TheNetworkEdge

The NF Network presents A quarterly research review
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***The Network Edge* brings you regular updates on the latest neurofibromatosis (NF) research and clinical advances from recent scientific publications. *The Network Edge* is organized into "bite sized" sections by specific subtopic, so you can focus on the information that interests you most.**

The Network Edge features…

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Highlights from Volume 10 of *The Network Edge*:

- New insights into how Bevacizumab is used to treat NF2 schwannoma and ependymoma tumors
- How a new UK Registry facilitates exploration of the causes and predictors of mortality in NF2
- Impact of migraine and pain on NF1 quality of life
- An analysis of teaching approaches to improve reading skills in children with NF1
- How NF2 schwannomas arise in the brain, and their possible relevance to treatment decisions
- NF2 biology updates: new drug candidates for meningiomas, understanding merlin biology
- The value of early life monitoring for NF1 optic pathway gliomas using MRI

The Network Edge: Volume 10 – Contents

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1. NF2 Clinical Trials

The Bottom Line: MRI imaging may predict persons with NF2-related vestibular schwannomas who are most likely to benefit from Bevacizumab treatment; the more rarely occurring NF2 tumor ependymoma may also benefit from Bevacizumab treatment.

a. Exploring Who Might Respond Best to Bevacizumab for NF2 Schwannoma Treatment

Bevacizumab has been widely utilized as a treatment for NF2 tumors. It acts by inhibiting the growth of new blood vessels within the tumor. Research continues to explore how Bevacizumab actually works. In addition, doctors are trying to detect as early in treatment as possible whether the drug is working and who is more likely to respond better. This is important because it would help in deciding whether to initiate or continue Bevacizumab treatment.

Li, Djoukhadar *et al.* *(United Kingdom)* conducted a trial of Bevacizumab in twelve individuals with a total of twenty NF2-related vestibular schwannomas between them. Participants were imaged prior to drug treatment using a technique called dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI). Drug was given at 5mg/kg every two weeks. After six months, twelve participants were found to have responded to treatment. In cases where tumor volume had stabilized, Bevacizumab dose shifted to 2.5 – 5mg/kg every four weeks.

Following the above treatment, four participants experienced improved hearing and word discrimination. This was attributed to reduction in tumor size, which could alleviate tumor pressure and impact on hearing nerve function. Eight participants showed no response to Bevacizumab, and treatments were discontinued for this group.

The DCE-MRI imaging completed at the start of the study provided information about the size, structure and cellular composition of each participant's tumors, including a view of the blood vessels within the tumor. Inhibiting the growth of these blood vessels is a primary effect of Bevacizumab on the tumor. When combined with the outcome of drug treatment, the DCE-MRI information showed that certain visual biological features were seen in those people who went on to respond to Bevacizumab. "Drug responsive"-associated features include a high level of edema (fluid build-up in the tumor) and

permeable blood vessels in the tumor (these would in part facilitate drug access to the tumor cells). In Bevacizumab-responsive tumors, these features reduced over the course of Bevacizumab treatment.

This fascinating study sheds further light on how Bevacizumab works on tumors. These findings also shed light on who might be most likely to benefit from Bevacizumab to help inform treatment decision making.

b. Bevacizumab's Effects on NF2 Ependymomas

Can Bevacizumab impact the growth of NF2-related ependymomas? These tumors grow in the spinal cord and affect around one-third of all individuals with NF2. Ependymomas are largely benign, but around one-quarter will become symptomatic (i.e. cause clinical problems). They can be treated by surgery or chemotherapy, but are they also good candidates for Bevacizumab treatment?

There has not been a clinical trial to look at this question, but **Farschtschi *et al.*** *(Germany, United States)* reviewed clinical data reporting Bevacizumab responses of symptomatic ependymomas in eight individuals with NF2 treated at three separate medical centers. The drug doses used varied. The data showed that all eight individuals experienced some positive effects after Bevacizumab treatment, with improvement in clinical measures such as pain level and bladder/bowel function. Interestingly, five participants were imaged using MRI, and only four showed visible reduction in tumor volume. This suggests benefits of Bevacizumab on ependymomas go beyond just tumor shrinkage, perhaps affecting the function of the cells in the tumor to alleviate symptoms.

The study also looked at the expression of the growth factor receptors VEGFR-1 and VEGFR-2 in these five imaged tumors. These receptors are involved in part with the growth of blood vessels within the tumor. Four tumors expressed both of these receptors; however, the tumor that did *not* show shrinkage did not express VEGFR-1. This suggests that perhaps VEGFR-1 is important in mediating Bevacizumab's effect on the ependymoma. Continued Bevacizumab treatment was needed to maintain the improvement seen in ependymomas.

Though not strictly a clinical trial, this report from a small case number sample of ependymomas presents interesting findings to inform the treatment of these less-studied tumors of NF2.

1. What's New in NF1 Biology?

The Bottom Line: Mouse models with plexiform neurofibromas respond well to the drug sunitinib malate; *Grb10* and *RunX1* are new potential candidate genes and drug targets in NF1 tumor growth; the genotype-phenotype link (connecting genetic mutations to specific manifestations of NF1) remains a challenge; researchers release updates on the molecular basis of bony abnormalities in NF1; new findings support the role of *NF1* gene in the normal development of the early nervous system.

a. Promising Drug for Controlling Plexiform Tumor Growth in Mice

In the search for treatments that impact the growth of plexiform tumors in NF1, **Ferguson *et al.*** NIH, CDMRP *(United States)* used a mouse model that was genetically engineered to have NF1 plexiform neurofibromas to assess the effects of the drug sunitinib malate on tumor growth. The specific mouse model used is especially helpful for testing drug therapies, as the tumors it grows along the spinal nerve roots are quite representative of human plexiform neurofibromas. The mice were allowed to develop established tumors and then treated with sunitinib malate, or with no drug at all, over a number of weeks. After treatment, the mice that had received the drug showed significantly smaller tumors than those that had received no drug. Positron emission tomography (PET) imaging was used to measure the level of metabolic activity in the tumors, and tumors that had received drug were less metabolically active, which suggests a slowing of tumor growth.

Sunitinib malate targets the cell signaling element Erk 1/2, and in the tumors treated here it seemed to be interfering with the activity of mast cells and fibroblasts around the tumors; these cells are promotors of plexiform tumor initiation and growth. These results suggest sunitinib malate could be promising for further investigation as a clinical treatment for plexiform neurofibromas.

b. New Genes and Potential Drug Targets Implicated in NF1

A recent study examined the role of an imprinted gene, called *Grb10,* in NF1 tumor growth. Imprinted gene biology is rather complex. In brief, only one "active" copy of an imprinted gene is inherited, either from an individual's mother or father. The other copy is rendered inactive. However, if there are complications at the molecular level in the cell, an individual can end up with either two active copies of the imprinted gene, or none at all. Either of these situations can lead to developmental abnormalities.

Grb10 is a signaling protein active along the Ras pathway, with normal functions in pathway regulation. **Mroue *et al*** NIH, FREE *(United States)* showed that in mouse cells and tumors, when the *Nf1* gene was knocked out, *Grb10* signaling disappeared and tumors grew. When *Grb10* function was genetically restored to mouse NF1 tumor cells, the tumor growth slowed. Conversely, when *Grb10* function was eliminated in mouse fibroblasts (a normal healthy cell type), the cells started to grow more rapidly, just as they did when *Grb10* was missing in the mouse cells lacking *Nf1*. This last finding was particularly important because it suggests that loss of *Grb10* loss alone in otherwise healthy cells may be sufficient to promote excessive growth. These findings highlight a role in NF1 tumor growth for *Grb10,* a member of the intriguing family of imprinted genes.

Li, Zhao *et al.* NIH *(United States)* studied genetic information from normal Schwann cells and from NF1 tumor cells using a technique called microarray analysis. This technique can be used to identify genes that are active at different levels between the two cell types, and it identified a gene called *RUNX-1* at high levels in the NF1 tumor cells and lower levels in the normal Schwann cells. The *RUNX-1* gene is involved in the normal development of the nervous system and of blood cells. A piece of human plexiform neurofibroma was examined, and RUNX-1 protein was present at high levels that were not seen in normal nerve tissue.

The investigators then took mouse tumor cells that were lacking normal *Nf1* gene function and grew them in a dish. These cells tended to multiply and form tumors. The cells were then treated with shRNA, a molecular tool that "silenced" or prevented activity of the *RUNX-1* gene in the cells. Following this treatment, the cells reduced their tumor-forming ability both in the dish, and in tumors in mice lacking normal *Nf1* gene function. These findings highlight RUNX-1 in helping to promoting NF1 tumor growth and as a possible future drug target for NF1 tumors.

When the *NF1* gene is not functioning normally, abnormal cell behavior can present in different ways. In the nervous system, it can lead to tumor growth, and in the skeleton, it can cause bony abnormalities. Cells lacking normal *NF1* gene function will show abnormal behavior in terms of adhering to their surroundings and migrating. These features can contribute to the formation of tumors in nerves or abnormal growth in the bones, as seen in individuals with NF1.

To explore this area, **Zhou *et al.*** CDMRP *(United States, China)* used a human cell type called mesenchymal stem/progenitor cells, in which *NF1* gene function has been disrupted. These are precursor cells that will eventually induce problems in - amongst other areas – the bony elements of the body. These cells were found to have increased ability to adhere to, and migrate within, their surroundings when NF1 protein function is deficient. Normal behavior can be restored if cells are treated with drugs that target the cell signals PI3-K and MAPK. This study highlights these specific signaling elements' role in controlling the abnormal cell behavior in the absence of a fully functional *NF1* gene and focuses on these elements as candidate drug targets for treating NF1 bony abnormalities.

In a detailed review article, **Ratner and Miller** NIH, CDMRP *(United States)* examine the evolution of NF1 biology and genetics, charting a timeline from Friedrich von Recklinghausen's initial 1882 report of NF1 as "von Recklinghausen's disease," to the present day, in which NF1 clinical trials are underway. They highlight the fact that genetic testing for NF1 clinical diagnosis is common in today's society. More than 1,400 individual *NF1* gene mutations have been reported, yet there are still no universal patterns linking gene mutations (genotype) to the type of tumors and manifestations that an individual will develop as a result of having NF1 (phenotype). The cell signaling pathways involved in the growth of NF1 tumors get more complex the more we learn about them, but they do at least open more doors to further points of entry within the cell for drug targeting.

Ratner and Miller also explore the crossover role for *NF1* gene mutations in other clinical syndromes. They remind us that *NF1* gene mutations can be identified in 5-10% of cancers in the general population where they seem to play a role in making tumors drug resistant. Overall, the authors of this detailed review remain hopeful about the progress of the field toward developing effective treatments.

c. A Role for the NF1 Gene in the Early Developing Nervous System

A clinical diagnosis of NF1 means that neurofibromin protein is not fully produced or working properly in all of an individual's cells. In the nervous system, this can cause tumors to grow. Different cell types can be affected by this, including both neurons (nerve cells) and glia (non-neuronal cells that perform multiple functions in maintaining the nervous system).

This discovery begs the question since the absence of neurofibromin protein can have such a profound impact, does the *NF1* gene have an important role in the *normal* developing nervous system? **Chen *et al.*** [NIH] *(United States)* explored this by studying neural stem cells, which are representative of the very earliest stages of nervous system development and can give rise to both neurons and glia. Studying neural stem cells in the developing mouse brain and in the dish, researchers show that the *Nf1* gene regulates neural stem cell multiplication through the signaling pathway of PI3K/AKT. When the stem cells turn into neurons and glia (the cells of the mature nervous system), *Nf1* gene regulates this process through signaling pathways MEK/Smad3/Jagged1/Hes1. These findings suggest there are indeed some key roles for *NF1* gene in normal development of the early nervous system.

2. What's New in NF2 Biology?

The Bottom Line: A large-scale screening study identifies new candidate drug targets for NF2 meningiomas that may also benefit meningiomas in the general population; in mice, a role is discovered for merlin protein in sperm maturation; researchers gain new understanding of how NF2 protein merlin functions in the cell.

a. Seeking New Drug Targets for Meningiomas

Meningiomas are the most common brain tumor in the general population and they are also common in people with NF2. Though largely benign, meningiomas can be life threatening.

Beauchamp *et al.* [NIH, CDMRP, FREE] *(United States)* focus on identifying drug therapies that will halt growth of meningioma cells. They conducted a high-throughput screen of drugs that target a group of cell signals called kinases to determine their effect on growth of meningioma cells in a dish. The drugs were designed to act by "silencing" synthesis of the kinases in the cell, through an approach called shRNA. Through this screen, a number of candidate drugs and drug targets for slowing meningioma cell growth were identified, including drugs to target cell signals AKT, SGK1, NDRG1 and PAK1.

The investigators further explored these signaling pathways by developing a second cell line also representative of meningiomas through genetic manipulation of brain arachnoid cells (the normal cells from which meningiomas arise). The most promising candidate treatment found for meningiomas was the mTORC1/mTORC2 inhibitor, AZD2014.

This study advances our understanding of meningiomas and potential ways to treat these challenging NF2 tumors. It is worth noting that in meningiomas in the general population, sixty percent are associated with mutations in the *NF2* gene. This means that research in this area could benefit the general population as well as people with NF2.

b. New Insights into Merlin Protein Roles and Biology

The *NF2* gene makes a protein called merlin; there are actually two different forms of this, called merlin isoforms. The purpose of these isoforms is not well understood. In an intriguing biological study,

Zoch *et al.* FREE *(Germany, United States)* developed mouse models lacking one or the other of the merlin isoforms in order to measure the impact. These mice did not form tumors, suggesting that when one merlin isoform is absent, the remaining active isoform compensates.

However, when one of the merlin isoforms is missing, spermatogenesis - the development of mature sperm – is not completed in the mice. Spermatogenesis seems to be dependent on the presence of the two fully functioning merlin isoforms, as neither isoform can compensate alone. Testicular atrophy is seen in aging mice lacking one of the isoforms, and there was a negative impact on reproduction and litter size when either of the isoforms was missing.

Further exploration suggested that the two merlin isoforms actually have different roles at different times in regard to development of the testes and sperm but that both isoforms are vital for normal sperm development. It is not yet clear whether this has bearing on humans with NF2, but the finding sheds further light on the important roles of the *Nf2* gene in normal development.

In a high-resolution crystal structure study of proteins, **Li, Zhou *et al.*** FREE *(China)* explored a cell signaling pathway called Hippo, which interacts with the NF2 protein merlin (it is not clear how). The investigators showed that merlin protein has a complex system of self-regulation to prevent the activation of the Hippo pathway. A molecule called angiomotin can bind to merlin and permit Hippo activation. This interaction is weakened when merlin is altered through a process called phosphorylation, a normal cell function that - in the healthy cell - regulates the activation of these pathways. In people with NF2, this signaling system is disrupted, which contributes to tumor cell growth. This study further unravels the fine details of the molecular structure of NF2 tumors.

3. NF Genetics Update

The Bottom Line: A new study focuses on individuals with NF1 who experience a genetic microdeletion; researchers attempt to understand genomic rearrangements in the *NF1* gene; muscle metabolism and function are directly impacted by the *NF1* gene mutation.

a. Largest Study of NF1 Microdeletion Cases

Around five percent of individuals with an NF1 diagnosis will experience a type of mutation in the *NF1* gene called a microdeletion. Individuals with an NF1 microdeletion are at increased risk of plexiform and cutaneous tumors, malignant peripheral nerve sheath tumors, and intellectual challenges. Though most people with NF1 tend to be smaller than their peers, an *NF1* gene microdeletion carries a risk of somatic overgrowth.

Ning *et al.* *(Canada, Germany, United States)* studied fifty-six individuals with NF1 and microdeletions and compared them to a larger group of two hundred and twenty-six individuals with NF1 and other types of gene mutations. Those with a microdeletion tended to be taller and heavier than the non-microdeletion group. The difference in height is seen as early as two years old but as

babies, all of the children with NF1 were the same length. This is the largest study conducted to date on microdeletion cases.

b. Exploring the Molecular Details of NF1 Gene Mutations

In a very complex genetics study, **Hsiao *et al.*** *(Austria, Belgium, Poland, United States)* explored the events that lead to genomic rearrangement, gene mutations, and – ultimately - to a diagnosis of NF1. The group scanned the genomes of eighty-five people with copy number variations in the *NF1* gene, to find their key genetic break points. Copy number variations can be quite diverse in the way they present, so this was a challenging undertaking. Nevertheless, the investigators determined that two specific events were involved in these individuals' genomic rearrangements: fork stalling and template switching (FoSTeS), and microhomology-mediated break-induced replication (MMBIR). In addition, the investigators identified a series of "hotspots" in the *NF1* gene that were likely targets for rearrangement and mutation.

4. Altered Brain Function in NF1

The Bottom Line: Unique signaling features seen in the NF1 "resting brain" may reflect intelligence.

a. Altered Signaling in the "Resting" NF1 Brain: A Link to IQ

It is well established that brain function and structure can be affected in people with NF1: learning difficulties of varying degrees are common, and enlarged brain size is a potential feature of NF1. These brain manifestations have been studied and attributed in part to overgrowth of brain cells, in a similar manner to the cell overgrowth in NF1 benign tumors. Like NF1 tumors, these brain features seem to be due to dysregulation of the RAS signaling pathway.

To further explore the functional impact of these brain differences in NF1, **Tomson *et al.*** [NIH, CDMRP] *(United States)* looked at the "resting brain" function and brain architecture of thirty adult men with NF1, of average age around twenty-eight, and compared them to thirty adult men without NF1, using the technique of functional magnetic resonance imaging (fMRI). The study found that the NF1 group had reduced connectivity along the anterior-posterior brain axis as well as other differences, including the way brain signaling organizes into modules. The extent of some of the differences in brain signaling seen in people with NF1 correlated with intelligence quotient (IQ) score.

The investigators speculated that the cellular basis of the connectivity differences in NF1 may be due to weaknesses in the glial cells that myelinate (insulate) the nerve fiber pathways in the brain. This study is the first to show there may be features unique to the resting brain function in individuals with NF1.

5. Social Challenges and Quality of Life in Neurofibromatosis

The Bottom Line: Pain can be a quality of life issue for children and teens with NF1; migraine is an often-overlooked complication of NF1 that requires attention from clinical caregivers; no link has been discovered between motor skills and language ability in NF1; the field of muscle function in NF1 is reviewed; quality of life in NF1 is explored as it affects individuals in Brazil.

a. Impact of Pain on Life Quality in Young People With NF1

Pain can be a significant consequence of living with NF1, depending on the location and size of tumors. **Wolters *et al.*** [NIH] *(United States)* surveyed a group of fifty-nine children and adolescents aged six to eighteen, all with NF1 and a diagnosis of plexiform neurofibroma. Caregivers of all fifty-nine participants completed a survey, along with forty-one of the young people (those over age ten).

The results were telling. Fifty-nine percent of the young people and seventy-three percent of their caregivers reported that NF1 pain interferes with daily life. Pain was especially common when the young people experienced large tumor volume and expressed symptoms of anxiety. The young people highlighted social stress as impacting their overall quality of life. The detrimental effect of pain was felt even when medication was utilized. In fact, to some extent medication had a negative effect: one-third of the young people reported using pain medication on a regular basis, which correlated with a lower quality of life, including symptoms of depression.

This important study highlights the significant impact that pain can have on the lives of young people with NF1 and the fact that pain can serve as an entry point into regular use of pain medication. This highlights the need for better awareness and understanding of the likelihood of pain in NF1 and how to better prevent and manage it.

b. Assessing the Impact of Migraine in NF1

Migraine and headache in NF1 can be a serious issue, but the link is not well understood. **Afridi *et al.*** *(United Kingdom)* noticed a high frequency of headache amongst their NF1 cases and wanted to find out more about the impact of these headaches on quality of life. This led to a survey of their NF1 cases on the subject of migraine and headache.

One hundred and fifteen individuals with NF1 aged sixteen to sixty-seven participated in surveys on the duration, severity and impact of their headaches. Responses varied: while twenty-five reported no headache at all, seventy-five participants experienced headaches that met the criteria for migraine, which had a significant impact on quality of life. Nine participants reported experiencing chronic daily migraine, and four of these individuals had brain abnormalities identified on MRI brain scanning.

Interestingly, the investigators said that the majority of their NF1 participants did not mention headache or migraine during regular clinical consultations, unless specifically asked about this topic. Speculating about the biological cause of headaches and migraines in NF1, the investigators note that mitochondria, which are the “energy source” of a cell, have been implicated in migraine. NF1 protein

neurofibromin actually has a role in normal mitochondria function; thus, it may be the case that lack of functional neurofibromin is detrimental to mitochondria function and contributes to headaches and migraine.

This study highlights an understudied area of NF1 and emphasizes the importance of regularly monitoring and assessing people with NF1 for headache and migraine and providing appropriate treatment, potentially including scanning for brain abnormalities when warranted.

c. No Link between Motor Skills and Language Ability in NF1

Iannuzzi *et al.* *(France)* explore the potential link between cognitive (learning) and motor (muscular) disorders in NF. They studied a group of forty-nine children with NF1 ages five to twelve and a comparison group of forty-nine children without NF1 but with a diagnosed "specific language disorder." The children with NF1 had more frequent and more severe motor impairment, particularly in the area of balance; however, their skills in manual dexterity were comparable to the non-NF1 group. In addition, there was no relationship between the level of motor skills and the level of language abilities in the NF1 group. The investigators speculated that this could mean that different brain regions are involved in these functions in children with NF1 and with the specific language disorder. More work remains to be done in this area.

d. More Insights into the Biology of NF1 Muscle Weakness in NF1

In recent years, a growing area of focus in NF1 research has been identifying and understanding the basis of muscular weakness. Much of this work has come from Australian NF research and clinical groups, and **Summers *et al.*** CDMRP, FREE *(Australia)* have published a thorough review of this topic. NF1 muscle deficits were initially thought to be due to an indirect impact of other NF1 manifestations such as tumor location. However, it has been demonstrated that the *NF1* gene activity in the muscle cells is affected in people with NF1, with a direct impact on muscle metabolism and function. Clinical studies have explored measures such as gait or level of interaction in physical activities in children with NF1. Biological studies have included the development of mouse models exhibiting a representation of the muscular deficits seen in people with NF1. This is a very useful and FREE review of this growing area of study in which much remains to be understood, including the nature of the interplay between muscular and nervous systems.

e. Impact of NF1 on Quality of Life: a Perspective from Brazil

Batista *et al.* FREE *(Brazil)* present a broad review of NF clinical management in Brazil, where NF affects an estimated eighty thousand people. The publication touches on a number of areas related to quality of life, including some less-frequently-discussed NF1 manifestations such as precocious puberty, seizures, and nutrition. From the perspective of social challenges, the paper highlights the fact that communication problems may be a concern not just in NF2 where hearing may be lost, but also in NF1

due to motor or language development issues. Another section highlights the impact of NF1 on psychosocial issues such as shyness, employability and personal relationships. Pain is also reviewed, with a detailed consideration of the types of pain that can occur in NF1.

One particularly helpful aspect of this review is that it lays out a number of "decision trees" and tables to clarify various discussion points. This comprehensive and FREE review of clinical management considerations in NF is well worth a look and focuses on many quality of life issues.

6. NF1 and the Eye: Optic Pathway Glioma and Other Features

The Bottom Line: Early life regular MRI screening may be valuable in prompt detection of OPGs.

a. Utility of MRI for Monitoring and Early Detection of OPGs

Prada *et al.* *(United States)* present a retrospective study of data captured over a twenty-year period from eight hundred and twenty-six children with NF1 ages one to nine. Subjects were monitored for the appearance of optic pathway gliomas (OPGs) using brain/orbital magnetic resonance imaging (MRI). Children diagnosed with NF1 were first scanned either at fifteen months or at the time of NF1 diagnosis if this came later. Annual MRIs followed unless an OPG was detected, in which case the children were imaged every three to six months. This screening was done in the context of a full-service NF clinic with the children receiving thorough clinical management including additional ophthalmological exams for visual acuity, etc.

Eighteen percent of the children were identified as having OPGs, with a median age of diagnosis of three years. Of those diagnosed with OPGs, fifteen percent progressed and required treatment (chemotherapy or surgery). The location of the tumor along the optic pathway was important: children with chiasmatic and post-chiasmatic tumors were at greater risk of progression than children with pre-chiasmatic tumors. In cases where children had already experienced visual decline at the time of OPG diagnosis, they were more likely to continue losing vision. However, when tumors were identified by MRI but there was no visual loss, the outcome was more hopeful.

Overall, this significant body of work highlights the usefulness of MRI in early detection of and timely treatment of OPGs.

7. Pheochromocytoma in NF1

The Bottom Line: NF1 signaling pathway elements as candidate drug targets for pheochromocytoma; pheochromocytoma tumors can present clinically in very different ways.

Pheochromocytomas are neuroendocrine tumors derived from the chromaffin tissue of the adrenal gland. Diagnosis of these tumors is important as they are linked to hypertension syndrome,

causing increased cardiovascular morbidity and mortality. These tumors occur in about five percent of NF1 cases, most prominently in the fourth decade of life. Largely benign, about ten percent of these tumors will become malignant. Pheochromocytomas are particularly associated with a few genetic disorders, including NF1. In the last few months a few papers have been published that examine and review this area of research. These are all FREE papers and well worth a look for those interested in these tumors.

a. New Insights into Pheochromocytoma Biology

Lam FREE *(Australia)* provides the most detailed review of pheochromocytoma biology and genetics. An important promotor of growth in these tumors is VEGF, the drug target of Bevacizumab a drug now fairly widely used for treatment of NF tumors. However like other NF1 tumors, pheochromocytoma growth is also driven by the familiar molecular elements (MEK, ERK, etc.) of the Ras signaling pathway which therefore also represent candidate drug targets for these tumors.

b. Case Reports Highlighting Diversity of Pheochromocytoma Presentation

Teasdale and Reda FREE *(Australia)* report on two different cases of NF1-related pheochromocytoma. The first tumor was found in a fifty-three-year-old man with inherited NF1 presenting with upper body discomfort. He had high adrenaline, rapid heart rate, and high blood pressure.

The second tumor was found in a twenty-nine-year-old woman also with inherited NF1 presenting with headache, palpitations and chest pain as well as upper body discomfort. She also had high adrenaline and rapid heart rate.

Both subjects had very different levels of noradrenaline, which was pinpointed as a reason for their different presentations, since the level of hormone will have different effects on blood pressure and other bodily responses. Both subjects were examined by computed tomography (CT) imaging of the abdomen and found to have adrenal tumor masses. Both had large tumors, which increases the likelihood of malignancy. There are no definitive markers for predicting or detecting malignancy in these tumors; therefore, both cases underwent surgery for tumor removal. The subjects received very different drug regimes to control their symptoms.

This study highlighted the fact that pheochromocytomas can present in different ways with varying characteristics and that there is still a need for better biological tools to monitor them.

c. Pheochromocytoma: A Historical Perspective

For further reading, a couple of papers provide a review of the pheochromocytoma field from different perspectives. **Costa *et al.*** FREE *(Brazil)* put pheochromoctyomas further into context genetically and biologically. Finally, **Else** FREE *(United States)* provides a historical perspective on pheochromocytomas, dating back to their initial description one hundred and fifty years ago.

8. NF1 Learning Disabilities

The Bottom Line: Comparing interventions for teaching reading to children with NF1 suggests more benefit from hands-on approaches; researchers explore new understanding of cell biology in NF1 learning disabilities.

a. Comparing Approaches for Teaching Reading to Children With NF1

Learning to read is a key life skill but can be a challenge for children with NF1 who have learning disabilities. However, it is not well reported whether children with NF1 and reading deficits benefit from remedial programs designed for the general population. This begs the question if they do not respond well to current programs, what kind of additional teaching support would be helpful to them? This issue is addressed by **Barquero *et al.*** [NIH] *(United States)* in a study of forty-nine children, half of whom have NF1 and reading deficits, and half of whom do not have NF1 but do have reading deficits. The children were randomly placed in one of two teaching groups. In one group, the children received the standard remedial treatment for learning deficits, which involved a focus on visuospatial interaction. For example, this might include being able to pick out word repetition patterns on a page. In the second group, more kinesthetic (hands-on) activities were used. For example, this might include using tiles with words on them as a teaching tool. The children from the general population responded equally well to both teaching methods; however, the children with NF1 particularly benefited from the "hands-on" treatment. This fits with the current understanding that children with NF1 can have deficits in visuospatial function, and opens up new approaches that may benefit this group when faced with reading difficulties.

b. Mouse Study Implications for Neuronal Excitability in NF1 Learning Disabilities

The effects of the *NF1* gene are far-reaching, and therefore when the gene is mutated as it is in people with NF1, it can affect many biological activities. **Wang *et al.*** *(The Netherlands)* report on the effects of gene mutation on synaptic transmission, which is one of the ways that brain cells "talk" to each other and share information. The investigators studied this in the brain of a mouse with just one functional copy of the *Nf1* gene *(Nf1+/-).* These mice are already known from many previous studies to have learning disabilities, and the investigators found that neurons (nerve cells) in the brains of these mice have increased excitability, which could contribute to learning or behavioral differences.

9. NF1 Clinical Management

The Bottom Line: Ensuring that people with NF1 receive lifelong care remains a challenge.

a. Exploring the Challenge of Receiving Lifetime Care for NF1

As NF1 is a chronic lifelong condition whose manifestations may change with age, it is critical that anyone with NF1 receives ongoing clinical monitoring. For children with NF1 this is fairly simple, as the child is under the care of the parent and more likely to go to the doctor for routine check-ups. However, for adults with NF1, more responsibility is placed on the individual to take care of himself and ensure that he undergoes check-ups to detect any changes in tumor size, etc. that may occur. Unfortunately, many individuals neglect their personal care. **Crawford *et al.*** *(Australia, United Kingdom)* set out to better understand the compliance of adults with NF1 in seeking clinical monitoring. The group completed a written survey/interview with ninety-four Australian adults with NF1 ages eighteen to forty.

The results showed that around half of participants did not seek regular monitoring. Top reasons given included not knowing where to seek health care, not knowing that regular clinical monitoring was necessary, feeling healthy and deeming clinical check-ups unnecessary, and feeling that monitoring was futile as nothing can be done for NF1 anyway. Further investigation revealed that many individuals were not even sure where to go for NF1 care; others were seeing either a general practitioner or still seeing the pediatrician who had managed their childhood NF1. This group covered a broad demographic in terms of NF1 severity and education level. Sixty-four percent were women and thirty-six percent men. However, responses did not correspond to any demographic subgroup. These findings suggest overall poor understanding among this NF1 population about how, where, and why to find NF1 clinical care; this is a priority to be addressed.

10. NF2 Clinical Management

The Bottom Line: A large UK Registry explores factors that might predict mortality in NF2 and identifies some potential indicators; a new study suggests NF2 vestibular schwannomas may arise from multiple sites in the brain, informing the diagnostic and treatment process; molecular markers may predict a schwannoma's size.

a. Largest Population Study to Date Identifying Predictors of Mortality in NF2

The United Kingdom has a National NF2 Registry that was established in 1990 and records all persons with NF2 receiving clinical care in the United Kingdom. This Registry allows for long-term collection of information from its participants. Facilitated by the UK's national healthcare system, by

2014, ninety-seven percent of people with NF2 in the United Kingdom were under the management of a NF2 Specialty Center, which meant they were included in the Registry. **Hexter *et al.*** (Canada, United Kingdom) utilized the Registry to determine whether there are either clinical features or molecular markers that can predict early death in people with NF2. This study was estimated as being three times larger than any other study of NF2 mortality completed to date.

The Registry has included one thousand, one hundred and ninety-two persons. Impressively, this study included a tracking of almost eleven thousand life years in total. Over the time of the Registry's existence to date (1990-2015), two hundred and forty-one individuals have died. All have been found to have NF2 as the definitive, probable, or possible cause of death, as determined by a search of death certificates.

In terms of clinical predictors of death, the two most prominent were: 1) younger age at diagnosis and 2) the presence of intracranial meningiomas. Sex (male/female) was not a predictor. Date of NF2 diagnosis was a predictor, with a decrease in mortality seen in more recent times, probably due to more individuals being consulted in Specialty Centers and diagnosed earlier with more possible treatment options. Certain types of *NF2* gene mutations predict higher mortality: nonsense or frame-shift. Missense mutations have lower mortality risk. A specific type of gene mutation called splice-site have higher mortality when in certain regions of the gene. Interestingly, the group found that when splice-site mutations are inherited within a family, causing NF2, family members can develop very different physical characteristics; the reason for this variability is unknown. Frameshift mutations seem to have a lower risk of mortality.

As this impressive Registry grows, it is anticipated that it will continue to yield very valuable information about the causes and hallmarks of NF2 and its progression.

b. New Insight into Brain Origins of NF2 Schwannomas

Stivaros *et al.* FREE *(United Kingdom, United States)* explore the question of which cellular regions in the brain NF2 vestibular schwannoma tumors actually originate from. Traditionally the tumors have been considered to originate from a discrete region of the vestibular nerve. Forty-one children and young individuals ages one to nineteen who had a diagnosis of NF2 were studied by magnetic resonance imaging (MRI) to determine where in the brain tumors were emerging. On imaging, thirty-four of the participants had some measurable disease (tumors). The study found that tumors could emerge from different regions of the vestibular nerve as well as on the adjacent cochlear nerve in various regions within the ear labyrinth. In some cases, tumors arise in more than one point on the nerve at the same time. This fits with the fact that early tumors can be described as a "bunch of grapes" appearance; these are likely multiple small tumors that eventually combine to form one big tumor. The investigators suggest too that when small tumors arise from multiple sites on the vestibular nerve, they are more likely to result in aggressive tumors.

This important study presents for the first time evidence that vestibular schwannomas can arise from multiple nerve sites and in different ways, and that the way in which they arise may be related to the severity of the tumor and its response to drug treatment.

c. Potential Molecular Predictors of Schwannoma Tumor Size

What causes NF2 vestibular schwannomas to grow to specific sizes, some bigger than others? A new report from **Mehrian-Shai *et al.*** *(Australia, Israel, United States)* looked at this question from a molecular perspective. The group assessed human tissue samples from twenty-seven large schwannomas and fifteen small schwannomas. These were screened against a microarray, a tool that allowed researchers to check for the presence of any genes that were expressed differently in large and small tumors. Eleven genes were identified to be expressed at variable levels depending on the sizes of the tumors. These were genes *GPX3*, *RPS19*, *CSRP1*, *PRSS2*, *PKP1*, *FRMD1*, *SFRS3*, *ELAVL4*, *NEFH*, *STMN2* and *SH3GL.* For example, *GPX3*, *RPS19* and CSRP1 expressed at higher levels in large schwannomas. These genes are known to be involved in tumor growth in cancers. Meanwhile, genes *FRMD1*, *ELAVL4, NEFH, PKP1* and *STMN* - which in the normal nervous system serve important roles in nerve cell function - were associated with small tumors, with more normal cell behavior. Though a small study, this investigation sheds light on the idea that there may be molecular differences between tumors destined to grow to different sizes. This information could be helpful in determining treatment strategies.

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