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JB Reflection and Perspectives
Kunihiko Suzuki and sphingolipidoses

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Kunihiko Suzuki is a neurologist by training whose research accomplishments range widely from basic research in brain lipids, their metabolism to genetic disorders involving the nervous system. Among them are identification of the enzymatic defect, the pathogenetic mechanism, and animal models of Krabbe's globoid cell leukodystrophy, the chemical and molecular pathologies of many glycosphingolipidoses, discovery of the abnormal accumulation of very long chain fatty acids in adrenoleukodystrophy, and elucidation of the complex metabolic interrelationship among sphingolipids with extensive use of the gene targeting technology. This reflections and perspectives highlight his accomplishments briefly.

Kunihiko Suzuki is Professor Emeritus, Neurology and Psychiatry, and Director Emeritus, the Neuroscience Center, School of Medicine, University of North Carolina (UNC) at Chapel Hill. Suzuki was born in Tokyo in 1932. He graduated from the College of General Education, the University of Tokyo, in 1955 with a degree in History and Philosophy of Science. Then, he graduated from the Faculty of Medicine, the University of Tokyo in 1959. After one year of the rotating internship at the United States Air Force Hospital Tachikawa, he crossed the Pacific to USA as a resident in clinical neurology at the Albert Einstein College of Medicine, Bronx, New York. He has written his accounts of his experience during his days of patient care as a clinical neurologist and also of the life in New York City in the early 1960's (*1*). After 4–5 years of patient care, Suzuki left clinical medicine and pursued his own research career at the Albert Einstein College of Medicine (1964–68), at the University of Pennsylvania School of Medicine (1969–72) and back at the Albert Einstein College of Medicine (1972–86). Then he was appointed Professor in the Departments of Neurology and Psychiatry and Director of the Brain and Development Research Center (1986–96) which was later re-named as the Neuroscience Center (1996–99), UNC at Chapel Hill, School of Medicine, where he continued his own research in molecular biology of genetic lysosomal diseases. After retiring from the UNC in 2002, he took up the position in Japan for him as Professor, Future Science and Technology Joint Research Center, Tokai University, and Director of the Institute of Glycotechnology (2003–07). He returned to Japan permanently in 2007 after 47 years in USA. Suzuki is the author of more than 350 peer-reviewed articles and book chapters and is the editor of three books. He was awarded the Japan Academy Prize in 2002 and elected to the membership of the Japan Academy in 2008. He is past President of the International Society for Neurochemistry (1993–95) and past President of the American Society for Neurochemistry (1985–87). He has served on the editorial boards of a number of scientific journals and was Chief Editor of the Journal of Neurochemistry, 1977–81. His laboratory always received young Japanese researchers for training as well as for collaboration, and their number reached 30. The following is a brief summary of the highlights of his research, performed in association with his colleagues.

Chemical pathology of glycosphingolipidoses

Kunihiko Suzuki started his research career by studying ganglioside metabolism (*2*, *3*) and studying the methods for analysing mammalian brain ganglioside patterns (*4–6*) at Albert Einstein College of Medicine. Those were the days when the thin-layer chromatography (TLC) had just appeared on the research scene as a powerful analytical tool. His method of analysing individual brain gangliosides combining the TLC and the colorimetric resorcinol method for sialic acid was widely used as the 'Suzuki method' (*4*). Abnormally high sulphatide content had been known in white matter of the brain of patients with metachromatic leucodystrophy (MLD). Suzuki isolated the abnormal inclusion bodies that stained metachromatically with cresyl violet and showed that they were the site of the sulphatide accumulation (*7*). They had a lamellar structure somewhat similar to the membranous cytoplasmic bodies found in neurons of Tay–Sachs disease brain. However, electron microscopic obersvations indicated that they were present primarily in the oligodendroglial cytoplasm. Analytically, they consisted of cholesterol, sulphatide and phospholipid in an approximately 1:1:1 ratio. This article was the first collaborative work with Kinuko Suzuki, who is a neuropathologist and the best co-worker in Suzuki's scientific life.

Since the first descriptions by Tay and also by Sachs in the late 19th century, Tay–Sachs disease had been the only known genetic ganglioside storage disorder for over a half century. The advance in the analytical methodology including the TLC allowed identification of a new ganglioside storage disorder, GM1 gangliosidosis, in the mid-1960's. While degradation of GM2 ganglioside is genetically impaired in Tay–Sachs disease, it is GM1 ganglioside the degradation of which is genetically blocked in GM1 gangliosidosis. Initially, the disease was called 'generalized gangliosidosis' because of an erroneous belief that GM1 ganglioside accumulated throughout the body in GM1 gangliosidosis while the accumulation of GM2 ganglioside was limited to the brain in Tay–Sachs disease. Kinuko and Kunihiko Suzuki found that the ultrastructure of the stored material in the liver of GM1-gangliosidosis patients was fibrillar and was entirely different from the membranous cytoplasmic bodies in neurons (*8*). His analytical studies showed that the abnormally accumulated material in the visceral organs in GM1 gangliosidosis was fragments of keratan sulphate-like glycosaminioglycans (*9*, *10*). It was tentatively identified as keratan sulphate on the basis of its behaviour in the preparative procedure, sugar composition and electrophoretic mobility. Because concentrations of these compounds were not increased in the viscera of patients with Tay–Sachs disease, he concluded that GM1 gangliosidosis is a combined cerebral gangliosidosis and visceral mucopolysaccharidosis. These materials in the visceral organs are fragments of partially degraded keratan sulphate with terminal galactose, which cannot be further degraded because of the genetic deficiency of GM1-ganglioside β-galactosidase (*11*). Suzuki proposed the systematic nomenclature of genetic ganglioside storage disorders, such as GM2 gnagliosidosis or GM1 gangliosidosis (*12*). It is now widely used as the standard nomenclature.

Demonstration of deficiency of galactocerebroside β-galactosidase in globoid cell leukodystrophy

Infantile globoid cell leukodystrophy (Krabbe disease, GLD) is one of the classical genetic white matter diseases transmitted as an autosomal recessive trait. The disease usually begins between ages 3 and 6 months with ambiguous symptoms, such as irritability or hypersensitivity to external stimuli, but soon progresses to severe mental and motor deterioration and progresses rapidly to a fatal outcome. The presence of numerous multinuclear globoid cells, almost total loss of myelin and oligodendroglia, and astrogliosis in the white matte are the characteristic pathological features.

A deficiency of a synthetic enzyme, cerebroside-sulphatide sulphotransferase, which synthesizes sulphatide from cerebroside had been proposed by Austin and his colleagues as the genetic cause of GLD. However, Suzuki found it difficult to reconcile other characteristics of this disease with the postulated lack of the sulphotransferase. For instance, Austin and his colleagues had found that the characteristic globoid cells contained high concentration of galactocerebroside and could be elicited experimentally only by galactocerebroside introduced into the brain. Suzuki labelled galactocerebroside with tritium at carbon 6 of the galactose moiety by oxidation with galactose oxidase and reduction with tritiated borohydride and established an assay method for galactocerebroside β-galactosidase (galactosylceramidase, GALC) based on the methodology provided to him by Radin. He and Yoshiyuki Suzuki found profound deficiency of the enzyme in the brains, liver and spleen of three patients with GLD (*13*). The deficiency was also confirmed in serum, leukocytes and fibroblasts from patients with GLD (*14*). They also showed that heterozygous carriers could be detected by their half-normal enzymatic activities. The first prenatal diagnosis of an affected fetus was soon accomplished using cultured aminiotic fluid cells (*15*). The discovery of the genetic cause of the human GLD also lead to identification of the dog model occurring among pure-bred Westhighland and Cairn terriers, which had been previously known on the basis of its pathology, as an enzymatically authentic model of the human GLD (*16*).

Psychosine hypothesis

The genetic deficiency of galactosylceramidase placed Krabbe disease among the inherited lysosomal disease together with the other classical leukodystrophy, metachromatic leukodystrophy. However, this created a new dilemma as to how to explain the lack of abnormal accumulation of its substrate, galactocerebroside, in the brain of patients in the presence of genetic defect in its degradation. The unique pathology of Krabbe disease is the rapid disappearance of the oligodendroglia. Because of the uniquely high concentration of galactosylceramide in myelin, the lack of accumulation of galactosylceramide in Krabbe disease can be explained phenomenologically by the total loss of myelin and the oligodendroglia. However, this merely shifts the question from galactosylceramide to myelin. What was needed was a mechanistic explanation. In 1972, Miyatake and Suzuki proposed a hypothesis which has been known as the psychosine hypothesis to explain the unusually rapid and complete destruction of the oligodendroglia in GLD (*17*). They found GALC also recognized psychosine (galactosylsphingosine) as a substrate and thus Krabbe patients also could not degrade psychosine. Psychosine, with its free amino group, is known to be highly cytotoxic (*18*). It is therefore conceivable that psychosine generated within the oligodendrocytes during the period of active myelination might reach a toxic level. Oligodendrocytes are selectively destroyed because psychosine formation occurs primarily in these cells. This hypothesis explains the early and selective destruction of oligodendrocytes and the resultant cessation of myelinaton. Analytical data on the tissue levels of psychosine in the brain in human GLD patients published several years later strongly supported the hypothesis (*19*, *20*). Igisu and Suzuki also developed

Fig. 1

sensitive and specific analytical procedure for determination of galactosylspingosine (psychosine) and found progressive accumulation in the brain of mouse model of galactosylceramidase deficiency (Twitcher mouse) and in the dog and human GLD patients (*21*, *22*). Galactosylsphingosine was undetectable in the brains of normal and heterozygous mice. The findings provided a strong support for the psychosine hypothesis as the biochemical pathogenetic mechanism of GLD. Kobayashi *et al.* (*23*) determined galactosylceramide (GalC) and galactosylsphingosine (psychosine) in tissues from infants and fetuses with GLD. They found a close relationship between the galactosylsphingosine accumulation and progression of the disease. A later work by Tohyama, then in Suzuki's laboratory, indicated that psychosine at a concentration in the range of 20 nM kills MOCH cells in culture (*24*). The MOCH cells derive from and exhibit many characteristics of the oligodendrocytes. Twenty nanomolar is the concentration of psychosine estimated to reach locally in the oligodendrocytes in GLD brain. History of the psychosine hypothesis has been reviewed by Suzuki (*25*, *26*).

Complex interrelationships between two β-galactosidases

There are two distinct acid β-galactosidases that hydrolyse glycosphingolipids with a terminal β-galactose residue. GLD is characterized by a genetic deficiency of GALC activity. The enzyme catalyses the lysosomal degradation of GalC to galactose and ceramide. The deficient activity of this enzyme can also be demonstrated with other natural substrates galactosylsphingosine (psychosine), lactosylceramide and monogalactosyldiglyceride. In GM1 gangliosidosis, on the other hand, GM1-ganglioside β-galactosidase activity is deficient. Tanaka and Suzuki (*27*, *28*) found that both β-galactosidases share lactosylceramide as a substrate, and consequently accumulation of lactosylceramide does not occur to any significant degree in either GLD or GM1 gangliosidosis. Suzuki (*29*) concluded that under these circumstances a specific disorder with accumulation of lactosylceramide would not occur in humans and showed, in collaboration with Wenger, that the patient earlier reported as having a lactosylceramidosis must have been in error (*30*). (This patient has subsequently been diagnosed as having Niemann–Pick type C disease and the underlying mutation has also been identified.) From these findings, Suzuki predicted that the genetic disease with abnormal accumulation of lactosylceramide could occur only when both GALC and GM1-ganglioside β-galactosidase were simultaneously deficient. This prediction was proven correct later when Tohyama in Suzuki's laboratory carried out a cross-breeding experiment between the twitcher mouse (see below) and the GM1-ganglioside β-galactosidase knockout mouse (*31*, *32*). The doubly deficient mice showed a massive accumulation of lactosylceramide most notably in the liver. Kobayashi *et al.* (*33*) demonstrated that under certain assay conditions, particularly in the presence of sodium cholate, GM1-ganglioside β-galactosidase could also hydrolyse GalC but not psychosine, while purified GALC could hydrolyse both GalC and psychosine (*34*). However, the capacity of GM1-ganglioside β-galactosidase to hydrozye GalC has only been demonstrated *in vitro* in the presence of artificial detergents but there is no evidence that it hydrolyses GalC *in vivo*. The unique lack of abnormal accumulation of the substrate (GalC) of the defective catabolizing enzyme (GALC) in GLD can be readily explained by the rapid disappearance of the oligodendrocytes, which are nearly the exclusive site of GalC synthesis, during the very early stage of active myelination period without postulating the *in vivo* GalC-hydrolysing activity of GM1-ganglioside β-galactosidase. These complex interrelationships of substrate specificity between the two lysosomal β-galactosidases are important in understanding the pathogenesis of GLD as demonstrated clearly by the cross-breeding experiments mentioned above between the two mouse strains each deficient in one or the other of the two β-galactosidases (*32*). Tohyama *et al.* (*32*) found that the gene dosage of acid β-galactosidase exerts an unexpected and paradoxical influence on the phenotype of the twitcher mouse. Twitcher mice with an additional complete deficiency of acid β-galactosidase exhibited the mildest phenotype with the longest lifespan and nearly rescued pathology in the central nervous system. In contrast, twitcher mice with a single functional acid β-galactosidase gene had the most severe disease with a shorter life span despite the fact that GM1 gangliosidosis carrier mice with an

otherwise normal genetic background are phenotypically normal. The observations may be interpreted that the acid β-galactosidase gene might function as a modifier gene for the phenotypic expression of genetic GALC deficiency.

Discovery of the twitcher mouse

A mouse strain that develop clinical signs at ~20 days, with stunted growth, twitching, hind leg weakness had been found at the Jackson Memorial Laboratory. The disease is fatal by 2 months. The principal pathological changes affect the myelin of both central and peripheral nervous systems. Duchen and his colleagues reported the neuropathology of degeneration of myelin sheaths and the presence of multinucleated macrophages with PAS-positive cytoplasm in this mutant, named twitcher, at a neuropathology meeting in the UK (*35*). The abnormalities closely resembled those found in human GLD. Suzuki initiated a collaboration with the UK group. Takuro Kobayashi, then working in Suzuki's laboratory, enzymatically studied brain and liver sent blindly from the UK laboratory and unequivocally demonstrated that the same GALC deficiency as in the human Krabbe disease is the underlying cause in this mutant (*36*). This mouse has since been used extensively in many laboratories in the world as an authentic model of human Krabbe disease and has provided important information in understanding the pathophysiology of GLD (*37–41*) including the already-mentioned demonstration of progressive accumulation of psychosine during the course of the disease (*22*), genetic and epigenetic effects on GLD phenotypes, and experimental treatments by enzyme replacement in grafted nerves of twitcher mouse into normal hosts (*42*), by bone marrow transplantation (*43*) or transgenic manipulation (*44*).

Accumulation of very long chain fatty acids in adrenoleukodystrophy

Adrenoleukodystrophy (ALD) is an X-linked recessive disorder. The most common initial manifestations are hyperactivity, which is noted between the ages of 4 and 8 years old and is often mistaken for an attention deficit disorder, and worsening school performance. Visual disturbances are often due to involvement of the cerebral cortex. Auditory discrimination is often impaired, although tone perception is preserved. This may be noticed by difficulty in using the telephone. Impaired cortisol response is present in 85% of patients. The condition tends to progress rapidly with increasing spasticity and paralysis, visual and hearing loss, and loss of ability to swallow. The patient becomes in the vegetative state after ~2 year from the initial symptom and may continue in this condition for 10 years or longer. A milder form often occurring in adult patients with predominant manifestations of the spinal cord and the peripheral nerves exists (adrenomyeloneuropathy, AMN).

Paul Schilder (1912–24) studied the postmortem brains from patients. He found severe loss of myelin associated with relative preservation of axons, and a perivascular accumulation of lymphocytes and fat-laden macrophages and glial cells. The condition was referred to as Schilder disease until ~40 years ago, when the name of ALD was introduced. When Igarashi and Suzuki started the biochemical studies of ALD, Powers and Schamburg (*45*, *46*) observed that adrenal cells of these patients contained characteristic lipid inclusions and these inclusions consisted of cholesterol esters. Igarashi *et al*. (*47*) reported an unusual abnormality in the fatty acid composition of cholesterol esters in the brain and adrenal cortex from the patients with ALD. Cholesterol ester in control brain contained mostly C16–C20 fatty acids, whereas cholesterol esters in ALD brain contained substantial amounts of very long chain fatty acids (C24–C30 or more), where they constituted 10–40% of total fatty acids, compared with 0–5% in controls. Increased proportions of the very long chain fatty acids were also found in galactosylceramide, sulphatide and gangliosides in the brain of patients (*47*, *48*) The abnormalities of cholesterol ester fatty acids were confirmed in all subsequent studies and the identification of this biochemical 'handle' led to the development of diagnostic assays for ALD using cultured fibroblasts, plasma and amniotic cells. This was the epoch-making discovery that initiated the new phase of investigations of the genetic diseases categorized as the peroxisomal disease of which ALD is one. The finding permitted precise clinical and prenatal diagnosis and has led to an identification of the biochemical defect involving the impaired function of peroxisomal lignoceroyl CoA ligase (*49*), the enzyme that catalyses the formation of the CoA derivative of very long chain fatty acids, and eventually to the discovery of the gene responsible for the disease, ABCD1 (*50*).

Molecular genetics of GM2 and GM1 gangliosidoses

Suzuki started his research career working primarily on analytical chemistry of human genetic diseases and shifted to enzymology as the field of genetic disorder moved. His sabbatical year in the laboratory of Elizabeth Neufeld at the NIH during the 1984–85 shifted the emphasis of his research to the molecular genetics. He sequenced the full-length human β-hexosaminidase α-chain cDNA at the NIH (*51*). After the sabbatical year, he moved to UNC at Chapel Hill where a series of molecular studies of human gangliosidoses emerged working with younger collaborators including identification of the mutations in GM2-gangliosidosis B1 variants (*52*, *53*), the classical Ashkenazi Jewish Tay–Sachs disease (*54*), non-Jewish Caucasian patients with GM2 gangliosidosis (*55*, *56*), Japanese Tay–Sachs disease (*57*, *58*) and Sandhoff disease (*59*, *60*). Nanba and Suzuki (*61*, *62*) isolated mouse acid β-galactosidase cDNA and compared with the human enzyme and then studied the genomic organization of mouse acid β-galactosidase gene. Then, Suzuki and collaborators analysed mutations in the acid β-galactosidase gene of Japanese patients with GM1 gangliosidosis (*63*) and studied the

effect of polymorphisms on the catalytic activities of acid β-galactosidase in different mouse strains (*64*). Suzuki's work on GM2-gangliosidosis AB variant which is due to genetic defects in GM2 activator protein will be described later in the section of sphingolipid activator proteins.

Generation of mice doubly deficient in synthesis and degradation of GalC

GalC is the most characteristic lipid in the myelin sheath. More than 95% of total GalC in the body is present as a myelin constituent. Its precise physiological function in the myelin sheath remains largely a matter of conjecture. GalC synthase (UDP-galactose: ceramide galactosyltransferase, CGT) synthesizes GalC from UDP-galactose and ceramide with either α-hydroxylated or non-hydroxylated fatty acids. CGT also synthesizes psychosine from UDP-galactose and sphingosine. This is the only known pathway to generate psychosine, since no enzymatic mechanism is known in mammals to generate psychosine by deacylation of GalC. Suzuki, in collaboration with Popko, cloned the cDNA (*65*) and then the gene that encode CGT (*66*). For the genetic analysis of galactolipid function, Coetzee *et al*. (*67*, *68*) generated CGT knockout mice (a null mutant deficient in CGT enzyme activity). The mice do not synthesize GalC or sulphatide but surprisingly form myelin sheaths of a normal ultrastructural appearance containing glucosylceramide, a lipid not previously identified in myelin. The inability to synthesize galactosylceramide is being compensated by glucosylceramide. Although the morphological appearance of myelin is normal, it appears to be metabolically and functionally unstable, and the mice exhibit severe tremor and with age develop progressive hind limb paralysis. On the other hand, GALC is responsible for physiological degradation of GalC and its genetic defect results in GLD. All CGT products known to date are degraded by GALC. This one-to-one relationship breaks down only for lactosylceramide, which is not synthesized by CGT but can be degraded by GALC. Ezoe *et al*. (*69*) generated mice doubly deficient in both synthesis and degradation of GalC by cross breeding the twitcher mice and the CGT knockout mice. The clinical and pathological phenotype of the doubly deficient mice were essentially indistinguishable from those of the CGT knockout mice but the life span was shorter. As expected from the lack of CGT, both GalC and psychosine were undetectable and the characteristic twicther pathology was never seen. These findings were expected because the lack of CGT results in no synthesis of the substrates that need to be degraded by GALC. However, neuronal pathology in the brain stem and spinal cord, which were not seen in the CGT knockout mice, was observed after 43 weeks. A speculation is that this phenomenon might have something to do with lactosylceramide metabolism as described above.

Biological functions of the sphingolipid activator proteins

Sphingolipid activator proteins are low molecular weight glycoproteins, which by themselves are catalytically inactive but are required as co-factors to facilitate interactions between membrane-bound hydrophobic sphingolipids and water-soluble exohydrolases in the lysosome. Two major groups exist. The GM2 activator protein is encoded by its own gene, while four sphigngolipid activator proteins, saposins A, B, C and D, are encoded by a single gene, which generates a common protein precursor, prosaposin. The prosaposin in turn is proteolytically processed to the four saposins. Human disease caused by genetic defects in GM2 activator protein is known as GM2-gangliosidosis AB variant. Its clinical, pathological phenotype is very similar to that of the classical Tay–Sachs disease. Human patients of prosaposin deficiency, specific saposin A, B or C deficiencies are known. Since the 1980's, Suzuki, in a close collaboration with Konrad Sandhoff, studied these activator proteins extensively. For example, Suzuki cloned and characterized the cDNA and the gene coding for the GM2 activator protein and identified a mutation responsible for the disease (*70*–*72*). A single prosaposin gene that encodes the four saposins was recognized when its cDNA was cloned and sequenced (*73*, *74*). Subsequently, the gene was characterized (*75*). Two sibs, affected by a rapidly fatal disorder with highly complex clinical and biochemical abnormalities, were found to have a mutation in the prosaposin gene. Abnormal accumulation of multiple sphingolipids was noted in the brain and systemic organs. The mutation responsible for this condition abolishes the intiation codon of the prosaposin gene (*76*) which causes a complete loss of the prosaposin as well as all four saposins. Saposin B deficiency leads to sulphatide accumulation and a metachromatic leukodystrophy-like disease with excess urinary excretion of sulphatide and globotriaosylceramide, indicating that saposin B is an *in vivo* activator of sulphatide and globotriaosylceremide by arylsulphatase A and α-galactosidase A, respectively. Saposin C deficiency leads to glucosylceramide accumulation and a Gaucher-like disease, indicating that saposin C is an *in vivo* activator of acid β-glucosidase. *In vivo* evidence exists to suggest that saposin D may be an activator for ceramidase (*77*). A mouse model of prosaposin deficiency generated by Fujita *et al*. (*78*) showed clinical, pathological, and biochemical abnormalities similar to human patients. Since human patients of saposin A or saposin D deficiency were not known, Matsuda and Suzuki generated specific knockout mouse models of saposin A (*79*) and saposin D (*80*). Then they found that saposin A is essential for *in vivo* degradation of GalC by GALC, which is the enzyme genetically deficient in GLD, because the saposin A-deficent mouse exhibited characteristics of late-onset Krabbe disease (*79*). Matsuda and Suzuki predicted on the basis of their finding that human disease should exist due to genetic deficiency of saposin A with a phenotype similar to that of GLD due to GALC deficiency (*79*).

This prediction came true three years later in 2005 when an Arabic patient was reported from Israel, who had a mutation in the coding sequence of saposin A (*81*). Furthermore, Matsuda *et al*. (*82*) made a potentially far-reaching observation that affected females that were continuously pregnant up to three pregnancies showed dramatically improved neurological symptoms compared to affected females that did not experience pregnancy, or affected males. The pathological hallmark of GLD, demyelination with infiltration of globoid cells, largely disappeared. Even a single event of pregnancy was sufficient to alleviate the clinical and pathological manifestations. They found that immune-related gene expression, such as MCP-1 and TNF-α was significantly down-regulated in the brain of affected mice during pregnancy. In addition, they found intense expression of the estrogen receptors on the globoid cells, activated astrocytes and microglia in the demyelinating area of the saposin A-deficient mice. They further demonstrated that estrogen supplement could largely mimic the effects of pregnancy and alleviate the disease not only in non-pregnant females but also affected males. These observations strongly suggest a close relationship between steroid hormones and the central nervous system.

The *in vivo* function of saposin D is less clear. Saposin D may be involved in hydrolysis of ceramide by acid ceremidase, since the mouse model with a mutation in saposin D showed an accumulation of α-hyroxyl fatty acid-containing ceramide in the kidney and the cerebellum (*80*). However, the main pathology is a massive hydronephrosis. There are conspicuous loss of the Purkinje cells in the cerebellum. These projects concerning the saposins are being followed up and further developed by Junko Matsuda, Suzuki's last student while he was in USA.

Perspectives

In addition to scientific contributions on sphingolipidoses, Suzuki contributed significantly to raising the Japanese leaders who have worked for lysosomal storage disorders in the field of Pediatrics, Neurology and Genetics. The list compiled by Suzuki includes 64 collaborators and 52 fellows and students from all over the world during the period between 1965 and 2003. Among them are 30 fellows from Japan. They all contributed to developments and advances of the field of genetic diseases in Japan. A half of them worked in universities and research institutions in Japan as equivalents of professors and associate professors after they returned from Suzuki's laboratory. He constantly provided them with support and encouragements from across the Pacific. The Kunihiko Suzuki Prize for promising young investigators was established in 2003 by the Japanese Society of Lysosomal Storage Diseases. It is and will remain as the source of guidance to pass on Suzuki's legacy down to future generations.

Epilogue

Meeting with Suzuki was the most fortunate event in my scientific life and I would like to express deep gratitude for having been given the chance. I wish Dr Suzuki good health and happiness for many years to come.

Conflict of interest
None declared.

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