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REVIEW ARTICLE

Metachromatic leukodystrophy: genetics, pathogenesis and therapeutic options

Volkmar Gieselmann (gieselmann@institut.physiochem.uni-bonn.de)

Institut für Physiologische Chemie, Rheinische-Friedrich-Wilhems Universität Bonn, Bonn, Germany

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Correspondence
V Gieselmann, Institut für Physiologische Chemie, Rheinische-Friedrich-Wilhems Universität Bonn, Nussallee 11, 53115 Bonn, Germany.
Tel: +49 228 73 2411 |
Fax: +49 228 72 2416 |
E-mail: gieselmann@institut.physiochem.uni-bonn.de

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Abstract

Metachromatic leukodystrophy is a lysosomal storage disease caused by the deficiency of arylsulphatase A (ASA). This leads to storage of the membrane lipid sulphatide, which is abundant in myelin. A pathological hallmark of the disease is demyelination, causing various and ultimately lethal neurological symptoms. Today more than 110 mutations in the *ASA* gene have been identified, of which only three are frequent. Patients homozygous for alleles, which do not allow for the synthesis of functional ASA always suffer from the severe form of the disease, whereas alleles allowing the expression of residual enzyme activity are associated with the later onset juvenile or adult forms of metachromatic leukodystrophy. In addition, there are other as yet unknown genetic or epigenetic factors modifying the phenotype substantially. ASA-deficient mice have been generated as a model of metachromatic leukodystrophy. These mice store sulphatide and show progressive neurological symptoms, but do not demyelinate. This animal model was recently improved using a transgenic approach, which generated mice in which sulphatide synthesis in myelin-producing cells is enhanced. This new animal model reflects the pathological characteristics of the human disease. ASA-deficient mice have been used in various therapeutic trials involving enzyme replacement, haematopoietic stem-cell-based gene therapy and direct injections of ASA-expressing viral vectors into the brain. These animal studies have paved the way for future clinical studies of enzyme replacement and gene therapy.

Conclusion: For many years this devastating disorder was considered untreatable and the outlook for patients was poor. Within a comparatively short period of time since the *ASA* gene was cloned in 1989, genetic and biochemical studies and data generated from newly developed animal models have led to the first clinical trials. It is hoped that these developments will prove beneficial for patients.

Metachromatic leukodystrophy is a lysosomal storage disease caused by the deficiency of the enzyme arylsulphatase A (ASA). This enzyme catalyzes the first step in the degradation pathway of the sphingolipid 3′-O-sulphogalactosylceramide, also known as sulphatide. The expression of this membrane lipid is restricted to certain cell types. Sulphatide is particularly abundant in the myelin of the nervous system, where it constitutes about 4% of all myelin lipids. Myelin is synthesized by oligodendrocytes in the central nervous system (CNS) or by Schwann cells in the peripheral nervous system. Myelin wraps around axons in a spiral fashion and is essential for electrical insulation and fast saltatory impulse conduction along the myelinated axon. Sulphatide is also found in other tissues, including the distal tubules of the kidney and bile duct epithelia. If ASA is deficient, sulphatide cannot be degraded and it accumulates. Functionally, this accumulation affects the nervous system in particular. Storage in gall bladder epithelia and renal tubules results in little or no functional impairment. The pathological hallmark of the disease is progressive demyelination, which results in a variety of neurological symptoms. Patients develop ataxia, an initially flaccid and later spastic paresis, optic atrophy and dementia. After years of suffering, they finally die in a decerebrated state (for a more detailed description of the disease see (1)).

Based on the age at disease onset, three clinical forms are distinguished: a late-infantile form, with the first symptoms developing around 2 years of age, a juvenile form, with patients presenting between 3 and 16 years of age, and an adult-onset form with manifestations appearing after 16 years of age. While this distinction is certainly clinically helpful, it is artificial, as disease severity is in fact a continuum. To date, there is no specific treatment for metachromatic leukodystrophy, although patients with juvenile- and adult-onset disease may benefit from allogeneic bone marrow transplantation in the early stages of disease. Based on results from animal studies, clinical studies of enzyme replacement therapy are already in progress and studies are planned in the near future to investigate the direct injection of ASA-expressing viral vectors into the brain or haematopoietic stem-cell-based gene therapy.

GENETICS OF METACHROMATIC LEUKODYSTROPHY

The human *ASA* gene is a small gene with eight exons encompassing only about 3 kilobases of genomic sequence (2).

Today, more than 110 different mutations causing metachromatic leukodystropy have been identified in the *ASA* gene (see the Human Gene Mutation Database). Genetically, the disease is heterogeneous: only three defective alleles are frequent (3,4). These include a splice donor-site mutation at the exon 2/intron 2 border, which has been found to account for about 25% of all alleles amongst European patients (3). A missense mutation causing a Pro246Leu substitution also accounts for about 25% (3), and a missense mutation causing an Ile179Ser substitution accounts for about 12%, of all defective alleles (4). All other mutant alleles have only been found in a few or single patients (e.g. (5)).

All types of mutations – deletions, insertions, splice site mutations, missense mutations, etc. – have been described. Missense mutations, however, are by far the most frequent type of defect found in the *ASA* gene (1).

As biochemical characterization of the consequences of missense mutations is rather laborious, only about a dozen of the missense mutations have been examined at the biochemical level (e.g. (5–8)). The results of these studies have revealed that the missense mutations can be divided into two classes: in most cases the amino acid substitutions caused by the missense mutations result in defective folding of the enzyme in the endoplasmic reticulum and subsequent rapid proteasomal degradation (8). For a small minority of mutations, the extent of folding still allows the enzyme to pass the quality control mechanisms of the endoplasmic reticulum and the enzyme reaches the Golgi apparatus and is correctly sorted to the lysosome. Due to the structural alterations, however, the defective ASA polypeptides are not stable in the lysosome and are rapidly degraded. One such example has been studied in great detail and even involved the crystallization of the defective enzyme (6).

As mentioned previously, the Pro426Leu substitution is one of the most frequent defective alleles, particularly in patients with the attenuated adult-onset form of disease. The Pro426Leu-substituted ASA has been shown to be synthesized normally and correctly sorted to the lysosome, where it is rapidly degraded. ASA is synthesized as a dimer. At the acidic pH of the lysosome, four of these dimers reversibly associate to form octamers. Interestingly, the interface between the dimers in the octamer contains a cathepsin L cleavage site. In the octamer, however, this site is inaccessible to the protease. From an evolutionary point of view, octamerization of ASA has probably developed to protect the enzyme from proteolytic attack by its fellow lysosomal enzymes. Proline residue 426 is involved in the lysosomal octamerization process of ASA. When this residue is substituted with leucine, the enzyme's ability to octamerize is greatly impaired and most of the enzyme remains in dimeric form in the lysosome. Thus, the cathepsin L cleavage site is exposed and the enzyme is rapidly degraded. This is one of few examples in metabolic diseases in general, in which the biochemical consequence of a missense mutation has been fully characterized at the molecular level. Such in-depth examinations are not only of academic interest they may also point to new therapeutic options. As the Pro426Leu substituted ASA is enzymatically active, stabilization of the enzyme through inhibitors of cathepsin L are, in principal, a therapeutic option.

Examination of the frequency of certain *ASA* alleles amongst patients with different forms of metachromatic leukodystrophy has revealed a genotype–phenotype correlation in metachromatic leukodystrophy (3,4). Patients who are homozygous for alleles that do not allow the synthesis of functional enzyme, always suffer from the most severe late-infantile form of metachromatic leukodystrophy. In these cases, the lack of functional enzyme results in rapid accumulation of sulphatide (9). Therefore, the onset of the disease is early and progression is usually rapid. If a patient carries one allele that allows for the expression of low amounts of residual enzyme activity – such as the abovementioned Pro426Leu allele – in most cases this results in the juvenile-onset form of disease (3). Many patients with adult-onset disease are in fact homozygous for alleles allowing for the expression of low amounts of functional enzyme. These residual activities prolong the process of accumulation and explain the late onset and less rapid progression of disease (9). Figure 1 summarizes data on genotype–phenotype correlations in metachromatic leukodystrophy, showing the relationship between age at disease onset and the different genotypes seen in patients. Interestingly, most patients carrying the Ile179Ser allele who have been diagnosed were heterozygous for this allele and a null allele (10). So far, not a single patient has been identified who is homozygous for this allele. Considering that this allele is the third most frequent amongst patients with metachromatic leukodystrophy, this is unexpected. It may be speculated that homozygosity for this allele may allow for normal sulphatide catabolism, so that homozygous individuals may remain healthy and are therefore not detected. This hypothesis is further supported by the results of the characterization of a few patients with very late-onset metachromatic leukodystrophy. For example, a patient developing the first symptoms at the age of 63 years was heterozygous for the Pro426Leu and Ile179Ser alleles (11). As the phenotype of patients with metachromatic leukodystrophy is substantially influenced by residual enzyme activity, this may be explained by a comparatively high residual enzyme activity associated with the Ile179Ser allele, which in combination the Pro426Leu allele, allows for an even later onset of disease, as seen in Pro426Leu homozygotes.

In addition to the correlation between genotype and age at disease onset, there is another genotype–phenotype correlation in metachromatic leukodystrophy, which associates the Pro246Leu and Ile179Ser alleles with different clinical phenotypes of patients. An international consortium has collected biochemical, genetic and clinical data from adult patients with metachromatic leukodystrophy (10). This collection revealed that patients homozygous for the Pro426Leu allele almost always present initially with progressive motor and sensory deficits. In contrast, in patients who carry an Ile179Ser allele the disease frequently starts with psychiatric symptoms and patients develop neurological symptoms only later in the course of the disease. Thus, there seems to be a correlation between genotype and initial clinical symptoms.

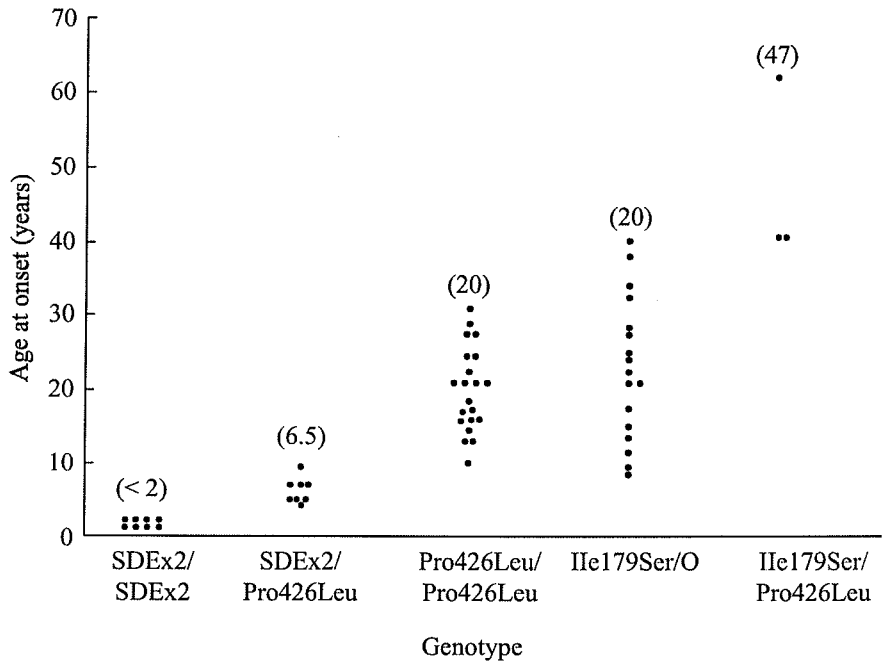

Figure 1 This figure shows the correlation between age at disease onset and the various arylsulphatase A (ASA) genotypes in patients with metachromatic leukodystrophy. SDEx2 denotes one of the frequent defective ASA alleles in which a splice donor site at the exon 2/intron 2 border is mutated (allele frequency ~25%). Pro426Leu is an allele of similar frequency, carrying the respective missense mutation (3). Ile179Ser accounts for about 12% of all defective alleles amongst patients with metachromatic leukodystrophy. O, depicts various null alleles. Dots give the age at onset for the various patients. Mean age is shown in brackets. Data were compiled from various publications and unpublished results (V Gieselmann, unpublished). Data for Ile179Ser/O patients are from (10).

When considering genotype–phenotype correlations in metachromatic leukodystrophy, one important issue should be emphasized. When patients of identical genotype are compared (e.g. Pro426Leu/Pro426Leu homozygotes), it becomes apparent that there is substantial variation occurring within the same genotype (Fig. 1). Variability can even occur within the same family (12,13). This shows that there are clearly other genetic or epigenetic factors, which influence the phenotype of the patient substantially. These factors are at present unknown. Nevertheless, the variation in phenotype observed in individuals with the same genotype precludes any prediction of clinical outcome based on genetic analysis. The genotype–phenotype correlation in metachromatic leukodystrophy is only significant when groups of patients are considered, and it does not allow for a precise individual prediction of the clinical course of the disease.

ANIMAL MODELS OF METACHROMATIC LEUKODYSTROPHY

Metachromatic leukodystrophy is a disease for which no naturally occurring animal model exists. The disease has only been described in humans. For that reason an ASA-deficient knockout mouse model was generated several years ago (14). These mice store sulphatide in tissues similar to humans (15). Sulphatide storage has been found in brain white matter, in Schwann cells, in the distal tubules of the kidney and in gall bladder epithelia. Thus, the pattern of sulphatide storage in ASA-deficient mice mimics that observed in humans with metachromatic leukodystrophy; however, the mice lack the pathological hallmark of the disease, which is demyelination (14,16). Even in very old (26 months) animals there are, at best, minimal signs of demyelination in the nervous system. As the animals do not develop demyelination in the central or peripheral nervous systems, they are of limited value in pathogenetic studies. Despite this, and the observation that lifespan is not reduced, the animals do develop progressive neurological symptoms (17). Compared with controls, ASA-deficient mice perform worse on a slowly rotating rod and various other behavioural tests. Neurological symptoms become apparent after about 6 months of life, and slowly worsen over time (17). As the mice do not show demyelination, it seems surprising that they develop neurological symptoms. Closer examination reveals that sulphatide storage in the nervous system is not restricted to the oligodendrocytes, but also occurs in many neurons (18). Therefore, it seems possible that the symptoms in the mice are caused by increasing sulphatide accumulation in neurons rather than oligodendrocytes. This may be extrapolated to humans. The disease has been classified as a leukodystrophy, based on pathological examinations of autoptic brains, in which storage has been described in neurons as well as glial cells (19). Whether neuronal storage also contributes to symptoms in patients during the early stages of disease is unknown, but the animal model suggests that this may be the case.

The reason for the lack of demyelination in the ASA-deficient mice seems to be insufficient sulphatide accumulation. When sulphatide storage in the CNS of

ASA-deficient mice was analysed at the age of 24 months, it was only elevated by about 1.4-fold compared with normal controls (20). In patients, a three- to sevenfold increase in sulphatide content was described in autoptic brains (1). This finding suggests that the low degree of sulphatide accumulation is responsible for the lack of demyelination in the mouse model. It should be mentioned that the turnover of sulphatide in myelin is very slow. Its half-life appears to be in the range of several months (21). As myelination is an evolutionary conserved process, the half-life of myelin lipids in men and mice is likely to be conserved too. As lysosomal accumulation requires sulphatide turnover, it may take as long as 2 years before symptoms develop. So, the 1.4-fold increase of sulphatide in mice possibly reflects the storage in humans of about the same age. The short lifespan of mice then limits the degree of accumulation to below the extent needed for the development of demyelination.

If the reason for the absence of demyelination in the ASA-deficient mouse is insufficient accumulation of sulphatide, then an increase in sulphatide should provoke loss of myelin. To achieve this, transgenic mice were generated, which express the sulphatide-synthesizing enzyme cerebroside sulphotransferase (CST) under the control of an oligodendrocyte and Schwann cell-specific proteolipid protein (PLP) promoter (M Eckhardt, unpublished). These transgenic mice were crossed with ASA-deficient mice. In the PLP-CST transgenic/ASA-knockout mice the enhanced synthesis of sulphatide should lead to enhanced accumulation. Analysis of sulphatide concentrations in the brain of these mice showed that, compared with the conventional ASA-knockout mice, sulphatide was increased about two- to threefold. Consequently, these mice developed demyelination in the peripheral nervous system accompanied by a substantial decrease in nerve conduction velocity. Also in the CNS, irregular myelin morphology and an increased frequency of hypomyelinated axons indicated demyelination. At the biochemical level this was reflected by a reduction of myelin basic protein. Compared with the peripheral nervous system, however, the degree of demyelination in the CNS was less pronounced. In addition, the pathology in the mice did not develop in the first months of life; it took more than a year before myelin pathology developed. Nevertheless, this new animal model is the first to reflect the pathology characteristic of the human disease.

THERAPEUTIC TRIALS

To date, no specific treatment is available for metachromatic leukodystrophy, but various therapeutic trials have been performed in the mouse model. Trials in humans have focused on haematopoietic stem cell transplantation. To understand the rationale of therapies that were so far applied it is helpful to briefly review the process by which ASA and lysosomal enzymes in general are sorted to the lysosome.

ASA is synthesized at the rough endoplasmic reticulum where it receives *N*-linked oligosaccharide side chains. Upon passage through the Golgi apparatus these oligosaccharide side chains receive mannose-6-phosphate residues, which represent the sorting signal specific for lysosomal enzymes. The residues bind to mannose-6-phosphate receptors, which mediate further vesicular transport to the lysosome. During this process, it is important to realize that binding to the receptors in the Golgi apparatus is incomplete and a considerable fraction of ASA is secreted. The secreted ASA can be recaptured by neighbouring cells because mannose-6-phosphate receptors are also present on the plasma membrane. ASA binding to these plasma membrane-located receptors is the basis of internalization and effective delivery to the lysosomes (22). Thus, it is either possible to provide the mannose-6-phosphate-containing recombinant ASA directly by intravenous injection, or to transplant ASA-expressing cells into the patient. These cells will secrete ASA, which can be endocytosed by the deficient cells of the patient.

Therapeutic trials in patients with metachromatic leukodystrophy

Based on this rationale, a number of patients were treated by haematopoietic stem cell transplantation (23,24). Importantly, monocytic cells of bone marrow can migrate into the brain and differentiate into microglial cells (25). Thus, these cells are able to cross the blood–brain barrier and should be able to deliver enzymes to oligodendrocytes and neurons. It is, however, still controversial to what extent patients with metachromatic leukodystrophy benefit from this treatment. It seems to be agreed that treatment of patients with the rapidly progressing late-infantile form of the disease is ineffective (26). These patients should not undergo transplantation. If transplantation is performed in the early disease stages in patients with late-onset forms of metachromatic leukodystrophy it seems that, at least in some patients, disease progression is slowed (23,24). Surprisingly, in these patients there is no improvement in peripheral nerve pathology (C Peters, personal communication and (27)).

Intravenous injection of recombinant ASA has been investigated in ASA-deficient mice. These mice were injected with very high doses – up to 40 mg/kg body weight – of ASA produced in Chinese hamster ovary cells (28). These mice showed a reduction in sulphatide storage in the kidneys and the peripheral nervous system. Surprisingly, after the fourth injection of high doses of ASA there was also a slight reduction in sulphatide storage in the brain. Importantly, the reduction was restricted to macrophages; a reduced accumulation in oligodendrocytes was not detected. This finding is important, as it is unclear how much sulphatide storage in macrophages contributes to the pathology in the disease. The histochemical *in situ* evaluation of storage was done with Alcian blue, which allows for a qualitative and, to a limited extent, quantitative evaluation. Therefore, it cannot be excluded that there may also have been a modest reduction in storage in the oligodendrocytes, but this has not yet been demonstrated. These unexpected positive results have led to the initiation of a phase I/II clinical study currently being performed in Denmark in patients suffering from the late-infantile form of the disease.

Therapeutic trials in ASA-deficient mice

Other studies performed in the mouse model involved direct injection of ASA-expressing recombinant viral vectors into the brain. As this technique is expected to yield rapidly increasing levels of ASA in the brain, it may be particularly suitable for patients suffering from the late-infantile form of metachromatic leukodystrophy, in which the rapid clinical deterioration requires an immediate increase of enzyme activity. One of the studies used lentiviral vectors, which were injected into the hippocampal fimbria of the mice (29). This study showed widespread distribution of enzyme activity in the brains of ASA-deficient mice. The study also showed protection against degeneration of hippocampal neurons, resulting in improved learning abilities in the mice.

Another study used adeno-associated viral vectors to express ASA in the brains of ASA-deficient mice (16,30). After injection, ASA was expressed in the brain, cerebellum and brainstem, and expression lasted for the entire observation period of up to 15 months post injection. The mice showed a substantial reduction of sulphatide storage, accompanied by an improvement in neuropathological findings, such as reduced microglial activation and neuronal degeneration. The study was performed with two sets of mice: in one set, the injection of the viral vectors was done in young animals before the development of symptoms. In the second, injection was in older animals that had already developed initial signs of neuromotor disability. Whereas sulphatide reduction and neuropathological correction was similar in both trials, the treatment did not prevent the progression of neuromotor disabilities in the older mice (30). The reason for this difference is unknown. It should be noted that treatment did not influence the levels of gangliosides GM2 and GD3 in the brain – which, for unknown reasons, also accumulate in the ASA-deficient mice – nor did it improve the reduction of galactosylceramide, which also occurs in ASA-deficient mice. Perhaps these uncorrected lipid abnormalities account for the persisting neurological symptoms in the mice. Nevertheless, the results of these studies are sufficiently promising that clinical studies are planned in the near future in the rapidly progressing late-infantile form of the disease.

A number of studies have used cell-based therapies in the ASA-deficient mouse. In these trials, different cell types were transplanted into the ASA-deficient mice. Because it can be expected that therapeutic efficacy will be better the more ASA these cells produce, some studies used cells that were genetically modified prior to transplantation to overexpress ASA. The latter involved studies in which haematopoietic stem cells were transduced with either retroviral or lentiviral vectors and subsequently transplanted into ASA-deficient mice. The outcome of these studies was quite different. Studies using retroviral vectors demonstrated only a marginal reduction of sulphatide storage and only marginal effects on neurological symptoms in the mice (31). When lentiviral vectors were used, there was a substantial improvement in demyelination, with almost full correction of decreased nerve conduction velocity (32). This therapeutic success is remarkable because none of the other groups working with the same animal model have been able to detect demyelination and corresponding alterations of electrophysiological parameters in the animals. The same group extended these experiments further and demonstrated the reversal of neurological damage in these animals upon haematopoietic stem cell transplantation (33). It must be kept in mind, however, that the neurological symptoms these mice display are not caused by demyelination, but most likely by neuronal storage. Thus, demonstration of reversal of neuropathology in these animals is of little relevance to metachromatic leukodystrophy, as these data do not allow the conclusion that demyelination in the disease can be reversed. In general, due to the lack of demyelination in the ASA-knockout mouse, this animal model does not allow the design of experiments addressing the reversibility of damage in this disease.

Amongst the cell-based therapy options in metachromatic leukodystrophy, haematopoietic stem-cell-based gene therapy is certainly the most feasible with respect to clinical applications. Therefore, based on the results obtained with the ASA-knockout mice, clinical trials are planned in patients with metachromatic leukodystrophy in the near future.

Other cell-based therapy options, which have been tried in the ASA-deficient mouse involve transplantation of oligodendroglial progenitor cells (34), neural progenitor cells (35) and glial precursor cells derived from embryonic stem cells (36). In studies of the transplantation of non-genetically modified oligodendroglial precursor cells, histochemical evaluation showed sulphatide storage to be reduced and the neurological symptoms of the mice were substantially improved. When neural progenitor cells, genetically modified to express large amounts of ASA, were transplanted into ASA-deficient mice, partial clearance in cells surrounding the transplanted cells was observed. The same applies to genetically modified glial precursor cells derived from embryonic stem cells. In this case, sulphatide storage was diminished only in the vicinity of the transplanted cells. Although these three studies are difficult to compare because the experimental settings were quite different, it is surprising that non-genetically modified oligodendroglial cells seem to be therapeutically more efficient than cells overexpressing ASA. This suggests that the properties of the cell type chosen for transplantation is important.

CONCLUSION

Metachromatic leukodystrophy is a lysosomal storage disease, which is well understood at the genetic level and for which a genotype–phenotype correlation exists when groups of patients are compared. The phenotypic variation amongst patients with the same genotype is, however, substantial, so it remains impossible to predict the individual disease course based on genetic data alone. The animal model currently available for the disease does not develop the widespread demyelination seen in patients, although the mice store sulphatide in the same tissues as humans. Therefore, the use of ASA-deficient mice in pathogenic studies and studies examining the prevention and reversibility of myelin degeneration is very limited. The mice enable proof of principle investigations, in which, primarily, the reduction of

sulphatide is demonstrated. Experimental studies in these animals have involved enzyme replacement therapies based on transplantation of cells and gene therapeutic trials. As a consequence, enzyme replacement therapy, haematopoietic stem-cell-based gene therapy and direct injection of adeno-associated viral vectors may soon be investigated in clinical trials.

CONFLICT OF INTERESTS STATEMENT

VG holds a limited number of stock options in the Danish company Zymenex A/S, which produces ASA for clinical application.

References

1. von Figura K, Gieselmann V, Jaeken J. Metachromatic leukodystrophy. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic and molecular bases of inherited disease*. New York: McGraw–Hill, 2001: 3695–724.
2. Kreysing J, von Figura K, Gieselmann V. Structure of the arylsulfatase A gene. *Eur J Biochem* 1990; 191: 627–31.
3. Polten A, Fluharty A, Fluharty CB, Kappler J, von Figura K, Gieselmann V. Molecular basis of different forms of metachromatic leukosdystrophy. *N Engl J Med* 1991; 324: 18–22.
4. Berger J, Loschl B, Bernheimer H, Lugowska A, Tylki-Szymanska A, Gieselmann V, et al. Occurrence, distribution, and phenotype of arylsulfatase A mutations in patients with metachromatic leukodystrophy. *Am J Med Genet* 1997; 69: 335–40.
5. Schestag F, Yaghootfam A, Habetha M, Poeppel P, Dietz F, Klein RA, et al. The functional consequences of mis-sense mutations affecting an intra-molecular salt bridge in arylsulphatase A. *Biochem J* 2002; 367: 499–504.
6. von Bulow R, Schmidt B, Dierks T, Schwabauer N, Schilling K, Weber E, et al. Defective oligomerization of arylsulfatase A as a cause of its instability in lysosomes and metachromatic leukodystrophy. *J Biol Chem* 2002; 277: 9455–61.
7. Hess B, Kafert S, Heinisch U, Wenger DA, Zlotogora J, Gieselmann V. Characterization of two arylsulfatase A missense mutations D335V and T274M causing late infantile metachromatic leukodystrophy. *Hum Mutat* 1996; 7: 311–17.
8. Poeppel P, Habetha M, Marcao A, Bussow H, Berna L, Gieselmann V. Missense mutations as a cause of meta-chromatic leukodystrophy. Degradation of arylsulfatase A in the endoplasmic reticulum. *Febs J* 2005; 272: 1179–88.
9. Conzelmann E, Sanfhoff K. Partial enzyme deficiencies: residual activities and the development of neurological disorders. *Dev Neurosci* 1983–4; 6: 58–71.
10. Rauschka H, Colsch B, Baumann N, Wevers R, Schmidbauer M, Krammer M, et al. Late-onset metachromatic leukodys-trophy: genotype strongly influences phenotype. *Neurology* 2006; 67: 859–63.
11. Perusi C, Lira MG, Duyff RF, Weinstein HC, Pignatti PF, Rizzuto N, et al. Mutations associated with very late-onset metachromatic leukodystrophy. *Clin Genet* 1999; 55: 130.
12. Clarke JT, Skomorowski MA, Chang PL. Marked clinical difference between two sibs affected with juvenile metachro-matic leukodystrophy. *Am J Med Genet* 1989; 33: 10–13.
13. Arbour LT, Silver K, Hechtman P, Treacy EP, Coulter-Mackie MB. Variable onset of metachromatic leukodystrophy in a Vietnamese family. *Pediatr Neurol* 2000; 23: 173–6.
14. Hess B, Saftig P, Hartmann D, Coenen R, Lullmann-Rauch R, Goebel HH, et al. Phenotype of arylsulfatase A-deficient mice: relationship to human metachromatic leukodystrophy. *Proc Natl Acad Sci USA* 1996; 93: 14821–6.
15. Schott I, Hartmann D, Gieselmann V, Lullmann-Rauch R. Sulfatide storage in visceral organs of arylsulfatase A-deficient mice. *Virchows Arch* 2001; 439: 90–6.
16. Sevin C, Benraiss A, Van Dam D, Bonnin D, Nagels G, Verot L, et al. Intracerebral adeno-associated virus-mediated gene transfer in rapidly progressive forms of metachromatic leukodystrophy. *Hum Mol Genet* 2006; 15: 53–64.
17. D'Hooge R, Van Dam D, Franck F, Gieselmann V, De Deyn PP. Hyperactivity, neuromotor defects, and impaired learning and memory in a mouse model for metachromatic leukodys-trophy. *Brain Res* 2001; 907: 35–43.
18. Wittke D, Hartmann D, Gieselmann V, Lullmann-Rauch R. Lysosomal sulfatide storage in the brain of arylsulfatase A-deficient mice: cellular alterations and topographic distribution. *Acta Neuropathol (Berl)* 2004; 108: 261–71.
19. Peng L, Suzuki K. Ultrastructural study of neurons in metachromatic leukodystrophy. *Clin Neuropathol* 1987; 6: 224–30.
20. Saravanan K, Schaeren-Wiemers N, Klein D, Sandhoff R, Schwarz A, Yaghootfam A, et al. Specific downregulation and mistargeting of the lipid raft-associated protein MAL in a glycolipid storage disorder. *Neurobiol Dis* 2004; 16: 396–406.
21. Jungalwala FB. Synthesis and turnover of cerebroside sulfate of myelin in adult and developing rat brain. *J Lipid Res* 1974; 15: 114–23.
22. Kornfeld S, Mellman I. The biogenesis of lysosomes. *Annu Rev Cell Biol* 1989; 5: 483–525.
23. Krivit W, Peters C, Shapiro EG. Bone marrow transplantation as effective treatment of central nervous system disease in globoid cell leukodystrophy, metachromatic leukodystrophy, adrenoleukodystrophy, mannosidosis, fucosidosis, aspartylglucosaminuria, Hurler, Maroteaux–Lamy, and Sly syndromes, and Gaucher disease type III. *Curr Opin Neurol* 1999; 12: 167–76.
24. Malatack JJ, Consolini DM, Bayever E. The status of hemato-poietic stem cell transplantation in lysosomal storage disease. *Pediatr Neurol* 2003; 29: 391–403.
25. Asheuer M, Pflumio F, Benhamida S, Dubart-Kupperschmitt A, Fouquet F, Imai Y, et al. Human CD34+ cells differentiate into microglia and express recombinant therapeutic protein. *Proc Natl Acad Sci USA* 2004; 101: 3557–62.
26. Bredius RG, Laan LA, Lankester AC, Poorthuis BJ, van Tol MJ, Egeler RM, et al. Early marrow transplantation in a pre-symptomatic neonate with late infantile metachromatic leukodystrophy does not halt disease progression. *Bone Marrow Transplant* 2007; 39: 309–10.
27. Sevin C, Aubourg P, Cartier N. Enzyme, cell and gene-based therapies for metachromatic leukodystrophy. *J Inherit Metab Dis* 2007; 30: 175–83.
28. Matzner U, Herbst E, Hedayati KK, Lullmann-Rauch R, Wessig C, Schroder S, et al. Enzyme replacement improves nervous system pathology and function in a mouse model for metachromatic leukodystrophy. *Hum Mol Genet* 2005; 14: 1139–52.
29. Consiglio A, Quattrini A, Martino S, Bensadoun JC, Dolcetta D, Trojani A, et al. *In vivo* gene therapy of metachromatic leukodystrophy by lentiviral vectors: correction of neuropath-ology and protection against learning impairments in affected mice. *Nat Med* 2001; 7: 310–16.
30. Sevin C, Verot L, Benraiss A, Van Dam D, Bonnin D, Nagels G, et al. Partial cure of established disease in an animal model of metachromatic leukodystrophy after intracerebral adeno-associated virus-mediated gene transfer. *Gene Ther* 2007; 14: 405–14.

31. Matzner U, Hartmann D, Lullmann-Rauch R, Coenen R, Rothert F, Mansson JE, et al. Bone marrow stem cell-based gene transfer in a mouse model for metachromatic leukodystrophy: effects on visceral and nervous system disease manifestations. *Gene Ther* 2002; 9: 53–63.
32. Biffi A, Capotondo A, Fasano S, del Carro U, Marchesini S, Azuma H, et al. Gene therapy of metachromatic leukodystrophy reverses neurological damage and deficits in mice. *J Clin Invest* 2006; 116: 3070–82.
33. Biffi A, De Palma M, Quattrini A, Del Carro U, Amadio S, Visigalli I, et al. Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. *J Clin Invest* 2004; 113: 1118–29.
34. Givogri MI, Galbiati F, Fasano S, Amadio S, Perani L, Superchi D, et al. Oligodendroglial progenitor cell therapy limits central neurological deficits in mice with metachromatic leukodystrophy. *J Neurosci* 2006; 26: 3109–19.
35. Kawabata K, Migita M, Mochizuki H, Miyake K, Igarashi T, Fukunaga Y, et al. *Ex vivo* cell-mediated gene therapy for metachromatic leukodystrophy using neurospheres. *Brain Res* 2006; 1094: 13–23.
36. Klein D, Schmandt T, Muth-Kohne E, Perez-Bouza A, Segschneider M, Gieselmann V, et al. Embryonic stem cell-based reduction of central nervous system sulfatide storage in an animal model of metachromatic leukodystrophy. *Gene Ther* 2006; 13: 1686–95.