

Exhibit E



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Venta et al.

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(54) **DNA ENCODING CANINE VON WILLEBRAND FACTOR AND METHODS OF USE**

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(52) **U.S. Cl.** **435/6; 435/91.2; 435/252.3; 435/325; 536/22.1; 536/24.3**

(58) **Field of Search** **435/6, 91.2, 252.3, 435/325; 536/22.1, 24.3**

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(57)

ABSTRACT

The complete sequence of the canine von Willebrand Factor cDNA and deduced amino acid sequence is provided. The mutation which causes von Willebrand's Disease in Scottish Terriers, Doberman pinschers, Shetland sheepdogs, Manchester terriers and Poodles are also provided. Methods for detecting carriers of the defective vWF gene are also provided.

16 Claims, 16 Drawing Sheets

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FIGURE 1A

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1  CATTAAAGG TCCTGGCTGG GAGCTTTTTT TTGGGACCAG CACTCCATGT TCAAGGGCAA
61  ACAGGGGCCA ATTAGGATCA ATCTTTTTTC TTTCTTTTTT TAAAAAAA AAATCTTCCC
121 ACTTTGCACA CGGACAGTAG TACATACCAG TAGCTCTCTG CGAGGACGGT GATCACTAAT
181 CAITTTCTCT GCTTCGTGGC AGATGAGTCC TACCAGACTT GTGAGGGTGC TGCTGGCTCT
241 GCGCCTCATC TTGCCAGGGA AACTTTGTAC AAAAGGGACT GTTGGAAAGT CATCGATGGC
301 CCGATGTAGC CTTCTGGGAG GTGACTTCAT CAACACTTTT GATGAGAGCA TGTACAGCTT
361 TGCGGGAGAT TGCAGTTACC TCCTGGCTGG GGACTGGCCG GAACACTCCA TCTCACTTAT
421 CGGGGGTTTC CAAAATGACA AAAGAGTGAG CCTCTCCGTG TATCTCGGAG AATTTTTCGA
481 CATTCAITTT TTTGTCAATG GTACCATGCT GCAGGGGACC CAAAGCATCT CCATGCCCTA
541 CGCCTCCATG GGGCTGTATC TAGAGGCCGA GGCTGGCTAC TACAAGCTGT CCAGTGAGGC
601 CTACGGCTTT GTGGCCAGAA TTGATGGCAA TGCCAACCTT CAAGTCCCTG TGTACAGACG
661 ATACTTCAAC AAGACCTGTG GGTGTGTGG CAACITTAAT ATCTTTGCTG AGGATGACTT
721 CAAGACTCAA GAAGGGACGT TGACTTCGGA CCCCTATGAC TTTGCCAATC CTTGGGCCCT
781 GAGCAGTGGG GAAACAAGGT GCAAACGGGT GTCCCTCCCT AGCAGCCCAT GCAATGTCTC
841 CTCTGATGAA GTGCAGCAGG TCCTGTGGGA GCACTGCCAG CTCCTGAAGA GTGCCTCGGT
901 GTTTCGCCGC TGCCACCCGC TGGTGGACCC TGAGCCTTTT GTGGCCCTGT GTGAAGGAC
961 TCTGTGCACC TGTGTCCAGG GGATGGAGTG CCGTTGTGGG GTCTCTCTGG AGTACGCCCG
1021 GGCCTGTGCC CAGCAGGGGA TTGTCTTGTG CGGCTGGACC GACCACAGCG TCTGCCGACC
1081 AGCATGCCCT GCTGGCATGG AGTACAAGGA GTGCGTGTCC CCTTGACCA GAACCTGCCA
1141 GAGCCTTCAT GTCAAAGAG TGTGTCAAGA CCAATGTGTA GATGGCTGCA GCTGCCCGCA
1201 GGGCCAGCTC CTGGATGAAG GCCACTGCGT GGGAAAGTCT GAGTGTCTCT GTGTGCATGC
1261 TGSSCAACGG TACCCTCCGG GCGCCTCCCT CTTACAGGAC TGCCACACCT GCATTTGCCG
1321 AATAGCCCTG TGGATCTGCA GCAATGAAGA ATGCCCAGGC GAGTGTCTGG TCACAGGACA
1381 GTCCCACTTC AAGAGCTTCG ACAACAGGTA CTTACCTTTC AGTGGGCTCT CCCACTACCT
1441 GCTGGCCGAG CACTGCCAGG ACCACACATT CTCTGTGTGC ATAGAGACTG TCCAGTGTGC
1501 CGATGACCTG GATGCTGTCT GCACCCGCTC GGTCAACGTC CGCTGCCTG GACATCACAA
1561 CAGCCTTGTG AAGCTGAAGA ATGSGGGAGG AGTCTCCATG GATGGCCAGG ATATCCAGAT
1621 TCCTCTCCTG CAGGCTGACC TCCGCATCCA GCACACCGTG ATGGCCTCCG TGCGCCTCAG
1681 CTACGGGGAG GACCTGCAGA TGGATTCCGA CGTCCGGGGC AGGCTACTGG TGACGCTGTA
1741 CCCCCTTAC GCGGGGAAGA CGTGCGGGCG TGCGGGGAAC TACAACGGA ACCGGGGGA
1801 CGACTTCTGT ACGCCGCGAG GCTGGCGGA GCGCCTGGTG GAGGACTTCG GGAACGCTG
1861 GAAGCTGTCT GGGGCTGCG AGAACCCTGA GAAGCAGCAC CGCGATCCCT GCAGCTCAA
1921 CCGCGCCGAG GCGAGTTTG CGGAGGAGGC GTGCGGCTG CTGACGTCT CGAAGTTCA
1981 GCGCTGCCAC CGAGCGGTGG GTCTCAGCC CTACGTGCG AGCTGCTCT ACAGCTCTG
2041 CTCTGTCTCC GACGGCAGAG ACTGTCTTTG CAGCGCCGTG GCTAACTACG CCGCAGCCGT
2101 GGGCCGGAGG GCGGTGCACA TCGCGTGGCG GGAGCCGGGC TTCTGTGGCG TGAGCTGCCC
2161 CCAGGGCCAG GTGTACCTGC AGTGTGGGAG CCGCTGCAAC ATGACCTGTC TCTCCTCTC
2221 TTACCCGGAG GAGGACTGCA ATGAGGTCTG CTTGGAAAGC TGCTTCTCCC CCCCAGGGCT
2281 GTACCTGGAT GAGAGGGGAG ATTGTGTGCC CAAGGCTCAG TGTCCCTGT ACTATGATGG
2341 TGAGATCTTT CAGCCCGAAG ACATCTTCTC AGACCATCAC ACCATGTGCT ACTGTAGGA
2401 TGGCTTCAAG CACTGTACCA CAAGTGGAGG CCTGGGAAGC CTGCTGCCCA ACCCGGTGCT
2461 CAGCAGCCCC CGGTGTACCC GCAGCAAAAG GAGCCTGTCC TGTGGGCCCC CCATGGTCAA
2521 GTTGTGTGT CCGCTGATA ACCCGAGGGG TGAAGGACTG GAGTGTGCCA AAACCTGCCA
2581 GAACATAGAC CTGCACTGCA TGAGCACAGG CTGTGTCTCC GGCTGCTCT GCGCCGAGG
2641 CATGGTCCGG CATGAAACA GGTGTGTGGC GCTGGAAAGA TGTCCCTGCT TCCACCAAGG
2701 CCAAGAGTAC GCGCCAGGAG AAACCGTGA AATTGACTGC AACACTTGT TCTGTGGGA
2761 CCGGAAGTGG ACCTGCACAG ACCATGTGTG TGATGCCACT TGCTCTGCCA TCGGCATGGC
2821 GCACTACCTC ACCTTCGAGG GACTCAAGTA CTTGTTCCT GGGGAGTCCC AGTATGTTCT
2881 GGTGCAGGAT TACTGCGGCA GTACCCCTGG GACCTTACCG ATCTTGGTGG GGAACGAGGG
2941 GTGCAGTAC CCGTCACTGA AATGCAAGAA GCGGGTCAAC ATCTTGGTGG AAGGAGGAGA
3001 GATTGAAGTG TTTGATGGGG AGGTGAATGT GAAGAAACC ATGAAGGATG AGACTCACTT
3061 TGAGGTGGTA GAGTCTGGTC AGTACGCTAT TCTCTGCTG GCAAGGCAC TCTGTGTGT
3121 CTGGGACCAC CGCCTGAGCA TCTGTGTGAC CCTGAAGCGG ACATACCAGG AGCAGGTGTG

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FIGURE 1B

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3181 TGGCCTGTGT GGGAAATTTG ATGGCATCCA GAACAATGAT TTCACCAGCA GCAGCCTCCA
3241 AATAGAAGAA GACCCTGTGG ACTTTGGGAA TTCTTGGAAA GTGAACCCGC AGTGTGCCGA
3301 CACCAAGAAA GTACCACTGG ACTCATCCCC TGCCGTCTGC CACAACAACA TCATGAAGCA
3361 GACGATGGTG GATTCCTCTT GCAGGATCCT CACCAGTCAT ATTTTCCAGG ACTGCAACAG
3421 GCTGGTGGAC CCTGAGCCAT TCCTGGACAT TTGCATCTAC GACACTTGCT CCTGTGAGTC
3481 CATTGGGGAC TGCACCTGCT TCTGTGACAC CATTGCTGCT TACGCCACAG TCTGTGCCCA
3541 GCATGGCAAG GTGGTAGCCT GGAGGACAGC CACATTCTGT CCCCAGAAAT GCGAGGAGCG
3601 GAATCTCCAC GAGAATGGGT ATGAGTGTGA GTGGCGCTAT AACAGCTGTG CCCCCTGCTG
3661 TCCCATCAGC TGCCAGCACC CCGAGCCCTT GGCATGCCCT GTACAGTGTG TTGAAGGTTG
3721 CCATGCCACG TGCCCTCCAG GGAATACTCT GGATGAGCTT TTGCAGACCT GCATCGACCC
3781 TGAAGACTGT CCTGTGTGTG AGGTGGCTGG TCGTGGCTTG GCCCCAGGAA AGAAAATCAT
3841 CTTGAACCCC AGTGACCCCTG AGCACTGCCA AATTTGTAAAT TGTGATGGTG TCAACTTCAC
3901 CTGTAAAGCC TCCAGAGAAC CCGGAAGTGT TGTGGTGGCC CCCACAGATG GCCCCATTGG
3961 CTCTACCACC TCGTATGTGG AGGACACGTC GGAGCCGGCC CTCCATGACT TCCACTGCAG
4021 CAGGCTTCTG GACCTGGTTT TCCTGTCTGA TGGCTCCTCC AAGCTGTCTG AGGACGAGTT
4081 TGAAGTGTG AAGGTCTTTG TGGTGGGTAT GATGGAGCAT CTGCACATCT CCCAGAGCG
4141 GATCCGCGTG GCTGTGGTGG AGTACCACGA CGGCTCCAC GCCTACATCG AGCTCAAGGA
4201 CCGGAAGCGA CCTCAGAGC TGCGGCGCAT CACCAGCCAG GTGAAGTACG CGGGCAGCGA
4261 GGTGGCCTCC ACCAGTGAGG TCTTAAAGTA CACGCTGTTT CAGATCTTTG GCAAGATCGA
4321 TCGCCCGGAA GCGTCTCGCA TTGCCCTGCT CCTGATGGCC AGCCAGGAGC CCTCAAGGCT
4381 GGGCCGGAAT TTGGTCCGCT ATGTGCAGGG CCTGAAGAAG AAGAAAGTCA TTGTATCCC
4441 TGTTGGGCAT GGGCCCCACG CCAGCCTTAA GCAGATCCAC CTCATAGAGA AGCAGGCCCC
4501 TGCAACAAG GCGTTTGTGT TCAGTGGTGT GGATGAGTTG GAGCAGCGAA GGGATGAGAT
4561 TATCAACTAC CTCTGTGACC TTGCCCCCGA AGCACCTGCC CCTACTCAGC ACCCCCCAAT
4621 GGGCCAGGTC ACGGTGGGTT CGGAGCTGTT GGGGGTTTCA TCTCCAGGAC CCAAAAGGAA
4681 CTCCATGGTC CTGGATGTGG TGTTTGTCTT GGAAGGGTCA GACAAAATTG GTGAGGCCAA
4741 CTTTAAACAA AGCAGGGAGT TCATGGAGGA GGTGATTGAG CGGATGGAGC TGGGCCAGGA
4801 CAGGATCCAC GTCAAGTGC TGCACTACTC GTACTGGTG ACCGTGGAGT ACACCTTCAG
4861 CGAGGCGCAG TCCAAGGCG AGGTCTTACA GCAGTGGCG GATATCCGAT ACCGGGGTGG
4921 CAACAGGACC AACACTGGAC TGGCCCTGCA ATACCTGTCC GAACACAGCT TCTGGTCCAG
4981 CCAGGGGGAC CGGGAGCAGG TACCTAACCT GGTCTACATG GTCACAGGAA ACCCCGCTTC
5041 TGATGAGATC AAGCGGATGC CTGGAGACAT CCAGGTGGTG CCCATCGGGG TGGGTCCACA
5101 TGCCAATGTG CAGGAGCTGG AGAAGATTGG CTGGCCCAAT GCCCCCATCC TCATCCATGA
5161 CTTTGAGATG CTCCCTCGAG AGGCTCTGTA TCTGGTGCTA CAGAGGTGCT GCTCTGGAGA
5221 GGGGCTGCAG ATCCCCACCC TCTCCCCCAC CCCAGATTGC AGCCAGCCCC TGGATGTGGT
5281 CCTCTCTCTG GATGGCTCTT CCAGCATTCG AGCTTCTTAC TTTGATGAAA TGAAGAGCTT
5341 CACCAAGGCT TTTATTTCAA GAGCTAATAT AGGGCCCCGG CTCACTCAAG TGTGGGTGCT
5401 GCAATATGGA AGCATCAGCA CTATCGATGT GCCTTGGAAAT GTAGCCTATG AGAAAGTCCA
5461 TTTACTGAGC CTTGTGGACC TCATGCAGCA GGAGGGAGGC CCCAGCGAAA TTGGGGATGC
5521 TTTGAGCTTT GCGGTGCGAT ATGTCACTTC AGAAGTCCAT GGTGCCAGGC CCGGAGCCTC
5581 GAAAGCGGTG GTTATCCTAG TCACAGATGT CTCGGTGGAT TCAGTGGATG CTGCAGCCGA
5641 GGGCGCCAGA TCCAACCGAG TGACAGTGTT CCCCATTGGA ATCGGGGATC GGTACAGTGA
5701 GGGCCAGCTG AGCAGCTTGG CAGGCCCAA GGTGGCTTCC AATATGGTAA GGCTCCAGCG
5761 AATTGAAGAC CTCCCCACCG TGGCCACCTT GGGAAATTC TCTTCCACA AGCTGTGCTC
5821 TGGGTTTGAT AGAGTTTGGC TGGATGAGGA TGGGAATGAG AAGAGGCCCG GGGATGTCTG
5881 GACCTTGCCA GACCAGTGGC ACACAGTGAC TTGCCTGCCA GATGGCCAGA CCTGTCTGAA
5941 GAGTCATCGG GTCAACTGTG ACCGGGGGCC AAGGCCCTCG TGCCCAATG GCCAGCCCCC
6001 TCTCAGGTA GAGGAGACCT GTGGCTGCCG CTGGACCTGT CCCTGTGTGT GCATGGGCGA
6061 CTCTACCCGG CACATCTGTA CCTTTGATGG GCAGAAATTC AAGCTGACTG GCAGCTGTTT
6121 GTATGTCTTA TTTCAAAACA AGGAGCAGGA CCTGGAGGTG ATTCTCCAGA ATGGTGCCTC
6181 CAGCCCTGGG CGGAAGCAGA CCTGCAATGA ATCCATTGAG GTGAAGCATG ACCGGCCTCTC
6241 AGTTGAGCTC CACAGTGACA TGCAGATGAC AGTGAAATGG AGACTAGTCT CCATCCATA
6301 TGTGGGTGGA CACATGGAAG TCAATGTTTA TGGGACCATC ATGTATGAGG TCAGATTCAA
6361 CCATCTTGGC CACATCTTCA CATTACCCCC CCAAAACAAAT GAGTTCAGC TGCAGCTCAG

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FIGURE 1C

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6421 CCCCAGGACC TTGCTTCCA AGACATATGG TCTCTGTGGG ATCTGTGATG AGAACCGAGC
6481 CAATGACTTC ATTCTGAGGG ATGGGACAGT CACCACAGAC TGGAGGGCAC TCATCCAGGA
6541 ATGGACCGTA CAGCAGCTTG GGAAGACATC CCAGCCTGTC CATGAGGAGC AGTGTCCTGT
6601 CTCGGAATTC TTCCACTGCC AGGTCTCTCT CTCAGAAATG TTTGCCGAGT GCCACAAGGT
6661 CCTCGCTCCA GCCACCTTTT ATGCCATGTG CCAGCCCCGAC AGTTGCCACC CGAAGAAAGT
6721 GTGTGAGGCG ATTGCTTGT ATGCCACCT CTGTGGGACC AAAGGGGTCT GTGTGGACTG
6781 CAGCAAGGCC AATTTCTGTG CTATGTATG TCCACCATCC CTGGTGTACA ACCACTGTGA
6841 GCATGGCTGC CCTGGCTCT GTGAAGGCAA TACAAGCTCC TGTGGGGACC AACCTCGGA
6901 AGGCTGCTTC TGCCCCCAA ACCAAGTCAT GCTGGAAGGT AGCTGTGTCC CCGAGGAGGC
6961 CTGTACCCAG TGCATCAGCG AGGATGAGT CCGGCACCAG TTCTTGAAA CCTGGGTCCC
7021 AGCCACCCAG CTTGCCAGA TCTGCACGT CCTCAGTGGG CGGAAGGTCA ACTGTACGTT
7081 GCAGCCCTGC CCCACAGCCA AAGCTCCAC CTGTGGCCCG TGTGAAGTGG CCGCCTCCG
7141 CCAGAACGCA GTGCAGTGT GCGCGAGTA CGAGTGTGTG TGTGACCTGG TGAGCTGTGA
7201 CCTGCCCCCG GTGCTCTCT GCGAAGATGG CTTCCAGATG ACCCTGACCA ATCTGGCGCA
7261 GTGCAGACCC AACTTCACT GTGCTGCAG GAAGGATGAA TGCAGACGGG AGTCCCCGCC
7321 CTCTTGTCCC CCGCACCGGA CGCCGGCCCT TCGGAAGACT CAGTGTCTGT ATGAGTATGA
7381 GTGTGCATGC AACTGTGTCA ACTCCACGGT GAGCTGCCCG CTTGGGTACC TGGCCTCGGC
7441 TGTACCAAC GACTGTGGCT GCACCACAC AACCTGCTTC CCTGACAGG TGTGTGTCCA
7501 CCGAGGCACC ATCTACCTG TGGGCCAGTT CTGGGAGGAG GCCTGTGACG TGTGCACTG
7561 CACGCACTTG GAGGACTCTG TGATGGGCTT GCGTGTGGCC CAGTGTCTCC AGAAGCCCTG
7621 TGAGGACAA TGCTGTCA GCTTCACTTA TGTCTTCAT GAGGGCGAGT GGTGTGAAG
7681 GTGTCTGCCA TCTGCTGTG AGGTGTCTAC TGGTTCACCA CGGGGCGAGC CCACTCTCA
7741 CTGGAAGAAT GTTGGCTCTC ACTGGGCTC CCCTGACAA CCCTGCCCTCA TCAATGAGTG
7801 TGTCCGAGTG AAGGAAGAGG TCTTTGTGCA ACAGAGGAAT GTCTCTGTC CCCAGCTGAA
7861 TGTCCCCACC TGCCCCACGG GCTTCCAGCT GAGCTGTAAG ACCTCAGAGT GTTGTCCAC
7921 CTGTCACTGC GAGCCCTGG AGGCCTGCTT GCTCAATGT ACCATCATG GSCCGGGAA
7981 AAGTCTGATG ATTGATGTGT GTACACCTG CCGCTGCACC GTGCCGGTGG GAGTCACTC
8041 TGGATTCAAG CTGGAGGGCA GGAAGACCAC CTGTGAGGCA TGCCCCCTGG GTTATAAGGA
8101 AGAGAAGAAC CAAGGTGAAT GCTGTGGGAG ATGTCTGCCT ATAGCTTGA CCATTCAGCT
8161 AAGAGGAGGA CAGATCATGA CACTGAAGCG TGATGAGACT ATCCAGGATG GCTGTGACAG
8221 TCACTTCTGC AAGGTCAATG AAGAGGAGA GTACATCTGG GAGAGAGAG TCAAGGTTG
8281 CCCACCTTTC GATGAACACA AGTGTCTGGC TGAGGGAGGA AAAATCATGA AATTCAGG
8341 CACCTGCTGT GACACATGTG AGGAOCCAG ATGCAAGGAT ATCATTGCCA AGCTGCAGCG
8401 TGTCAAAGTG GGAGACTGTA AGTCTGAAGA GGAAGTGGAC ATTCACTACT GTGAGGTAA
8461 ATGTGCCAGC AAGGCCGTGT ACTCCATCCA CATGGAGGAT GTGCAGGACC AGTGCTCTCT
8521 CTGCTCGCCC ACCCAGACGG AGCCCATGCA GGTGGCCCTG CGCTGCACCA ATGGCTCCCT
8581 CATCTACCAT GAGATCCTCA ATGCCATGCA ATGCAGGTGT TCCCCAGGA AGTGCAGCAA
8641 GTGAGGCCAC TGCTTGGATG CTACTGTCTG CTGCTTACC CGACCTCACT GCACTGGCCA
8701 GAGTGTCTCT CAGTCTCTCT CAGTCTCTCT CCTGCTCTGC TCTTGTGCTT CCTGATCCCA
8761 CAATAAAGGT CAATCTTCA CCTTGAJAAA AAAAAAAAAA AA

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Human Dog	MIPARFAGVLLALALILPGLTCAEGTRGRSSTARCSLFGSDFVNTFDGSMYSFAGYCSYL -S-T-LVR-----K-TK--V----N-----L-G--I----E-----D----	60
Human Dog	IAGGCQFRSFSYIGDFQNGKRVSLSVYLGEFFDIHLFVNGTIVTQGDQRVSMPIYASKGLYL ---D--EH-I-L-G--D-----KL--T-SI-----N----	120
Human Dog	ETEAGVYKLSGEAYGFVARIDGSGNFQVLLSDRYFNITCGLCGNFNIFAEDDFMTQEGTL -A-----S-----N-----K-----	180
Human Dog	TSDPYDFANSHALSSGEQNCERASFPSSSCNISSGEMQKGLMEQCQLLKSTSVFARCHPL -----R-X-V-----P--V--D-V-QV-----A-----	240
Human Dog	VDPEFFVALCEKTLCECAGGLECACPALLEYARTCAQEGHVLVYGNTHSACSFPVCPAGME -----R---T-VQ-M--P-AV-----A---Q-I-----V-R-A-----	300
Human Dog	YROCVPACARTQSLHINEMCOERCVDGSCPEGQLLDEGLCVESTECPCVHSGKRYPPG -KE-----T-----VK-V--Q-----H--G-A--S---A-Q-----	360
Human Dog	TSLSRDNTICIRNSQWICSNEECPGECVLTQGSFHSFDNRYFTFSGICQVLLARDCOD A--LQ--H-----L-----V-H---Q----	420
Human Dog	HSFSIVIEIVQCADRDVCTRSVTVRLPGLHNSLVKLYEGAGVAEDGQDVQLPLKGD -T--V-----L-----R-----N-G--S-----I-Y--Q---	480
Human Dog	RIQHTVITASVRLSYGEDLQNDWGRGRLLVKLSPVYAGKTCGLCGNYNGQDDFLTPSG -----M-----S-V-----T-Y-A-----RG-----R---V--A-	540
Human Dog	LAEPRVEDFGNAWKLGCCQDLQKQHSDPICALNPFMTTFSEEACAVLTSPTFEACHRAVS ---L-----L-A-EN-----R---S---QA--A---L--SK--P-----G	600
Human Dog	PLPYLRNCRYDVCSGSGRECLCGALASYAACAGRGVRVAKREPGRCELNCPKQGVYLO -Q--VQ--L-----D---S-V-N---V-R--NI-----F-A-S--Q-----	660
Human Dog	CGTPCHLTCSRSLSYFDEECHEACLEGCPFPGLVDERGECVPAKQCPCYDGEIFQPED -----M--L-----E-D--V--S--S---L-----	720
Human Dog	IFSDHNTMCYCEDGFMCTMSGVPGSLLPDAVLSSPLSHRSKRSLSRPPMVKLVCPADN -----T--GL-----NP-----RC-----	780
Human Dog	LRAEGLECTKTCQNYDLECMHGCVSQCCLPPGNVRHENACVALERCPCFHQKEYAPGE P-----A-----Q---T-----Q-----Q-----	840
Human Dog	TVKIGCNTCVCRDRKNCIDHVCATCSTIGHMYLTFDGLKYLFPGECCQVVLVQDYCGS ---D-----T-----A-----	900
Human Dog	NPGTFRILVGNKGCSEHPSVKCIGRVITLVEGGEIELFDGEVNVKRPKDETHFEVVESGR ---L-----E--Y-----K-----Q-----	960
Human Dog	YIILLGKALSVVWDRHLSISVVLKQTYQEKVCGLCGNFDGIONNDLTSSNLQVEEDFVD -V-----HR-----T--R---Q-----F--S--I-----	1020
Human Dog	FGSNKVVSSQCADTRKVPDSSPATCHNNHXQTHVDSSCRILTSDVFQDCNKLVDPEPY -----NP-----K-----V-----I-----R-----F-----	1080

FIGURE 2A

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Human Dog	LDVCIYDTCSCEISIGDCACFCOTIAAYARVCAQHGKVVTHRTATLCPQSCEENLRENGY --I-----T-----A-----F--N-----K----	2140
Human Dog	ECENRYNSCAPACQVTCQHFELACFVQCVEGCHANCPPGKILDELLQTCVDPEDCPVCE -----PI-----I-----	2200
Human Dog	VAGRRFASGKVKTLKPSDFEHCOICHCDVVMKTCEACQEPGGLVVPFDAPVSPTILYVE -----L-P--II-----N--G--F--K--R--SV-----G-IGS--S---	2260
Human Dog	DISEPPLKDFYCSRLDLVFLDGSSRLSEAEFEVLKAFVWDMERLRISQKVRVAVVE -T-----H-----K--D-----V--G--H-K--RI-----	2320
Human Dog	YHDSKRAYIGLNDKRPSELRRISQVKYAGSQVASTSEVLKXITLFOIFS KIDRPEASKI -----E-----T-----E-----G-----	2380
Human Dog	ALLMASQEPQKMSRNFVRYVQGLKKKKVIVIPVGIGPEANLKQIRLIEKQAPENKAFVL -----S-LA--L-----S-----H-----F	2440
Human Dog	SSVDELEQQRDEIVSYLCLAFAPPTLPENAGQVTGPGLLGVSTLGPKNMSVLDVA -G-----R--IN-----A--QH-P-----SE-----SP-----V	2500
Human Dog	FVLEQSDKIGEADFNRSKEFMEEVIQRMDVGQDSIRVTVLQYSKMTVEYFFSEAQSKGD -----N--K-R-----R-----T-----E	2560
Human Dog	ILQVRREIRYQGGNRTNTGLALRYLSDHSFLVSQGDREQAPNLVYMTGNPASDEIKPLP V--Q--D--R-----Q--E--S-----V-----M-	2620
Human Dog	GDIQVVPICVGPANAVQELERIGWPNAPILIQDFETLPREAPDLVLRCCSGEGLQIPTL -----H-----K-----H--M-----	2680
Human Dog	SPAPDCSQPLDVILLDDGSSSFPASYFDEKSFAXAFISKANIGPRLTQVSVLQYGSITT --T-----V-----I-----T-----R-----	2740
Human Dog	IDVPFNVPPEKAHLLSLVDVMQREGGFSQIGDALGFVAVRYLTSEMHGARGASKAVVILV -----AY--V-----L--Q-----E-----S-----V--V-----	2800
Human Dog	TDVSVDSVDAADAARSNRVTVPFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDLPTM -----E-----SE--SS--KAG--H-R-----V	2860
Human Dog	VTLGNSFLKRLCSGFVRICMDEEDGNEKRPDGVWTLPDQCHTVTCQPDGQTLLKTHRVNCD A-----F-----D-V-V-----L-----S-----	2920
Human Dog	RGLRPSCPNSQSPVKVEETCGCRHTCPVCCTGSSTRHTVTFDQGNFKLTGSCSYVLQNK --P-----G-P-LR-----M-----	2980
Human Dog	EQDLEVILNGACSPGARQGCCHKSEIVCHSALSVELHSDMEVTNGLVSVVPYVGGMMEV -----Q-----KET-----DG-----QH-----I-----D---	3040
Human Dog	NVYGAINGEVRFNHLGHIFFTPQNNFQLQLSPKTFASKTYGLCGICDENGANDFMRLD ----T--Y-----R-----I---	3100
Human Dog	GTVTIDWKTLVQEWTVQRPQOTCOPILEEQCLVFDSSHCOVLLPLFAECHKVLAPATFY -----A-I-----QL-K-S--VH--P-SEFF-----SE-----	3160

FIGURE 2B

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Human	AICQQDSCHQEQVCEVTASYAHLCRTNGVCVDWRTPDFCAMSCPPSLVYNHCEHGCPRHG	2220
Dog	-M--P-----PKK---A--L-----K-----RAN-----L-	
Human	DGNVSSCGDHPSEGCFCPPDKVMLEGSVPPEACTQCIGEDGVQHQFLEAWVPDHPQCI	2280
Dog	E--T-----Q-----NQ-----S-----R-----T---A-----	
Human	CTCLSGRKVNCTTQPCPTAKAPTCLCEVARLRQADQCCPEYECVCDPVSCDLPFVPHG	2340
Dog	-----L-----P-----V-----L-----P-	
Human	ERGLQPTILTNPGECPNFTICACRKEECKRVSPFSCPPHRLPTLRKTQCCDEYECACNCVN	2400
Dog	-D--H-----D--R-E-----T-A-----	
Human	STVSCPLGYLASTATNDGCGTTTTCLDPKVCVHRLSTIYFVGQFWEEGCDVCTCTMEDAV	2460
Dog	-----AV-----F-----G-----A-----L--S-	
Human	MGLRVAQCSQKPCEDSCRSGFYVLHEGECGGRCLPSACEVVTGSHRGLSQSSWKSVMGSD	2520
Dog	-----N-L-----A--H--N--H	
Human	WASPENPCLINECVRVKEEVFIQQRNVSCPOLEVPCPSGFQLSCNTSACCPSCRCEPME	2580
Dog	---D-----V-----N--T--T-----E--T-H--PL-	
Human	ACHLNGTVIGPGKTVMIDVCTTCRCMVQGVVISGFKLECRKITCNPCPLGYKEENNTGEC	2640
Dog	--L---I---SL-----T-P-----G-----EA-----K-Q---	
Human	CGRCLPTACTIQLRGGQINTLKRDETLDGCDTHFCKVNERGEYFWKRVGTGCPFFDEHK	2700
Dog	-----I-----I-----S-----I-----	
Human	CLAEGGKIMKIFGTCCDTCEEPECNDITARLQYVVGVSCKSEVEVDIHYCQGXKASKAMY	2760
Dog	-----X--I-K--R---D---E-----E-----V-	
Human	SIDINDVQDQCSCCSPTRTEPMQVALHCTNGSVVYHEVLNWECKKCSPRKCSK	2813
Dog	--HME-----Q-----R-----LI---I---I--R-----	

FIGURE 2C

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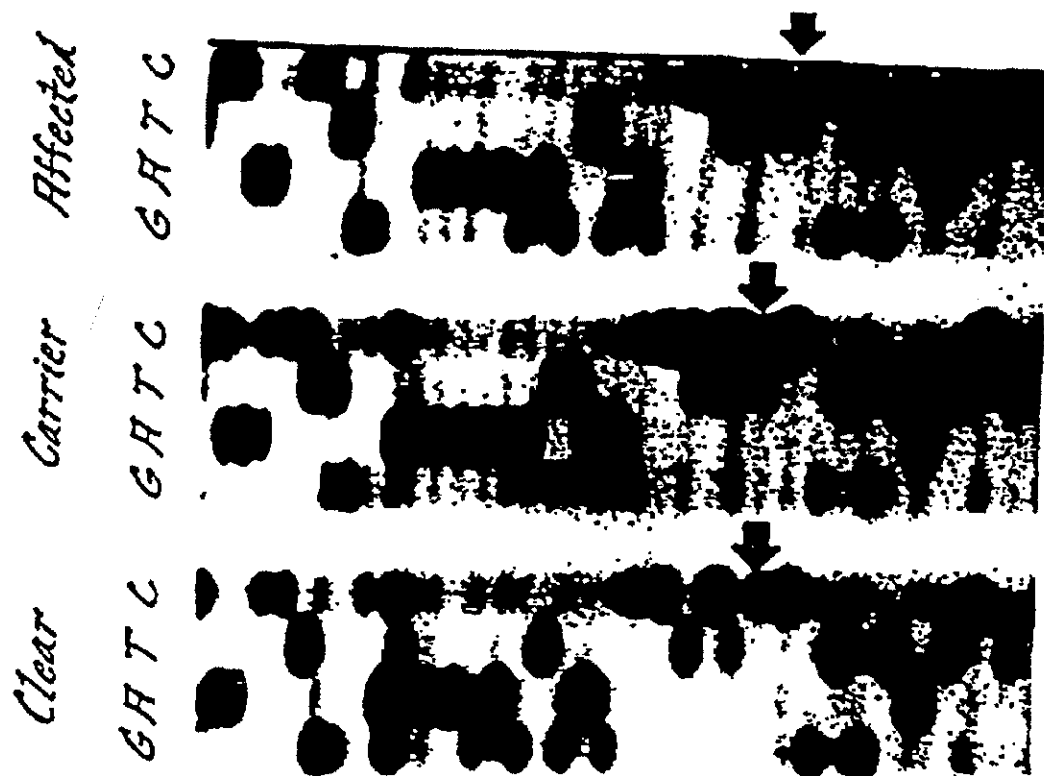


FIG. 2

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exon 4 AAATGACAAAAGAGTGAGCCCGTC*

AGGGGTTTTCCAAAATGACAAAAGAGTGAGCCTCTCCGTGTATCTCGGAGAATTTTTCGA
G G F Q N D K R V S L S V Y L G E F F D

CATTCATTTGTTTGTCAATGGTACCATGCTGCAGGGGACCCAAAGGTAAGTCAGAAGCCC
I H L F V N G T M L Q G T Q R

GAATGTTCAGGTTAATATGGACCCCTGGGGATCACTTTGCAACCCCCTTGTTTTTTTCAGAT

GAGGGAGCCGGGGCCAGAGACAGGAAGTAAATGTGCCCAGGGAAAGTGAGTGGCAGGAC

TGGGTGAAAGCCCCATATCCCGACTCCTGGTCAAGGAGACTTTGCACCAAGGTCCCAGCC
3'-GGGCTGGCGACCAAGTTCCTCTGAA-5'

CTGGAGCATGGGGTTGGGGTTGGAAGGTGGAGGGACATGGAGGAAATGCATGAGAAGCAC

exon 5

GCTTCCTGAGCTCCTCCTTGTCACCAGCATCTCCATGCCCTACGCCTCCAATGGGC
I S M P Y A S N G

FIGURE 4

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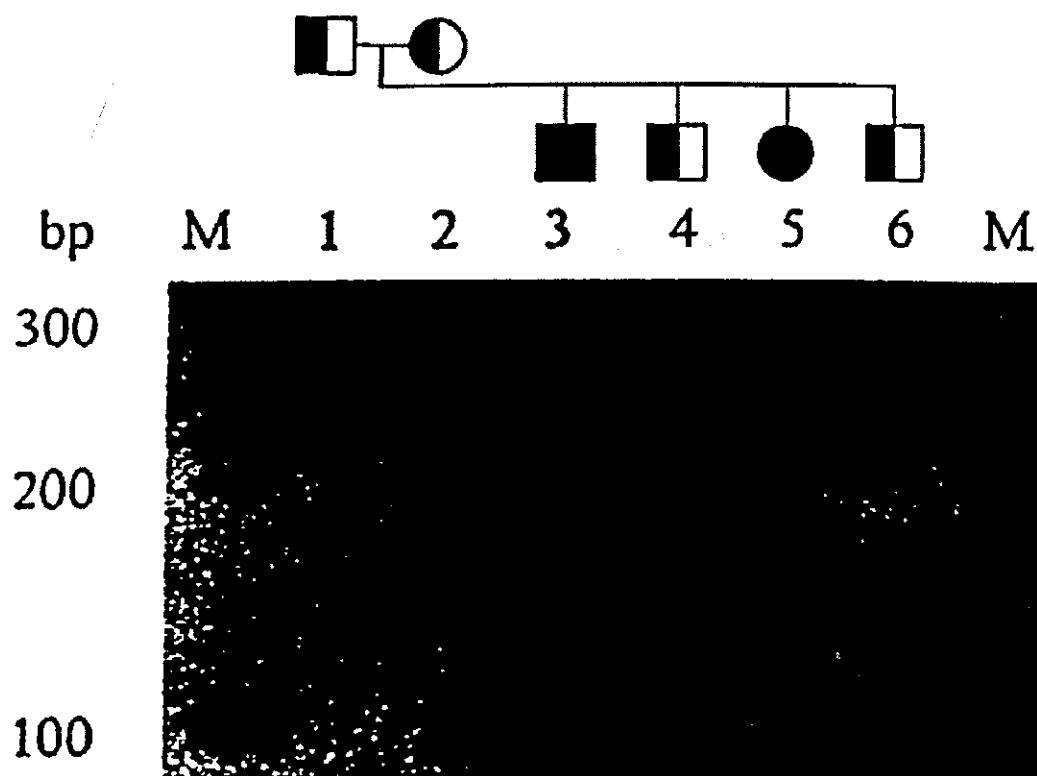


FIG. 5

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Normal Allele

Exon 43

Intron 43

Exon 44

★
AGGACAAC**TGCCTGCCTGTC**Ggtgagtggg ... GGCTTCACTTAT
|||||||
AGGTRAGT Donor Consensus

Mutant Allele

★
AGGACAAC**TGCCTGCCTgtc**agtgagtggg ... GGCTTCACTTAT
|| |||
AGGTRAGT Donor Consensus

Figure 6

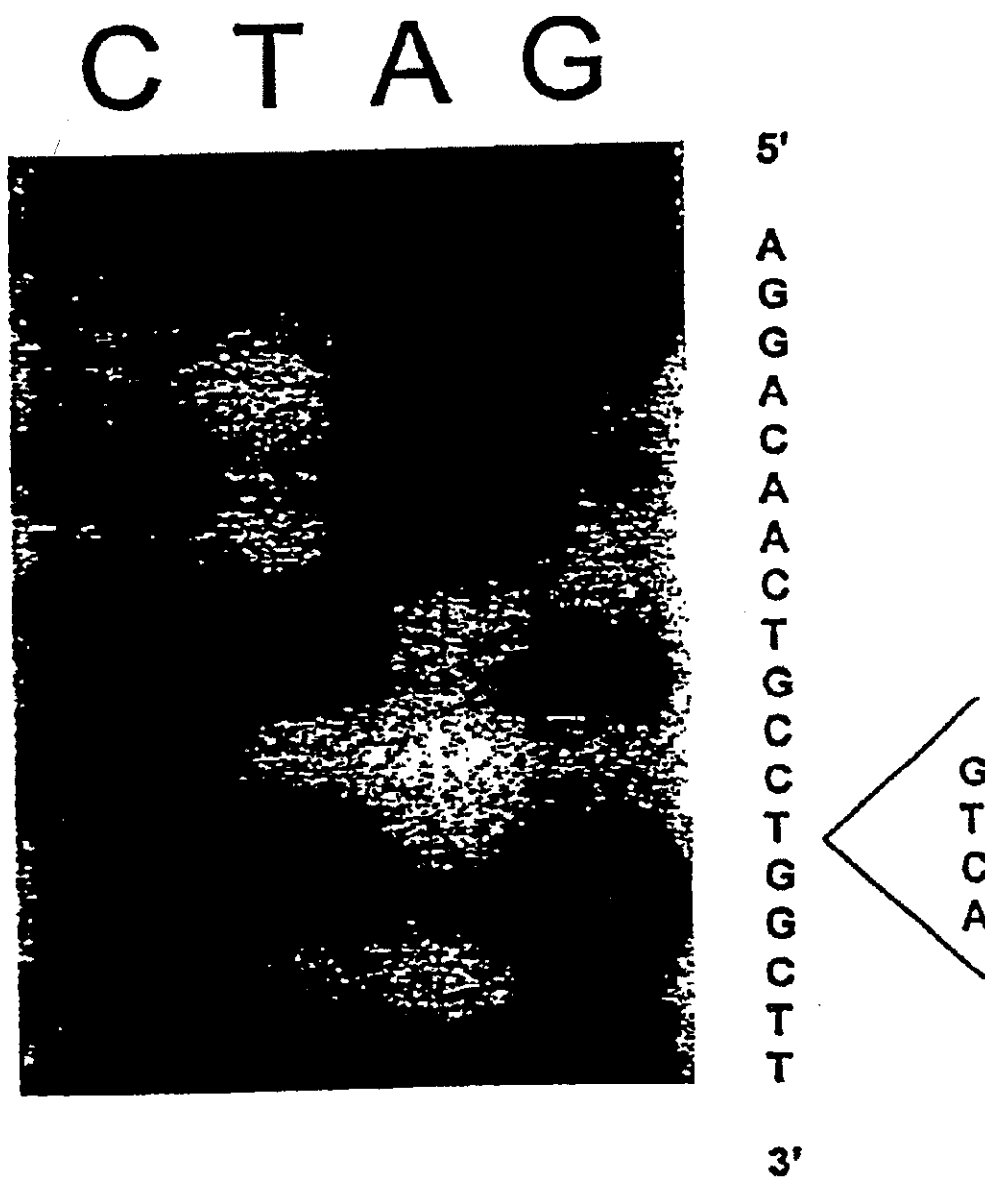
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Figure 7



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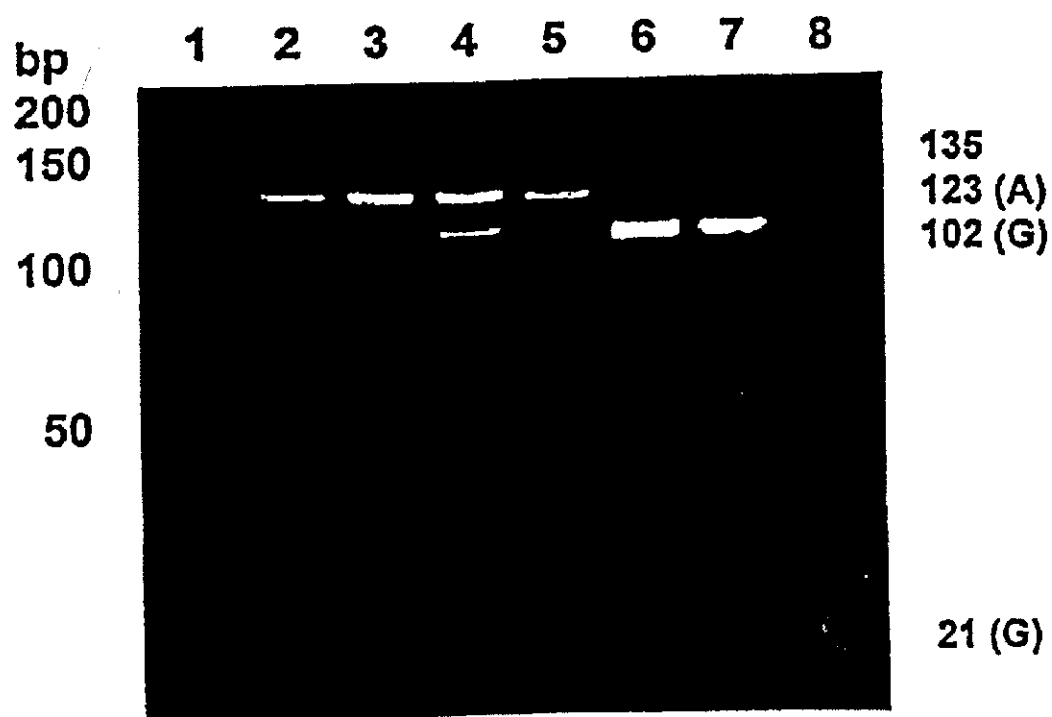


Figure 8

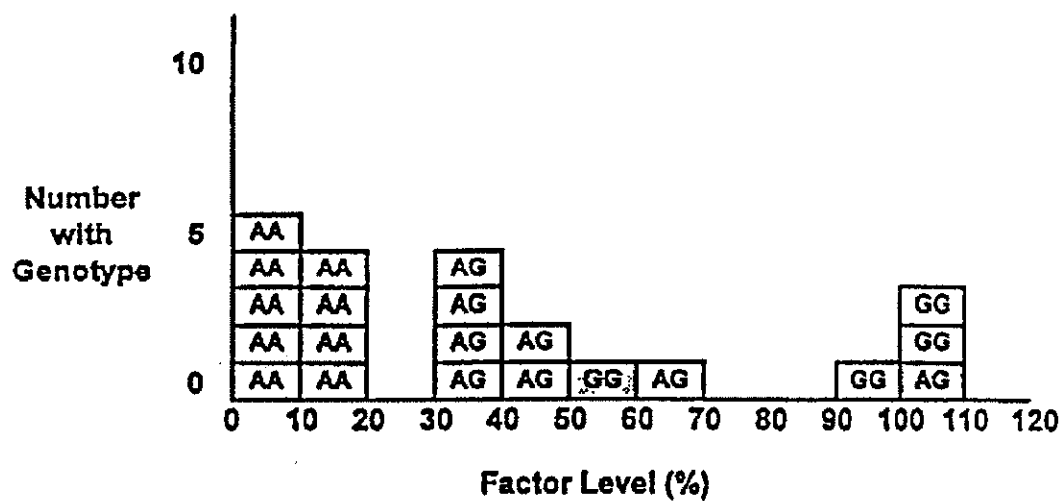
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Figure 9



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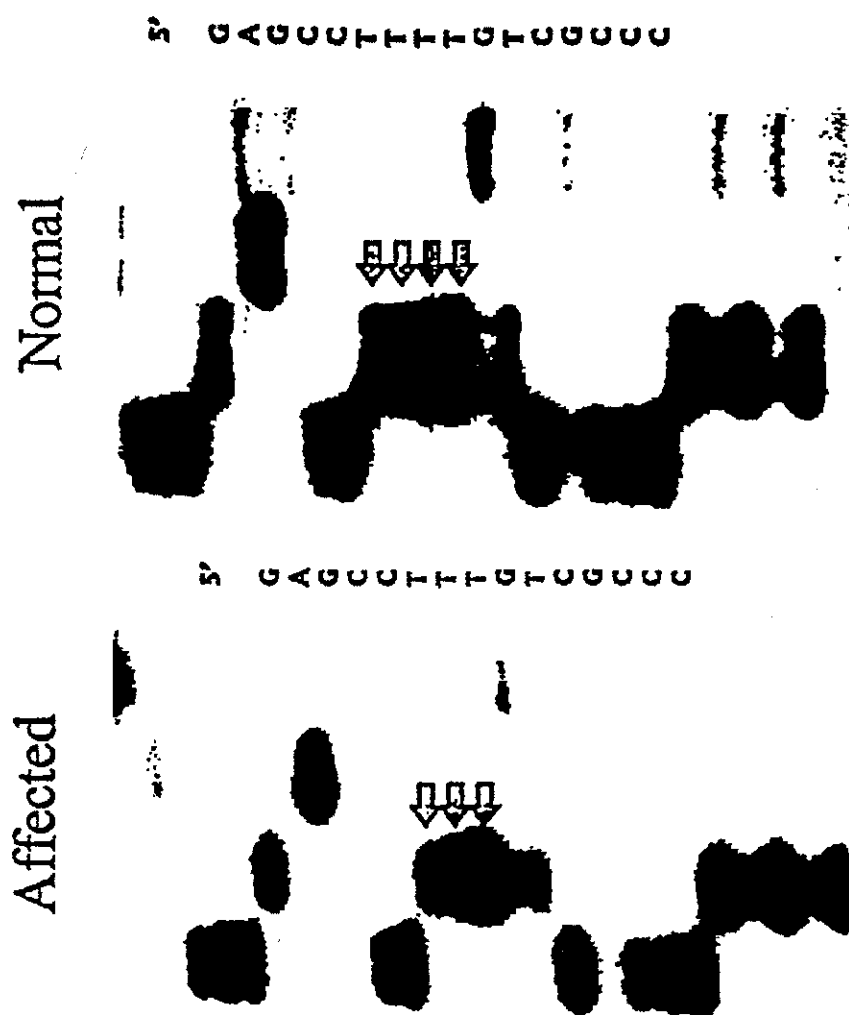


Figure 10

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Exon 7

V L W E Q C Q L L K S A S V F A R C H P L V
 GTCCTGTGGAGCAGTGCCAGCTCCTGAAGAGTGCCCTGGTGTTCCTCCCTGCCACCCGCTGGTG
 TCCTGTGGAGCAGTGCCAG
 DVWFEX7D GCNNNNNNGC Mwo I

D P E P F V A L C E R T L C T C V Q G M E C
 GACCTGAGCCCTTTGTGCGCCCTGTGTGAAGGACTCTGTGCACCTGTGTCCAGGGGATGGAGTGC
 GCNNNNN-NNNGC Mwo I
 A735

P C A V L L E Y A R A C A Q Q G I V L Y G W
 CCTTGTGGGTCCCTCCTGGAGTAGCCCGGCTGTGCCAGCAGGAATTGTCTGTACGGCTGG
 ATGCCGACC

T D H S V C R
 ACCGACCACAGCGTCTGCCG
 TGGCTGGTG-5'
 DVWFEX7U

Figure 11

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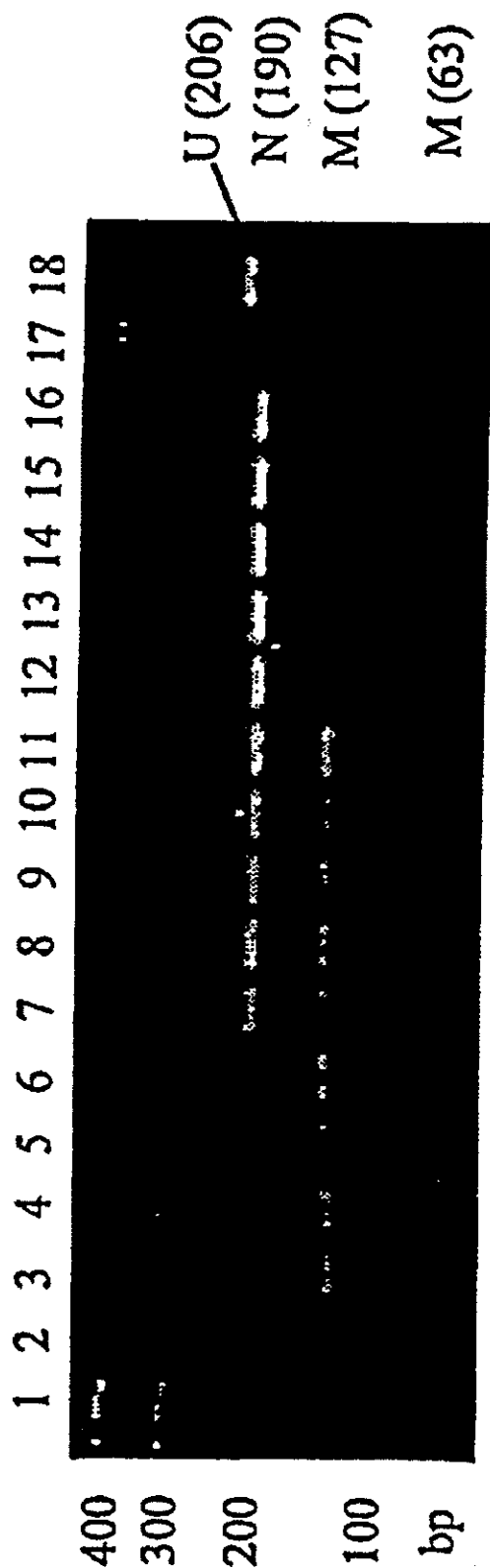


Figure 12

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DNA ENCODING CANINE VON WILLEBRAND FACTOR AND METHODS OF USE

RELATED APPLICATIONS

The present invention is a continuation-in-part of U.S. Ser. No. 08/896,449, filed Jul. 18, 1997, now U.S. Pat. No. 6,040,143 which claims priority from U.S. Ser. No. 60/020,998, filed Jul. 19, 1996, both hereby expressly incorporated by reference.

The present application claims priority to PCT patent application serial number PCT/US99/18153, filed on Aug. 10, 1999, which claims priority to U.S. Pat. No. 6,074,832, issued Jun. 13, 2000.

FIELD OF THE INVENTION

This invention relates generally to canine von Willebrand factor (vWF), and more particularly, to the gene encoding vWF as well as a genetic defect that causes canine von Willebrand's disease.

BACKGROUND OF THE INVENTION

In both dogs and humans, von Willebrand's disease (vWD) is a bleeding disorder of variable severity that results from a quantitative or qualitative defect in von Willebrand factor (vWF) (Ginsburg, D. et al., *Blood* 79:2507-2519 (1992); Ruggeri, Z. M., et al., *FASEB J* 7:308-316 (1993); Dodds, W. J., *Mod Vet Pract* 681-686 (1984); Johnson, G. S. et al., *JAVMA* 176:1261-1263 (1988); Brooks, M., *Probl In Vet Med* 4:636-646 (1992)). This clotting factor has two known functions, stabilization of Factor VIII (hemophilic factor A) in the blood, and aiding the adhesion of platelets to the subendothelium, which allows them to provide hemostasis more effectively. If the factor is missing or defective, the patient, whether human or dog, may bleed severely.

The disease is the most common hereditary bleeding disorder in both species, and is genetically and clinically heterogenous. Three clinical types, called 1, 2, and 3 (formerly I, II, and III; see Sadler, J. E. et al., *Blood* 84:676-679 (1994) for nomenclature changes), have been described. Type 1 vWD is inherited in a dominant, incompletely penetrant fashion. Bleeding appears to be due to the reduced level of vWF rather than a qualitative difference. Although this is the most common form of vWD found in most mammals, and can cause serious bleeding problems, it is generally less severe than the other two types. In addition, a relatively inexpensive vasopressin analog (DDAVP) can help alleviate symptoms (Kraus, K. H. et al., *Vet Surg* 18:103-109 (1989)).

In Type 2 vWD, patients may have essentially normal levels of vWF, but the factor is abnormal as determined by specialized tests (Ruggeri, Z. M., et al., *FASEB J* 7:308-316 (1993); Brooks, M., *Probl In Vet Med* 4:636-446 (1992)). This type is also inherited in a dominant fashion and has only rarely been described in dogs (Turrentine, M. A., et al., *Vet Clin North Am Small Anim Pract* 18:275 (1988)).

Type 3 vWD is the most severe form of the disease. It is inherited as an autosomal recessive trait, and affected individuals have no detectable vWF in their blood. Serious bleeding episodes require transfusions of blood or cryoprecipitate to supply the missing vWF. Heterozygous carriers have moderately reduced factor concentrations, but generally appear to have normal hemostasis.

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Scottish terriers have Type 3 vWD (Dodds, W. J., *Mod Vet Pract* 681-686 (1984); Johnson, G. S. et al., *JAVMA* 176:1261-1263 (1988)). Homozygotes have no detectable vWF and have a severe bleeding disorder. Heterozygotes have reduced levels of the factor, and are clinically normal (Brooks, M. et al., *JAVMA* 200:1123-1127 (1992)). The prevalence of vWD among Scottish terriers including both heterozygotes and homozygotes has been variously estimated from 27-31% (Stokol, T. et al., *Res. Vet. Sci.* 59:152-155 (1995); Brooks, M., *Proc. 9th ACVIM Forum* 89-91 (1991)).

Currently, detection of affected and carrier Scottish terrier dogs is done by vWF antigen testing (Benson, R. E. et al., *Am J Vet Res* 44:399-403 (1983); Stokol, T. et al., *Res. Vet. Sci.* 59:152-155 (1995)) or by coagulation assays (Rosborough, T. K. et al., *J. Lab. Clin. Med.* 96:47-56 (1980); Read, M. S. et al., *J. Lab. Clin. Med.* 101:74-82 (1983)). These procedures yield variable results, as the protein-based tests can be influenced by such things as sample collection, sample handling, estrous, pregnancy vaccination, age, and hypothyroidism (Strauss, H. S. et al., *New Eng J Med* 269:1251-1252 (1963); Bloom, A. L., *Mayo Clin Proc* 66:743-751 (1991); Stirling, Y. et al., *Thromb Haemostasis* 52:176-182 (1984); Mansell, P. D. et al., *Br. Vet. J.* 148:329-337 (1992); Avgeris, S. et al., *JAVMA* 196:921-924 (1990); Panciera, D. P. et al., *JAVMA* 205:1550-1553 (1994)). Thus, for example, a dog that tests within the normal range on one day, can test within the carrier range on another day. It is therefore difficult for breeders to use this information.

It would thus be desirable to provide the nucleic acid sequence encoding canine vWF. It would also be desirable to provide the genetic defect responsible for canine vWD. It would further be desirable to obtain the amino acid sequence of canine vWF. It would also be desirable to provide a method for detecting carriers of the defective vWF gene based on the nucleic acid sequence of the normal and defective vWF gene.

SUMMARY OF THE INVENTION

The present invention provides a novel purified and isolated nucleic acid sequence encoding canine vWF. Nucleic acid sequences containing the mutations that cause vWD in Scottish terriers, Doberman pinschers, Shetland sheepdogs, Manchester terriers and Poodles are also provided. The nucleic acid sequences of the present invention may be used in methods for detecting carriers of the mutation that causes vWD. Such methods may be used by breeders to reduce the frequency of the disease-causing allele and the incidence of disease. In addition, the nucleic acid sequence of the canine vWF provided herein may be used to determine the genetic defect that causes vWD in other breeds as well as other species.

Additional objects, advantages and features of the present invention will become apparent from the following description, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and by referencing the following drawings in which:

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FIGS. 1A-1C is the nucleic acid sequence of the canine von Willebrand factor of the present invention (SEQ ID NO: 1);

FIGS. 2A-2C is a comparison of the human and canine prepro-von Willebrand factor amino acid sequences (SEQ ID NO: 2);

FIG. 3 provides nucleotide sequencing ladders for the von Willebrand's disease mutation region for normal (clear), carrier, and affected Scottish terriers, the sequences being obtained directly from PCR products derived from genomic DNAs in exon 4;

FIG. 4 illustrates the results of a method of the present invention used to detect the Scottish terrier vWD mutation (SEQ ID NOS: 3-13);

FIG. 5 shows the Scottish terrier pedigree, which in turn illustrates segregation of the mutant and normal vWF alleles;

FIG. 6 is an illustration showing the splice site comparison between normal and mutant Doberman pinscher vWF alleles (SEQ ID NOS: 14-17);

FIG. 7 is a photograph of a sequencing ladder showing the cryptic splice site from the mutant allele (SEQ ID NO: 18);

FIG. 8 is a photograph of an agarose gel showing representative results of the PCR-based diagnostic test;

FIG. 9 is a histogram of genotypes versus reported vWF values;

FIG. 10 is a photograph of a sequencing gel showing the mutation region between a vWD affected and a homozygous normal Shetland sheepdog (SEQ ID NOS: 19 and 20);

FIG. 11 is a diagram illustrating the Mwo I diagnostic test for the Shetland sheepdog Type 3 vWD mutation (SEQ ID NOS: 21-25); and

FIG. 12 is a photograph of an agarose gel showing the results of the diagnostic test for the Shetland sheepdog Type 3 vWD mutation.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The cDNA encoding canine von Willebrand Factor (vWF) has been sequenced, and is set forth in FIGS. 1A-1C and SEQ ID NO: 1. The deduced amino acid sequence is set forth in FIGS. 2A-2C and SEQ ID NO: 2. In one embodiment, the mutation of the normal vWF gene which causes von Willebrand's Disease (vWD) in Scottish terriers, a deletion at codon 88 of the normal gene resulting in a frameshift, is provided. In another embodiment, a splice junction mutation at nucleotide position 7639 of the normal gene, which causes vWD in Doberman pinschers, Manchester terriers and Poodles, is provided. In yet another embodiment, a single base deletion at nucleotide position 937 of the normal gene, causing vWD in Shetland sheepdogs, is provided. The nucleic acid sequences of the present invention may be used in methods for detecting homozygous and heterozygous carriers of the defective vWF gene.

In a preferred method of detecting the presence of the von Willebrand allele in canines, DNA samples are first collected by relatively noninvasive techniques, i.e., DNA samples are obtained with minimal penetration into body tissues of the

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animals to be tested. Common noninvasive tissue sample collection methods may be used and include withdrawing buccal cells via cheek swabs and withdrawing blood samples. Following isolation of the DNA by standard techniques, PCR is performed on the DNA utilizing pre-designed primers that produce enzyme restriction sites on those DNA samples that harbor the defective gene. Treatment of the amplified DNA with appropriate restriction enzymes such as BsiE I thus allows one to analyze for the presence of the defective allele. One skilled in the art will appreciate that this method may be applied not only to Scottish terriers, Doberman pinschers, Shetland sheepdogs, Manchester terriers and Poodles, but to other breeds such as Dutch Kooikers, as well.

The presence of the von Willebrand allele in canines can also be detected utilizing ligation amplification reaction technology (LAR) known to those skilled in the art. LAR is a method analogous to PCR for DNA amplification wherein ligases are employed for elongation in place of polymerases used for PCR. Another alternate method for detecting the presence of the canine von Willebrand allele also known to those skilled in the art, is allele specific oligonucleotide hybridization, wherein an oligonucleotide of about 20 bp containing the contiguous nucleotides of the allele of interest is hybridized to the canine DNA.

The present invention provides breeders with an accurate, definitive test whereby the undesired, defective vWF gene may be eliminated from breeding lines. The current tests used by breeders are protein-based, and as noted previously, the primary difficulty with this type of test is the variability of results due to a variety of factors. The ultimate result of such variability is that an inordinate number of animals fall into an ambiguous grouping whereby carriers and noncarriers cannot be reliably distinguished. The present invention obviates the inherent limitations of protein-based tests by detecting the genetic mutation which causes vWD. As described in the Specific Examples, the methods of the present invention provide an accurate test for distinguishing noncarriers, homozygous carriers and heterozygous carriers of the defective vWF gene.

It will be appreciated that because the vWF cDNA of the present invention is substantially homologous to vWF cDNA throughout the canine species, the nucleic acid sequences of the present invention may be used to detect DNA mutations in other breeds as well. In addition, the canine vWF sequence presented herein potentially in combination with the established human sequence (Genbank Accession No. X04385, Bonthron, D. et al., *Nucleic Acids Res.* 14:7125-7128 (1986); Mancuso, D. J. et al., *Biochemistry* 30:253-269 (1989); Meyer, D. et al., *Throm Haemostasis* 70:99-104 (1993)), may be used to facilitate sequencing of the vWF gene and genetic defects causing vWD, in other mammalian species e.g., by using cross-species PCR methods known by those skilled in the art.

It is also within the contemplation of this invention that the isolated and purified nucleic acid sequences of the present invention be incorporated into an appropriate recombinant expression vector, e.g., viral or plasmid, which is capable of transforming an appropriate host cell, either eukaryotic (e.g., mammalian) or prokaryotic (e.g., *E. coli*). Such DNA may involve alternate nucleic acid forms, such as cDNA, gDNA, and DNA prepared by partial or total chemical synthesis. The DNA may also be accompanied by additional regulatory elements, such as promoters, operators

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and regulators, which are necessary and/or may enhance the expression of the vWF gene product. In this way, cells may be induced to over-express the vWF gene, thereby generating desired amounts of the target vWF protein. It is further contemplated that the canine vWF polypeptide sequence of the present invention may be utilized to manufacture canine vWF using standard synthetic methods.

One skilled in the art will appreciate that the defective protein encoded by the defective vWF gene of the present invention may also be of use in formulating a complementary diagnostic test for canine vWD that may provide further data in establishing the presence of the defective allele. Thus, production of the defective vWF polypeptide, either through expression in transformed host cells as described above for the active vWF polypeptide or through chemical synthesis, is also contemplated by the present invention.

The term "gene" as referred herein means a nucleic acid which encodes a protein product. The term "nucleic acid" refers to a linear array of nucleotides and nucleosides, such as genomic DNA, cDNA and DNA prepared by partial or total chemical synthesis from nucleotides. The term "encoding" means that the nucleic acid may be transcribed and translated into the desired polypeptide. "Polypeptide" refers to amino acid sequences which comprise both full-length proteins and fragments thereof. "Mutation" as referred to herein includes any alteration in a nucleic acid sequence including, but not limited to, deletions, substitutions and additions.

As referred to herein, the term "capable of hybridizing under high stringency conditions" means annealing a strand of DNA complementary to the DNA of interest under highly stringent conditions. Likewise, "capable of hybridizing under low stringency conditions" refers to annealing a strand of DNA complementary to the DNA of interest under low stringency conditions. In the present invention, hybridizing under either high or low stringency conditions would involve hybridizing a nucleic acid sequence (e.g., the complementary sequence to SEQ ID NO: 1 or portion thereof), with a second target nucleic acid sequence. "High stringency conditions" for the annealing process may involve, for example, high temperature and/or low salt content, which disfavor hydrogen bonding contacts among mismatched base pairs. "Low stringency conditions" would involve lower temperature, and/or higher salt concentration than that of high stringency conditions. Such conditions allow for two DNA strands to anneal if substantial, though not near complete complementarity exists between the two strands, as is the case among DNA strands that code for the same protein but differ in sequence due to the degeneracy of the genetic code. Appropriate stringency conditions which promote DNA hybridization, for example, 6xSSC at about 4520 C., followed by a wash of 2xSSC at 50° C. are known to those skilled in the art or can be found in Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989), 6.31-6.3.6. For example, the salt concentration in the wash step can be selected from a low stringency of about 2xSSC at 50° C. to a high stringency of about 0.2xSSC at 50° C. In addition, the temperature in the wash step can be increased from low stringency at room temperature, about 22° C., to high stringency conditions, at about 65° C. Other stringency parameters are described in Maniatis, T., et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring N.Y., (1982), at pp.

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387-389; see also Sambrook J. et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Volume 2, Cold Spring Harbor Laboratory Press, Cold Spring, N.Y. at pp. 8.46-8.47 (1989).

SPECIFIC EXAMPLE 1—SCOTTISH TERRIERS

Materials And Methods

Isolation of RNA. The source of the RNA was a uterus from a Scottish Terrier affected with vWD (factor level <0.1% and a clinical bleeder), that was surgically removed because of infection. Spleen tissue was obtained from a Doberman pinscher affected with vWD that died from dilated cardiomyopathy (factor level 7% and a clinical bleeder). Total RNA was extracted from the tissues using Trizol (Life Technologies, Gaithersburg, Md.). The integrity of the RNA was assessed by agarose gel electrophoresis.

Design of PCR primer sets. Primers were designed to a few regions of the gene, where sequences from two species were available (Laverigne, J. M. et al., *Biochem Biophys Res Commun* 194:1019-1024 (1993); Bakhshi, M. R. et al., *Biochem Biophys Acta* 1132:325-328 (1992)). These primers were designed using rules for cross-species' amplifications (Venta et al., "Gene-Specific Universal Mammalian Sequence-Tagged Sites: Application To The Canine Genome" *Biochem. Genet.* 34:321-341 (1996)). Most of the primers had to be designed to other regions of the gene using the human sequence alone (Mancuso, D. J. et al., *Biochemistry* 30:253-269 (1991)). Good amplification conditions were determined by using human and canine genomic DNAs.

Reverse Transcriptase-PCR. Total RNA was reverse transcribed using random primers (Bergenhem, N. C. H. et al., *PNAS (USA)* 89:8789-8802 (1992)). The cDNA was amplified using the primer sets shown to work on canine genomic DNA.

DNA Sequence Analysis. Amplification products of the predicted sizes were isolated from agarose gels by adsorption onto silica gel particles using the manufacturer's method (Qiagen, Chatsworth, Calif.). Sequences were determined using ³³P-5' end-labeled primers and a cycle sequencing kit (United States Biochemical Corp., Cleveland, Ohio). The sequences of the 5' and 3' untranslated regions were determined after amplification using Marathon™ RACE kits (Clontech, Palo Alto, Calif.). Sequences were aligned using the Eugene software analysis package (Lark Technologies, Houston, Tex.). The sequence of the canine intron four was determined from PCR-amplified genomic DNA.

Design of a Diagnostic Test. PCR mutagenesis was used to create diagnostic and control BsiE I and Sau96 I restriction enzyme sites for the test Amplification conditions for the test are: 94° C., 1 min, 61° C., 1 min, and 72° C., 1 min, for 50 cycles using cheek swab DNA (Richards, B. et al., *Human Molecular Genetics* 2:159-163 (1992)).

Population Survey. DNA was collected from 87 Scottish terriers from 16 pedigrees. DNA was isolated either from blood using standard procedures (Sambrook, J. et al., Cold Harbor Spring Lab, Cold Harbor Spring N.Y., 2nd Edition, (1989)) or by cheek swab samples (Richards, B. et al., *Human Molecular Genetics* 2:159-163 (1992)). The genetic status of each animal in the survey was determined using the BsiE I test described above.

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Results

Comparison of the canine and human sequences. The alignment of the canine and human prepro-von Willebrand Factor amino acid sequences is shown in FIGS. 2A-2C (SEQ ID NO: 2). The location of the Scottish terrier vWD mutation is indicated by the "*". Potential N-glycosylation sites are shown in bold type. The known and postulated integrin binding sites are boxed. Amino acid numbers are shown on the right side of the figure. The human sequence is derived from Genbank accession number X04385.

Overall, 85.1% sequence identity is seen between the prepro-vWF sequences. The pro-region is slightly less conserved than the mature protein (81.4% vs. 87.5%). There were no other noteworthy percentage sequence identity differences seen in other regions of the gene, or between the known repeats contained within the gene (data not shown). Fourteen potential N-linked glycosylation sites are present in the canine sequence, all of which correspond to similar sites contained within the human sequence. The two integrin binding sites identified in the human vWF protein sequence (Lankhof, H. et al., *Blood* 86:1035-1042 (1995)) are conserved in the canine sequence as well (FIGS. 2A-2C; SEQ ID NO: 2). The 5' and 3' untranslated regions have diverged to a greater extent than the coding region (data not shown), comparable to that found between the human and bovine sequences derived for the 5' flanking region (Janel, N. et al., *Gene* 167:291-295 (1995)). Additional insights into the structure and function of the von Willebrand factor can be gained by comparison of the complete human sequence (Genbank Accession No. X04385; Bonthron, D. et al., *Nucleic Acids Res.* 14:7125-7128 (1986); Mancuso, D. J. et al., *Biochemistry* 30:253-269 (1989); Meyer, D. et al., *Thromb Haemostasis* 70:99-104 (1993)) and the complete canine sequence reported here.

The sequence for most of exon 28 was determined (Mancuso, D. J. et al., *Thromb Haemost* 69:980 (1993); Porter, C. A. et al., *Mol Phylogenet Evol* 5:89-101 (1996)). All three sequences are in complete agreement, although two silent variants have been found in other breeds (Table 1, exon 28). Partial sequences of exons 40 and 41 (cDNA nucleotide numbers 6923 to 7155, from the initiation codon) were also determined as part of the development of a polymorphic simple tandem repeat genetic marker (Shibuya, H. et al., *Anim Genet* 24:122 (1994)). There is a single nucleotide sequence difference between this sequence ("T") and the sequence of the present invention, ("C") at nucleotide position 6928.

Scottish Terrier vWD mutation. FIG. 3 shows nucleotide sequencing ladders for the vWD mutation region for normal (clear), carrier, and affected Scottish terriers. The sequences were obtained directly from PCR products derived from genomic DNAs in exon 4. The arrowheads show the location of the C nucleotide that is deleted in the disease-causing allele. Note that in the carrier ladder each base above the point of the mutation has a doublet appearance, as predicted for deletion mutations. The factor levels reported for these animals were: Normal, 54%; Carrier, 34%; Affected, <0.1%.

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As a result of the deletion, a frameshift mutation at codon 88 leads to a new stop codon 103 bases downstream. The resulting severely truncated protein of 119 amino acids does not include any of the mature vWF region. The identity of the base in the normal allele was determined from an unaffected dog.

Development of a diagnostic test A PCR primer was designed to produce a BsiE I site in the mutant allele but not in the normal allele (FIG. 4; SEQ ID NOS 3 and 10). The position of the deleted nucleotide is indicated by an asterisk. The altered nucleotides in each primer are underlined. The normal and mutant allele can also be distinguished using Sau96 I. The naturally occurring Sau96 I sites are shown by double underlines. The highly conserved donor and acceptor dinucleotide splice sequences are shown in bold type.

In order to ensure that the restriction enzyme cut the amplified DNA to completion, an internal control restriction site common to both alleles was designed into the non-diagnostic primer. The test was verified by digestion of the DNA from animals that were affected, obligate carriers, or normal (based on high factor levels [greater than 100% of normal] obtained from commonly used testing labs and reported by the owners, and also using breeds in which Type 3 vWD has not been observed). The expected results were obtained (e.g., FIG. 5). Five vWD-affected animals from a colony founded from Scottish terriers (Brinkhous, K. M. et al., *Ann. New York Acad. Sci.* 370:191-203 (1981)) were also shown to be homozygous for this mutation. An additional unaffected animal from this same colony was found to be clear.

It would still be possible to misinterpret the results of the test if restriction enzyme digestion was not complete, and if the rates of cleavage of the control and diagnostic sites were vastly different. The rates of cleavage of the two BsiE I sites were thus examined by partially digesting the PCR products and running them on capillary electrophoresis. The rates were found to be very nearly equal (the diagnostic site is cut 12% faster than the control site).

The mutagenesis primer was also designed to produce a Sau96 I site into the normal allele but not the mutant allele. This is the reverse relationship compared to the BsiE I-dependent test, with respect to which allele is cut. Natural internal Sau96 I sites serve as digestion control sites (shown in FIG. 4). The test using this enzyme produced identical genotypic results compared to the BsiE I for all animals examined (data not shown).

Mendelian Inheritance. One test often used to verify the correct identification of a mutant allele is its inheritance according to Mendel's law of segregation. Three pedigrees were examined in which the normal and mutant alleles were segregating, as shown in FIG. 5. Exon four of the vWF gene was PCR-amplified from genomic DNA. The PCR products were examined for the presence of the normal and mutant vWF alleles by agarose gel electrophoresis after digestion with BsiE I (see FIG. 5). The affected animals are homozygous for the mutant allele (229 bp; lanes 3 and 5). The other animals in this pedigree are heterozygotes (251 bp and 229 bp; lanes 1, 2, 4, and 6), including the obligate carrier parents.

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TABLE 1

Differences Between Scottie and Doberman Pinscher
Protein And Nucleotide von Willebrand Factor Sequences
With Comparison To The Human Sequences

Exon	A.A. ¹	Amino Acid			Codon		
		Human	Scottie	Doberman	Human	Scottie	Doberman
5' UT ²	nuc-35 ³	N/A ⁴	N/A	N/A	N/A	A	G
4	85	S	S/F.Shift ⁵	S	TCC	TCC/TC	TCC
5	173	M	R	K	ATG	AGG	AAG
11	422	S	T	T	TCC	ACA	ACC
21	898	C	C	C	TGC	TGT	TGC
21	905	F	F	L	TTT	TTC	TTA
24	1041	S	S	S	TCA	TCA	TCG
24	1042	S	S	S	TCC	TCC	TCA
28	1333	D	D	E	GAC	GAC	GAG
28	1349	Y	Y	Y	TAT	TAT	TAC*
42	2381	P	L	P	CCC	CTG	CCG
43	2479 ⁶	S	S	S	TCG	TCG	TCA
45	2555	P	P	P	CCC	CCC	CCG
47	2591	P	P	P	CCC	CCT	CCC
49	2672	D	D	D	GAT	GAT	GAC
51	2744	E	E	E	GAG	GAG	GAA

¹Amino acid residue position²Untranslated region³Nucleotide position⁴Not Applicable⁵Frameshift mutation⁶Splice site mutation for Doberman pinscher, Manchester terrier and Poodle

Boxed residues show amino acid differences between breeds

*This site has been shown to be polymorphic in some breeds

The mature VWF protein begins in exon 18

The alleles, as typed by both the BsiE I and Sau96 I tests, showed no inconsistencies with Mendelian inheritance. One of these pedigrees included two affected animals, two phenotypically normal siblings, and the obligate carrier parents. The two parents were found to be heterozygous by the test, the two affected animals were found to be homozygous for the mutant allele, and the normal siblings were found to be heterozygotes.

Population survey for the mutation. Cheek swabs or blood samples were collected from 87 animals in order to determine the incidence of carriers in the U.S. Scottish terrier population. Although an attempt was made to make the sample as random as possible, these dogs were found to come from 16 pedigrees, several of which are more distantly interconnected. This is due to some ascertainment bias, based on ownership (as opposed to phenotypic ascertainment bias). In these 87 animals, 4 affected and 15 carrier animals were found.

Discussion

These results establish that the single base deletion found in exon four of the vWF gene causes vWD in the Scottish terrier breed. The protein produced from the mutant allele is extremely short and does not include any of the mature vWF protein. Four Scottish terriers known to be affected with the disease are homozygous for the mutation. Five other mixed-breed dogs descended from Scottish terriers, and affected with vWD, are also homozygous for the mutation. No normal animals are homozygous for the mutation. Unaffected obligate carriers are always heterozygous for the mutation.

The gene frequency, as determined from the population survey, appears to be around 0.13 resulting in a heterozygote frequency of about 23% and expected frequency of affected animals of about 2%. Although the sample size is relatively small and somewhat biased, these data are in general agree-

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ment with the protein-based surveys (Stokol, T. et al., *Res Vet Sci* 59:152-155 (1995); Brooks, M., *Probl In Vet Med* 4:636-646 (1992)), in that the allele frequency is substantial.

All data collected thus far indicate that this mutation may account for essentially all of the von Willebrand's disease found in Scottish terriers. This result is consistent with the results found for other genetic diseases, defined at the molecular level, in various domestic animals (Shuster, D. E. et al., *PNAS (USA)* 89:9225-9229 (1992); Rudolph, J. A. et al., *Nat Genet* 2:144-147 (1992); O'Brien, P. J. et al., *JAVMA* 203:842-851 (1993)). A likely explanation may be found in the pronounced founder effect that occurs in domestic animals, compared to most human and wild animal populations.

Published data using the protein-based factor assays have shown that, at least in several instances, obligate carriers have had factor levels that would lead to a diagnosis of "clear" of the disease allele. For example, in one study an obligate carrier had a factor level of 78% (Johnson, G. S. et al., *JAVMA* 176:1261-1263 (1980)). In another study, at least some of the obligate carriers had factor levels of 65% or greater (Brinkhous, K. M. et al., *Ann. New York Acad. Sci.* 370:191-203 (1981)). In addition, the number of animals that fall into an equivocal range can be substantial. In one study, 19% of Scottish terriers fell in this range (50-65% of the normal vWF antigen level) (Stokol, T. et al., *Res Vet Sci* 59:152-155 (1995)). Thus, although the protein-based tests have been useful, the certainty of the DNA-based test described herein should relieve the necessity of repeated testing and the variability associated with the protein-based assays.

The mutation is present in the pre-vWF part of the molecule. This part of the molecule is processed off prior to delivery of the mature protein into the plasma. This pre-portion of the molecule is important for the assembly of the mature vWF protein (Verwiej, L. et al., *EBMO J* 6:2885-2890 (1987); Wise, R. J. et al., *Cell* 52:229-236 (1988)). With the Scottish terrier frameshift vWD mutation, neither this pre-portion nor any of the mature factor is ever produced, in keeping with the fact that no factor has ever been detected in the blood of affected dogs.

The determination of the complete canine vWF cDNA sequence will have an impact upon the development of carrier tests for other breeds and other species as well. Currently, Shetland sheepdogs (see Specific Example 3) and Dutch Kooikers are known to have a significant amount of Type 3 vWD (Brooks, M. et al., *JAVMA* 200:1123-1127 (1992); Slappendel, R. J., *Vet-Q* 17:S21-S22 (1995)). Type 3 vWD has occasionally been seen in other breeds as well (e.g., Johnson, G. S. et al., *JAVMA* 176:1261-1263 (1980)). All Type 3 vWD mutations described in humans to date have been found within the vWF gene itself. The availability of the canine sequence will make it easier to find the mutations in these breeds. In addition, at least some Type 1 mutations have been found within the human vWF gene, and thus Type 1 mutations may also be found within the vWF gene for breeds affected with that form of the disease. The availability of two divergent mammalian vWF cDNA sequences will also make it much easier to sequence the gene from other mammalian species using cross-species PCR methods (e.g., Venta et al., *Biochem. Genet.* 34:321-341 (1996)).

The test described herein for the detection of the mutation in Scottish terriers may be performed on small amounts of DNA from any tissue. The tissues that are the least invasive to obtain are blood and buccal cells. For maximum convenience, a cheek swab as a source of DNA is preferred.

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SPECIFIC EXAMPLE 2—DOBERMAN PINSCHER

Materials and Methods

RT-PCR and DNA Sequence Analysis. RNA was isolated by using Trizol (Life Technologies, Gaithersburg, Md.) from the spleen of a Doberman pinscher that was affected with vWD (factor value of 7% of normal) and that had died from dilated cardiomyopathy. RT-PCR was performed as previously described using primers to the canine vWF cDNA. Most PCR products were determined directly using a cycle sequencing kit (Amersham Corp, Chicago, Ill.). A minor band containing the four base deletion (see Results) was subcloned into a plasmid vector prior to sequence analysis. The five kb intron 43 was amplified using a commercially available kit for long PCR (Boehringer-Mannheim, Indianapolis, Ind.). The cycling times and temperatures were as follows: initial denaturation, 93° C., 2 min; 10 cycles of 93° C., 15 sec, 62° C., 30 sec, 68° C., 4 min; 20 cycles of 93° C., 15 sec, 62° C., 30 sec, 68° C., 4 min with 20 additional sec per cycle. This was followed by a final extension at 68° C. for 7 min. The sequences of the primers used were: exon 43 (sense primer), 5'-TCTACCCTGTGGGCCAGTTC-3' (SEQ ID NO: 26), and exon 44 (antisense primer), 5'-GACCACCTCACAGGCAGAT-3' (SEQ ID NO: 27).

PCR-Based Mutation Test. PCR mutagenesis was used to create an Msp I site in the normal allele but not in the mutant allele. An internal Msp I digestion control site was also created by PCR mutagenesis within the anti-sense primer, whose target is within intron 43. The control site is contained within the amplification products of both alleles. The sequences of the primers are: diagnostic (sense) primer, 5'-CTGTGAGGACAACCTGCCTGCC-3' (SEQ ID NO: 28); and common (anti-sense) primer, 5'-TGGCCCTGAACCGGAAATTACTCAAG-3' (SEQ ID NO: 29) (the altered bases within each primer are underlined). A 'touchdown' PCR protocol was used for the amplification. The amplification conditions are: 94° C., 30 sec, 63 to 55° C. 40 sec, and 72° C., 50 sec, for the first 8 cycles, with the annealing temperature dropping one degree per cycle. Twenty-eight additional cycles were run, with the annealing temperature held at 55° C. The DNA was digested with Msp I after PCR amplification.

Population Survey. Owners who participated in a population survey supplied cheek swabs from their dogs for genotype analysis. Richards, B. et al., *Hum. Mol. Genet.* 2:159 (1992). A number of these dogs had associated vWF values that were determined by various testing laboratories that provide this service to breeders.

Results

During the sequence analysis of the vWF mRNA from an affected Doberman pinscher, a significant nucleotide difference from the Scottish terrier sequence was discovered. This change was found at the last base of exon 43 (nucleotide 7437 from the initiation codon, at amino acid position Ser 2479; G in Scotties, A in the affected Doberman) (Table 1). Although this is a silent amino acid change, it causes the splice junction to be less similar to the mammalian splice junction consensus. Nakai, K. et al., *Gene* 141:171 (1994); Krawcsak, M. et al., *Genet.* 90:41 (1992). Just upstream of the normal splice junction is another sequence that also has significant similarity to the consensus, which is increased by the A at nucleotide position 7437 (FIG. 6; SEQ ID NOS: 14-17). The A at the end of exon 43 could cause the normal

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splice junction to be used less frequently, and that the upstream cryptic splice site becomes the one predominantly used. Comparison of the splice sites by a devised statistical method (Shapiro, M. B. et al., *Nucleic Acids Res.* 15:7155 (1987)) gave the following scores: normal splice position with the wild-type allele (G at 7536), 83.9; cryptic splice site with the wild-type allele, 60.6; normal splice position with the mutant allele (A at 7437), 72.2; cryptic splice site with the mutant allele, 70.5. Higher scores represent a greater likelihood of splicing potential. The scores for the normal and cryptic splice sites are quite different with the wild-type allele, but are very close with the mutant allele. These results support the probability of a decreased likelihood for splicing at the normal site, and an increased potential for splicing at the cryptic site with the mutant allele.

A faint RT-PCR band just below the major band from which the variant nucleotide had been detected was observed. This minor band was missing the four bases at the end of exon 43 as confirmed by sequence analysis (FIG. 7; SEQ ID NO: 18). The position of the four deleted bases is shown on the right side of FIG. 7 (SEQ ID NO: 18).

A PCR-based test was developed to detect the nucleotide difference in genomic DNA as described herein in Materials and Methods. The results of the test for several animals with a spectrum of factor values yield a significant correlation between genotype and factor value as shown in FIG. 8. Lane 1 contains a 50 bp ladder as a size marker. The uncut PCR product is 135 bp (lane 8). Both alleles contain a common Msp I restriction site that serves as an internal digestion control. The mutant (A) and normal (G) alleles are represented by the 123 bp and 102 bp bands, respectively. Reported factor levels and deduced genotypic status for dogs represented in the additional lanes are as follows: 2, 12%, affected (AA); 3, 8%, affected (AA); 4, 39%, carrier (AG); 5, 68%, carrier (AG); 6, 125%, homozygous normal (GG); 7, 136%, homozygous normal (GG). A survey of 21 randomly ascertained animals with associated factor values showed a strong correlation between genotype and factor level as presented in the histogram of FIG. 9. The shaded boxes indicate predicted genotypes based on factor levels that are not consistent with the genotypes deduced from the PCR-based diagnostic test. Larger factor value-only surveys (Johnson et al., *Vet. Clin. North Am. Small Anim. Pract.* 18:195-229 (1988); Moser et al., *Am. J. Vet. Res.* 57:1288-1293 (1996); Stokol et al., *Aust. Vet. J.* 72:257-262 (1996)) indicate substantial overlap between genotypes based upon the protein-based methods. A larger survey on 67 additional Dobermans contained in 10 independently ascertained pedigrees was performed to obtain an estimate of the mutant allele frequency within the breed. Of the total of 88 animals, 40 were M, 35 were AG, and 13 were GG. From these results, the A allele frequency was estimated to be 0.64.

Discussion

The splice junction mutation at the end of exon 43 is the cause of recessive Type 1 vWD found within the Doberman pinscher breed. The mutation decreases the similarity between the normal splice junction and the mammalian consensus while at the same time increasing the similarity of the cryptic splice site found just upstream of the normal splice site (FIG. 6; SEQ ID NOS: 14-17). The calculated Shapiro-Senapathy splice site values (Shapiro, M. B. et al., *Nucleic Acids Res.* 15:7155 (1987)) are very similar for the normal and cryptic splice sites when an A is present at nucleotide position 7536. The Shapiro-Senapathy calculation is probably not completely accurate in determining the

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relative amount of splicing that can occur between different sites. Therefore, it is not inconsistent to find that the cryptic splice site is used more often than the normal site, in the mutant allele.

The sequence of the minor amplification product seen just below the main amplification band exactly matches that predicted by the use of the cryptic splice site (FIG. 7; SEQ ID NO: 18). The fact that there is less cryptically spliced mRNA than normally spliced mRNA present in the cytoplasm can be explained by the relative instability of the cryptically spliced message. The cryptically spliced mRNA produces a shift in the translational reading frame, resulting in the formation of a premature stop codon. It is well known that mRNAs that produce truncated proteins are unstable, perhaps because ribosomes do not remain attached to the message to protect it from degradation by intracellular RNases or because of the incomplete assembly of spliceosomes on mutant splice sites. Maquat, L. E., *Am J Hum Genet* 59:279 (1996). The average amount of vWF protein present in affected animals is roughly 10% of the normal canine value. Thus, each mutant allele should produce about 5% of the normal amount of vWF mRNA and protein. From this, it can be predicted that the average heterozygous Doberman should produce 55% of the average canine vWF value. The vWF mRNA estimated in affected animals has been shown to be roughly 20% of normal by densitometry scans of northern blots. Meinkoth, J. H. et al., *Am. J. Vet. Res.* 56:1577 (1995). This mRNA is predicted to consist primarily of the correctly spliced transcript.

The mutation has been shown to be linked to the vWF locus (FIG. 9 and Holmes, N. G. et al., *J. Small An. Prac* 37:307 (1996). Most human Type 1 vWD, in which there is a true clinical bleeding problem, appears to be inherited in a dominant, incompletely penetrant fashion. Ginsburg, D. et al., *Blood* 79:2507 (1992). Although a few Type 1 mutations have been found within the vWF locus (see, e.g., Siguret, V. et al., *Hum. Genet.* 93:95 (1994); Eikenboom, J. C. J. et al., *Blood* 88:2433 (1996)); it has been argued that another locus or loci may also cause some Type 1 vWD. Ginsburg, D. et al., *Blood* 79:2507 (1992). In fact, one murine Type 1 vWD has been mapped to locus that is not linked to the vWF gene. Nichols, W. C. et al., *Blood* 83:3225 (1994). The data show that a least a proportion of Type 1 vWD in humans might also be caused by the exon 43 mutation, or other leaky splice junction mutations. The mode of inheritance for this type of mutation is recessive, but it might appear to be dominant in certain situations, such as that of the Doberman pinscher. The number of splice site mutations of the type described herein are significantly below the number that would be predicted to occur, suggesting that these types of mutations are more difficult to detect or have been overlooked in the past. Krawcsak, M. et al., *Hum. Genet.* 90:41 (1992). This might be because they produce a less severe phenotype than other types of mutations that cause a complete loss of function.

SPECIFIC EXAMPLE 3—SHETLAND SHEEPDOG

Total DNA was isolated from material obtained from a spay of an affected Shetland sheepdog (Sheltie). This animal had been tested for the vWF antigen, and was reported to have a 0% value by a laboratory skilled in this testing (Diagnostic Laboratory, Comparative Hematology Section, College of Veterinary Medicine, Cornell University). The owner had decided to have the spay done after obtaining this result, and donated the removed tissues. The entire RT-PCR coding region of this mutant gene was sequenced as

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described in Specific Example 1, to identify the mutation that causes vWD. A mutation was found in the vWF gene that appears to be responsible for most or all of the type 3 vWD found in the Sheltie breed. A deletion of a single T was found at nucleotide position 735 of the encoding region (FIG. 10; SEQ ID NOS: 19 and 20). The arrows in FIG. 10 indicate the series of T nucleotides in which one T has been deleted in the DNA of the affected animal compared to the normal animal. This deletion, present in the equivalent of human exon 7, would cause a shift in the reading from of the vWF-encoding region, and result in a severely truncated protein. A diagnostic test was designed to detect this mutation (FIG. 11; SEQ ID NOS: 21-25). The deletion causes the creation of an Mwo I restriction site and thus, the Mwo site is found in the mutant allele, but not in the normal allele. The sequence shown in FIG. 11 (SEQ ID NOS: 21 and 22) is that of the canine gene that corresponds to the human vWF exon 7. The single letter code for amino acids is shown above the nucleotide sequence and the primer sequences are shown below the gene sequence. The Mwo I sites are also indicated. An internal digestion control site is present in the non-diagnostic primer region. Reagent concentrations for this test were: 100 μ M dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2 mM $MgCl_2$, 0.05 to 0.1 μ g target DNA, 15 μ M of each primer (SEQ ID NOS: 23 and 25), and 0.025 U Taq DNA polymerase. Cycling conditions were: 94° C., 4 min, one cycle, followed by 50 cycles of 94° C., 30 sec, 63° C., 40 sec, and 72° C., 40 sec. The relatively low Taq concentration (compared to generally accepted conditions) with the high number of cycles prevents the amplification of non-specific PCR bands. One microliter of Mwo I restriction enzyme (New England Biolabs, Inc.) and 2 μ l of 50 mM $MgCl_2$ were added directly to the PCR reaction after amplification, and incubated at 60° C. for 1 hr. Digestion products were then observed after gel electrophoresis on a 1.5% agarose gel and the results shown in FIG. 12. Lanes 1 and 17 show a one hundred bp ladder. Lanes 2-6 show the results from an affected animal, lanes 7-11 show the results from a carrier animal, and lanes 12-16 show the results from a homozygous normal animal. Lane 18 shows an undigested control PCR product. The duplicate samples demonstrate the reproducibility of the test. Numbers on the left side of the gel show the sizes of the standard bands, and numbers on the right side of the gel show the sizes of the uncut product (U), the normal allele (N), and the two bands for the mutant allele (M).

A survey of Shelties was conducted to determine the frequency of the mutation within the U.S. population. Of a total of 103 animals, 14 were carriers, giving a carrier frequency of 13.6%. This frequency is less than the value of 28% reported for the breed in 1988 for 730 animals when using the factor antigen test. Brooks, M. et al., *J. Am. Vet. Med. Assoc.* 200:1123-1127 (1992). One third of these carriers are thought to be due to Type 1 vWD also present in the breed. Still, the value of 13.6% would be lower than the calculated value of 18.7% from the antigen test. This difference could be due to either ascertainment biases in either study, a true decrease in the frequency of the disease in this breed, one or more additional Type 3 mutations in the breed, or a combination of these possibilities. Whatever the reason for the difference, most or all of the Type 3 disease in the Sheltie is probably caused by this one mutation. This is based on the understanding of the importance of the Founder effect (or populate sire effect) on the increase in the

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frequency of specific genetic diseases in purebred populations of domestic animals. A 17 member pedigree of Shelties, in which the mutation was segregating was tested for normal Mendelian inheritance of the allele. There were no differences from what would be expected under co-dominant inheritance of the two alleles.

SPECIFIC EXAMPLE 4

In an effort to find mutations that cause vWD in other canine breeds, affected animals were surveyed, as diagnosed by low levels of vWF antigen, for the three mutations set forth herein. In the case of the Manchester terrier breed, it was found that at least a portion of the affected animals had the identical mutation that causes vWD in the Doberman pinscher. The test described supra for the Doberman pinscher was utilized to test an affected Manchester terrier, plus several related animals. The affected animal was found to be homozygous for the mutant allele (Table 2). In addition, several animals who had vWF values in the carrier range were found to be carriers at the genotypic level.

TABLE 2

Manchester terrier vWF values vs. DNA genotype		
Dog	vWF value ^a	Genotype ^b
MT1	200%	normal
MT2	76%	normal
MT3	42%	carrier
MT4	19%	carrier
MT5	NT	carrier
MT6	NT	carrier
MT7	10%	affected

^aFactor values as reported from a testing lab (Cornell CVM, Hematology Lab).

^bGenotype for the leaky splice mutation originally found in the Doberman pinscher.

SPECIFIC EXAMPLE 5

In an effort to locate mutations that cause vWD in other canine breeds, affected animals as diagnosed by low levels of vWF antigen, were surveyed for the three mutations set forth herein. The test described supra for the Doberman pinscher was utilized and, in the case of the Poodle breed, it was found that the affected animals had the identical mutation that causes vWD in the Doberman pinscher. The affected animals were found to be homozygous for the mutant allele. In addition, several animals who had vWF values in the carrier range were found to be carriers at the genotypic level.

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention.

All patents and other publications cited herein are expressly incorporated by reference.

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SEQUENCE LISTING

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			500					505					510		
Tyr	Pro	Ala	Tyr	Ala	Gly	Lys	Thr	Cys	Gly	Arg	Gly	Gly	Asn	Tyr	Asn
		515					520					525			
Gly	Asn	Arg	Gly	Asp	Asp	Phe	Val	Thr	Pro	Ala	Gly	Leu	Ala	Glu	Pro
	530					535					540				
Leu	Val	Glu	Asp	Phe	Gly	Asn	Ala	Trp	Lys	Leu	Leu	Gly	Ala	Cys	Glu
	545				550					555					560
Asn	Leu	Gln	Lys	Gln	His	Arg	Asp	Pro	Cys	Ser	Leu	Asn	Pro	Arg	Gln
				565					570					575	
Ala	Arg	Phe	Ala	Glu	Glu	Ala	Cys	Ala	Leu	Leu	Thr	Ser	Ser	Lys	Phe
			580					585						590	
Glu	Pro	Cys	His	Arg	Ala	Val	Gly	Pro	Gln	Pro	Tyr	Val	Gln	Asn	Cys
		595					600					605			
Leu	Tyr	Asp	Val	Cys	Ser	Cys	Ser	Asp	Gly	Arg	Asp	Cys	Leu	Cys	Ser
	610					615					620				
Ala	Val	Ala	Asn	Tyr	Ala	Ala	Ala	Val	Ala	Arg	Arg	Gly	Val	His	Ile
	625				630					635					640
Ala	Trp	Arg	Glu	Pro	Gly	Phe	Cys	Ala	Leu	Ser	Cys	Pro	Gln	Gly	Gln
			645					650						655	
Val	Tyr	Leu	Gln	Cys	Gly	Thr	Pro	Cys	Asn	Met	Thr	Cys	Leu	Ser	Leu
		660					665						670		
Ser	Tyr	Pro	Glu	Glu	Asp	Cys	Asn	Glu	Val	Cys	Leu	Glu	Ser	Cys	Phe
	675					680						685			
Ser	Pro	Pro	Gly	Leu	Tyr	Leu	Asp	Glu	Arg	Gly	Asp	Cys	Val	Pro	Lys
	690					695					700				
Ala	Gln	Cys	Pro	Cys	Tyr	Tyr	Asp	Gly	Glu	Ile	Phe	Gln	Pro	Glu	Asp
	705				710				715					720	
Ile	Phe	Ser	Asp	His	His	Thr	Met	Cys	Tyr	Cys	Glu	Asp	Gly	Phe	Met
			725						730					735	
His	Cys	Thr	Thr	Ser	Gly	Gly	Leu	Gly	Ser	Leu	Leu	Pro	Asn	Pro	Val
			740				745						750		
Leu	Ser	Ser	Pro	Arg	Cys	His	Arg	Ser	Lys	Arg	Ser	Leu	Ser	Cys	Arg
		755					760					765			
Pro	Pro	Met	Val	Lys	Leu	Val	Cys	Pro	Ala	Asp	Asn	Pro	Arg	Ala	Glu
		770				775					780				
Gly	Leu	Glu	Cys	Ala	Lys	Thr	Cys	Gln	Asn	Tyr	Asp	Leu	Gln	Cys	Met
	785				790					795					800
Ser	Thr	Gly	Cys	Val	Ser	Gly	Cys	Leu	Cys	Pro	Gln	Gly	Met	Val	Arg
			805					810					815		
His	Glu	Asn	Arg	Cys	Val	Ala	Leu	Glu	Arg	Cys	Pro	Cys	Phe	His	Gln
		820					825						830		
Gly	Gln	Glu	Tyr	Ala	Pro	Gly	Glu	Thr	Val	Lys	Ile	Asp	Cys	Asn	Thr
		835					840					845			
Cys	Val	Cys	Arg	Asp	Arg	Lys	Trp	Thr	Cys	Thr	Asp	His	Val	Cys	Asp

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850	855	860
Ala Thr Cys Ser Ala Ile Gly Met Ala His Tyr Leu Thr Phe Asp Gly		
865	870	875 880
Leu Lys Tyr Leu Phe Pro Gly Glu Cys Gln Tyr Val Leu Val Gln Asp		
	885	890 895
Tyr Cys Gly Ser Asn Pro Gly Thr Leu Arg Ile Leu Val Gly Asn Glu		
	900	905 910
Gly Cys Ser Tyr Pro Ser Val Lys Cys Lys Lys Arg Val Thr Ile Leu		
	915	920 925
Val Glu Gly Gly Glu Ile Glu Leu Phe Asp Gly Glu Val Asn Val Lys		
	930	935 940
Lys Pro Met Lys Asp Glu Thr His Phe Glu Val Val Glu Ser Gly Gln		
	945	950 955 960
Tyr Val Ile Leu Leu Leu Gly Lys Ala Leu Ser Val Val Trp Asp His		
	965	970 975
Arg Leu Ser Ile Ser Val Thr Leu Lys Arg Thr Tyr Gln Glu Gln Val		
	980	985 990
Cys Gly Leu Cys Gly Asn Phe Asp Gly Ile Gln Asn Asn Asp Phe Thr		
	995	1000 1005
Ser Ser Ser Leu Gln Ile Glu Glu Asp Pro Val Asp Phe Gly Asn Ser		
	1010	1015 1020
Trp Lys Val Asn Pro Gln Cys Ala Asp Thr Lys Lys Val Pro Leu Asp		
	1025	1030 1035 1040
Ser Ser Pro Ala Val Cys His Asn Asn Ile Met Lys Gln Thr Met Val		
	1045	1050 1055
Asp Ser Ser Cys Arg Ile Leu Thr Ser Asp Ile Phe Gln Asp Cys Asn		
	1060	1065 1070
Arg Leu Val Asp Pro Glu Pro Phe Leu Asp Ile Cys Ile Tyr Asp Thr		
	1075	1080 1085
Cys Ser Cys Glu Ser Ile Gly Asp Cys Thr Cys Phe Cys Asp Thr Ile		
	1090	1095 1100
Ala Ala Tyr Ala His Val Cys Ala Gln His Gly Lys Val Val Ala Trp		
	1105	1110 1115 1120
Arg Thr Ala Thr Phe Cys Pro Gln Asn Cys Glu Glu Arg Asn Leu His		
	1125	1130 1135
Glu Asn Gly Tyr Glu Cys Glu Trp Arg Tyr Asn Ser Cys Ala Pro Ala		
	1140	1145 1150
Cys Pro Ile Thr Cys Gln His Pro Glu Pro Leu Ala Cys Pro Val Gln		
	1155	1160 1165
Cys Val Glu Gly Cys His Ala His Cys Pro Pro Gly Lys Ile Leu Asp		
	1170	1175 1180
Glu Leu Leu Gln Thr Cys Ile Asp Pro Glu Asp Cys Pro Val Cys Glu		
	1185	1190 1195 1200
Val Ala Gly Arg Arg Leu Ala Pro Gly Lys Lys Ile Ile Leu Asn Pro		
	1205	1210 1215
Ser Asp Pro Glu His Cys Gln Ile Cys Asn Cys Asp Gly Val Asn Phe		
	1220	1225 1230
Thr Cys Lys Ala Cys Arg Glu Pro Gly Ser Val Val Val Pro Pro Thr		
	1235	1240 1245
Asp Gly Pro Ile Gly Ser Thr Thr Ser Tyr Val Glu Asp Thr Ser Glu		
	1250	1255 1260
Pro Pro Leu His Asp Phe His Cys Ser Arg Leu Leu Asp Leu Val Phe		
	1265	1270 1275 1280

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Leu Leu Asp Gly Ser Ser Lys Leu Ser Glu Asp Glu Phe Glu Val Leu
 1285 1290 1295
 Lys Val Phe Val Val Gly Met Met Glu His Leu His Ile Ser Gln Lys
 1300 1305 1310
 Arg Ile Arg Val Ala Val Val Glu Tyr His Asp Gly Ser His Ala Tyr
 1315 1320 1325
 Ile Glu Leu Lys Asp Arg Lys Arg Pro Ser Glu Leu Arg Arg Ile Thr
 1330 1335 1340
 Ser Gln Val Lys Tyr Ala Gly Ser Glu Val Ala Ser Thr Ser Glu Val
 1345 1350 1355 1360
 Leu Lys Tyr Thr Leu Phe Gln Ile Phe Gly Lys Ile Asp Arg Pro Glu
 1365 1370 1375
 Ala Ser Arg Ile Ala Leu Leu Leu Met Ala Ser Gln Glu Pro Ser Arg
 1380 1385 1390
 Leu Ala Arg Asn Leu Val Arg Tyr Val Gln Gly Leu Lys Lys Lys Lys
 1395 1400 1405
 Val Ile Val Ile Pro Val Gly Ile Gly Pro His Ala Ser Leu Lys Gln
 1410 1415 1420
 Ile His Leu Ile Glu Lys Gln Ala Pro Glu Asn Lys Ala Phe Val Phe
 1425 1430 1435 1440
 Ser Gly Val Asp Glu Leu Glu Gln Arg Arg Asp Glu Ile Ile Asn Tyr
 1445 1450 1455
 Leu Cys Asp Leu Ala Pro Glu Ala Pro Ala Pro Thr Gln His Pro Pro
 1460 1465 1470
 Met Ala Gln Val Thr Val Gly Ser Glu Leu Leu Gly Val Ser Ser Pro
 1475 1480 1485
 Gly Pro Lys Arg Asn Ser Met Val Leu Asp Val Val Phe Val Leu Glu
 1490 1495 1500
 Gly Ser Asp Lys Ile Gly Glu Ala Asn Phe Asn Lys Ser Arg Glu Phe
 1505 1510 1515 1520
 Met Glu Glu Val Ile Gln Arg Met Asp Val Gly Gln Asp Arg Ile His
 1525 1530 1535
 Val Thr Val Leu Gln Tyr Ser Tyr Met Val Thr Val Glu Tyr Thr Phe
 1540 1545 1550
 Ser Glu Ala Gln Ser Lys Gly Glu Val Leu Gln Gln Val Arg Asp Ile
 1555 1560 1565
 Arg Tyr Arg Gly Gly Asn Arg Thr Asn Thr Gly Leu Ala Leu Gln Tyr
 1570 1575 1580
 Leu Ser Glu His Ser Phe Ser Val Ser Gln Gly Asp Arg Glu Gln Val
 1585 1590 1595 1600
 Pro Asn Leu Val Tyr Met Val Thr Gly Asn Pro Ala Ser Asp Glu Ile
 1605 1610 1615
 Lys Arg Met Pro Gly Asp Ile Gln Val Val Pro Ile Gly Val Gly Pro
 1620 1625 1630
 His Ala Asn Val Gln Glu Leu Glu Lys Ile Gly Trp Pro Asn Ala Pro
 1635 1640 1645
 Ile Leu Ile His Asp Phe Glu Met Leu Pro Arg Glu Ala Pro Asp Leu
 1650 1655 1660
 Val Leu Gln Arg Cys Cys Ser Gly Glu Gly Leu Gln Ile Pro Thr Leu
 1665 1670 1675 1680
 Ser Pro Thr Pro Asp Cys Ser Gln Pro Leu Asp Val Val Leu Leu Leu
 1685 1690 1695

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Asp Gly Ser Ser Ser Ile Pro Ala Ser Tyr Phe Asp Glu Met Lys Ser	1700	1705	1710
Phe Thr Lys Ala Phe Ile Ser Arg Ala Asn Ile Gly Pro Arg Leu Thr	1715	1720	1725
Gln Val Ser Val Leu Gln Tyr Gly Ser Ile Thr Thr Ile Asp Val Pro	1730	1735	1740
Trp Asn Val Ala Tyr Glu Lys Val His Leu Leu Ser Leu Val Asp Leu	1745	1750	1755
Met Gln Gln Glu Gly Gly Pro Ser Glu Ile Gly Asp Ala Leu Ser Phe	1765	1770	1775
Ala Val Arg Tyr Val Thr Ser Glu Val His Gly Ala Arg Pro Gly Ala	1780	1785	1790
Ser Lys Ala Val Val Ile Leu Val Thr Asp Val Ser Val Asp Ser Val	1795	1800	1805
Asp Ala Ala Ala Glu Ala Ala Arg Ser Asn Arg Val Thr Val Phe Pro	1810	1815	1820
Ile Gly Ile Gly Asp Arg Tyr Ser Glu Ala Gln Leu Ser Ser Leu Ala	1825	1830	1835
Gly Pro Lys Ala Gly Ser Asn Met Val Arg Leu Gln Arg Ile Glu Asp	1845	1850	1855
Leu Pro Thr Val Ala Thr Leu Gly Asn Ser Phe Phe His Lys Leu Cys	1860	1865	1870
Ser Gly Phe Asp Arg Val Cys Val Asp Glu Asp Gly Asn Glu Lys Arg	1875	1880	1885
Pro Gly Asp Val Trp Thr Leu Pro Asp Gln Cys His Thr Val Thr Cys	1890	1895	1900
Leu Pro Asp Gly Gln Thr Leu Leu Lys Ser His Arg Val Asn Cys Asp	1905	1910	1915
Arg Gly Pro Arg Pro Ser Cys Pro Asn Gly Gln Pro Pro Leu Arg Val	1925	1930	1935
Glu Glu Thr Cys Gly Cys Arg Trp Thr Cys Pro Cys Val Cys Met Gly	1940	1945	1950
Ser Ser Thr Arg His Ile Val Thr Phe Asp Gly Gln Asn Phe Lys Leu	1955	1960	1965
Thr Gly Ser Cys Ser Tyr Val Leu Phe Gln Asn Lys Glu Gln Asp Leu	1970	1975	1980
Glu Val Ile Leu Gln Asn Gly Ala Cys Ser Pro Gly Ala Lys Glu Thr	1985	1990	1995
Cys Met Lys Ser Ile Glu Val Lys His Asp Gly Leu Ser Val Glu Leu	2005	2010	2015
His Ser Asp Met Gln Met Thr Val Asn Gly Arg Leu Val Ser Ile Pro	2020	2025	2030
Tyr Val Gly Gly Asp Met Glu Val Asn Val Tyr Gly Thr Ile Met Tyr	2035	2040	2045
Glu Val Arg Phe Asn His Leu Gly His Ile Phe Thr Phe Thr Pro Gln	2050	2055	2060
Asn Asn Glu Phe Gln Leu Gln Leu Ser Pro Arg Thr Phe Ala Ser Lys	2065	2070	2075
Thr Tyr Gly Leu Cys Gly Ile Cys Asp Glu Asn Gly Ala Asn Asp Phe	2085	2090	2095
Ile Leu Arg Asp Gly Thr Val Thr Thr Asp Trp Lys Ala Leu Ile Gln	2100	2105	2110
Glu Trp Thr Val Gln Gln Leu Gly Lys Thr Ser Gln Pro Val His Glu			

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2115	2120	2125
Glu Gln Cys Pro Val Ser Glu Phe Phe His Cys Gln Val Leu Leu Ser		
2130	2135	2140
Glu Leu Phe Ala Glu Cys His Lys Val Leu Ala Pro Ala Thr Phe Tyr		
2145	2150	2155
Ala Met Cys Gln Pro Asp Ser Cys His Pro Lys Lys Val Cys Glu Ala		
2165	2170	2175
Ile Ala Leu Tyr Ala His Leu Cys Arg Thr Lys Gly Val Cys Val Asp		
2180	2185	2190
Trp Arg Arg Ala Asn Phe Cys Ala Met Ser Cys Pro Pro Ser Leu Val		
2195	2200	2205
Tyr Asn His Cys Glu His Gly Cys Pro Arg Leu Cys Glu Gly Asn Thr		
2210	2215	2220
Ser Ser Cys Gly Asp Gln Pro Ser Glu Gly Cys Phe Cys Pro Pro Asn		
2225	2230	2235
Gln Val Met Leu Glu Gly Ser Cys Val Pro Glu Glu Ala Cys Thr Gln		
2245	2250	2255
Cys Ile Ser Glu Asp Gly Val Arg His Gln Phe Leu Glu Thr Trp Val		
2260	2265	2270
Pro Ala His Gln Pro Cys Gln Ile Cys Thr Cys Leu Ser Gly Arg Lys		
2275	2280	2285
Val Asn Cys Thr Leu Gln Pro Cys Pro Thr Ala Lys Ala Pro Thr Cys		
2290	2295	2300
Gly Pro Cys Glu Val Ala Arg Leu Arg Gln Asn Ala Val Gln Cys Cys		
2305	2310	2315
Pro Glu Tyr Glu Cys Val Cys Asp Leu Val Ser Cys Asp Leu Pro Pro		
2325	2330	2335
Val Pro Pro Cys Glu Asp Gly Leu Gln Met Thr Leu Thr Asn Pro Gly		
2340	2345	2350
Glu Cys Arg Pro Asn Phe Thr Cys Ala Cys Arg Lys Asp Glu Cys Arg		
2355	2360	2365
Arg Glu Ser Pro Pro Ser Cys Pro Pro His Arg Thr Pro Ala Leu Arg		
2370	2375	2380
Lys Thr Gln Cys Cys Asp Glu Tyr Glu Cys Ala Cys Asn Cys Val Asn		
2385	2390	2395
Ser Thr Val Ser Cys Pro Leu Gly Tyr Leu Ala Ser Ala Val Thr Asn		
2405	2410	2415
Asp Cys Gly Cys Thr Thr Thr Thr Cys Phe Pro Asp Lys Val Cys Val		
2420	2425	2430
His Arg Gly Thr Ile Tyr Pro Val Gly Gln Phe Trp Glu Glu Ala Cys		
2435	2440	2445
Asp Val Cys Thr Cys Thr Asp Leu Glu Asp Ser Val Met Gly Leu Arg		
2450	2455	2460
Val Ala Gln Cys Ser Gln Lys Pro Cys Glu Asp Asn Cys Leu Ser Gly		
2465	2470	2475
Phe Thr Tyr Val Leu His Glu Gly Glu Cys Cys Gly Arg Cys Leu Pro		
2485	2490	2495
Ser Ala Cys Glu Val Val Thr Gly Ser Pro Arg Gly Asp Ala Gln Ser		
2500	2505	2510
His Trp Lys Asn Val Gly Ser His Trp Ala Ser Pro Asp Asn Pro Cys		
2515	2520	2525
Leu Ile Asn Glu Cys Val Arg Val Lys Glu Glu Val Phe Val Gln Gln		
2530	2535	2540

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Arg Asn Val Ser Cys Pro Gln Leu Asn Val Pro Thr Cys Pro Thr Gly
 2545 2550 2555 2560
 Phe Gln Leu Ser Cys Lys Thr Ser Glu Cys Cys Pro Thr Cys His Cys
 2565 2570 2575
 Glu Pro Leu Glu Ala Cys Leu Leu Asn Gly Thr Ile Ile Gly Pro Gly
 2580 2585 2590
 Lys Ser Leu Met Ile Asp Val Cys Thr Thr Cys Arg Cys Thr Val Pro
 2595 2600 2605
 Val Gly Val Ile Ser Gly Phe Lys Leu Glu Gly Arg Lys Thr Thr Cys
 2610 2615 2620
 Glu Ala Cys Pro Leu Gly Tyr Lys Glu Glu Lys Asn Gln Gly Glu Cys
 2625 2630 2635 2640
 Cys Gly Arg Cys Leu Pro Ile Ala Cys Thr Ile Gln Leu Arg Gly Gly
 2645 2650 2655
 Gln Ile Met Thr Leu Lys Arg Asp Glu Thr Ile Gln Asp Gly Cys Asp
 2660 2665 2670
 Ser His Phe Cys Lys Val Asn Glu Arg Gly Glu Tyr Ile Trp Glu Lys
 2675 2680 2685
 Arg Val Thr Gly Cys Pro Pro Phe Asp Glu His Lys Cys Leu Ala Glu
 2690 2695 2700
 Gly Gly Lys Ile Met Lys Ile Pro Gly Thr Cys Cys Asp Thr Cys Glu
 2705 2710 2715 2720
 Glu Pro Glu Cys Lys Asp Ile Ile Ala Lys Leu Gln Arg Val Lys Val
 2725 2730 2735
 Gly Asp Cys Lys Ser Glu Glu Glu Val Asp Ile His Tyr Cys Glu Gly
 2740 2745 2750
 Lys Cys Ala Ser Lys Ala Val Tyr Ser Ile His Met Glu Asp Val Gln
 2755 2760 2765
 Asp Gln Cys Ser Cys Cys Ser Pro Thr Gln Thr Glu Pro Met Gln Val
 2770 2775 2780
 Ala Leu Arg Cys Thr Asn Gly Ser Leu Ile Tyr His Glu Ile Leu Asn
 2785 2790 2795 2800
 Ala Ile Glu Cys Arg Cys Ser Pro Arg Lys Cys Ser Lys
 2805 2810

<210> SEQ ID NO 3
 <211> LENGTH: 60
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris

<400> SEQUENCE: 3

aggggggtttc caaaatgaca aaagagtgg cctctccgtg tatctcggag aatttttoga 60

<210> SEQ ID NO 4
 <211> LENGTH: 60
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris

<400> SEQUENCE: 4

cattcatttg ttgtcaatg gtaccatgct gcaggggacc caaaggtaa tcagaagccc 60

<210> SEQ ID NO 5
 <211> LENGTH: 60
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris

<400> SEQUENCE: 5

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gaatgttcag gttaatatgg accctgggga toactttgca accccttgt tttttcagat 60

<210> SEQ ID NO 6
 <211> LENGTH: 60
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 6

gagggagccg gggccagag acaggaagta aatgtgccca gggaaagtga gtggcaggac 60

<210> SEQ ID NO 7
 <211> LENGTH: 60
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 7

tgggtgaaag ccccatatcc cgactcctgg tcaaggagac ttgcaccaa ggtcccagcc 60

<210> SEQ ID NO 8
 <211> LENGTH: 60
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 8

ctgggagcatg ggggtggggg tggaagggtg agggacatgg aggaatgca tgagaagcac 60

<210> SEQ ID NO 9
 <211> LENGTH: 58
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 9

gcttctgag ctctccttg tccaccagc atctccatgc cctacgcctc caatgggc 58

<210> SEQ ID NO 10
 <211> LENGTH: 24
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 10

aaatgacaaa agagtgaacc ggtc 24

<210> SEQ ID NO 11
 <211> LENGTH: 24
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 11

aagtctcctt gaccagcggg cggt 24

<210> SEQ ID NO 12
 <211> LENGTH: 35
 <212> TYPE: PRT
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 12

Gly Gly Phe Gln Asn Asp Lys Arg Val Ser Leu Ser Val Tyr Leu Gly
 1 5 10 15

Glu Phe Phe Asp Ile His Leu Phe Val Asn Gly Thr Met Leu Gln Gly
 20 25 30

Thr Gln Arg
 35

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<210> SEQ ID NO 13
 <211> LENGTH: 9
 <212> TYPE: PRT
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 13
 Ile Ser Met Pro Tyr Ala Ser Asn Gly
 1 5

<210> SEQ ID NO 14
 <211> LENGTH: 31
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 14
 aggacaactg cctgcctgtc ggtgagtggg g 31

<210> SEQ ID NO 15
 <211> LENGTH: 31
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 15
 aggacaactg cctgcctgtc agtgagtggg g 31

<210> SEQ ID NO 16
 <211> LENGTH: 8
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 16
 aggttagt 8

<210> SEQ ID NO 17
 <211> LENGTH: 12
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 17
 ggcttcactt at 12

<210> SEQ ID NO 18
 <211> LENGTH: 18
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 18
 aggacaactg cctggctt 18

<210> SEQ ID NO 19
 <211> LENGTH: 15
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 19
 gagcctttgt cgccc 15

<210> SEQ ID NO 20
 <211> LENGTH: 16
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris
 <400> SEQUENCE: 20

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gagccttttg tcgccc 16

<210> SEQ ID NO 21
<211> LENGTH: 218
<212> TYPE: DNA
<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 21

gtcctgtggg agcagtgcca gctcctgaag agtgccctcg tgtttgcccg ctgccaccgg 60
ctgggtggacc ctgagccttt tctgcacctg tgtgaanagga ctctgtgcac ctgtgtccag 120
gggatggagt gcccttgtgc ggtcctcctg gactacgccc gggcctgtgc ccagcaggga 180
attgtgctgt acggtctggac cgaccacagc gtctgcgg 218

<210> SEQ ID NO 22
<211> LENGTH: 73
<212> TYPE: PRT
<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 22

Val Leu Trp Glu Gln Cys Gln Leu Leu Lys Ser Ala Ser Val Phe Ala
1 5 10 15
Arg Cys His Pro Leu Val Asp Pro Glu Pro Phe Val Ala Leu Cys Glu
20 25 30
Arg Thr Leu Cys Thr Cys Val Gln Gly Met Glu Cys Pro Cys Ala Val
35 40 45
Leu Leu Glu Tyr Ala Arg Ala Cys Ala Gln Gln Gly Ile Val Leu Tyr
50 55 60
Gly Trp Thr Asp His Ser Val Cys Arg
65 70

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 23

tcctgtggga gcagtgccag 20

<210> SEQ ID NO 24
<211> LENGTH: 11
<212> TYPE: DNA
<213> ORGANISM: Canis familiaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: 3-9
<223> OTHER INFORMATION: n=a,c,t, or g

<400> SEQUENCE: 24

gennnnnnng c 11

<210> SEQ ID NO 25
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 25

gtggtcggtc cagccgta 18

<210> SEQ ID NO 26
<211> LENGTH: 20

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<212> TYPE: DNA
 <213> ORGANISM: Canis familiaris

<400> SEQUENCE: 26

tataacctgt gggccagttc

20

<210> SEQ ID NO 27
 <211> LENGTH: 19
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris

<400> SEQUENCE: 27

gaccacctca caggcagat

19

<210> SEQ ID NO 28
 <211> LENGTH: 21
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris

<400> SEQUENCE: 28

ctgtgaggac aactgcctgc c

21

<210> SEQ ID NO 29
 <211> LENGTH: 26
 <212> TYPE: DNA
 <213> ORGANISM: Canis familiaris

<400> SEQUENCE: 29

tggccctgaa cgggaaatta ctcaag

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We claim:

1. An isolated nucleic acid molecule comprising a nucleotide sequence encoding mutated canine von Willebrand Factor polypeptide which causes canine von Willebrand's disease, wherein the nucleotide sequence is capable of hybridizing under high stringency conditions to the complementary sequence of the sequence of SEQ ID NO. 1 having a mutation at nucleotide 937.

2. A vector comprising the nucleic acid molecule of claim 1.

3. A cell comprising the vector of claim 2.

4. The isolated nucleic acid molecule of claim 1, wherein the mutation at nucleotide 937 is a base deletion.

5. A method of detecting a canine von Willebrand Factor gene in a sample comprising the steps of:

- a) contacting the sample with an oligonucleotide comprising contiguous nucleotides of the nucleic acid sequence of SEQ ID NO. 1 or complement thereof, having a mutation at nucleotide 937, and capable of specifically hybridizing with the canine von Willebrand Factor gene, under conditions favorable for hybridization of the oligonucleotide to any complementary sequence of nucleic acid in the sample; and
- b) detecting hybridization, thereby detecting a canine von Willebrand Factor gene.

6. The method of claim 5, further comprising the step of:

- c) quantifying hybridization of the oligonucleotide to the complementary sequence.

7. The method of claim 5, wherein the mutation at nucleotide 937 is a base deletion.

8. An assay kit for screening for a canine von Willebrand factor gene comprising:

- a) an oligonucleotide comprising contiguous nucleic acids of the nucleotide sequence of SEQ ID NO. 1 having a mutation at nucleotide 937, and capable of hybridizing with the nucleotide sequence encoding canine von Willebrand factor;

- b) reagents for hybridization of the oligonucleotide to a complementary nucleic acid sequence; and

- c) container means for a)-b).

9. The assay kit of claim 8, wherein the mutation at nucleotide 937 is a base deletion.

10. An assay kit for screening for a canine von Willebrand Factor gene comprising:

- a) a oligonucleotide comprising contiguous nucleic acids of the nucleotide sequence that is complementary to the sequence of SEQ ID NO. 1 having a mutation at nucleotide 937, and capable of specifically hybridizing to the complementary nucleotide sequence;

- b) reagents for hybridization of the oligonucleotide to a complementary nucleic acid sequence; and

- c) container means for a)-b).

11. The assay kit of claim 10, wherein the mutation at nucleotide 937 is a base deletion.

12. A method for detecting a mutated canine von Willebrand Factor gene in a canine DNA sample comprising the steps of:

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a) amplifying the DNA sample by polymerase chain reaction to produce polymerase chain reaction products, wherein the polymerase chain reaction uses primers that produce a restriction site in a mutant allele but not in a normal allele, wherein the mutation in the mutant allele is a base deletion at nucleotide 937 of the gene encoding canine von Willebrand Factor (SEQ ID NO. 1);

b) digesting the polymerase chain reaction products with a restriction enzyme specific to the restriction site of the restriction site primer to produce DNA fragments; and

c) detecting the DNA fragments, thereby detecting a mutated canine von Willebrand Factor gene.

13. The method of claim 12, wherein the DNA fragments are detected by gel electrophoresis.

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14. The method of claim 12, wherein the primers comprise the sequence of SEQ ID NOS: 23 and 25.

15. The method of claim 12, wherein the restriction enzyme is Mwo I.

16. An oligonucleotide probe capable of detecting a mutation associated with canine von Willebrand's disease, wherein the mutation is a base deletion at nucleotide 937 of the nucleotide sequence encoding canine von Willebrand Factor polypeptide, wherein the nucleotide sequence is capable of hybridizing under high stringency conditions to the complementary sequence of the sequence of SEQ ID NO. 1.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO. : 6,780,583 B1
APPLICATION NO. : 09/662478
DATED : August 24, 2004
INVENTOR(S) : Patrick J. Venta et al.

Page 1 of 1

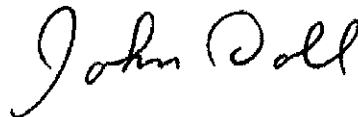
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

ON COVER PAGE, ITEM 63

“Continuation-in-part of application No. 08/896,449, Jul. 18, 1997, now Pat. No. 6,040,143.” should be --Divisional of Application No. 09/132,652, filed Aug. 11, 1998, now Pat. No. 6,074,832, which is a Continuation-in-part of Application No. 08/896,449, filed on Jul. 18, 1997, now Pat. No. 6,040,143.--

Signed and Sealed this

Ninth Day of June, 2009

A handwritten signature in cursive script that reads "John Doll".

JOHN DOLL
Acting Director of the United States Patent and Trademark Office