FULL PAPER *Internal Medicine*

Clinical and Clinico-Pathologic Characteristics of Shiba Dogs with a Deficiency of Lysosomal Acid β-Galactosidase: A Canine Model of Human GM1 Gangliosidosis

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(Received 19 July 2002/Accepted 7 November 2002)

ABSTRACT. The present study was conducted to determine the clinical and clinico-pathologic characteristics of Shiba dogs with GM1 gangliosidosis, which is due to an autosomal recessively inherited deficiency of lysosomal acid β-galactosidase activity. Clinical and clinico-pathological features were investigated in 10 homozygous Shiba dogs with GM1 gangliosidosis. The age at onset was 5 to 6 months and the dogs manifested progressive neurologic signs including loss of balance, intermittent lameness, ataxia, dysmetria and intention tremor of the head. The dogs were unable to stand by 10 months of age due to a progression of ataxia and spasticity in all limbs. Corneal clouding, a visual defect, generalized muscle rigospasticity, emotional disorder and a tendency to be lethargic were observed at 9 to 12 months. The dogs became lethargic from 13 months of age. The survival period seemed to be 14 to 15 months. As a clinico-pathologic feature, lymphocytes with abnormally large vacuoles were observed in peripheral blood (30 to 50% of total lymphocytes) through the lifetime of the dogs. The clinical and clinico-pathologic characteristics of this animal model are useful for not only the development and testing of potential methods of therapy, but also the diagnosis of affected homozygous Shiba dogs in veterinary clinics.

KEY WORDS: acid β-galactosidase deficiency, β-galactosidosis, GM1 gangliosidosis, lysosomal storage disease, Shiba dog.

J. Vet. Med. Sci. 65(2): 213–217, 2003

GM1 gangliosidosis, a lysosomal storage disease that affects the brain and multiple systemic organs, is due to an autosomal recessively inherited deficiency of acid β-galactosidase activity [15]. The potential of new therapeutic methods, i.e., enzyme replacement, tissue transplantation and gene therapy, is now under consideration. However, any potential treatment must first be shown to be efficacious and safe using animal models. Models of GM1 gangliosidosis have been recorded in cats, dogs, sheep, and calves, all of which are domestic animals with spontaneously occurring storage disease [15]. In addition, a mouse model lacking a functional β-galactosidase gene has been generated by homologous recombination and embryonic stem cell technology by two groups of investigators [4, 5]. Among the various models of GM1 gangliosidosis, the canine disease probably best resembles the human disease genetically, clinically, biochemically and pathologically [15]. In dogs, the disease has been described in mixed-breed beagles [9], English springer spaniels [2], Portuguese water dogs [11, 12], Alaskan huskies [6, 7], Shiba dogs [16, 20] and a cross-bred dog [18]. Molecular defects that cause the disease have been identified in Portuguese water dogs [17] and Shiba dogs [19]. Therefore, these canine breeds are expected to provide versatile *in vivo* systems for testing gene therapy.

To consider the potential of new therapeutic programs and accurately estimate the efficacy of trials, a knowledge of the clinical and clinico-pathologic features shown originally by the animal model is essential. The purpose of the present study is to characterize these features in Shiba dogs with GM1 gangliosidosis using enough numbers of affected homozygous animals, and discuss the usefulness of this animal model of human GM1 gangliosidosis.

MATERIALS AND METHODS

Animals: Ten homozygous Shiba dogs with GM1 gangliosidosis were used in the present study. The dogs, born from 1997 to 2002, belonged to the family pedigree shown in Fig. 1. The pedigree was reported previously [20], and updated to 2002 in the present study. The affected dogs were diagnosed based on clinical signs, and the results of an enzyme assay [14] and a DNA mutation assay [19].

Clinico-pathologic study: A complete blood count was made using an automatic cell counter (Celltac α, Nihon Kohden, Tokyo, Japan). An analysis of general blood chemistry was performed by using a Cobas Mira autoanalyzer (Roche, Basel, Switzerland). The blood chemistry included total protein, glucose, urea nitrogen, creatinine, total cholesterol, triglyceride, calcium, inorganic phosphorus, total bilirubin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase and creatinine kinase. A morphological analysis of leukocytes was carried out microscopically using a blood smear treated with Giemsa stain.

RESULTS

Clinical features: The clinical signs manifested by the homozygous dogs with GM1 gangliosidosis are summarized in Table 1. The dogs were clinically normal at birth

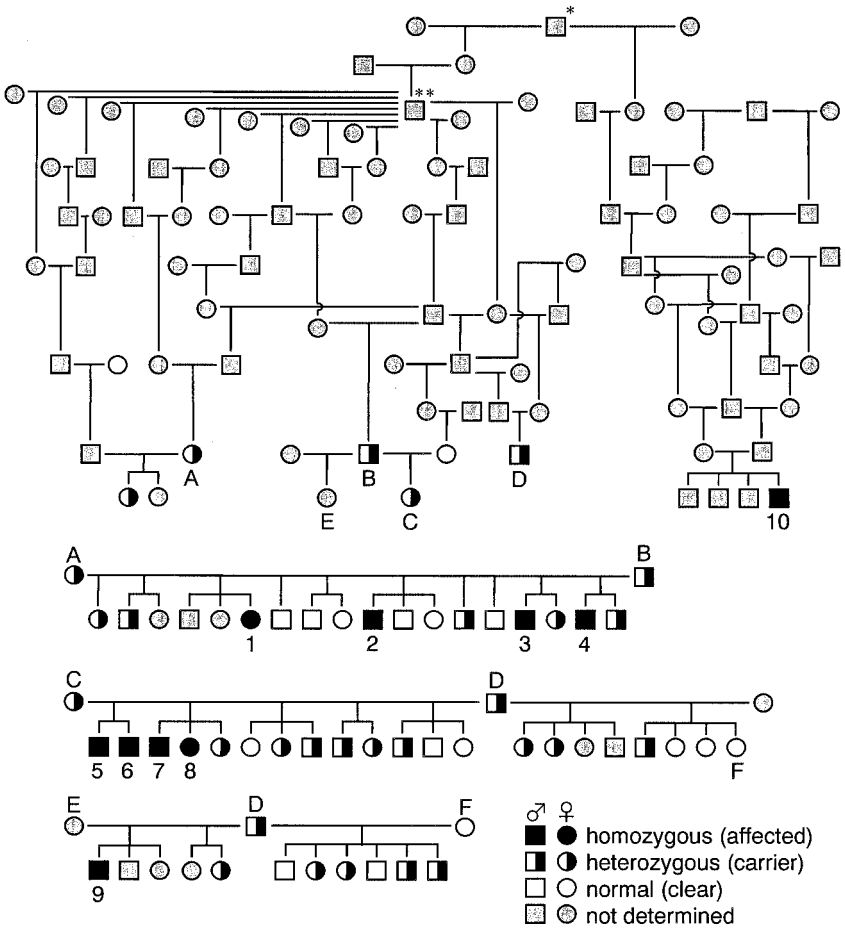

Fig. 1. The pedigree of Shiba dogs with GM1 gangliosidosis. Genotypes were determined using the DNA mutation assay described by Yamato *et al.* [19]. Dogs 1 and 2 were reported previously as the first cases in the Shiba breed [20]. Dog 10 possessing a forefather (*) in common with the other affected dogs (Dogs 1–9), was also reported previously [16]. ** A suspected carrier stud dog that is a forefather of Dogs 1–9.

Table 1. Clinical signs of Shiba dogs with GM1 gangliosidosis

Age (months)	Clinical signs
<5	No clinical or neurological signs
5–6	Loss of balance, Lameness (intermittent), Ataxia (mild to moderate), Dysmetria (mainly hypermetria), Head tremor (intention tremor)
7–8	Ataxia (severe), Toppling gait, Exaggeratedly startled response
9–10	Atactic abasia, Astasia, Corneal clouding, Visual defect, Muscle rigospasticity in limbs and crest, Emotional disorder
11–12	Generalized muscle rigospasticity, Tonic spasm, Tendency to be lethargic, Unresponsive to sounds, Weight loss
13<	Lethargy, Death (after 14 months)*

* One dog died with orthothanasia at 14.3 months of age, and the other dogs were euthanased humanely by 15 months of age.

and healthy up until 5 months of age. Clinical signs of GM1 gangliosidosis appeared at age 5 to 6 months. During that period, the dogs manifested neurologic signs including loss of balance, intermittent lameness in mainly the pelvic limbs, mild to moderate ataxia, dysmetria and intention tremor of the head. The gait was characterized by ataxia with hyper-

metric thoracic limbs. However, the dogs were alert and attentive.

These manifestations progressed, and the dogs frequently fell down at 7 to 8 months of age. Furthermore, the dogs showed an exaggeratedly startled response to stimuli such as sounds and touch. The dogs became unable to walk at about 9 months and to stand by 10 months of age. Mild clouding in the central of cornea and a visual defect not due to the corneal clouding were observed at 9 to 10 months. A visual defect was evaluated using dropping cotton balls and menace testing. The dogs sometimes became aggressive and barked without reason in this period.

Muscle rigospasticity in the limbs and crest was observed at about 10 months, and progressed to be generalized at age 11 to 12 months. The dogs sometimes showed tonic spasms, and tended to be lethargic. They were unresponsive to sounds and calls during this period. The weight of the dogs tended to decrease after 10 months of age although the food intake hardly changed. After 13 months of age, the dogs were constantly lethargic. One dog died with orthothanasia at age 14.3 months while the other dogs were euthanased humanely by 15 months of age owing to a poor prognosis and postmortem analyses for other studies.

In addition, there were not dysmorphic changes and dwarfism in the dogs. Radiographic evaluations of the vertebral column, long bones, and visceral organs including liver and spleen were normal through their lifetime. Furthermore, generalized seizures were not observed.

Clinico-pathologic features: The complete blood count and the values for general blood chemistry in the homozygous dogs were all within the normal limits through their lifetime (data not shown). However, a Giemsa-stained blood smear revealed the presence of abnormally large vacuoles in lymphocytes (Fig. 2). The vacuolated lymphocytes accounted for 30 to 50% of all lymphocytes, and the count hardly changed throughout the lifetime of Shiba dogs with GM1 gangliosidosis. The vacuolated lymphocytes varied morphologically with some having vacuoles of various sizes (Fig. 2A), one to a few large vacuoles (Fig. 2B), and many vacuoles of moderate size (Fig. 2C). Other types of periferal leukocytes, i.e., neutrophils, eosinophils and monocytes, were morphologically normal (data not shown).

DISCUSSION

In humans, GM1 gangliosidosis occurs mainly in early infancy (type 1; the infantile form), but the clinical spectrum of the disease has been expanded to include a late infantile/juvenile form (type 2) affecting older children and an adult/chronic form (type 3) with milder symptoms and survival through adulthood (Table 2) [15]. In infantile cases, the symptoms appear at birth and include progressive neurological deterioration, developmental arrest, generalized rigospasticity, psychointellectual dysfunctions, seizures, peripheral edema, facial dysmorphism, hepatosplenomegaly and generalized skeletal dysplasia. In contrast, dysmorphic changes and hepatosplenomegaly are less prominent or absent in the late infantile/juvenile and adult/chronic forms. Neither the type nor location of a mutation in the gene is correlated to the clinical phenotype, but the difference in clinical, pathological and biochemical features among patients represents different mutations of the gene for acid β-galactosidase in humans.

The clinical features in the canine models of GM1 gangliosidosis are varied (Table 2). The disease in mixed-breed beagles has features comparable with those observed in the human late infantile/juvenile form, the symptoms being a normal postpartum development with the subsequent appearance of progressive motor dysfunction, an absence of hepatosplenomegaly, and no abnormalities of the face and bones at necropsy [9, 10]. The disease in English springer spaniels and Portuguese water dogs has similar features including age of onset, clinical course and survival period, bone dysplasia and lymphocyte vacuolations [1], but dif-

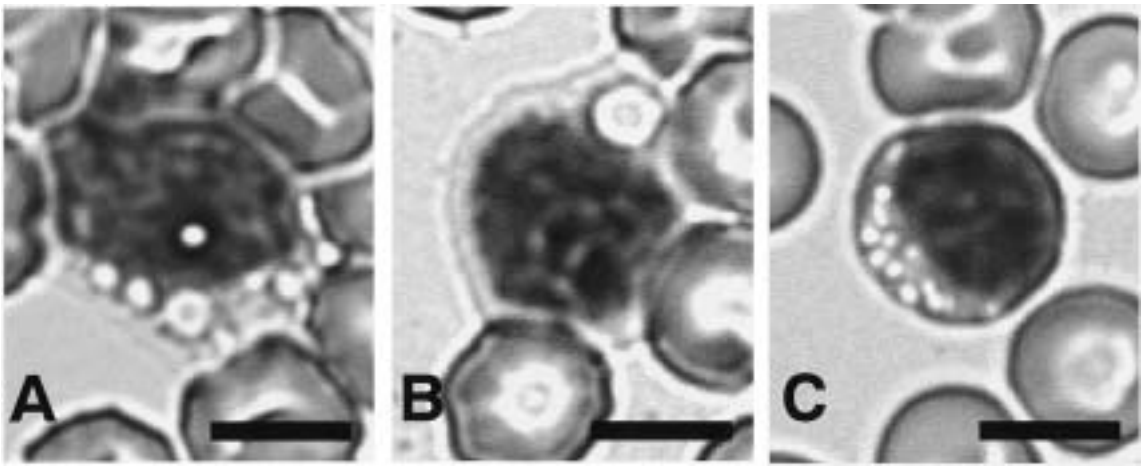

Fig. 2. Peripheral lymphocytes with abnormal vacuoles in homozygous Shiba dogs with GM1 gangliosidosis. Photos show lymphocytes with vacuoles of various sizes (A), one markedly large vacuole (B), and many vacuoles of moderate size (C). Bar=5 μm.

Table 2. Comparison of clinical features of human and canine GM1 gangliosidosis

	Human GM1 gangliosidosis[8,15]			Canine GM1 gangliosidosis				
Features	Type 1	Type 2	Type 3	Shiba dogs	MB[9,10]	ESS[1,2]	PWD[1,11,12]	AH[6,7]
Age at onset*	0–6 mo	7 mo–3 yr	3–30 yr	5–6 mo	2 mo	4–5 mo	4–5 mo	6–8 wk
Clinical course	<2 yr	1–5 yr	10–30 yr	5–14 mo	2–10 mo	4–9 mo	4–9 mo	1–7 mo
Dwarfism	+	–	–	–	–	+	–	+
Facial distortion	+	–	–	–	–	+	–	–
Bone deformities	+	+/–	–	–	–	+	+	+/–
Hepatosplenomegaly	+	+/–	–	–	–	NC	–	–
Tremors	NC	NC	NC	+	+	+	+	+
Ataxia	–	+	+	+	+	+	+	+
Spasticity	–	+	+	+	+	NC	+	NC
Dysmetria/hypermetria	NC	NC	NC	+	+	NC	+	+
Seizures	+	+	–	–	–	NC	–	+/–
Vision loss	Early	Late	–	+	–	Possible	+	–
Corneal clouding	+/–	–	–	+	–	NC	+/–	NC
Strabismus	NC	+/–	NC	Possible	+ External	NC	NC	+ Internal
Nystagmus	NC	NC	NC	–	NC	+	+	+ Positional
Dysarthria	+	+	+	–	NC	NC	–	NC
Dysphagia	+	NC	NC	–	+	NC	NC	NC
Startled response to sound	+	+	–	+	NC	NC	NC	NC
Hyperreflexia of limbs	+	+	NC	+	+	NC	Possible	NC
Psychointellectual dysfunctions	+	+	+/–	Possible	NC	NC	NC	NC
Vacuolated lymphocytes	+	+	+/–	+	NC	+**	+	+**

Superscripts represent reference numbers. Types 1–3 represent infantile, late infantile/juvenile and chronic/adult forms in human GM1 gangliosidosis, respectively. Abbreviations of canine breeds, MB: mixed-breed beagles; ESS: English springer spaniels; PWD: Portuguese water dogs; AH: Alaskan huskies. +: present; –: absent; NC: not commented on in the reference. * The onset of the disease represents ages at which affected patients first manifested neurologic signs. ** The abnormal vacuolations are present in neutrophils, eosinophils and monocytes as well as lymphocytes in the English springer spaniels and Alaskan huskies.

fered with respect to the presence of coarse facial features, which were observed only in English springer spaniels [2]. The disease in English springer spaniels is therefore comparable with the human infantile form, whereas that in Portuguese water dogs has many features similar to the human late infantile/juvenile form. In addition, the disease in Alaskan huskies is similar to the human late infantile/juvenile form with respect to age of onset, temporal evolution and a lack of hepatosplenomegaly, but one of three dogs examined showed a retarded enchondral ossification of the lumbar vertebral epiphyses and abnormally wide intervertebral disc spaces [6], which are features frequently observed in patients with the human infantile form [15].

In Shiba dogs with GM1 gangliosidosis, a slightly older age of onset and longer survival period were observed as compared with other canine models (Table 2). Furthermore, no morphological changes including dwarfism, facial and bony deformities and hepatosplenomegaly were observed. These clinical features in the affected Shiba dogs are also similar to those in the human late infantile/juvenile form, but seem to be mildest in the canine GM1 gangliosidoses.

The phenotypic differences among the canine models mentioned above suggest distinct mutations in the β-galactosidase gene like in the human disease. The homozygous recessive mutation in the Portuguese water dogs was first identified at nucleotide $G^{200}\rightarrow A$ in exon 2 resulting in an $Arg^{60}\rightarrow His$ substitution in the β-galactosidase, but this was not responsible for the disease in the English springer spaniels [17]. Second, the mutation in the Shiba dogs was identified as a deletion of nucleotide C^{1668} in exon 15, which shifts the translational reading frame at amino acid residue 550 [19]. Accordingly, to consider the potential of new therapeutic programs including gene therapy using a canine model, a detailed knowledge of the clinical features as well as the molecular defect proper is essential.

In veterinary clinics, many lysosomal storage diseases in animals are not investigated to a complete diagnosis, both because their devastating neurologic features make them difficult candidates for such an investigation and because there are few centers capable of providing practical diagnostic assistance [13]. This is also the reason that GM1 gangliosidosis in Shiba dogs had not been reported until 2000 [20] although some suspected carrier studs left many offspring and seemed to transmit the mutant allele years ago (Fig. 1). After the first occurrence (Dogs 1 and 2), an affected Shiba dog (Dog 10) which is a homozygote possessing the mutant allele and does not belong to the family pedigree genetically controlled by us was reported in 2001 [16], suggesting the mutant allele is distributed widely in breeding colonies of Shiba dogs all over Japan. Furthermore, the disease might exist outside of Japan because this traditional Japanese breed has been imported to North America, Europe and Australia [3]. However, if veterinary practitioners encounter an affected Shiba dog, a knowledge of the clinical features of this disease (Table 1) including lymphocyte vacuolations (Fig. 2) will obviously make a practical diag-

nosis easier, leading to a complete diagnosis in the laboratory and therefore, diagnostic assistance.

In conclusion, the clinical and clinico-pathologic characteristics of the animal model employed in the present study are useful for the development and testing of potential methods of therapy for the human form of the disease. Furthermore, in veterinary clinics, the characteristics will also be useful for the practical diagnosis of affected homozygous Shiba dogs prior to a laboratory diagnosis using the enzyme assay and/or the DNA mutation assay.

ACKNOWLEDGEMENTS. This research was supported by a grant from the Suhara Memorial Foundation and a Grant-in-Aid for Scientific Research (No. 14560258) from the Ministry of Education, Culture, Sports, Science and Technology.

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