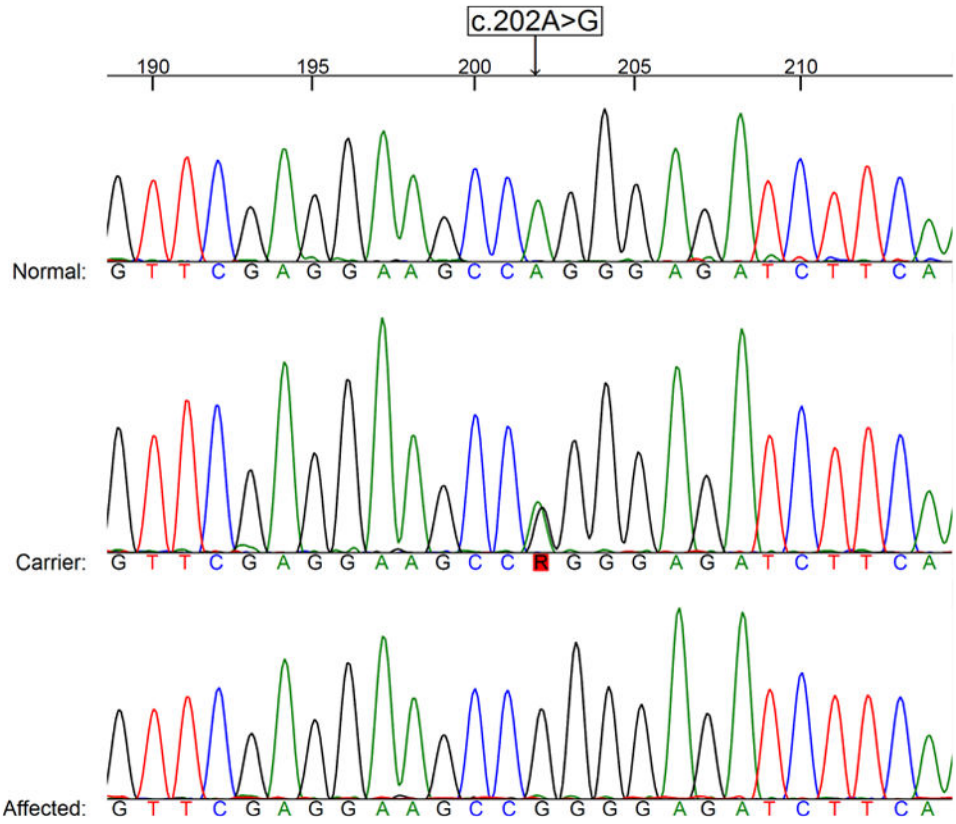

Figure 2.

Sequence chromatograms of the *F7* region surrounding the c.202A>G mutation site in Asian elephants (*Elephas maximus)* showing sequences for normal (homozygous for wild-type allele), carrier (heterozygous) and affected (homozygous for mutant allele) types. R refers to double peaks with an A and G. Numbers refer to the base position in the coding sequence.

Figure 3.

Homology between vertebrate species with respect to the Factor VII (FVII) protein. Numbers represent amino acid positions either side of the location of a p.Arg68Gly missense mutation in an Asian elephant (*Elephas maximus*) with FVII deficiency. Amino acids identical in 5 or more species are shaded with a grey background to demonstrate the highly conserved nature of this area in the gene.

Table 1

Primer sequences used for PCR amplification and Sanger sequencing of the Factor VII (*F7*) gene in Asian elephants (*Elephas maximus*) to enable investigation of an individual with a demonstrated deficiency in circulating Factor VII coagulation protein.

Exon	Forward Primer Sequence (5′-3′)	Reverse Primer Sequence (5′-3′)
1	GAGCAGCTGAGGAACTTAGCC	CCCACTTTCCAGATTTGAGG
2	TACAAGCCAGGAGAAGGAGC	ATGGACTCCAGGAGACATGG
3	AGAAGGCTCTGAGAGGGAGG TCTGTGGCTGACTTGTTTGC	TGTTGGTGAGTTTGCTGAGG AGAAGGGGGTGAGGTAGGG
4	AACTCACCGCCATCTCTCC TCAACCCGTGCCAGAATG	CTGTCTCCCACCAGCTTCC TCAACACTCTCAGATTGGAAGG
5	CTGTACCAGCTGCTTTTCCC	TCAGTAAAGGTTATGCCCGC
6	AGCTCAGGCAGATGTAACCC	GCTGACCTGCCATTTTTCTC
7	GCCAGATAAGAGGGCAGTTG	CGATAGCAGAGAGGTTTGCC
8	TGACAGGCCAAAGACACAAC ACGTAGTGCCCCTCTGTTTG TCTCCCGGTACATTGAGTGG	GTCCCATCCAGGTAGCCAG GCAGCAGCAGCTTTATTTCC GACGTCCATCTCTCTCAGCC

J Wildl Dis. Author manuscript; available in PMC 2018 April 01.

Table 2

Plasma coagulation profiles and coagulation factor (FII, FV, FVII and FX) activity values in Asian elephants (*Elephas maximus*) from Australian zoos. The elephant designated as the case individual was the subject of an investigation into Factor VII coagulation protein deficiency.

Animal	Age (yr)	PT[a] (s)	PTT[b] (s)	FII activity (%)	FV activity (%)	FVII activity (%)	FX activity (%)	Genotype c.202A> G	Genotyping Method[d]
Case[c]	41	54[c]	17[c]	129	260	2	148	GG	gS, T
Male 2	14	11	17	__[e]	—	73	—	AA	gS, T
Male 3	25	8	11	181	325	100	236	AA	gS
Female 1	20	7	14	133	344	76	138	AA	gS, T
Female 2	14	7	11	101	246	56	115	AA	gS, T
Female 3	12	7	17	90	215	71	116	-	-
Female 4	22	11	—	—	—	69	—	AA	gS, T
Female 5	21	13	23	—	—	50	—	AA	gS, T
Female 6	14	10	16	—	—	64	—	AA	gS, T
Female 7	42	11	17	117	253	94	124	—	—
Female 8	25	8	11	152	325	108	184	—	—
Female 9	57	8	10	133	260	95	146	—	—
Female 10	15	11	17	—	—	60	—	AA	gS, T
Calf 1	4	9	17	107	316	44	134	AG	gS, T
Calf 2	4	11	18	95	275	42	120	AG	gS, T
Calf 3	—	—	—	—	—	—	—	AG	cS
Calf 5	5	12	—	—	—	47	—	—	—
Calf 6	4	13	18	—	—	45	—	AA	gS, T
Calf 7	6	11	19	—	—	59	—	AA	T

[a] Prothrombin time

[b] Partial thromboplastin time

[c] PT and PTT are mean values (n=25)

[d] gS=gDNA sequencing, T=TaqMan assay, cS=cDNA sequencing

[e] —=test not performed

Table 3

Polymorphisms found in the Factor VII (*F7*) gene coding regions of Asian elephants (*Elephas maximus*) held in Australian zoos and their predicted effects, using the *F7* gene in the African elephant (*Loxodonta africana*) as a reference source (NCBI RefSeq ID: NM_001330481.1).

SNP[a]	Codon	Amino acid	SIFT[b]	PROVEAN[b]	Occurrence of SNP
c.202A>G	AGG>GGG	Missense p.Arg68Gly	Not tolerated	Deleterious	Case and his offspring (*n* = 4)
c.300G>C	CTG>CTC	Silent	NA[*]	NA	All sequenced individuals (*n* = 13)
c.386T>A	CTG>CAG	Missense p.Leu129Gln	Tolerated	Neutral	All sequenced individuals (*n* = 12)
c.489T>C	GAT>GAC	Silent	NA	NA	All sequenced individuals (*n* = 12)
c.1161T>C	AGT>AGC	Silent	NA	NA	All sequenced individuals (*n* = 12)

[a] SNP=Single Nucleotide Polymorphism

[b] Software prediction tools: SIFT (http://sift.bii.a-star.edu.sg), PROVEAN (http://provean.jcvi.org/index.php)

[*] NA=not applicable