**Report of the UK Scientific Workshop on Krabbe Disease**

**15th and 16th October 2013**

**Author.Editorial:** Pat Roberts, Save Babies Through Screening Foundation UK, Dr Guy Besley.

**Abstract**

Globoid-cell leukodystrophy or Krabbe disease is an autosomal recessive lysosomal storage disease due to the inborn deficiency of β-galactosylceramidase (GALC) activity, critical for myelin integrity. Insufficient GALC leads to accumulation of the toxic substance psychosine with demyelination in the brain impacting the central and peripheral nervous system. The most aggressive form of the disease is among infants and young children. Treatment is generally confined to Haematopoietic Cell Transplantation which has a greater chance of benefit if the child is pre-symptomatic. In the UK children are generally diagnosed when symptoms have already developed and therefore it is too late to receive effective treatment. Children with the early infantile form receive palliative care until death, generally before the age of 2 years.

**Background to the Scientific Workshop**

The UK Scientific Workshop on Krabbe disease was organized to bring together International clinical and scientific experts in Krabbe disease, myelin, neurology and transplantation with a focused aim of:

- Sharing existing knowledge and experiences of researching, screening, diagnosing and treating Krabbe disease or similar disorders.

- Identifying and prioritising any new clinical and scientific questions to improve the diagnosis, screening and treatment of Krabbe disease.

- Initiate and support new international clinical and scientific research collaborations to develop the capacity to enable new knowledge to be generated with regard to the diagnosis, screening, treatment and care of children with Krabbe disease.

Some key yet challenging success criteria for the scientific workshop were developed which included a number of areas:

- A recognition that Krabbe disease is elevated in terms of medicine in the UK. That the scientific workshop has provided a good platform to ensure everyone is up to speed together on the current situation on Krabbe disease by sharing existing knowledge and experiences of diagnosing, screening and treating Krabbe disease. To develop a real strategic process for moving forward.

- That the workshop has enabled clinicians, scientists, researchers and the patient organization, who are currently not perhaps investing in developing scientific enquiry and clinical care in Krabbe disease to generate networks and to work together to get from research to therapies and clinical trials. That we are able to identify and prioritise the current and future clinical and scientific research questions to improve the diagnosis, screening and treatment of Krabbe disease.

- That we have initiated and can support new international clinical and scientific research collaborations to develop the capacity to enable new knowledge to be generated with regard to the diagnosis, screening, treatment and care of children with Krabbe disease. A programme of ongoing international meetings will be developed (independent or alongside existing international meetings).

- That we have developed meeting proceedings and information that can be submitted for publication in appropriate journals or websites.
Save Babies UK are grateful for the attendance and contributions made by so many experts from the UK and across the world. Particularly thanks go to Dr Guy Besley, Professor Tim Cox, Dr Colin Steward and Dr Simon Jones for acting as an advisory board for the workshop and for leading the sessions. Sincere thanks also to our speakers who have kindly contributed to this report.

A Family Perspective of Krabbe disease

**Ms Sarah Dudko, Trustee of Save Babies UK**

Ms Sarah Dudko, family member, delivered the opening address, sharing her experiences as the mother of Daisy who died from Krabbe disease in 2011. Sarah courageously spoke about the difficulty in initially getting clinicians to accept that there was something more fundamentally wrong with Daisy than just day to day childhood illness. Of her experience, following many tests and scans of finally receiving the diagnosis of Krabbe disease, initially in the mail and the family then establishing via the internet that Daisy was terminally ill. Sarah gave a very moving account, taking us through some of the difficulties in caring for a Krabbe child, in striving to make every day of Daisy's life meaningful and precious and in dealing with the many different and difficult problems that arose during Daisy's short life. Sarah spoke about trying to cope with Daisy's death and also shared some observations on what life was like for her family on an ongoing basis following the death of a child.

Krabbe Disease: an overview, with special reference to chemical pathology

**Dr Marie T Vanier, INSERM and Hôpitaux de Lyon, Lyon, France**

The timeline in history of Krabbe disease starts in 1916 with the clinical and pathological description of 5 patients by the Danish paediatrician Knud Krabbe, followed by the discovery of a deficiency in the lysosomal enzyme galactosylceramidase (1970) and cloning of the GALC gene (1993). Important pathological features are the presence of "globoid cells", and the early and near complete loss of oligodendrocytes. Early studies (1960s) showed that galactosylceramide can elicit experimental globoid cells in cell cultures and in living animals. While the classical infantile form has long been considered to constitute 80% of the cases or more, the recent Worldwide registry (122 cases) only found 63%. In France the data for the periods 1975-89 (77%) and 1990-2012 (64%) are in good agreement with the registry. The apparent increased incidence of late onset forms in recent years likely reflects a better awareness. The different methods for measuring galactosylceramidase activity were critically discussed, as well as the most common GALC gene mutations - primarily the large 30Kb deletion associated to a c. 502C>T polymorphism - and some "adult-onset" mutations. A very rare form of Krabbe disease (1 published and 1 personal cases) is due to the deficiency of saposin A, encoded by the PSAP gene. Brain lipids have been extensively studied in the 70s in autopsy material from patients with the infantile form (8-32 months old). While an essentially normal composition has been observed in cerebral cortex, cerebral white matter was remarkable by a very low concentration of all myelin lipids and abnormal myelination indexes. The myelin content was only 2% of that in a 1 year old child, but its composition was normal. A high galactosylceramide/sulfatide ratio was found, in line with the known storage in globoid cells. But the overall amount of galactosylceramide was very low, equivalent to that seen in the white matter of a child aged 2-4 months. Therefore, Krabbe disease is a paradoxical galactosylceramide storage disease, with a low content of the accumulated substrate in the target tissue. To explain the unique features of Krabbe disease as compared to metachromatic leukodystrophy, and following their finding that galactosylceramidase could also hydrolyse "lysogalactosylceramide" / "psychosine", Miyatake and Suzuki developed in 1972 the "psychosine hypothesis". This very cytotoxic compound was shown to kill oligodendrocytes in culture at very low concentrations. A significant increase of psychosine (normally present in minute amounts) was then demonstrated, first in
white matter of Krabbe disease patients (1976), and later in the various animal models (mice, dog, monkey). This lipid is synthesised mainly in the oligodendrocytes, by the same galactosyltransferase as galactosylceramide. The existence of a galactosylceramide deacylase has not been demonstrated (a sphingomyelin and glucosylceramide deacylase has been recently described in epidermis). According to the hypothesis, the local psychosine increase will lead to oligodendrocyte dysfunction then death, followed by myelination arrest. Indeed, many deleterious effects of psychosine have been described, such as inhibition of PKC, activation of caspases, of cytokines, of phospholipase A2, disruption of rafts or membranes, axonal defects on cultured neurons, even microglial response. There is however an important limitation: psychosine has only been studied in the brain of infantile patients. Its level in the late infantile/adult-onset forms is unknown, and could be normal or below a critical threshold. There are still many unanswered questions regarding pathophysiology of Krabbe disease. Whether blood levels of psychosine could be used as a biomarker is a timely question. Modulation of the disease by immunological factors will likely be discussed later in the meeting.

Krabbe disease in infancy and later in life – Clinical and diagnostic aspects among the leukodystrophies, with some remarks on management

Prof. Alfred Kohlschuetter, University Medical Centre Eppendorf, Hamburg, Germany

The clinical consequences of galactocerebrosidase deficiency, the basic defect in Krabbe disease, are mainly related to the progressive destruction of myelin in the central nervous system, and to a variable degree also the peripheral nerves. The spectrum of types of manifestations consists of two major groups that differ not only by age at appearance of first symptoms but also by certain biological characteristics. The early-onset form of the disease typically starts in previously healthy infants with unexplained crying, irritability, stiffness and loss of motor activity at 4 to 6 months of age, followed by a rapid neurological deterioration and death before the age of very few years.

The suspicion of a leukodystrophy should arise in any patient with progressive spasticity associated with peripheral neuropathy. Infantile Krabbe disease is suggested by its dramatic progression in the absence of other plausible causes; alternative diagnostic possibilities include infantile forms of ceroid lipofuscinoses or gangliosidoses. Neuroimaging typically shows lesions of the central cerebral white matter with a streaky appearance caused by perivenular clusters of globoid cells. The myelin damage shows a predilection for the corticospinal tracts. Lesions of cerebellar white matter, swelling of the optical nerves and progressive brain atrophy are also seen. The diagnosis is confirmed by appropriate enzyme tests.

The late-onset forms are characterized by a wide range of the age at first manifestation. A slowly progressive spastic gait disorder, associated with a variable peripheral neuropathy and beginning at almost any age after the infantile period, is a non-specific first manifestation. Visual failure, due to involvement of the optic nerves and cerebral visual pathways, appears in many patients initially or later during the course. The neurological progression is usually slow and the survival time after appearance of the first symptoms many years. Good natural history data, however, are not available. Single cases with rapid deterioration after the onset have been observed. Results of neuroimaging are similar to those seen in the infantile form but the streaky pattern of the cerebral white matter is not so prominent. The posterior limbs of the inner capsule can be affected. Suspecting late-onset Krabbe disease, particularly in adults, can be puzzling, and the diagnosis is probably missed in many cases.

While discussions of the chances and risks of experimental treatment are ongoing and evaluation of new therapies in late-onset forms is difficult in view of the widely unknown variability of the natural course,
optimizing palliative treatment modalities remains important. In patients with infantile Krabbe disease, irritability and pain are prominent problems. Some of these seem due to an inflammatory component of the disease process and are amenable to antiphlogistic medication. Convulsions in such infants are not necessarily of an epileptic nature but may represent reactive painful spasms induced by sudden external stimuli. Recognizing such seizure-like conditions is helpful, as they do not respond to anticonvulsive drugs but rather to adequate handling.

**Diagnosis of Krabbes Disease, genetic mutations, treatment trials in animal models and current research and viral gene therapy trials in the dog model.**

*Prof David Wenger, Thomas Jefferson University, Philadelphia USA*

Krabbe disease is a genetic leukodystrophy caused by mutations in the galactocerebrosidase (GALC) gene. While most patients present initially as infants, older individuals are also diagnosed. There may be a delay in obtaining a diagnosis in some patients until certain symptoms initiate testing. In this laboratory, most patients are diagnosed by measuring GALC activity using leukocytes. A delay in diagnosis may limit the success of any treatment aimed at preventing or repairing damage to the nervous system. In order to obtain a diagnosis earlier, newborn screening for Krabbe disease was instituted in 2006 in New York State. Newborns with a low GALC value after re-testing are tested using a conventional test and mutation analysis of the GALC gene is also done. All individuals who have lower than normal GALC activity do have mutations in the GALC gene. Some of these mutations are clearly disease-causing (they have been found in patients confirmed to have Krabbe disease or result in a greatly altered GALC enzyme), some have been found in late-onset patients, some have never been seen before and some are normal polymorphisms. We have recently catalogued 147 mutations found in patients with Krabbe disease. The finding of either previously unreported mutations or mutations found in late-onset patients causes considerable uncertainties for both the family and the physician. The determination of when and if the individual might develop Krabbe disease is critically important to the success of the program. When therapy should be started requires careful clinical evaluation and neurodiagnostic studies. Environmental or additional genetic factors may precipitate the onset of symptoms in older individuals. Those newborns with very low GALC activity and two mutations in the GALC gene that have been found in patients with an infantile presentation are offered hematopoietic stem cell transplantation (HSCT) within one month of life. When HSCT is performed in pre-symptomatic or very mildly affected infants it can extend the lives of these individuals. However, these patients may have significant motor and cognitive deficits. Better treatments are needed.

There are several animal models that also have low GALC activity. Many studies have been done using the mouse model. Some treatments only resulted in a small increase in lifespan and some were found to be not well tolerated by the mice. Attempts have been made to supply the missing enzyme by gene therapy. We tested a viral vector, called AAVrh10 containing the GALC gene. Injecting this vector into the brain and into a blood vessel has shown promise in significantly prolonging the lives of treated mice (from about 40 days to 150+ days). These mice are fertile and show few signs of the disease until very late in their lives. However, for some reason these mice suddenly become weak and die. Why? Studies in the mice are continuing. At this time we have initiated studies using AAVrh10 to treat the dog model of Krabbe disease. This larger animal will serve as a bridge between studies in mice and humans. More than one approach may be needed to prevent and to correct the pathology seen in both the CNS and PNS in this disease.
Six Years of Screening for Krabbe Disease in New York State

Dr Joseph Orsini, New York State Department of Health, Wadsworth Centre, Albany, USA

We have screened approximately 1.6 million infants for Krabbe disease as of 7th March 2013 (start date was 7th August 2006). We have referred 291 infants, of these we only have 5 real Krabbe cases (infantile, symptomatic, eligible for cord blood transplant). We break into risk categories based on diagnostic lab activity value. As of March, 2013, we have 14 high risk cases (high risk are infants that had activity <\= 0.15 nmol/h per mg protein). In David Wenger's lab, one of these infants had activity measured at a later date that would have place him/her into moderate risk group. All of these infants had two mutations. Next we have 27 moderate risk patients, these have dx activity from 0.16-0.29 nmol/h per mg protein. Approximately 20 of these infants had two mutations, the remaining 7 look like carriers, but we could have missed a deletion or duplication. Another way people look at these, is how many of our 291 infants had two mutations. The problem with this is that we do not always know if the variant we have detected in a gene is disease causing or just a polymorphism. We find a fair number of novel variants, some appear to be polymorphisms. Anyway, of our 291 infants referred, 59 have two mutations and 226 have only one mutation.

So the screen positive rate is low for this test and the positive predictive value is low as well. However, with genotyping, we can indicate how serious the referral is. Obviously someone with two infantile mutations is much more serious a referral than infant that is detected with on a single mutation. Many of the variants we have detected are novel, so we do not know how serious they are until we get diagnostic lab test results back.

As for lab set up and costs, we started off running my lab with three tandem mass spectrometers to screen 250,000 infants/year (about 280,000 samples). Additionally we purchased two robotic liquid handlers to process the samples. This was about a million dollars of equipment. We have had this equipment since Dec. 2005 and we started live screening in August 2006, Hence the equipment cost would be about 1 million/1.75 million children or about $0.57/baby. We set up DNA testing as part of our algorithm. This helps reduce the number of referrals by around 40% as many of our screen positive infants detected by enzyme alone, end up having only polymorphisms detected in the GALC gene. So without this, we would have referred around 500 infants.

The three major issues are: 1. Only 5 true cases found in 1.75 million screened, gives low incidence in our state (1/350,000, which is lower than was predicted). 2. Some infants have low enzyme activity with the diagnostic lab and two mutations that are asymptomatic, but we do not know when/if these children will develop Krabbe disease. We are finding that some reported mutations look like polymorphisms, so this adds to the complexity. 3. Perhaps the biggest issue is that of the five infants that we detected with infantile Krabbe disease, four have had transplants, and two have died due to transplant complications. Of the two surviving infants, one is not doing well at all. The good news is that the other surviving infant just started kindergarten, this is great (he is bi-lingual, seems happy, but cannot walk without assistance - I am not sure what kind of assistance). A difficulty is that the transplant centre with the most experience (Duke) is far away for some families and for some families it is too disruptive for the family to undergo transplant this far from home, and make decision so quickly. The success rate at the other transplant centres is more of an unknown.
Predicting Krabbe Disease onset after newborn screening

Dr Maria Escolar, Programme for the Study of Neurodevelopment in Rare Disorders, Children’s Hospital of Pittsburgh of UPMC, USA

We have studied the natural history of early and late infantile Krabbe disease, and developed a staging system that evaluates the disease burden based on symptoms. The transplant outcomes correlated well with the staging at the time of treatment in both early and late infantile. Other tests such as brain imaging and neurophysiological studies were found to reflect disease progression but are not predictive of outcomes after transplantation. In the early infantile onset, babies benefit from unrelated umbilical cord blood transplantation only if the patients are diagnosed before the onset of symptoms. Newborn screening has been available in New York State since August of 2006 and more recently it is also available in Illinois. Several states have passed legislation and are considering adding Krabbe to the expanded newborn screening programs. Therefore, there is an urgent need to establish predictors of disease onset since it is not known whether babies who screen positive will develop disease as a baby or as an adult. Our group has studied and developed new methodologies to identify differences in the internal capsule using brain MRI tractography. We found that our new tool distinguishes between babies who have early-onset disease from those who will develop symptoms much later. The tool provides very consistent FA values that can detect small regional differences and age-related changes in the first weeks of life. In addition to this methodology we have established a tissue repository and evaluated potential biomarkers of disease onset. Both tools have the potential to be key components of a population-screening program to help determine who should receive treatment before symptoms develop.

The Professor Ed Wraith Memorial Lecture

Ed wraith was the very first consultant to advise Save Babies UK when we were examining the feasibility of founding the Charity way back in 2008. His assessment of the situation for Krabbe patients and also for newborn screening generally, together with advice on the difficulties, issues, benefits, realistic goals and timescales was invaluable. More recently Ed, and Mrs Christine Lavery of the MPS Society, discussed with SBUK the benefits that could be achieved in holding a scientific workshop in the UK on Krabbes disease, confirming some of the success criteria and advising of the key people that we needed to attend and also to lead the workshop. It is clear from the successful outcomes that his assessment of the situation and the benefits that could come out of such a workshop were absolutely correct. We are grateful for Ed’s support during the past 5 years and were extremely pleased that Dr Rob Wynn agreed to use this opportunity to dedicate his presentation in memory of Ed.

Treatment of LSDs and Leukodystrophies by HSCT; Experience in one UK centre

Robert Wynn, Honorary Clinical Professor of Paediatric Haematology and BMT. Director of Blood and Marrow Transplant Programme, Royal Manchester Children’s Hospital

This talk is dedicated to the memory of Ed Wraith. Ed fostered the development of transplant in metabolic disorder. He was a friend and colleague to many. I am lucky to have arrived in Manchester for Paediatric Haematology and transplant training and to have come under his tutelage. We in Manchester miss him very much but will strive to honour his memory by continuing the work that he started in improving therapies for children with metabolic disease.

The Manchester experience of blood and marrow transplant of metabolic disorders is similar to the international, registry experience. Over the last years transplantation techniques and the increased
availability of well-matched donors have greatly improved transplant survival rates. In the last 8 years overall survival rates are about 90%. Full intensity conditioning therapy with individually guided busulfan dosing and avoidance of in vitro T cell depletion are important in achieving engraftment. Cord blood is the preferred cell source since it is available quickly and the time from diagnosis to transplant is reduced and engraftment is more complete than following transplant using other cell sources.

Surviving the transplant is only part of the story in metabolic disease stem cell transplant. Increasingly the transplant and metabolic teams have looked at disease outcomes following intervention. It is clear that many different factors influence outcome following transplant. Some of these factors cannot be influenced – the disease and its genotype for example. Others are amenable to influence. Specifically the time to transplant and the efficacy of any therapy at delivering enzyme and reducing substrate are both potentially controllable.

Patients with advanced disease have poor transplant and disease-related outcomes. Patients transplanted earlier in their disease courses have improved outcomes. Earlier diagnosis – for example through screening – will improve outcomes. The use of cord blood will also reduce the interval between diagnosis and actual transplant.

Some therapies deliver more enzyme to host tissue than other therapies. BMT is more effective than ERT in CNS disease since enzyme delivered by ERT does not cross the blood brain barrier. Using a carrier family donor will deliver less enzyme in transplant than a wild type donor who is not a genetic carrier of the disease. We have been able to show that unrelated donor transplant recipients have better clearance of residual stored substrate than family donor transplant recipients. Cord blood is particularly good because it is always unrelated and it is more often fully engrafted than other cell sources.

There has been particular interest in gene-modified autologous stem cell approaches to metabolic disorders. Enzyme delivery can be increased beyond that possible with a wild type donor and because the donor is self then the risk of the procedure is reduced. The current optimal cellular therapy solution through delivered enzyme is autologous gene-modified stem cell transplant of an infant diagnosed early in life following neonatal screening. Such an approach will both improve the outcome in those conditions that are currently “transplantable” such as Hurler disease and will make other such as Hunter and Krabbe more responsive to a cellular therapy approach.

**RIP3 as a novel potential therapeutic target for Gaucher disease and for Krabbe disease**

Prof. Anthony H. Futterman, Department of Biological Chemistry, Weizmann Institute of Science, Rehovot 76100, Israel.

Krabbe disease resembles neuronal forms of Gaucher disease (nGD) inasmuch as it causes acute neurodegeneration in infants, and is also caused by the inability to hydrolyze a simple mono-glycosylated glycosphingolipid (galactosylceramide in the case of Krabbe disease and glucosylceramide in case of nGD). Moreover, infiltration of the central nervous system by multinucleated giant cells of macrophage lineage (known as Gaucher cells in GD and globoid cells in Krabbe) is unique to these glycosphingolipidoses. In addition, the cognate deacylated metabolites, 1-β-glucosyl- and 1-β-galactosylphingosine (‘psychosine’) have been implicated in the neuropathology of both diseases. We now demonstrate similarities in the pathological pathways involved in both Krabbe and nGD, since both show elevated activities of the receptor-interacting protein kinase (Rip) pathway.

Levels of RIP1 and RIP3 were both markedly elevated in the brains of a genetic model of symptomatic nGD mice and in the brains of the Twitcher mouse. Levels of RIP1 were also elevated in the one available brain of a human patient who succumbed to nGD. nGD was induced in Rip3-deficient mice by daily injections of a chemical inhibitor of glucocerebrosidase, CBE. Levels of RIP1 and RIP3 were markedly elevated in the brains of
CBE-treated mice. However, whereas control mice injected with CBE displayed typical manifestations of murine nGD (i.e. weight loss and loss of motor coordination), the signs of disease in Rip3\(^{-/-}\) mice injected with CBE were dramatically ameliorated. Notably, the life-span of Rip3\(^{-/-}\) mice injected with CBE was significantly extended to >150 days, with survival to 180 days in some animals, whereas no Rip3\(^{-/-}\) mice survived beyond 40 days of age. Importantly, the improvements in motor coordination and life-span were observed prior to the appearance of neuronal loss, but after the appearance of neuroinflammation, and were accompanied by markedly fewer activated microglia in layer V of the cortex. These results directly implicate the RIP3 kinase pathway in neuroinflammation, and indicate that this pathway might be a molecular target for therapeutic intervention in nGD and in Krabbe disease.

Our results show that RIP kinases are directly involved in the pathway of the pathological events which induce the relentless neuroinflammatory changes and tissue injury that are characteristic of nGD. Moreover, it appears that RIP1 and RIP3 are also implicated in the acute neuropathological changes that occur throughout the central nervous system in Krabbe disease. The apparently preferential activation of the RIP pathway in these biochemically cognate disorders, but not in other LSDs which affect the brain and display neuroinflammation, suggests the existence of a specific mechanism of neuronal death and/or microglia activation related to the nature of the accumulating substrates, rather than a common mechanism of cell death in all LSDs. No inhibitors of the RIP3 pathway displaying in vivo activity have been identified, but development of such inhibitors may provide an alternative therapy for n GD and potentially also for Krabbe disease, for which innovative treatment is urgently required.

**UK Krabbe Disease Workshop - ‘Overcoming Therapeutic Obstacles in Krabbe Disease’**

**Prof. Timothy M Cox. Neena Kim, M Begoña Cachón-González, Department of Medicine, University of Cambridge UK**

**Introduction**

This, the first UK Krabbe workshop was held propitiously almost exactly 100 years after Dr Knud Krabbe made the first description of the disease. It is salutary to see what this first Century of investigation has provided and how it might be a launch pad for improved care, and if possible the near-miracle we all seek – effective therapy.

**Commercial update**

Substantially, the pharmacopoeia for ultra-rare diseases is empty; but commercial investment in orphan drugs has been profitable in the lysosomal diseases, and at least two companies, Zymenex and Shire have attempted to develop enzyme therapy for Krabbe disease.

While neither company attended, Dr Aidan Gill of Shire UK, kindly supplied an in-depth presentation about initial incursions into the field: negotiations with the Food and Drug Administration, required Shire to develop a study of the natural course of Krabbe disease (HGT-GLD-056). While we would have been interested to see the pre-clinical programmes of work in experimental animals, the presentation showed how this would provide contemporaneous data on disease progression in infants with Krabbe disease. HGT-GLD-056 planned primary endpoints in growth parameters from baseline and, as a surrogate measure for survival, the onset date the infant or child became dependent on oral nutrition, hydration and/or ventilatory support. Secondary objectives, based on prior studies, were put in place to evaluate clinical parameters using the neurological examination and infant distress scales; it would have examined the utility of Bengt Hagberg’s original clinical staging for the disease, as published in 1962. Time to death or absolute survival and adverse event experiences would have been included. The
newborn screening programme for Krabbe disease in the State of New York from August 2006 onwards, allowed incidence data for an American population to be estimated.

Six months from kick-off and with HGT-GLD-056 approved, only one eligible was patient identified, but the family declined participation. Estimating the disease frequency at <1:300,000 live births, the natural history study was withdrawn, since it was unlikely that any clinical data to support a therapeutic programme would be available for comparative purposes. While Shire confirm there was a high, unmet need for Krabbe disease, challenges to feasibility, including the inability to recruit subjects, the lower than predicted incident of infantile Krabbe disease and its rapidly progressive course, led to a commercial decision not to proceed. Shire now states that it has no current projects in Krabbe disease.

Controversial aspects - and value - of neonatal screening

At the meeting, we heard how better reproductive choices and greater clarity of management can be obtained through neonatal screening programmes, even though such programmes remain controversial and are not introduced outside the US States of Missouri and New York. If effective treatments beyond neonatal, umbilical-cord haematopoietic stem cell transplantation (HSCT) were introduced, this would mandate further consideration by the regulatory screening authorities. We were all moved by the accounts of families as to how earlier diagnosis improved care and wellbeing generally in families afflicted by severe Krabbe disease, and the matter clearly remains an area of active consideration.

Haematopoietic Stem-cell Transplantation

In relation to early- or late-infantile onset patients, it is worth noting that few such patients, who were diagnosed and treated in the pre-symptomatic phase by HSCT, actually died of Krabbe disease, and the fascinating work of Dr Maria Escolar and the assessment of 16 such infants and the long-term follow-up show what can be achieved, even in the face of such a severe disease. However, despite the courageous efforts of Shire and its ultimate withdrawal from a therapeutic programme in Krabbe disease, hard objective thinking is needed. The development of a natural history programme must have been predicated on extensive pre-clinical data in animal models showing efficacy of enzymatic augmentation – but this information has not been made available.

The Battleground – knowing the enemy

Krabbe disease clearly has daunting characteristics: it is diffuse and fulminating; it affects infants and young children, causing rapid death, principally as a result of loss of oligodendroglia which support the integrity of the myelin around axons in the brain, spinal cord, peripheral nerves and their roots. Although very rare, there is a consensus that apart from Dr Orsini’s figures from New York State, its global birth frequency is approximately 1:10⁵. The neuropathology is not fully understood, but it is clear that the diffusible sphingolipid metabolite, galactosylsphingosine (‘psychosine’), is responsible for much of the neurotoxicity. We are fortunate that Dr Marie Vanier, who first described the elevated brain psychosine concentrations when working in the laboratory of Professor Lars Svennerholm, attended: not only is psychosine toxic at micromolar concentrations, but recent studies from Professor Marco Presta and Mirella Belleri of Brescia University found effects on angiogenesis in Krabbe disease, not only in the Twitcher mouse and the human Krabbe disease brain, but in vitro, as a result of exposure to quasi-pathological concentrations of this sphingolipid. The defective in vivo angiogenic response shown in subcutaneous Matrigel implants in twitcher mice strongly implicates a diffusible toxin like psychosine.

Amassing a Therapeutic offensive

The platforms for the future of Krabbe disease are rich and ripe for exploration: we have authentic small and large animal models in the form of the Krabbe mouse, the twitcher mouse and the West Highland terrier dog; we
have an impressive repertoire of reagents developed from the biochemical genetics of Krabbe disease, and from studies of its pathogenesis through the release of psychosine and our knowledge of myelin pathophysiology; there are emerging studies of cell death control, as presented for the first time in unpublished data from Professor Futerman, and in the medium- to long-term future the striking advances in contemporary regenerative medicine as applied to neuroscience.

Here I focus also on the unique properties of the lysosome, as recognized from its first description by Christian de Duve – namely accessibility of the lysosomal compartment, implicated in Krabbe disease, to corrective enzymes delivered in the fluid phase.

**Substrate inhibitors**

This brings us to another potential therapy, commonly known as “substrate reduction therapy”, in which a selective inhibitor of the first committed step of galactosphingolipid biosynthesis could inhibit formation of toxic galactosylpsychosine and its congener galactocerebroside in all tissues. The therapeutic target is the enzyme that brings about the transfer of UDP-galactose to the ceramide backbone to generate principally monogalactosylceramide – this enzyme (2-hydroxyacylsphingosine 1-β-galactosyltransferase, EC 2.4.1.45) is present on the endoplasmic reticulum (ER) luminal membrane surface. No inhibitors of this enzyme have yet been identified for therapeutic exploration, but an analogous selective inhibitor of the related enzyme positioned on the cytosolic leaflet of the ER UDP-glucosylceramide transferase is a proven therapeutic target in Gaucher disease, and where there is already an approved therapy (N-butyldeoxyxojirimycin, miglustat, and another, eliglustrat tartrate, in late-phase clinical development with strong clinical efficacy). Either as a primary therapy for late-onset forms of Krabbe disease or as an adjunctive therapy for combination with other agents, including gene therapy for functional complementation, a search for a selective inhibitor of galactosphingolipid biosynthesis would be an important priority in Krabbe disease.

**Controlling neuronal death responses**

Given the challenges of acute infantile Krabbe disease and the slow therapeutic progress beyond H SCT or limited indications in the disease, there is clearly a need to search “off-field”. In this context, the presentation by Professor Tony Futerman from the Weizmann Institute, Israel, is of telling significance. In the biochemically related condition, neuronopathic Gaucher disease, Einat Vitner working with Professor Futerman found evidence of activation of the receptor-interacting serine-threonine kinases, RIP 1 and 3. These kinases, which are an active focus of contemporary molecular cell biology, control the switch between programmed and necrotic cell death. As explained by Tony Futerman, Dr Vitner has shown that neuro-inflammation and neuronal death in Gaucher mice is abrogated by the absence of RIP 3. Initial studies indicated that Type II Gaucher disease was a unique neurodegenerative model of this RIP pathway, but having heard of this information, it seemed to us that the chemical relatedness of Krabbe disease might render it a comparable target. In careful studies, Krabbe disease rather than other lysosomal disorders affecting the brain, was found to share the same mechanism of activation of necrotic cell death – so-called necroptosis. Collaborative experiments are under way using selective inhibitors for investigation with the Weizmann Institute to determine whether the RIP pathway can be developed as a therapeutic target in human Krabbe disease.

**Gene Therapy – single and combinatorial**

Already, following the lines of enzymatic complementation and gene therapy, great strides have been made even in Krabbe disease, as shown by Dr Wenger in his presentation, using combinatorial approaches to provide complementing gene peripherally and at the same time into the brain of neonatal mice. This has extended the life from approximately 40 days to well over 100 or 150 days in many cases. In comparable
work conducted by Dr Neena Kim in our own laboratory, has obtained similar results using a different serotype of recombinant adeno-associated viral vectors (rAAV) expressing the GALC protein. Drs Kim and Cachón-González have generated a system for large-scale production of GALC, and in collaboration with Professor Randolph Read at the Cambridge Institute for Medical Research, the crystal structure of GALC was solved by Janet Deane at atomic resolution for the first time in 2011. This at last has allowed the mapping and interpolation of disease-causing mutations onto the GALC structure, and may also assist Dr Joseph Orsini in understanding the effects of some of the complex polymorphisms and haplotype associations that are found in populations screened at the DNA level as part of his neonatal diagnostic work-up in mass population analyses.

**Cell therapies now emerging**

While HSCT based on umbilical cord blood transplantation in the neonatal period for young patients with Krabbe disease has a currently unique place in management, as shown by Dr Escolar, there is no doubt that gene therapy for neurodegenerative disease has an emerging position for clinical application to human diseases. Publication this year by Dr Alessandra Biffi in the San Raffaele Telethon Institute in Milan of successful gene therapy based on transduced autologous HCT reinjected into patients with presymptomatic metachromatic leukodystrophy, a disease closely related to Krabbe disease and with similar devastating effects, gives grounds for real hope in this field. Other groups, particularly in Paris, are shortly to start comparable gene therapy initiatives using rAAV vectors injected directly into the brain, with the same objective of widespread functional correction by harnessing the secretion-recapture phenomenon related to lysosomal proteins.

**Treating widespread disease**

In our own work, using rAAV, expressing GALC in the brains of twitcher mice, we showed strong therapeutic effects which also took account of the peripheral disease, which as Dr Escolar has described, remains a focus for disability in those patients who survive HSCT in the infantile phase of the disease, but whose motor function and development often deteriorate as a result of peripheral neuropathy and spinal-root demyelination. High-resolution imaging was shown to image dorsal root ganglia and the sciatic nerves of untreated mice with Krabbe disease. Combination therapy was shown to prevent the neuro-inflammation and enlargement of the nerves and the cognate ganglia, indicating systemic benefit. We have yet to try a combination of systemic and intracerebral therapy with enzymatic complementation, and await with interest the results of Dr Wenger’s planned experiments using the large animal model of Krabbe disease in the West Highland terrier. All are convinced, however, that the salutary outcomes of gene therapy include improved survival and weight maintenance, as well as motor performance, high induction of enzyme activity, with reduction of neuro-inflammatory changes, restoration of myelin thickness and myelin integrity. This stratagem has yet to be applied to patients - but is an impressive start.

**Regenerative Medicine**

Space will not allow thorough consideration of spectacular developments in regenerative medicine, initiated principally by the work of the two Nobel laureates of 2012: Sir John Gurdon and Shinya Yamanaka, for the discovery that mature cells can be re-programmed to become pluripotent. The application of regenerative medicine to neuroscience has clear therapeutic opportunities: the striking development of a self-organising, three-dimensional brain organoid culture published last month by Lancaster et al from labs in Edinburgh and Cambridge deserve mention. They used a system based on embryonic stem cells and, later, induced pluripotential stem cells ultimately derived from human skin. The complexity, function and even spontaneous development of a primitive eye, as well as the organisation of the different layers and differentiation of the cerebral cortex and other cerebral structures emerging from these
discoveries, has captured the imagination of biological scientists everywhere – it provides unequivocal inspiration for human therapeutic neuroscience.

**Clinical Application**

We have a strong platform of discovery, a new ‘toolkit’ – and the will to do it. So many advances in challenging conditions that were thought way beyond any form of treatment until recently have come about through the combined efforts and strategic thinking of patient-based charities and advocacy groups, in combination with work by scientists and clinicians in academic centres to show what might be done to inspire investment from pharmaceutical companies.

Time and again, an individual approach and non-profit organisations have beaten the path to allow the pharmaceutical incentive to drive through. This first meeting of your charity has been a moving one, setting out an overwhelming case of unmet need for patients and their families afflicted by Krabbe disease. By bringing together disparate clinicians, scientists and other interested parties from different fields, you have done something unique in the UK. All of us see a new focus of unmet need and sense the serious challenge for professional investment.

One hundred years after its first description, Krabbe disease still remains for most an abandoned battleground; but through the new toolkits of biomedical science, innovative reagents and unique opportunities are available for those courageous explorers who cannot leave progress and a better future by stepping aside.

**Conclusion of the Scientific Workshop**

The scientific workshop has allowed for significant engagement, networking and discussion between clinicians and scientists. There was an appreciation of the varying presentations, the securing of new knowledge and the opportunity for more detailed discussion. Positive feedback given in respect of the opportunity to meet as a small group to learn together, to network and to find opportunities for cross collaboration. Delegates would ideally similarly wish to meet in the UK every 2 years.

The diagnosis and treatment of Krabbe disease remains challenging. However it became clear through presentations and discussion that we can possibly utilize some of the research work and findings in the USA to develop some practical information for health professionals in the UK to aid the earlier diagnosis of Krabbe disease. This may not only improve diagnosis but also provide better information and quality of care. Earlier diagnosis would enable palliative care services to be secured much sooner than seems to happen now in the UK.

Scientific discussion has enabled a real opportunity and the possibility of further collaboration on Krabbe research programme between the UK and overseas laboratories. At the meeting Professor Tony Futerman spoke about the work of Einat Vitner and other colleagues at the Weizmann Institute of Science on the brain injury that occurs in severe forms of another lysosomal disorder, Gaucher disease. The process, necroptosis, appeared to be selective for Gaucher but Prof Cox had seen the work earlier and seen a likely connection with Krabbe disease. Preliminary studies confirmed that this seemed to be the case so that the laboratories in Israel and Cambridge have now set up a collaboration to work on this aspect of Krabbe disease. Since several biopharmaceutical companies have active programmes of research into necroptosis as a clinical target for new drugs in other diseases where cell death and inflammation occur, there is a credible chance that this path of work will have relevance (and benefit) in Krabbe. While correction of the genetic (enzyme) defect in Krabbe disease is likely to be fundamental to recovery, there were several groups pursuing gene therapy internationally - including blood stem-cell based approaches - and more was needed. With this in mind, the scientific collaboration offered the chance of developing a therapy for Krabbe disease which would control existing injury to the nervous system and
combine this with gene transfer to correct the underlying defect.

Discussion has created a further opportunity for SBUK to learn more of the diagnostic and screening laboratories, the challenges for screening for LSDs generally including Krabbes disease to enable a greater understanding of what exactly needs to be done in the UK.

A resultant opportunity for us now to look at ways we could better engage with the UK network and there was a proposal of the benefit in having a named contact in every specialist centre.

Media interest in Scotland was significant throughout the scientific workshop, thereby raising awareness of Krabbe disease and the need for further research in order to deliver better diagnosis and improved treatment.
### List of Krabbe Scientific Workshop Attendees

<table>
<thead>
<tr>
<th>Name</th>
<th>Position and Affiliation</th>
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<tr>
<td><strong>Dr Guy Besley</strong></td>
<td>Retired Consultant Clinical Scientist, Willink Biochemical Genetics Unit, Manchester, UK</td>
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<tr>
<td><strong>Dr Annette Bley</strong></td>
<td>Inherited Metabolic Disorders Clinician, University Medical Centre Eppendorf, Hamburg, Germany</td>
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<tr>
<td><strong>Dr Alexander Broomfield</strong></td>
<td>Consultant Metabolic Paediatrician, Great Ormond Street Hospital, London, UK</td>
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<tr>
<td><strong>Mr Derek Burke</strong></td>
<td>Chief Biomedical Scientist, Enzyme Unit, Chemical Pathology, Great Ormond Street Hospital, London, UK</td>
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<td><strong>Dr Begoña Cachón-González</strong></td>
<td>Department of Medicine, Addenbrooke's Hospital, Cambridge, UK</td>
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<td><strong>Dr Anupam Chakrapani</strong></td>
<td>Consultant Paediatric in Inherited Metabolic Disorders, Birmingham Children's Hospital, Birmingham, UK</td>
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<tr>
<td><strong>Dr Heather Church</strong></td>
<td>Clinical Scientist, Willink Biochemical Genetics Unit Regional Genetics Laboratory, St Mary's Hospital, Manchester UK</td>
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<td><strong>Prof. Timothy Cox</strong></td>
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<td><strong>Dr Anupam Chakrapani</strong></td>
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<td><strong>Ms Sarah Dudko</strong></td>
<td>Trustee of Save Babies Through Screening Foundation UK</td>
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<tr>
<td><strong>Dr Maria Escolar</strong></td>
<td>Director: Program for the Study of Neurodevelopment in Rare Disorders Children's Hospital of Pittsburgh of UPMC, USA</td>
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<tr>
<td><strong>Prof. Anthony Futerman</strong></td>
<td>Department of Biological Chemistry, Weizmann Institute of Science, Rehovot, Israel.</td>
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<td><strong>Prof. Simon Heales</strong></td>
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<td><strong>Dr Marie Jackson</strong></td>
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<td><strong>Dr Simon Jones</strong></td>
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<td><strong>Dr John Livingston</strong></td>
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<td><strong>Dr Joseph J Orsini</strong></td>
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<td><strong>Prof. Hugh Perry</strong></td>
<td>Centre for Biological Sciences, University of Southampton, UK.</td>
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<td><strong>Dr Germain Pierre</strong></td>
<td>Paediatric Metabolic Consultant, Bristol Royal Hospital for Children, UK</td>
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<td><strong>Dr Uma Ramaswami</strong></td>
<td>Consultant Metabolic Paediatrician, Royal Free Hospital, London UK</td>
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<td><strong>Prof. Neil Scolding</strong></td>
<td>University of Bristol Institute of Clinical Neurosciences, Department of Neurology, Frenchay Hospital, Bristol, UK</td>
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<td><strong>Ms Louise Simmons</strong></td>
<td>Metabolic Specialist Nurse, Birmingham Children's Hospital, Birmingham, UK.</td>
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<td><strong>Dr Colin Steward</strong></td>
<td>Consultant in BMT, Genetic and Metabolic diseases, Royal Hospital for Children, Bristol and Reader in Stem Cell Transplantation, School of Cellular &amp; Molecular Medicine, Bristol, UK</td>
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<tr>
<td><strong>Dr Marie Vanier</strong></td>
<td>Director of Research (emeritus), INSERM, Lyon, France</td>
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<td>Name</td>
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<tr>
<td>Dr David Wenger</td>
<td>Lysosomal Diseases Testing Laboratory, Department of Neurology, Thomas Jefferson University, Philadelphia, USA</td>
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<tr>
<td>Prof. Bryan Winchester</td>
<td>Emeritus Professor of Biochemistry, Clinical and Molecular Genetics Unit, UCL Institute of Child Health, University College London, UK</td>
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<tr>
<td>Dr Robert (Rob) Wynn</td>
<td>Consultant Paediatric Haematologist, Director, Blood and Marrow Transplant Unit, Royal Manchester Children's Hospital, UK</td>
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