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Minireview

The laboratory diagnosis of mucopolysaccharidosis III (Sanfilippo syndrome): A changing landscape

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ABSTRACT

Mucopolysaccharidosis type III (MPS III) is characterized by progressive neurological deterioration, behavioral abnormalities, a relatively mild somatic phenotype, and early mortality. Because of the paucity of somatic manifestations and the rarity of the disease, early diagnosis is often difficult. Therapy targeting the underlying disease pathophysiology may offer the greatest clinical benefit when started prior to the onset of significant neurologic sequelae. Here we review current practices in the laboratory diagnosis of MPS III in order to facilitate earlier patient identification and diagnosis. When clinical suspicion of MPS III arises, the first step is to order a quantitative assay that screens urine for the presence of glycosaminoglycan biomarkers using a spectrophotometric compound (e.g., dimethylmethylene blue). We recommend testing all patients with developmental delay and/or behavioral abnormalities as part of the diagnostic work-up because quantitative urine screening is inexpensive and non-invasive. Semi-quantitative urine screening assays using cationic dyes on filter paper (e.g., spot tests) have relatively high rates of false-positives and false-negatives and are obsolete. Of note, a negative urinary glycosaminoglycan assay does not necessarily rule out MPS because, in some patients, an overlap in excretion levels with healthy controls may occur. All urine samples that test positive for glycosaminoglycans with a quantitative assay should be confirmed by electrophoresis, thin layer chromatography, or tandem mass spectrometry, which further improves the sensitivity and specificity. The gold standard for diagnosis remains the enzyme activity assay in cultured skin fibroblasts, leukocytes, plasma, or serum, which can be used as a first-line diagnostic test in some regions. Molecular genetic analysis should be offered to all families of patients to allow genetic counseling for informed family planning. For a small number of variants, genotype–phenotype correlations are available and can offer prognostic value. Prenatal testing via enzyme activity assay in chorionic villi or amniotic fluid cells is available at a limited number of centers worldwide, but whenever possible, a molecular genetic analysis is preferred for prenatal diagnosis. To conclude, we discuss the development of newborn screening assays in dried blood spots and high-throughput methods for sequencing the protein-coding regions of the genome (whole exome sequencing) and their relevance to future changes in the MPS III diagnostic landscape.

Contents

Abbreviations: DMB, dimethylmethylene blue; GAG, glycosaminoglycan; HPLC, high-performance liquid chromatography; LC, liquid chromatography; MPS, mucopolysaccharidosis; MS/MS, tandem mass spectrometry; NRE, non-reducing end; uGAG, urinary glycosaminoglycan.

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1. Introduction

The mucopolysaccharidoses (MPS) belong to the group of more than 50 inherited lysosomal storage disorders [1]. The MPS are caused by impaired catabolism of glycosaminoglycans (GAGs), leading to the accumulation of GAGs in lysosomes, resulting in cellular, tissue, and organ damage [2]. Clinically, the MPS are progressive, multi-systemic disorders characterized by a variable age of onset, marked clinical variability, significant morbidity, and early mortality [1].

Mucopolysaccharidosis type III (MPS III, Sanfilippo syndrome) is reported to be the most commonly occurring of the MPS [3]. Together, the cumulative reported incidence of all subtypes of MPS III varies between 0.28 and 4.1 per 100,000 live births [4]. There are four different confirmed subtypes of MPS III: type A (OMIM #252900), type B (OMIM #252920), type C (OMIM #252930), and type D (OMIM #252940), all of which are inherited in an autosomal recessive manner (Table 1). Each subtype is caused by a deficiency in a different enzyme in the catabolic pathway for heparan sulfate [1]. Recent research suggests that the lysosomal enzyme arylsulfatase G is also required to complete the degradation of heparan sulfate, since deficient mice accumulate heparan sulfate in visceral organs and in the central nervous system and develop neuronal cell death and behavioral deficits. It has been tentatively suggested that arylsulfatase G deficiency, not yet described in humans, may be termed MPS IIIE when identified [5].

MPS III presents a diagnostic challenge, particularly during the early disease course, because it primarily manifests as a neurological disorder [6]. Somatic symptoms, while present, tend to be milder than those seen in the other MPS [4]. Delays of 1–9 years between onset of signs and symptoms and diagnosis have been reported [7–11]. While such delays have generally not raised concerns in the past, recent therapeutic developments for patients with MPS III have highlighted the need for earlier and more timely diagnoses [12]. Developments in the clinical trial stage include substrate reduction therapy [13,14], gene therapy [15] (NCT01474343), and intrathecally delivered enzyme replacement therapy (NCT01155778, NCT01299727). Although the pathogenesis of the neurological decline in MPS III is not well understood, it has been suggested (but not proven) that the best chance for optimum patient outcome will occur when therapies that alter the disease pathophysiology are initiated before extensive and/or irreversible neurological damage has occurred [12]. In addition, an early diagnosis allows a patient's family to receive genetic counseling and make an informed decision about future family planning, which may be of particular importance in small communities showing a “founder” effect [5] or communities in which consanguineous partnerships are more common [16].

In this review, we briefly describe the clinical phenotype of MPS III, then discuss the various laboratory assays suitable for screening, enzymatic diagnosis, and molecular genetic confirmation, including techniques that are currently under development. Based on the published literature and our own clinical experience, we highlight the benefits and pitfalls associated with each method.

2. Clinical phenotype

MPS III is a progressive disease with a continuous spectrum of clinical symptoms, from the “classic” or “severe” phenotype to a more attenuated disease course. The pathology of the disease has historically been divided in into three phases [4]. Because the degree of severity and the timing of each phase vary significantly between individuals and disease subtypes, this description is meant to provide an overview of the disease process rather than specific time points for pathogenesis. In the classic description, the first phase generally begins between the ages of 1 and 3 years with a slowing or plateauing of cognitive development. In some patients, isolated speech delay may be the earliest finding [17,18]. Motor development is usually not affected during this phase. Characteristic somatic signs and symptoms, such as coarse facial features, hearing loss, recurrent ear infections, frequent upper respiratory infections, and orthopedic manifestations (scoliosis, kyphosis, lumbar lordosis, hip dysplasia and pain, carpal tunnel syndrome, trigger digits), may begin to emerge during this phase, but it should be noted that these are generally mild and highly variable [6]. At around 3–4 years of age, the second phase begins. Children begin to show progressive cognitive decline, behavioral abnormalities, and sleeping disturbances. Behavioral difficulties, including hyperactivity, impulsivity, obstinacy, anxious behaviors, and autistic-like behaviors, worsen over time and can become extreme [19–21]. The duration of the second phase is quite variable depending upon the patient's phenotype. Those patients with the severe phenotype usually remain in the second phase for about 7–10 years, while those with an attenuated phenotype may remain in this phase well into adulthood [8,22]. The onset of severe dementia and motor function decline heralds entrance into the third phase. Patients lose locomotion, which is associated with a decline in behavioral disturbances, and swallowing difficulties and spasticity emerge. Patients eventually regress to a fully bedridden and vegetative state. Death occurs in the second or beginning of the third decade of life in patients with the severe form of the disease [7–10,23,24] and in the fourth to sixth decade of life in patients with the attenuated form [8,22,25]. As mentioned above, however, these phases are subjective in nature, and disease progression in MPS III can be heterogeneous.

Because MPS III is primarily a neurological disease, young patients are often misdiagnosed with attention-deficit/hyperactivity disorder, autism spectrum disorders, pervasive developmental disorder, or idiopathic developmental or speech delay [6]. A key factor in early diagnosis is the ability of clinicians to entertain the possibility of an inherited metabolic disease in cases of developmental delay, sleeping disturbances,

Table 1
The subtypes of mucopolysaccharidosis III.

Subtype	Deficient enzyme	Gene	Locus	Number of exons	Size of gene
MPS IIIA	Heparan-N-sulfatase	*SGSH*	17q25.3	8	11 kb
MPS IIIB	N-alpha-D-acetylglucosaminidase	*NAGLU*	17q21.2	6	8.3 kb
MPS IIIC	Heparan acetyl-CoA:alpha-glucosaminide N-acetyltransferase	*HGSNAT*	8p11.21	18	62.4 kb
MPS IIID	N-acetylglucosamine-6-sulfatase	*GNS*	12q14.3	14	46 kb

MPS, mucopolysaccharidosis.