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X-LINKED

Relationship of X-Linked Retardation to Fragile X

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TITLE: THE CELL BIOLOGY OF NON-SPECIFIC MENTAL RETARDATION AND FRAGILE X

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ABSTRACT:

Mental retardation (MR) is a developmental disability which is highly heterogeneous in terms of underlying causes and manifestations. Common to all these syndromic and non-syndromic forms of mental retardation are the limited cognitive abilities and the onset early in life. In spite of more than three decades of scientific research, we still do not know the neurobiological mechanisms responsible for the cognitive deficits in MR. Neuropathological studies in the 1970's revealed that various forms of MR are associated with abnormalities in the complexity of dendrites and in the density and shape of dendritic spines. These nerve cell structures are prominently involved in neuronal information processing and communication. Thus, in many cases MR may result from abnormal development of the connectivity of the brain or a limited ability of brain circuitry to adapt to environmental demands. As a result, human information processing is limited.

Recent clinical genetic studies have produced important clues about the cellular mechanisms that may lead to the abnormal connectivity underlying MR. In the case of non-specific MR, it turned out that three of the eight genes, which cause MR when mutated, are directly involved in cellular signaling through Rho GTPases. These Rho GTPases are key molecular switches, which integrate the numerous signals that neurons receive during development, into coordinated actions of the cytoskeleton necessary for directed outgrowth and connectivity. Understanding the cellular mechanisms by which Rho GTPases mediate the development and adaptation of nervous system connectivity may provide the key to understanding the mechanisms underlying MR. In the long run, this understanding may lead to the development of pharmacological means to ameliorate the cognitive deficits in MR. These mechanisms may also be relevant to fragileX as a connection between fraX and Rho GTPases has recently been established.

Roundtable Panel: X-Linked Mental Impairment

Chairmen: *Vincent des Portes, MD and Roger Stevenson, MD*

Speakers: *Giovanni Neri, MD, Silvia Russo, PhD,*

Vincent des Portes, MD, Roger Stevenson, MD, Ger Ramakers Ph.D.

Summary written by Vincent des Portes, July 24, 2002.

In addition to the fragile X syndrome, a lot of new genes involved in X-linked Mental retardation have been discovered recently. Up-to-date clinical and genetic data related to these new neurogenetic conditions were discussed. Especially, issues concerning clinical approach to diagnosis were emphasized, during an informal and interactive "round table".

Giovanni Neri, MD drew a global picture of the X-Linked Mental retardation genes and showed an updated classification of XLMR conditions.

“X-linked mental retardation (XLMR) is a specific type of mental retardation due to mutant genes located on the X chromosome. It is expressed in hemizygous males more than in heterozygous females and it contributes to the 25% excess of males among the mentally retarded. Its prevalence in the general population has been estimated at 1.8/1,000 males, with a carrier frequency of 2.4/1,000 females. The most common and best known among XLMR syndromes is the fragile X syndrome. However, many other syndromes are known, their number exceeding 130 at a recent count. Each one of them poses diagnostic and counseling problems, given that less than 30 genes have been identified so far as responsible for these conditions. Additionally, there exist more than 70 families with so called non-specific XLMR (MRX), each one potentially identifying a new gene. Only 10 of these MRX genes have been cloned so far, contributing powerfully to understanding the molecular mechanisms that are responsible for the malfunctioning of the central nervous system cells and that ultimately cause the appearance of mental retardation.”

Giovanni Neri pointed out three recent data: cloning of the gene involved in the Borjeson-Fossman-Lehman syndrome, XNP gene deletion identified in a Chudley-Lowry family, and a mutation found in a family affected with MR+psychosis+spastic paraplegia.

Silvia Russo presented a linkage analysis of four unrelated families affected with Mental Retardation in males. This presentation was a good illustration of the strategy and pitfalls encountered in gene mapping and search for a mutation in candidate genes.

The most important work concerned the family MRX72, whose gene was located in Xq28. Ten genes were screened for mutation, but no discrete mutation could be exhibited so far.

Vincent des Portes' first presentation focused on clinical data available for a practical approach to XLMR.

“Only a few families share a mutation in one of the twelve new XLMR genes already discovered, and only little clinical data are available to date. Most of the genes involved in so called “non-specific” MR are also responsible for syndromic conditions, demonstrating a continuum between “syndromic” and “non specific” mental retardation. Nevertheless, in some published families, males bearing a mutation still seem to be affected with pure MR, without any striking clinical hallmarks. For some of those families, thorough clinical assessments were undertaken to try to point out some specific features leading to the screening of one or another gene. No comprehensive “flow chart” is available so far, but several relevant clinical, imaging,

and biological features have already been identified and may prove useful for the clinician. For instance, natural history of early development and cognitive level is informative: hypotonia in infancy is usually associated with *OPHN-1* and *SLC6A8* (Creatin Transporter) mutations. A short and transitory period of regression may be noticed in *MECP2* affected males. Mutations in most of the *MRX* genes lead to a moderate to severe MR in males, except for *MECP2* and *FMR2*, which are involved in mild MR. Psychiatric features can be prominent, like atypical schizophrenia with *MECP2*. Autism has been described sporadically in many families and is not specific to any gene. Carrier females with mutation in *rab-GDI*, *FACL4* or *SCL6A8* may show mild cognitive impairment. Neurologic features are also good landmarks: spasticity has been described in patients with *MECP2* mutation; dystonia is observed in families with mutation in *ARX* and *SCL6A8*; infantile spasms, partial motor seizures, and undefined seizure, may occur in children with a mutation in *ARX*, *OPHN-1*, and *SCL6A8*, respectively. Bilateral strabismus is a constant feature in *OPHN-1* mutated patients. New imaging techniques are also powerful: a striking vermian hypoplasia with partial agenesis of lobules VI and VII was seen in two revisited *OPHN-1* families; creatine deficiency due to mutation in *SLC6A8* is exhibited on brain proton magnetic-resonance spectroscopy. Finally, a complete skewed X-inactivation pattern in obligate carrier females is always associated with mutations in *ARHGEF6* and *FACL4*, as it has been described previously in *XNP* mutations (*ATR-X* syndrome).”

Roger Stevenson talked about the clinical aspects of three *XLMR* genes. The first one, *Rsk2*, which is commonly involved in Coffin-Lowry syndrome, has also been demonstrated as responsible for non-specific forms of MR. In these cases, mutations did not involve the Kinase domain. The two other genes were recently identified as responsible for MR. Mutations in *SLC6A8* gene, that encodes for the Creatin Transporter, lead to a progressive cognitive impairment with IQ worsening in elder patients. Recently, *AGTR2* (Angiotensin II-Receptor type 2) has been demonstrated as involved in non-syndromic MR, and seems to be a frequently mutated gene.

Vincent des Portes showed new clinical and radiological data exhibited in two unrelated families with a known mutation in the *Oligophrenin-1* gene. Clinical data shared by affected individuals were neonatal hypotonia with motor delay but no obvious ataxia, early onset complex partial seizures, marked strabismus and moderate to severe mental retardation. Detailed neuropsychological assessment in two individuals showed a deficit in short-term memory and motor coordination with a striking slowness for execution of sequential movements. Brain MRIs performed in three individuals exhibited a unique vermian dysgenesis including an incomplete sulcation of anterior and posterior vermis with the most prominent defect in lobules VI and VII (partial agenesis of declive, folium and tuber). A mild dysgenesis was observed in the cerebellar hemispheres. The brainstem was normal. A non specific cortico-subcortical atrophy was seen with mild dilatation of lateral ventricles and subarachnoidal spaces. Clinical and radiological features observed in *OPHN-1* mutated subjects can be considered a clinico-radiological syndrome, useful for a practical clinical approach to mental retardation diagnosis. In addition, *OPHN-1* inactivation should be considered a relevant model of developmental vermis disorganisation, leading to a better understanding of the role of cerebellum and cortico-cerebello-cortical loops in non-specific mental retardation.

Ger Ramakers, as a scientist working on the pathophysiology of MR, emphasized the importance of investigating human brain tissue. He called both scientists and parents to help make post-mortem brain tissue available so we can establish in a better way than before what is wrong in the brains of MR people. This would not be limited to non-specific MR, but also include syndromic MR.

During this session, the need of more multisite studies and standardized clinical assessment was pointed out, in order to improve the clinical diagnosis to mental retardation of unknown etiology.

TITLE: OPHN-1 (Oligophrenin-1) GENE MUTATION LEADS TO A SPECIFIC CEREBELLAR DYSGENESIS, INCLUDING A PARTIAL AGENESIS OF VERMIAN LOBULES VI AND VII

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ABSTRACT:

In order to find a specific clinical profile for mutation in OPHN-1 (Oligophrenin 1) gene, clinical studies including physical examination, cognitive assessment, 3D brain MRI, and positron emission tomography (PET) were performed in three individuals from two unrelated families with a known mutation in OPHN-1 (Billuart et al., 1998): one 18-year-old female with an X; 12 balanced translocation encompassing OPHN-1, and two males (18 and 34 years old) from the same family (MRX 60) with a frameshift mutation in OPHN-1.

Clinical data shared by affected individuals, including the t(x;12) female, were neonatal hypotonia with motor delay, mild partial complex seizures and oculomotor impairment with strabismus and abnormal visually guided saccades without oculomotor apraxia. Detailed neuropsychological assessment showed mild to moderate mental retardation, a deficit in verbal and visuo-spatial short-term memory, in learning capacities, and in motor coordination tasks with a striking slowness for execution of sequential movements and right hand intention tremor. Brain MRIs performed in the three individuals exhibit an original and strikingly identical vermian dysgenesis including an incomplete sulcation of both anterior and posterior vermis and partial agenesis of lobules VI and VII (declive, folium and tuber). A mild dysgenesis was observed in the cerebellar hemispheres. Brainstem seemed normal. A non specific cortico-subcortical atrophy was seen with mild dilatation of lateral ventricles and subarachnoidal spaces. PET images were analysed with the statistical parametric mapping (SPM) software and compared to 10 normal controls, showing a significant hypoperfusion in the right temporal cortex, in the right cerebellum and in the right striatum.

Clinical and radiological features shown in OPHN-1 mutated subjects are discussed and compared to Joubert syndrome and other cerebellar dysplasia. In addition to cerebellar abnormalities, cerebral cortical structures are likely to be involved in some of the clinical features described in these three subjects. OPHN-1 inactivation should be considered as a relevant model of developmental vermian impairment, leading to a better understanding of the role of cerebellum and cerebellar-cortical pathways in cognition.

TITLE: NON SPECIFIC X-LINKED MENTAL RETARDATION (MRX):
IS A CLINICAL APPROACH TO DIAGNOSIS AVAILABLE YET?

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ABSTRACT:

The discovering of new MRX genes is a breakthrough that had lead to new understanding of the aetiology of some unexplained familial cases of mental retardation (MR). However, only a few families share a mutation in one of the twelve new genes already discovered, and only little clinical data are available to date. Most of the genes involved in so called “non-specific” MR are also responsible for syndromic conditions, demonstrating a continuum between “syndromic” and “non specific” mental retardation. Nevertheless, in some published families, males bearing a mutation still seem to be affected with pure MR, without any striking clinical hallmarks. For some of those families, thorough clinical assessments were undertaken to try to point out some specific features leading to the screening of one or another gene. No comprehensive “flow chart” is available so far, but several relevant clinical, imaging, and biological features have already been identified and may prove useful for the clinician.

For instance, natural history of early development and cognitive level is informative: hypotonia in infancy is usually associated with OPHN-1 and SLC6A8 (Creatin Transporter) mutations. A short and transitory period of regression may be noticed in MECP2 affected males. Mutations in most of the MRX genes lead to a moderate to severe MR in males, except for MECP2 and FMR2, which are involved in mild MR. Psychiatric features can be prominent, like atypical schizophrenia with MECP2. Autism has been described sporadically in many families and is not specific to any gene. Carrier females with mutation in rab-GDI, FAFL4 or SCL6A8 may show mild cognitive impairment. Neurologic features are also good landmarks: spasticity has been described in patients with MECP2 mutation; dystonia is observed in families with mutation in ARX and SCL6A8; infantile spasms, partial motor seizures, and undefined seizure, may occur in children with a mutation in ARX, OPHN-1, and SCL6A8, respectively. Bilateral strabismus is a constant feature in OPHN-1 mutated patients. New imaging techniques are also powerful: a striking vermian hypoplasia with partial agenesis of lobules VI and VII was seen in two revisited OPHN-1 families; creatine deficiency due to mutation in SLC6A8 is exhibited on brain proton magnetic-resonance spectroscopy. Finally, a complete skewed X-inactivation pattern in obligate carrier females is always associated with mutations in ARHGEF6 and FAFL4, as it has been described previously in XNP mutations (ATR-X syndrome).

Despite important progress in clinical delineation of X-linked MR, mutations in genes like RabGDI, PAK3, and ARX still seem to lead to a “non-specific MR condition. Thorough and standardised clinical, neuropsychological and radiological investigations are undertaken for these families. For this core of remaining “non specific” MR, it is likely that technological advances in mutation detection devices, as HPLC or DNA chips, will allow implementation of large routine screening for dozens of genes. However, a wide molecular screening will never replace a good clinical assessment of the child and his family, which remains the most powerful tool for an accurate and reliable approach to mental retardation diagnosis.

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TITLE: SEGREGATION ANALYSIS IN X LINKED MENTAL RETARDATION FAMILIES.

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POSTER ABSTRACT:

Since 1995 we have screened FRAXA and FRAXE mutations on a sample of mentally impaired patients, 545 males and 130 females, detecting 65 full mutated males (FM) and 5 FM females, 7 males mosaics for premutation PM/FM and 1 deleted patients. Among patients negative for FRAXA/FRAXE test, we collected families with apparent X linked segregation of mental retardation. We report on three different families in which non syndromic mild to moderate mental retardation segregates as an X-linked form. One of them was previously reported as MRX72, segregation analysis allowed to map the candidate gene in Xq28, telomerically to DXS1073 microsatellite marker, while sequencing analysis excluded the involvement of positional and functional candidate genes, GDI, NEMO, IRAK and VBP-1. Restriction of the associated interval and occurrence of rearrangements for the genes previously sequenced will be performed.

The second MRX family consists of a two generation pedigree with three affected males; linkage analysis by microsatellite markers was ruled out association to the whole X chromosome apart a region in Xq25-q26.3 between DXS8009 and DXS1192. Two point analysis did not evidence a LOD>2, probably because of the limited number of meioses in the family. Other markers within this interval will be studied and functional and positional candidate genes sequenced in affected patients from this family.

The third family shows a three generation pedigree with two mentally impaired and two mentally sane males in the second generation. More than 25 markers spanning the whole X chromosome were studied excluding association apart markers DXS1047 (Xq25) and DXS1226 (Xp22). Further markers have to be tested in order to define a single association region.

Frax-e

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Jonathan and Kathy Doring

A Tale of Two Fragile X Sites

In the days shortly after identification of the CGG repeat expansion in the FMR1 gene, it became clear that what we knew as fragile X syndrome was really two different disorders caused by defects in two different genes, each affected by CGG repeats. It turned out that some of the families that were being studied as having fragile X syndrome did not show the repeat expansion in the FMR1 gene, in spite of having a fragile site on their X chromosomes in what was apparently the same place. Laboratories quickly determined that these families had a fragile site that was a short distance away from the one in the FMR1 gene, and this newly recognized fragile site became known as FRAXE (FRAXA being the first fragile site on the X chromosome, and FRAXE being the 5th to have been found).

As families were sorted into having either FRAXA or FRAXE, it became clear that FRAXE disease was rather rare (maybe 1/100 as common as FRAXA), and that the disease was somewhat milder, with few, if any, of the physical features found in patients with FRAXA. Yet the repeat expansions were very similar. Both involved CGG repeats that became very large in affected individuals.

It took a few more years before the gene affected by repeat expansion at FRAXE was characterized. This gene is now known as FMR2. It is not at all like FMR1. It makes a completely different protein that is found very early in fetal development and at low levels in the brains of adults. The FMR2 protein is found in the nuclei of cells, and appears to be involved in regulating the activity of genes, possibly working to help cells determine what type of cell they will become. Less is known about the FMR2/FRAXE gene and its protein than those of FMR1/FRAXA, although recent advances have begun to shed light on FMR2's normal function and the consequences of its absence in individuals with FRAXE. Very recently, a mouse model for FRAXE was developed, and this should provide significant new data about the disease in humans.

FRAXE families have been involved in fragile X research and in the various foundations dedicated to support of families and individuals with fragile X syndrome since the beginning of these efforts. Although their form of the fragile X syndrome is somewhat different, they are no less welcome in our community. They have a great deal to contribute to both our scientific and social understanding of developmental disability, and suffer from a very similar (and nearby!) disorder.

This was a rousing and inspirational session presided over by Jonathan Doring, a young man with the Fragile X E disorder. Jonathan described his rather rich full life in as a recent high school graduate and student in junior college. He described his interests and hobbies along with some of his disappointments. He fielded questions with aplomb, demonstrating his training.

Jonathan's mother, Kathleen Doring then spoke about the challenges and rewards of raising Jonathan, with an emphasis on the confusing issues surrounding his diagnosis with Fragile X E. She emphasized the value of inclusion with the greater fragile X community, despite the differences between the two disorders (Fragile X syndrome--the "A" type—and Fragile X E). Since Jonathan was studied cytogenetically and later by molecular methods, he has had several diagnoses with consequent confusion for the family.

David Nelson then provided an overview of Fragile X E disease. He compared and contrasted the disorder with the typical Fragile X syndrome. He explained that the mutational mechanisms are nearly identical, which results in a similar fragile site, while the genes affected are quite different. This difference leads to the different outcome for the diseases.

The session provided a better understanding of the molecular aspects of Fragile X E disease while offering an opportunity to hear about the disorder from a family living with it. Both parents and researchers came away with a much improved understanding of the disease.