© 1998 Oxford University Press

Human Molecular Genetics, 1998, Vol. 7, No. 6 **1047–1052**

A dinucleotide mutation in the endothelin-B receptor gene is associated with lethal white foal syndrome (LWFS); a horse variant of Hirschsprung disease (HSCR)

G. C. Yang, D. Croaker, A. L. Zhang, P. Manglick, T. Cartmill and D. Cass*

Department of Surgical Research, Royal Alexandra Hospital for Children, Westmead, NSW 2145, Australia

Received February 10, 1998; Revised and Accepted March 19, 1998

DDBJ/EMBL/GenBank accession no. AF019072

Lethal white foal syndrome (LWFS) is a congenital anomaly of horses characterized by a white coat colour and aganglionosis of the bowel, which is similar to Hirschsprung disease (HSCR). We decided to investigate possible mutations of the endothelin-B receptor gene (*EDNRB*) in LWFS as recent studies in mutant rodents and some patients have demonstrated EDNRB defects. First, we identified a full-length cDNA for horse *EDNRB*. This cDNA fragment contained a 1329 bp open reading frame which encoded 443 amino acid residues. The predicted amino acid sequence was 89, 91 and 85% identical to human, bovine and mouse as well as rat EDNRB respectively, but only 55% identical to the human, bovine and rat endothelin A receptor (EDNRA). Secondly, sequence analysis, together with allele-specific PCR and the amplification-created restriction site (ACRS) technique, revealed a dinucleotide TC→AG mutation, which changed isoleucine to lysine in the predicted first transmembrane domain of the EDNRB protein. This was associated with LWFS when homozygous and with the *overo* phenotype when heterozygous.

INTRODUCTION

Hirschsprung disease (HSCR) is a congenital human disorder characterized by the absence of enteric ganglion cells (aganglionosis) in the distal gastrointestinal tract (1). The disease is thought to be multifactorial, modified by sex, and there is a positive family history of HSCR in 15% of patients. Recent studies in HSCR patients have demonstrated gene mutations in two membrane receptor–ligand pairs: *RET*–glial-derived neurotrophic factor (*GDNF*) (2–4), and endothelin-B receptor (*EDNRB*)–endothelin 3 (*EDN3*) (5,6), as well as a transcription factor *SOX10* (7). All these are considered to be loss-of-function mutations.

Aganglionosis occurs in rodents (8,9), cats (10), pigs (11) and horses (12–14). The rodent models also have a characteristic coat colour pattern suggesting that the genetic defect resides in a common mechanism involved in regulation of development of two neural crest cell-derived cell lineages, namely the enteric nervous system (ENS) and epidermal melanocytes. When the *RET* gene was functionally deleted in mice, the animals produced had extensive aganglionosis and a pigmented coat (15); whereas when *EDNRB* or *EDN3* genes were 'knocked out' the mice produced had colonic aganglionosis and changes in the coat colour resulting in a phenotype identical to *piebald-lethal* and *lethal spotting* mice. Subsequent studies identified an absence of EDNRB in *piebald-lethal* mice, and a point mutation of pro-EDN3 in *lethal spotting mice* (16). The spotting lethal rat has a 301 bp deletion in *EDNRB* exon–intron 1 (17). It too has a white coat and aganglionosis, which often extends into the ileum.

The combination of white coat with little or no patches of colour and extensive aganglionosis has also been described in the congenital abnormality, lethal white foal syndrome (LWFS), which presents as a fatal neonatal intestinal obstruction (12,13). LWFS most commonly results from mating *overo* × *overo* paint horses. The similarity in clinical, histological and pigmentary phenotype to that observed in rodent models led us to suspect that a defect in the *EDNRB* gene may cause LWFS.

To decide whether an EDNRB defect causes the LWFS, we first isolated and identified a full-length cDNA for *EDNRB* from standard bred horses. When we compared this sequence with that of LWFS horses, we found a TC→AG dinucleotide mutation in the *EDNRB* gene of LWFS horses, changing the amino acid isoleucine to lysine in the predicted first transmembrane domain of the EDNRB protein. Association analysis confirmed the homozygous mutation was present in all LWFS.

RESULTS

Complete cDNA sequence for the horse *EDNRB*

As no sequence data or gene structure information were available for horse *EDNRB*, we applied a strategy of cross-species RT-PCR combined with a technique for isolation of full-length cDNA to determine the coding sequence of horse *EDNRB*. Two pairs of primers, ho1/pr4 and pr3/ho2, were selected and designed so as to have high homology with *EDNRB* cDNA in species including human, rodents, bovine and pig, but weak homology with corresponding sequences of *EDNRA*. The central part of a 1282 bp cDNA framed by oligonucleotide sequence ho1 and ho2 was isolated in two cDNA fragments (ho1/pr4 and pr3/ho2) from random-primed first-strand cDNA (Fig. 1). Sequences determined

*To whom correspondence should be addressed. Tel: +61 2 9845 3059; Fax: +61 2 9845 3180; Email:dannyc@nch.edu.au

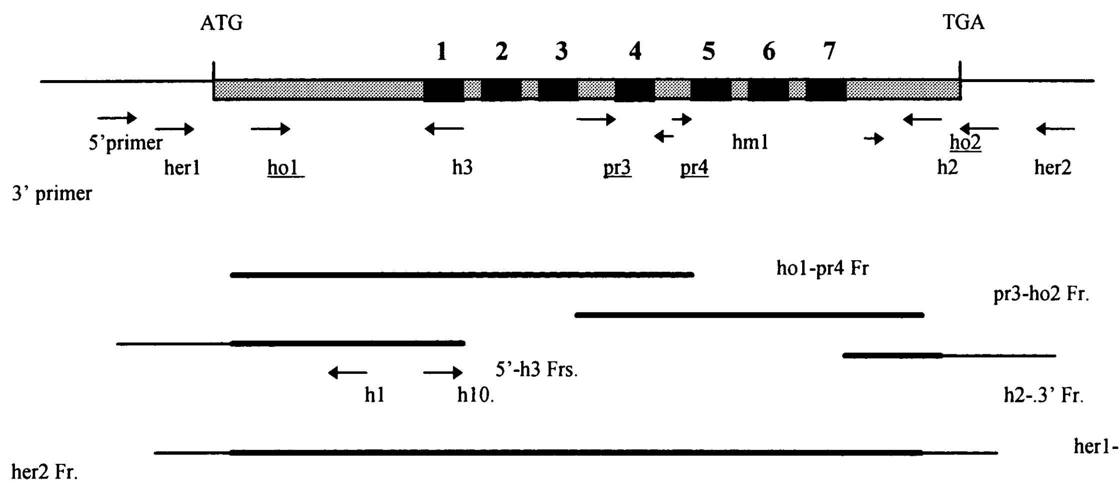

Figure 1. Schematic diagram showing the location on cDNA and direction of primers utilized in RT-PCR and inverse PCR used for isolation of full-length horse *EDNRB* cDNA. The translated region is shown by the box. The non-translated region is a single line. The sequences encoding the seven predicted transmembrane domains are displayed as black areas.

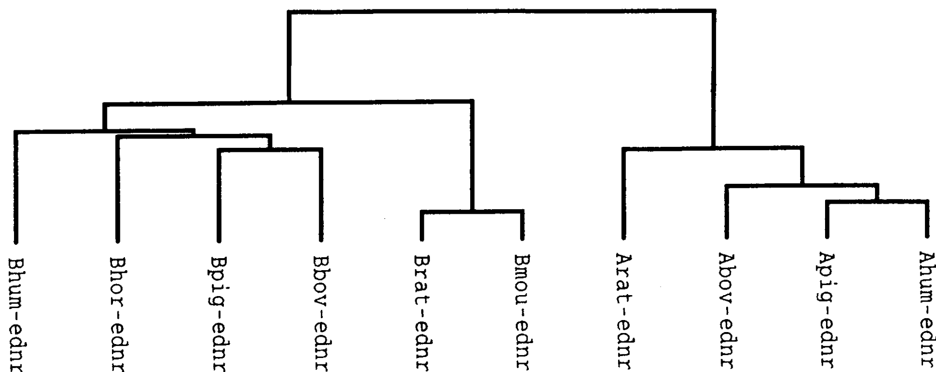

Figure 2. A dendrogram of multiple sequence alignment showing homology of amino acid sequence of horse EDNRB with other species. Sequences were extracted from the database using the Entrez network server. The notations are as follows: ednr = endothelin receptors; the prefix A = EDNRA, prefix B = EDNRB, hum = human, hor = horse, bov = bovine, mou = mouse.

in the ho1/pr4 cDNA fragment and the pr3/ho2 cDNA fragment overlapped. The isolated 5′- and 3′-terminal cDNA contained 130 bp of 5′- and 228 bp of 3′-untranslated regions respectively. Sequence analysis indicated that these sequences also overlapped with the initially isolated central part of the cDNA segment framed by oligonucleotides ho1 and ho2. In order to isolate the whole molecule of cDNA, a pair of primers, her1 and her2, were generated from the 5′- and 3′-untranslated region and used to amplify the cDNA sequence from the first strand cDNA pool. A 1667 bp cDNA fragment was isolated and cloned into plasmid vector. The complete cDNA sequence is composed of a 1329 bp open reading frame which encodes a 443 amino acid peptide. By using a probe which contained a 113 bp 5′-untranslated region and a 237 bp 5′-coding region, a 5 kb mRNA band was found on northern blot hybridization (data not shown). In the 3′-non-coding region of this sequence, there were two potential polyadenylation signals AATAA and ATTAAA which were at 123 and 24 bp upstream from the end of this sequence where there is thought to be a polyadenylation site for EDNRB mRNA. Amino acid sequence analysis showed that this peptide consists of seven hydrophobic domains, a typical feature of the G protein-coupled receptor family (GPCR). The DNA and deduced amino acid sequence were aligned with *EDNRB* and *EDNRA* from several species. Sequence comparison revealed that the coding region sequence of the cDNA has most homology with *EDNRB*, with 85% identity in humans. The predicted amino acid sequence shares 91% identity with bovine, 89% with humans, and 85% with rat and mouse EDNRB; but has only 55% homology to human, bovine and rat EDNRA. A dendogram of receptor sequences showed that our putative horse EDNRB best aligned with the EDNRB family (Fig. 2).

TC→AG mutation in *EDNRB* cDNA of horse LWFS

To determine whether mRNA for *EDNRB* is produced and whether there is a mutation in the *EDNRB* gene in LWFS, we performed RT-PCR to isolate cDNA by using different pairs of primers located along the cDNA. The cDNA fragments obtained

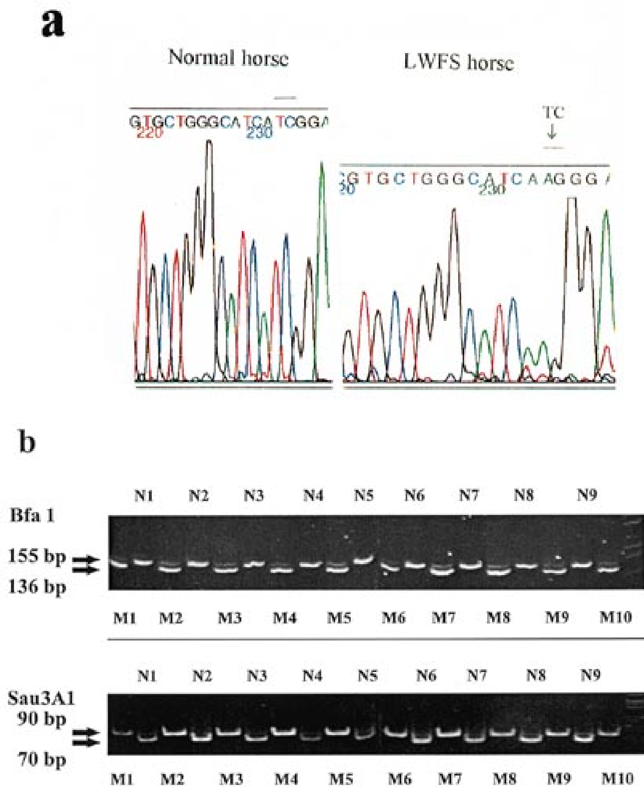

Figure 3. (**a**) The dinucleotide mutation detection by automatic sequencing. The sequence was determined from two representative clones harbouring the normal and LWFS alleles, respectively. The dinucleotide TC→AG mutation is indicated by the arrow. (**b**) Association of the dinucleotide mutation with LWFS. Analysis was performed by use of the ACRS approach. An 8% polyacylamide gel electrophoresis showing the restriction enzyme *Bfa*I digestion pattern of PCR products from 10 LWFS horses (M1–M10) and nine normal horses (N1–N9) by using designed primer ps2 and hex1 primer (top). There are indigestible 155 bp DNA bands (normal controls) and digested 136 bp bands (LWFS). On the bottom is the *Sau*3AI digestion pattern of the PCR products primed with ps4 and ps5. The indigestible 90 bp DNA band occurs only in LWFS, whereas the digested 70 bp DNA band occurs only in normal controls.

were compared with that of control horses. The results showed only a single cDNA band in each PCR and no obvious size difference compared with the normal horse (data not shown). We then cloned the whole cDNA including the 5′- and 3′-untranslated regions. Sequence analysis was performed on three different clones from both directions. This revealed a dinucleotide TC→AG mutation at nucleotide position 353–354 in LWFS which changes isoleucine to lysine in the predicted first transmembrane domain of the horse EDNRB protein (Fig. 3a). Neither this mutation nor the control DNA sequence resulted in a restriction enzyme digestion site that would allow genotyping.

Association of the mutation in the *EDNRB* gene with horse LWFS

We carried out allele-specific PCR to confirm the mutation in the genomic DNA and to determine whether this mutation is present in other LWFS animals. When mutant allele-specific primer hm4 was used, a 152 bp PCR band was produced in six LWFS horses; however, no PCR product was found in normal horses. In contrast, when a wild-type allele-specific primer hs1 was used, only normal horses produced 152 bp DNA bands (data not shown). These results indicated that all LWFS horses were homozygous for the mutation.

In parallel to the allele-specific PCR, we adapted the amplification-created restriction site (ACRS) technique (18) to perform association analysis of the dinucleotide mutation with the LWFS. Two ACRS primers, ps2 and ps4, were designed to distinguish and identify mutant and wild-type alleles. All 155 bp PCR DNA bands amplified by ps2/hex1 primers from 10 LWFS horses were all digestible with restriction enzyme *Bfa*I, giving two fragments with sizes of 136 and 19 bp. All DNA bands produced by PCR with the same primers from nine normal horses failed to be cut. In contrast, the 90 bp DNA band amplified by ps4/ps5 primers from DNA of 10 LWFS horses could not be cut by restriction enzyme *Sau*3AI; however, the DNA band produced by PCR from nine normal horses was digested by this enzyme to give two fragments with sizes of 70 and 20 bp (Fig. 3b).

To elucidate how the dinucleotide mutation was inherited from the parents of affected foals, a family analysis was carried out by PCR using ACRS primers, ps2 and ps4 in eight horse families (a total of 22 animals). All available sires and dams of LWFS horses were heterozygous for the mutation (Fig. 4a).

To confirm the co-occurrence of the dinucleotide mutation with LWFS and to determine the genetic basis of the *overo* phenotype, 15 further *overo* horses were subjected to ACRS analysis. All were heterozygous for the dinucleotide mutation (Fig. 4b). This suggested that a single dose of mutant allele was responsible for the *overo* phenotype. Altogether 48 chromosome pairs were analysed. All 10 chromosome pairs that were associated with LWFS were homozygous for the mutation, and of the 27 chromosome pairs from *overo* horses or parents of LWFS progeny, 27 chromosomes were normal (TC), and 27 chromosomes were mutant (AG), i.e. all animals were heterozygous. All 11 normal horse chromosome pairs (nine selected controls and two from a second sire and offspring in family 2) had the TC sequence.

DISCUSSION

In this study, we isolated full-length cDNA for horse *EDNRB*. The deduced amino acid sequence, consisting of 443 amino acid residues, has seven transmembrane domains with a typical sequence and topographical structure representing a GPCR. So far two isoforms of endothelin receptors, EDNRB and EDNRA, have been found in humans and several animals. Both of the receptors belong to the GPCR family and show different binding activities to the three endothelin peptides, endothelins 1, 2 and 3 (19). Sequence comparison of our predicted protein with human, rat, mouse and bovine endothelin receptors showed 85–91% identity with EDNRB, which is a high degree of homology, but only 55% identity with the EDNRA of several species, suggesting that this protein does represent EDNRB rather than EDNRA. To be certain that this protein is the horse EDNRB, it will be necessary to perform expression and affinity studies in the future. However, it was the similarity of the LWFS phenotype to that shown in other aganglionosis animals which had defects in the EDNRB that prompted us to isolate the horse *EDNRB* gene and search for mutations in it. Therefore, the identification of a dinucleotide mutation in this gene that is associated with LWFS

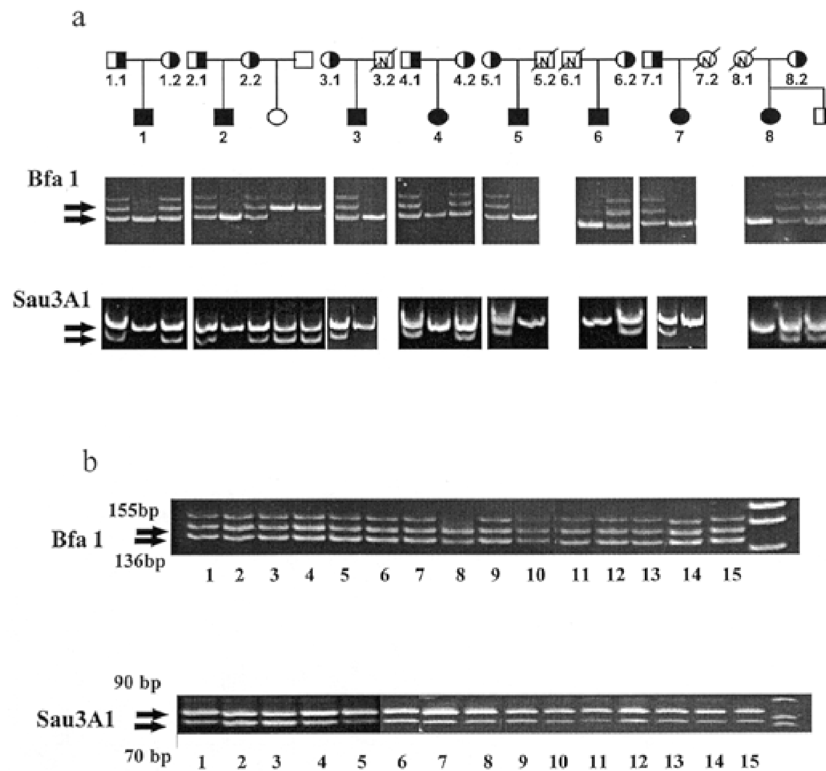

Figure 4. (**a**) Dinucleotide mutation in eight horse families with LWFS progeny. ACRS primer pairs ps2/hex1 and ps4/ps5 gave a PCR product from all the members of the eight horse families which were then digested with *Bfa*I or *Sau*3AI and analysed by electrophoresis on an 8% polyacylamide gel. Digested and undigested DNA bands are indicated by arrows (the third DNA band, seen at the top, is the result of heteroduplex DNA from heterozygotes, produced in the PCR reaction primed with ps2/hex1). The eight horses with LWFS are numbered 1–8. The digestion pattern is directly under each notated animal. The parents from which we could not get a sample are designated by the hatched N. (**b**) Determination of heterozygosity of the dinucleotide mutation in *overo* paint horses. PCR products, amplified by using ACRS primers ps2/hex1 and ps4/hex1 from 15 *overo* horses, were digested with restriction enzyme *Bfa*I and *Sau*3AI and analysed by electrophoresis on an 8% polyacylamide gel. Undigested and digested DNA bands are indicated by upper and lower arrows respectively.

further supports our conclusion that the isolated cDNA codes for horse EDNRB.

The dinucleotide mutation associated with LWFS was identified initially by sequence comparison and then confirmed by PCR with an allele-specific primer. However, it remained possible that the dinucleotide genetic variation might be a single nucleotide mutation (responsible for LWFS) along with a one nucleotide variation that represented a sequence polymorphism. This mutation plus polymorphism might not be detected by the allele-specific primer technique. To rule out this possibility, the ACRS method was applied (18). As only the co-presence of the dinucleotide mutation (AG) permitted the sequence to be recognized by the restriction enzyme *Bfa*I and hence digested; the fact that there was complete digestion of amplified PCR product from LWFS horses suggested that all LWFS horses were homozygous for the dinucleotide mutation. Dinucleotide mutations have been found occasionally; for example in the human hepatictriglyceride lipase (*HTGC*) gene (20) and the plant dihydrodipicolinate synthase gene (21).

The observation of a dinucleotide mutation in *EDNRB* in LWFS horses, together with rodent mutations in *EDN3*, *EDNRB* provides four animal models displaying congenital aganglionosis involving the EDN3–ENDRB signal transduction system. Accumulated evidence suggests that this system interacts with the GDNF–RET signal system (22) and could be interfered with by other unidentified signal systems (23,24) which may represent the molecular basis of HSCR. The recent location in the *Dom* mouse of mutations in SOX10 (SRY-like HMG box family of transcription factors) (25) raises the possibility that this is another interrelated factor.

LWFS is produced most often by mating horses with a specific colour pattern, known as *overo* (13). Our results explain and extend this observation. All 11 parents of LWFS and 15 additional *overo* horses were heterozygous carriers. The single dose of the mutant allele is enough to produce the characteristic coat colour but the presence of at least one normal allele protects against the development of aganglionosis (the LWFS phenotype). This explanation was confirmed in family 8 where a sibling was heterozygous for the dinucleotide mutation but was unaffected (Fig. 4a). To develop the LWFS phenotype, the foal must be homozygous for the mutant allele, inheriting one from each heterozygous parent. This inheritance is similar to that in rodents (8,9). However, in humans, heterozygous as well as homozygous *EDNRB* mutations are found in HSCR patients (22). This could be explained by a dose effect of gene expression. In rodent models, the single *EDNRB* gene of heterozygous animals may be expressed over and above the requirement for development of the ENS. Therefore, even in the heterozygous state, expression of one copy of the gene could be sufficient for normal ENS development but could lead to some diminution in the number of melanocytes.

In contrast, in humans, the *EDNRB* gene could be regulated to be expressed at a lower level so that expression of both genes is required to ensure normal ENS development.

In contrast to the *piebald lethal* mouse, where the whole *EDNRB* gene and possibly other genes are deleted, and the *spotting lethal* rat, where 301 bp of *EDNRB* are deleted, in the LWFS only a single amino acid is changed. Comparison of amino acid sequence of horse EDNRB with mouse, rat, pig, bovine and human EDNRB revealed conservation of the isoleucine residue at amino acid position 118 on the first transmembrane domain in all species (data not shown), suggesting that the change of the isoleucine residue to lysine at position 118 of the receptor protein may impair receptor function. Therefore, the LWFS may provide valuable material for the study of domain function and molecular events occurring in signal transduction.

MATERIALS AND METHODS

DNA and RNA preparation

DNA was extracted from horse peripheral blood lymphocytes. RNA was prepared from horse liver tissue using TR1 Reagent as described by the manufacturer (Molecular Research Centre).

Isolation of full-length cDNA for the horse *EDNRB* gene

First-strand cDNA was synthesized through reverse transcription by using random primers and avian myeloblastosis virus (AMV) reverse transcriptase according to the manufacturer's description (Boehinger Mannheim). In order to isolate the central part of the cDNA, two segments as shown in Figure 1 were isolated by PCR amplification using two pairs of primers (ho1/pr4 and pr3/ho2) (Table 1). Primer sequences for pr3 and pr4 were identical to highly conserved *EDNRB* gene sequences in several species. The degenerate primers ho1 and ho2 were designed to approximate EDNRB sequences found in several species at the 5′ and 3′ ends of the gene respectively. The two cDNA fragments were cloned into plasmid and sequenced by automatic sequencing.

Table 1. PCR primers used in isolation of full-length cDNA for horse *EDNRB*

5′ primer	5′-TGCTGCGAGAAGACGACAGAAT-3′
her1	5′-GCGTTCCAGGGAAGAAAG-3′
ho1	5′-CGGACGCGCCC/TTGGTT/GGCGCTG3′
h1	5′-CGCCGTCCTCCCTGCTTT-3′
h10	5′-GTCCTGCCTAGTGTTCGTG-3′
h3	5′-CACGAACACTAGGCAGGACAC-3′
pr3	5′-TATCGAGCTGTTGCTTCTTG-3′
pr4	5′-CTGCATGAAGGCTGTTTTCT-3′
hm1	5′-ACAAGAATGCTAAGGATTGG-3′
h2	5′-ACAGTCCTTGGAAGACAAGC-3′
ho2	5′-GGAACGGAAGTTGTCATATCCGTG-3′
her2	5′-GGCTTTCACAATGAGGCTT-3′
3′ primer	5′-oligo(dt)$_{30}$N-1N-3′

Inverse PCR was performed to isolate the 5′ cDNA. The first-strand cDNA was made by reverse transcription using modified oligo(dT) primers and switching at the 5′ end by capswitch oligonucleotide provided in Capfinger PCR kit (Clontech). From this first-strand cDNA, the 5′ primer and h3 primer were used to amplify the 5′ end sequences. Then 50 µl of the PCR product was blunt ended with T4 DNA polymerase at 16°C for 30 min and heated at 72°C for 10 min. The DNA fragments were then recovered with ethanol precipitation and subjected to T4 polynucleotide kinase reaction at 37°C for 30 min. After phenol and chloroform treatment, the phosphorylated DNA was recovered further by ethanol precipitation and self-ligated with T4 DNA ligase to produce circular molecules. A pair of primers, h1/h10, were used in the subsequent inverse PCR with this circular molecule as a template. Only one DNA band was finally obtained and cloned for sequencing.

To isolate the 3′ part of the cDNA, a PCR reaction with 3′ primer and hm1 primer was set up from the oligo(dT)-primed first-strand cDNA and followed by nested PCR with 3′ primer and h2 primer. The DNA band was located on a 2% low melting temperature agarose gel, excised, reamplified and cloned to determine the sequence. To isolate complete *EDNRB* cDNA molecules, a pair of primers, her1 and her2, were generated from 5′- and 3′-untranslated regions. The full-length cDNA was cloned into a vector and automatically sequenced. The cDNA sequence was deposited in GenBank under accession no. AF019072.

Database searches and computer analysis

Sequence database searches and homology analysis were performed using Entrez and BLAST programs [National Center for Biotechnology Information (NCBI)]. The GCG program Pileup was used for multiple sequence alignment, and the dendogram was displayed by use of the GCG utility figure. The PROSITE database was selected for pattern-based sequence analysis (http://www.expasy.ch/sprot/scnpsite.html).

Allele-specific PCR and amplification-created restriction site approach (ACRS)

In allele-specific PCR, mutant allele primer hm4 (5′gttcgtgctgggcatcatc3′) or wild-type allele primer hs1 (5′gttcgtgctgggcatcaag3′) were designed with the dinucleotide mutation and corresponding normal sequence located at the 3′ end of the primer.

ACRS primers ps2 (5′agtgttcgtgctgggcatc3′) and ps4 (5′agtgttcgtgctgggcatga3′) were designed to contain a nucleotide mismatch with the normal sequence at the 3′ terminus of the primer so as to create a *Bfa*I site next to the AG mutation sequence and a *Sau*3AI site on the sequence next to the normal TC sequence, respectively. Amplification of DNA was by nested PCR using ACRS primers with the ho11/hex fragment as the template. The PCR reaction was: 3 min at 94°C followed by 30 cycles of 94°C for 40 s, 63°C [for ps2/hex1 (5′cttgtagacattgatggggat3′) primer] or 61°C [for ps4/ps5 (5′tcaagatattaggccgttcc3′) primer] for 40 s and 72°C for 40 s. The last cycle was followed by an extension step of 8 min at 72°C. Then 25 µl of PCR product was purified by ethanol precipitation. The recovered DNA was incubated with *Bfa*I (Biolabs, New England) or *Sau*3AI at 37°C overnight. The

digested DNA was separated on an 8% polyacylamide gel by electrophoresis in TBE buffer.

ACKNOWLEDGEMENTS

We are grateful to the Australian Paint Horse Society, especially to Mr Murray Stevens, Mr Bruce Ramsay and Mrs Leanne Millard for help, advice and encouragement, as well as all those breeders who sent us samples. We also gratefully acknowledge Professor Reuben Rose of Sydney University Veterinary Faculty for advice and control DNA and Dr T.K. Bell of The University of Queensland, Australian Equine Blood Typing Research Laboratory for control specimens. D.C. was supported by a grant from Royal Australasian College of Surgeons and the Department of Paediatric Surgery, University of Sydney.

REFERENCES

1. Cass, D.T. (1986) Hirschsprungs disease: a historical review. *Prog. Pediatr. Surg*., **20**, 199–214.
2. Edery, P., Lyonnet, S., Mulligan, L.M., Pelet, A., Dow, E., Abel, L., Holder, S., Nihoul-Fekete, C., Ponder, B.A.J. and Munnich, A. (1994) Mutations of the *RET* proto-oncogene in Hirschsprungs disease. *Nature*, **367**, 378–379.
3. Romeo, G., Ronchetto, P., Yin, L., Barone, V., Seri, M., Ceccherini, I., Pasini, B., Bocciardi, R., Lerone, M., Kaariainen, H. and Martucciello, G. (1994) Point mutations affecting the tyrosine kinase domain of the *RET* proto-oncogene in Hirschsprungs disease. *Nature*, **367**, 377–378.
4. Angrist, M., Bolk, S., Halushka, M., Lapchak, P.A. and Chakravarti, A. (1996) Germline mutations in glial cell line-derived neurotrophic factor (GDNF) and RET in a Hirschsprung disease patient. *Nature Genet*., **14**, 341–344.
5. Puffenberger, E.G., Hosoda, K., Washington, S.S., Nakao, K., deWit, D., Yanagisawa, M. and Chakravarti, A. (1994) A missense mutation of the endothelin-B receptor gene in multigenic Hirschsprung disease. *Cell*, **79**, 1257–1266.
6. Edery, P., Attie, T., Amiel, J., Pelet, A., Eng, C., Hofstra, R.M.W., Martelli, H., Bidaud, C., Munnich, A. and Lyonnet, S. (1996) Mutation of the endothelin-3 gene in the Waardenburg–Hirschsprung disease (Shah–Waardenburg syndrome). *Nature Genet*., **12**, 442–444.
7. Pingault, V., Bondurand, N., Kuhlbrodt, K., Goerich, D., Prehu, M.O., Puliti, A., Herbarth, B., Hermans-Borgmeyer, I., Legius, E., Matthijs, G., Ariel, J., Lyonnet, S., Ceccherini, I., Romeo, G., Smith, J., Read, R., Wegner, M. and Goossens, M. (1998) *SOX10* mutations in patients with Waardenburg–Hirschsprung disease. *Nature Genet*., **18**, 171–173.
8. Lane, P.W. (1966) Association of megacolon with two recessive spotting genes in the mouse. *J. Hered*., **57**, 181–183.
9. Ikadai, H., Fujita, H., Agematsu, Y. and Imamichi, T. (1979) Observation of congenital aganglionosis rat (Hirschsprung's disease rat) and its genetical analysis. *Congen. Anom*., **19**, 31–36.
10. Dietzmann, V.U. (1968) Uber das Vorkommen des kongenitalen Megakolons (Hirschsprungsches Megakolon) bei der Katz. *Mh Verterinermed*., **23**, 349–352.
11. Kernkamp, H.C.H. and Kanning, H.H. (1955) Primary megacolon (Hirschsprung's disease) in swine. *North Am. Veterinarian*, **36**, 642–643.
12. Hultgren, B.D. (1982) Ileocolonic aganglionosis in white progeny of overo spotted horses. *J. Am. Vet. Med. Assoc*., **180**, 289–292.
13. McCabe, L., Griffin, L.D., Kinzer, A., Chandler, M., Beckwith, J.B. and McCabe, E.R.B. (1990) Overo lethal white foal syndrome: equine model of aganglionic megacolon (Hirschsprung disease). *Am. J. Med. Genet.*, **36**, 336–340.
14. Dyke, T.M., Laing, E.A. and Hutchins, D.R. (1990) Megacolon in two related Clydesdale foals. *Aust. Vet. J*., **67**, 463–464.
15. Schuchardt, A., D'Agati, V., Larsson-Blomberg, L., Costantini, F. and Pachnis, V. (1994) Defects in the kidney and enteric nervous system of mice lacking the tyrosine kinase receptor Ret. *Nature*, **367**, 380–383.
16. Hosoda, K., Hammer, R.E., Richardson, J.A., Greenstein Baynash, A., Cheung, J.C., Giaid, A. and Yanagisawa, M. (1994) Targeted and natural (piebald-lethal) mutations of endothelin-B receptor gene produce megacolon associated with spotted coat color in mice. *Cell*, **79**, 1–20.
17. Ceccherini, I., Zhang, A.L., Matera, I., Yang ,G., Devoto, M., Romeo, G. and Cass, D.T. (1995) Interstitial deletion of the endothelin-B receptor gene in the spotting lethal (sl) rat. *Hum. Mol. Genet*., **4**, 2089–2096.
18. Eiken, H.G., Odland, E., Boman, H., Skjelkvale, L., Engebretsen, L.F. and Apold, J. (1991) Application of natural and amplication created restriction sites for the diagnosis of PKU mutations. *Nucleic Acids Res*., **7**, 1427–1430
19. Sakurai, T., Yanagisawa, M. and Masaki, T. (1992) Molecular characterisation of endothelin receptors. *Trends Pharmacol. Sci*., **13**, 103–108.
20. Hadzopoulou-Cladaras, M. and Cardot, P. (1993) Identification of a *cis*-acting negative DNA element which modulates human hepatic triglyceride lipase gene expression. *Biochemistry*, **32**, 9657–9667.
21. Ghislain, M., Frankard, V. and Jacobs, M. (1995) A dinucleotide mutation in dihydrodipicolinate synthase of *Nicotiana sylvestris* leads to lysine overproduction. *Plant J*., **8**, 733–743.
22. Chakravarti, A. (1996) Endothelin receptor-mediated signalling in Hirschsprung disease. *Hum. Mol. Genet*., **5**, 303–307.
23. Puffenberger, E.G., Kauffman, E.R., Bolk, S., Matise, T.C., Washington, S.S., Angrist, M., Weissenbach, J., Garver, K.L., Mascari, M., Ladda, R., Slaugenhaupt, S.A. and Chakravarti, A. (1994) Identity-by-descent and association mapping of a recessive gene for Hirschsprung disease on human chromosome 13q22. *Hum. Mol. Genet*., **3**, 1217–1225.
24. Romeo, G. and Mckusick, V.A. (1994) Phenotypic diversity, allelic series and modifier genes. *Nature Genet*., **7**, 451–453.
25. Southard-Smith, E.M., Kos, L. and Pavan, W.J. (1998) *Sox10* mutation disrupts neural crest development in *Dom* Hirschsprung mouse model. *Nature Genet*., **18**, 60–64.