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Review

Current Aspects of Clinical Genetic Diagnosis in Werdnig-Hoffman Spinal Muscular Atrophy

Axinia Corches

Clinic of Psychiatry and Neurology for Children and Adolescents, Timisoara, Romania

REZUMAT

Aspecte actuale de diagnostic clinico-genetic în Atrofia musculară spinală Werdnig-Hoffman

Atrofia musculară spinală (AMS) tip I Werging-Hoffman reprezintă cea mai severă formă de atrofie musculară spinală, cu determinism genetic, transmitere autosomal recesivă și cauzată de mutația genei SMN situată la nivelul cromozomului 5q. Defectul genetic situat la acest nivel, determină precoce atrofii musculare prin degenerescența progresivă a motoneuronilor spinali. Diagnosticul precoce în AMS tipI, Werding-Hoffman este important pentru demersul terapeutic, evoluția bolii fiind rapidă și speranța de viață situându-se în jurul vârstei de 6 luni. Simptomatologia clinică este specifică din primele zile de viață. Copilul trebuie monitorizat în serviciul de terapie intensivă pentru susținerea funcțiilor cardio-respiratorii. Aspectul caracteristic de copil cu hipotonie generalizată și fenomenele de insuficiență cardio-respiratorie impun investigația genetică din primele zile de la naștere pentru depistarea defectului genetic la nivelul genei SMN, cromozomul 5q. Sunt sintetizate în lucrare caracteristicile clinico-genetice ale bolii în conformitate cu literatura actuală de specialitate. Se subliniază importanța diagnosticului și instituirea managementului precoce, atrofia musculară spinală tip I Werging-Hoffman fiind o boală neurologică invalidantă. Sunt importante sfatul genetic și susținerea familiei în demersul terapeutic care se impune în conformitate cu recomandările actuale din literatura medicală de specialitate.

Cuvinte cheie: atrofia musculară spinală tip I, Werding-Hoffman, defect genetic, diagnostic precoce, simptomatogie clinică, demers terapeutic

ABSTRACT

Spinal muscular atrophy (SMA) Type I, Werdnig-Hoffman is the most severe form of spinal muscular atrophies with genetic determinism, autosomal recessive transmission and it is caused by genetic disorder in the SMN gene located on chromosome 5q, which results in early muscle atrophy due to progressive degeneration of spinal motor neurons. SMA Werdnig-Hoffman is an emergency in pediatric neurology and early diagnosis is important for the therapeutic approach, the rapid progression of the disease, life expectancy being situated around the age

Corresponding author: Axinia Corches MD, PhD
Paediatric neurology and psychiatry,Head of Department, Clinic of Psychiatry and Neurology for Children and Adolescents, Timisoara
e-mail: axiniacorches@yahoo.com

of 6 months. The clinic symptomatology is specific in the first days of life. The child should be monitored in the intensive care service for the support of cardio-respiratory functions. The characteristic appearance of a child with generalized hypotonia and cardio-respiratory failure phenomena require genetic investigation starting with the first days after birth to detect the genetic defect in the SMN gene, in chromosome 5q. The work summarizes the clinical genetic characteristics of the disease according to current professional literature. It emphasizes the importance of early diagnosis and of early initiation of management, because type I spinal muscular atrophy is a neurological emergency.

Key words: spinal muscular atrophy type I, Werdnig-Hoffman, genetic defect, early diagnosis, clinical symptomatology, therapeutic approach

DEFINITION

Type I Werdnig-Hoffman spinal muscular atrophy (SMA) is a progressive neuromuscular disease with recessive autosomal transmission characterized by muscular weakness and atrophy caused by degeneration of motor neurons in the spinal cord and in the brainstem nuclei.

HISTORY

In the early 1980s, Werdnig and Hoffman described a disorder with childhood onset that was characterized by progressive muscle weakness. In terms of histopathology, it showed loss of neurons in the previous horns of the spinal cord. (Katirji B, 2002).

Several families with multiple affected members have been described, some of them with severe, others with benign evolution. Clinical findings summarized in the 1950s led to the classification of spinal muscular atrophies (SMA) into three clinical forms, Type I, II and III, depending on the patient's age. In the 1990s, advances in molecular genetics have facilitated the understanding of muscular atrophies by associating these diseases with the long arm of chromosome 5, following research studies on genetic linkage.

In 1995, journal Cell published data on two genes that were supposed to carry the mutations which cause SMA. These are the Survival Motor Neuron-SMN gene and Neuronal Apoptosis Inhibitory Protein -NAIP. Both genes are located in two copies on the chromosome 5 (Menkes John H, 2000).

EPIDEMIOLOGY

As frequency of hereditary diseases, SMA is consecutive to cystic fibrosis, with an incidence of about 1 / 10,000 new-borns. The Type I acute, infantile, Werdnig-Hoffman form, represents about ´O of cases. (Zerres K, Rudnik-Schoneborn S-Natural history in proximal spinal muscular atrophy. ArchNeurol, 1995 May; 52(5)518-523).

CLASSIFICATION

Over time, the nosology of muscle atrophies underwent some changes. Currently, there are two major classifications, the first one in that it has long been in use and worthwhile in practice, the second one because it uses new information related to the genetics of these diseases, leading to a more rigorous division of the forms of the disease.

The first classification belongs to the International Spinal Muscular Atrophy Consortium-ISMAC.

According to this classification, SMA is divided into four types: (Table 1).

In 1993, Harding introduced the concept of HMN (Hereditary Motor Neuropathy). (Hanemann C. Ludolph A, 2002). The concept introduced by Harding tries to cover the whole spectrum of hereditary diseases affecting the spinal and bulbar motor neurons without sensory phenomena. This

Table 1. SMA Classification according to ISMAC (Tsao Bryan, Armon C, 2001)

1.	SMA I or the severe, infantile form, Werdnig Hoffman disease
2.	SMA II or intermediary form
3.	SMA III or mild, juvenile, Kugelberg- Welander form
4.	SMA IV or adult form, including more entities

concept includes proximal HMNs, forms in which the motor deficit is proximal (these forms partially overlap with the forms described in the ISMAC classification) and distal HMNs, forms in which the motor deficit of the lower limbs is predominantly distal and they are called distal muscular atrophies.

For practical and didactic purposes, SMA is currently classified according to an important genetic criterion, namely, their relationship with locus 5q12.2-q13.3. This classification is presented in **Table 2**.

Table 2. Genetic Classification of SMAs

I.	Autosomal recessive forms related to locus 5q12.2-q13.3
	1. SMA type I Werdnig Hoffman
	2. SMA type II intermediate form
	3. SMA type III Kugelberg-Welander
	4. SMA type IV adult form
II.	Forms that are not relatedto locus 5q12.2-q13.3
	1. Distal SMAs
	2. Segmentation SMAs
	3. Special shapes SMAs

Genetics of 5q autosomal recessive SMA

The progress of molecular biology in the second half of the twentieth century allowed the description the pathogenesis of genetic amyotrophies.

The determining genetic mechanism is similar in the three forms of 5q autosomal recessive SMA, the locus involved being the long arm of this chromosome in the region q12.2-q13.3. There are two genes involved in the disease: SMN 1, located in the telomere area and SMN 2 located in the centromere area. The acronym SMN stands for survival motor neuron gene. The most important is the SMN1, because 95% of the cases of SMA are caused by a mutation in this gene. (Fenichel Gerald M, 2001). SMN 2 does not cause the disease directly but it contributes to the severity of the disease by the number of copies that are present. The more numerous, the milder form of the disease, having late onset and slower growth.

SMN1 gene is situated on chromosome 5, in two copies with variable location: in most normal individuals (96%) there is a copy in each chromosome 5. 18% of normal individuals have both copies of the gene on one of the two chromosomes 5. It is made up of 9 exons and encodes a protein composed of 294 amino acids. There is a high homology between SMN 1 and SMN 2 although the two genes are not identical. The specificity is located in the exons 7 and 8 where the two genes differ by a few short nucleotide sequences. (HanemannC, Ludolph A, 2002).

SMN2 gene is located centromerically to SMN1 gene. Unlike SMN1, in SMN2, exon 7 is not shifted, this means that it is not expressed in the final product of transcription. The number of SMN 2 copies is variable. 88% of normal people have one or two copies. All individuals, whether healthy, carrying or sick with SMA, have at least one copy of SMN 2. In sick individuals, the number of genes is in inverse proportion to the severity of the condition. In conclusion, in patients with SMA, SMN2 gene is considered "protective" against neuronal damage caused by mutations in the SMN1gene. The higher the number of SMN 2 copies, the smaller the effects of the mutation and less severe the disease. Recent studies have shown that through methods of genetic therapy, the SMN2 gene could be induced to produce an almost identical protein as the one encoded by the SMN 1, which could provide protection against the effects of the mutation in SMN1 gene in patients with SMA. (Neuromuscular Disease Center, Washington University School of Medicine.

SMN Protein (Survival Motor Neuron Protein) has a role that currently is not completely elucidated. It is located in the nucleus and in the cytoplasm and is part of a complex consisting of several proteins that serves to protect the cell from apoptosis induced by certain proteins. SMN is located in the nervous and muscular tissue, including the myocardium. The deletion of this protein makes it inoperable, the motor neurons will suffer apoptosis leading to their loss, thus producing neurological deficit at clinical level.

Neuronal apoptosis inhibitory protein (NAIP) is a protein encoded by a gene located very close to SMN 1. This protein is absent in patients with SMA, the more frequent, the more severe the disease. The absence of this protein has a phenotypic effect only in females.

Several mutations in the SMN1and SMN2 genes have been described that correlate differently with the neuromuscular phenotypic configuration. In the SMN1 gene, deletions occur affecting particularly exons 3, 6, 7, and 8. The exon 7 deletion causes 95% of all cases of SMA in any form. The higher the deletion, the more severe the phenotype. (M Gerald Fenichel, 2001)

SMN2 gene shows a variable number of copies

that correlates with the severity of the phenotype. In Form I, 2-3 copies are described. Mutations of the deletion type at the level of neuronal apoptosis inhibitory protein NAIP occur more frequently in patients with Form I of the disease. The absence of this protein may lead to a severe phenotype.

Transmission in SMAs related to locus 5q12.2-q13.3 is autosomal recessive. Carrier frequency in the population is 1% -1.8%. The disease occurs when both copies of SMN1are mutant and the resulting protein is non-functional. Three mechanisms are described by which a product of conception may acquire the mutation of SMN1 gene in homozygous state:

1. Each parent has one mutant gene in the pair (is a carrier). In this case, the risk of recurrence of the disease in the next child is 25%.
2. One parent is a carrier - one of the two copies of SMN1 is mutant and the normal copy from the other parent suffers a spontaneous mutation after conception. The child will have no normal copy of the gene and will be sick. The risk of recurrence is low, since the probability of occurrence of such a situation is low.
3. One parent has the two copies of SMN1 on a single allele, on one of the two chromosomes
4. There is a risk to transmit the chromosome to the child without any copy. If the other parent transmits a mutant copy to the child, the latter will have no normal copy of SMN1 and will be sick. The risk of recurrence in this case is 25% (M Gerald Fenichel, 2001).

Symptomatology

SMA Type I is the most severe form of SMA from the point of view of disease evolution and prognosis. The onset of the disease is situated around the age of 6 months. However, in more than 95% of the patients, symmetrically, progressive muscular weakness and hypotonia or atonia are noted before the age of 3 months. Moreover, in about 30% of cases, cyanosis, arthrogryposis or skeletal (scoliosis) and joint deformities can be observed at birth, as a result of hypotonia present even in intrauterine life.

Based on clinical examination, one can establish the diagnosis of the peripheral motor neuron syndrome. On inspection, we see a flaccid, hypotonic-looking child, with an aspect of "floppy baby" ("rag doll"). The damage to a lesser extent of the distal region of the limbs that allow distal movement of the fingers is obvious, while the major proximal

Figure 1. The appearance of "floppy baby"

damage leaves the limb roots at the level of the bed, the hips in flexion-abduction-external rotation, knees in semi-flexion, foot in equinus, arms in internal rotation, elbows bent, forearms in pronation, fists in extension, all these defining the batrachian position ("frog-like position"). The child cannot keep his head in the trunk axis unless he is sat up, it falls forward. The child does not support himself on the legs. Key diagnostic feature is that these children do not acquire the sitting position (Zerres K, 1999).

At the limb level, the proximal dominant hypotonia is observed with flaccid muscle masses, lacking tone, atrophic (more pronounced proximally, evident in the thighs, arms, shoulders) and the lack of opposition to passive movements.

Motor deficit at limb level is clearly proximal, symmetric and severe. Movements of the small muscles of hand and foot are possible. Motor deficit is gradually evolving upward. Atrophy and paralysis evolve rapidly, initially in the back muscles, then progressively at the girdle muscles, finally targeting the distal parts of the limbs. The deficit is generalized in a few weeks, leading to the emergence of a symmetrical flaccid quadriparesis. Muscle atrophy can be masked sometimes by adipose tissue. (Dubowitz, 1995)

Neck muscles are affected, too, as well as the paravertebral muscles. The damage of the muscles leads to skeletal deformities: pectus excavatum, scoliosis, and kyphoscoliosis.

Osteotendinous reflexes are abolished following the peripheral motor neuron lesion helping to distinguish from other causes of central hypotonic syndrome.

The cardiac damage is not obvious, but there

may be a specific amendment to the basic ECG rhythm due to fasciculation of the limbs and of the chest wall muscle. However, fasciculation is often limited to the tongue and are difficult to distinguish from normal movements. (Wang C, 2007)

Intercostal muscle paralysis is constant, infant breathing only with the diaphragm (breathing paradox). Crying is short and without force. Swallowing disorders appear, too. Mental development of these children is always normal.

Patients do not show impairment of extra-ocular muscles and facial muscle weakness is often minimal or absent.

Diagnostic methods

Spinal muscular atrophy type I diagnosis arises only in the presence of symptoms. Thorough neurological examination and medical history play a crucial role. Doctors investigating children presenting hypotonia and muscular weakness should maintain a high degree of suspiciousness to this diagnosis. Certain physical characteristics are readily identifiable. Muscle weakness is usually symmetrical and more proximal than distal, tendon reflexes are absent or diminished. (Wang C, 2007)

In most cases, accurate diagnosis is established based on a genetic test that determines whether the body has at least one copy of the SMN1 gene. The genetic test determines whether there are unique sequences of the gene (distinguishing them from SMN2 gene-like sequences) of exons 7 and 8. This test has an accuracy of 95% (95% of patients will show abnormal results) and is virtually 100 % specific (if a person manifests muscular weakness, and genetic test cannot find a copy of the SMN1 gene, the diagnosis of SMA is absolutely sure). (Lefebvre S, 1998)

A negative test should be followed by clinical reassessment for atypical features (contractures, diaphragmatic eventration, and congenital absence of certain muscles) and by other diagnostic tests (CK, EMG, NCV). If they suggest, however, SMA, a genetic testing is performed to detect the number of SMN1 copies and a sequencing to detect point mutations in patients with a single copy. If present two SMN1 copies are present, inquiries must be directed to other neuro-muscular disease. (Zerres K 1999).

Recommendations for genetic testing

- The confirmation of the diagnosis of SMA in children with clinical symptoms.
- Identification of carrier state (heterozygous) by testing in asymptomatic adults at high risk. Note that testing does not have utility for predicting age of onset, severity, type or modality of disease progression. ((Prior T., 2006; Tsao B, 2013).
- Antenatal diagnosis in high-risk pregnant women. It is performed by analyzing DNA extracted from fetal cells obtained by amniocentesis or by biopsy of chorionic villus sampling. (T Prior, 2006; Tsao B, 2013).

Test methods

- identifying deletions of exons 7 and 8 of SMN1 gene by MLPA (multiplex ligation-dependent probe amplification) tests;
- whole sequencing of SMN1 gene to identify intragenic mutations;
- quantitative PCR to determine the number of copies of the SMN2 gene;
- PCR-based gene dosage analysis to identify the number of copies of the SMN1gene (detection of carrier state).

When the genetic test cannot be performed, other tests are indicated:

1. Determination of creatine kinase (normal or slightly elevated)
2. Electrophysiological tests (EMG, NCV, repetitive stimulation). Electromyographic characteristics in SMA type I show features denervation and decreased motor action potential, spontaneous activity type positive sharp waves, fibrillation and occasionally twitching occurs most frequently.
3. Muscle biopsy may be necessary if electrophysiological tests show patterns characteristic for diseases of muscles, nerves or neuromuscular junction (for differential diagnosis). (M.R Lunn, 2008). Selection of the muscle for biopsy should be done according to clinically affected muscles but not so affected that degeneration should make the interpretation irrelevant. (Tsao B., 2013).
4. Imaging tests: Although in SMA Werdnig - Hoffman there is usually no CNS involvement, non - specific changes (cerebral atrophy or other ischemic changes) can be observed, associated with prematurity or birth asphyxia.

Management of newly identified patients with SMA

Many care issues arise when a patient is newly diagnosed with spinal muscular atrophy. Physicians should address various aspects of care issues as soon as possible.

Education and family counseling

Due to the complexity of medical problems associated with the diagnosis of SMA, it is advised that healthcare providers should designate a contact person for the family. This person is usually a pediatric neurologist or a geneticist.

During the first meeting with the family, it is important to provide data on disease progression and prognosis, physio-pathological processes and phenotypic classification. At the same time, an interdisciplinary intervention plan should be established with the family. It includes, in general, genetic, pulmonary, gastroenterology and nutrition checkups. (Wang C., 2007).

There is no cure treatment for children with Werdnig-Hoffmann disease

Therapeutic target is not exactly clear (there is no international consensus on the level of care), aimed at improving the treatment of specific symptoms. Some therapies can be perceived as placing the quality of life in conflict with life span, by prolonging the life and not healing the suffering of these patients. Thus, there is no international consensus on the level of care, while the level of experience and training, and the resource availability have major influence on the recommendations. Finally, the family is the one who takes the decisions on the aggressiveness of the supportive therapies. (Bach JR, 2003).

SMAs affect the muscles used for chewing, sucking and swallowing. This can lead to severe reductions in weight, difficulty in swallowing saliva and fostering aspiration pneumonia. Small, frequent meals, aspiration of the pharynx for saliva and, if necessary, feeding through gastrostomy are recommended.

Pulmonary disease is the major cause of morbidity and mortality in SMA type 1.

Key respiratory problems in SMA type I are:

- recurrent cough (reduced clearance ability of lower airway secretions),
- hypoventilation during sleep,
- underdeveloped lungs and chest wall,
- recurrent infections (exacerbate muscle weakness).

Treatment options range from failure to provide respiratory support to the use of non-invasive procedures (intermittent positive pressure ventilation) or to long-term invasive procedures (tracheotomy). Special attention must be given to the use of mechanical ventilation in acute crises due to increased risk of addiction. (Dubowitz V, 1995).

Treatment of life-threatening respiratory complications is controversial. Any treatment decision should be taken after consultation between parents and the entire medical team. (Zerres K 1999).

Physical therapy is useful to minimize contractures and to help the patient / carers to develop compensatory strategies; Muscle strengthening is not a reason for therapy.

Several studies have been conducted on various curative drugs, but none showed a significant difference from placebo. Study drugs included: Gabapentin, Phenylbutyrate, Albuterol, Riluzole, Valproate, Aclarubicin, Myostatin

Currently there are five drugs in various stages of clinical trials (one in phase three - Olesoxime).

Table 1 based on data released in June 2014, which includes the drugs under study http://www.smafoundation.org/development/pipeline/

Evolution

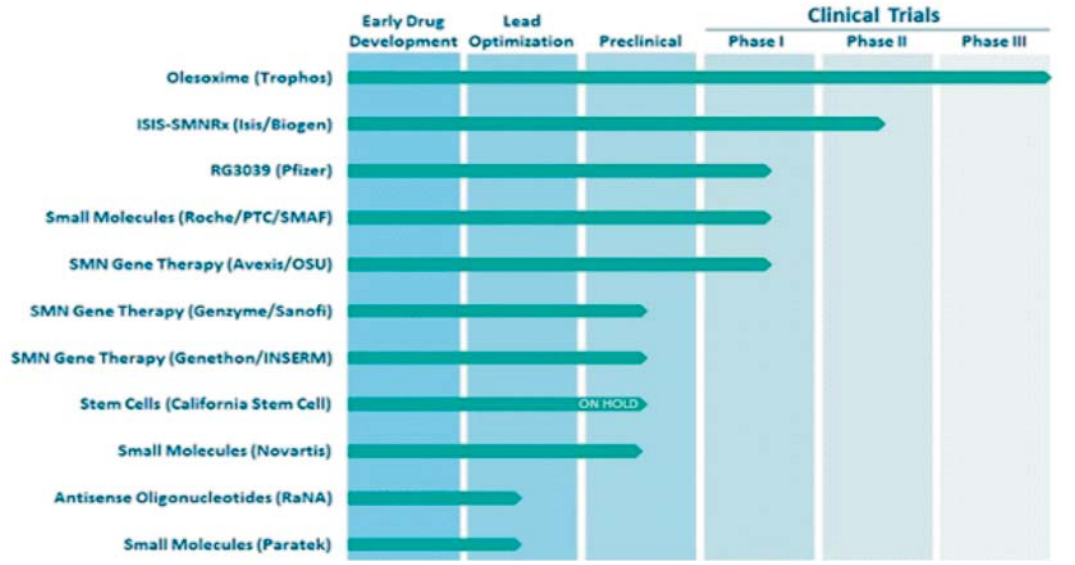
In 95% of cases, children die before the age of 18 months due to complications of the disease, but they may live up to 2 years if they receive respiratory support. (Tsao B., 2013).

There are disease cases of fulminant muscle weakness, where the bulbar dysfunction occurs early. In their situation, the average survival is 5.9 months. (Prior T., 2006).

CONCLUSIONS

- Werdnig-Hoffman spinal muscular atrophy is a disabling neuromuscular disease with genetic determinism.
- Early diagnosis is essential for initiating the management as early as possible.
- The appearance of "floppy baby" is indicative for clinical diagnosis.
- Genetic testing is necessary to confirm the diagnosis.

Table 1.



- There is no curative treatment. Currently, information about possible curative medicines is released into mass media.
- There is no international consensus on the level of care, decisions about the aggressiveness of supportive therapies are taken by the family. (Bach JR, 2003).

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