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Newborn Screening for Lysosomal Storage Disorders
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Multiplex Newborn Screening for Lysosomal Storage Disorders: Feasibility Is Growing but Prediction of Phenotype Needs Elucidation

Lysosomal storage disorders (LSDs) are a group of over 45 genetic diseases caused by the absence or impaired function of specific lysosomal enzymes. The combined birth prevalence of LSDs is at least 1:7,700. Most patients are clinically normal at birth. LSDs are chronic and progressive, with a variety of symptoms. If severely affected patients are not treated, they often die by their teens. Despite the absence of a cure, treatments have been developed for some of the LSDs using bone marrow transplantation or enzyme replacement therapy (ERT). Several other types of therapy are in a preclinical phase of development.

The efficacy of a therapy for the prevention of irreversible neuropathologies will depend on early diagnosis and prompt treatment. Early detection can also provide the advantages of timely genetic counseling, a shorter period of stress and uncertainty about the diagnosis and, in the case of bone marrow transplantation, a greater chance for successful engraftment due to the naturally suppressed immune system of the neonate.

If there is no family history of LSDs, the only feasible way to diagnose asymptomatic individuals is by newborn screening. Ideally a universal screening method should be developed using dried blood spots (Guthrie cards) collected from neonates. This paper demonstrates proof of this principle by describing a protein-based multiplex assay for 11 lysosomal proteins which retrospectively identified individuals with 1 of 11 LSDs.

The patient samples were either from newborns subsequently diagnosed with an LSD or from patients after a diagnosis of LSD. Controls were newborns and adults. The principle of the assay was immune quantification of protein. Newborn controls had higher median values and a greater range for most of the proteins measured compared to adult controls. All patient samples were outside the respective control ranges, except for Pompe's disease (sensitivity 90%, specificity >99%) and Gaucher disease (sensitivity 50%, specificity 98%). Protein profiles, i.e. plotting various protein markers against each other, improved discrimination but

did not resolve it completely. Taken together, all patients with mucopolysaccharidosis types I (n = 4), II (n = 19), IIIA (n = 13), and VI (n = 2), as well as all patients with metachromatic leukodystrophy (n = 4), Niemann-Pick disease type A/B (n = 5), and multiple sulfatase deficiency (n = 1) had lower specific protein levels than controls. All mucopolipidosis II/III cases (n = 10) could be distinguished by their higher levels of a combination of proteins. Most but not all Fabry (n = 11), Pompe (n = 30) and Gaucher (n = 12) patients were identified by single protein values or profiles of multiple proteins. Second tier confirmation assays based on enzyme activity could be performed in borderline cases. With further validation, including a larger, prospective study and the determination of population-specific reference ranges, protein profiling with a combination of markers could be affordably implemented for newborn screening for LSD.

Comment

In addition to multiplex protein-based screening, a multiplex assay using tandem mass spectrometry to directly measure enzymatic activity has previously been described for Gaucher, Niemann-Pick A/B, Pompe, Fabry and Krabbe diseases [1]. It was shown that patients of various ages could be readily distinguished from healthy controls, including heterozygous carriers. It was suggested that this method could be affordably incorporated into newborn screening programs.

Screening on the basis of enzymatic activity is in principle more sensitive than screening for protein, since some patients have 'normal' amounts of inactive enzyme. In early studies for Pompe it was shown that those infants who responded best to ERT in fact belonged to this group. This is attributed to the fact that such patients have little or no immune response to supplemented enzyme. Because protein-based screening might miss those patients who would benefit most from ERT, the Genzyme Corporation has chosen to develop neonatal screening reagents for 5 LSDs on the basis of enzymatic activity. A higher specificity for Gaucher than the 98% reported for the protein-based assay would also be an advantage.

In 2005 the Health Council of the Netherlands recommended expansion of the public newborn screening program. Although 4 LSDs were among the diseases considered, none of them were finally recommended. Pompe's disease came closest to being recommended but was excluded because of uncertainty about the availability and the effectiveness of treatment [2]. Just 1 year later, in 2006, ERT for Pompe received marketing approval in Europe and the United States, increasing the likelihood that Pompe will soon be added to screening programs. However, another issue should be addressed first. At present, the prevalence of late-onset Pompe is much higher than the infantile form of the disease [unpublished data from the Dutch Association for Neuromuscular Diseases]. This will probably remain so even after the introduction of treatment, as the birth prevalence of late-onset Pompe has

been estimated to be more than double that of infantile disease [3]. Although screening may be aimed at identifying newborns who would benefit from immediate treatment, it may unwittingly identify late-onset patients at a presymptomatic stage. A recent article mentions that data will soon be published showing that this is in fact the case [4]. Before further implementation of screening for treatable LSDs such as Pompe, research is needed on the prediction of phenotype for newborns identified with low protein or enzyme levels. The Dutch Association for Neuromuscular Diseases is strongly in favor of screening, as this would facilitate timely initiation of treatment for all patients. At the same time research on phenotype prediction as well as on the expected impact of presymptomatic screening is warranted.

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Informed Choice in Antenatal Down Syndrome Screening: A Cluster-Randomised Trial of Combined versus Separate Visit Testing

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Informed Choice on Prenatal Down Syndrome Screening?

It is generally recognized that undergoing prenatal screening should be the result of an informed choice. In the UK prenatal screening for Down syndrome is offered to pregnant women either as part of a combined visit together with other prenatal tests, or as a separate test-specific visit to a hospital. It is however unknown which policy facilitates or impedes informed decision making. Possibly, a separate visit leads to more informed choices because it allows people to evaluate more carefully whether they want to undergo a prenatal test. On the other hand, a combined visit might lead to more informed choices by removing practical

barriers (an extra visit). An earlier study of this group found that a combined visit facilitated informed choice in those accepting prenatal screening without impairing informed choice in those rejecting it. This study was aimed to test the hypothesis that conducting prenatal screening at a combined visit results in more informed choices to accept a prenatal test than at a separate visit.

In a cluster-randomized trial, community midwives were allocated either to the combined visit group or to the separate visit group. All participants were offered the second trimester maternal serum screening test. The study sample contained 982 pregnant women visiting one of the 37 participating midwives. Informed choice was measured using a classification system that is based on both knowledge and value consistency (the multidimensional measure of informed choice).

Less than half of the women (43.5%) made an informed choice about prenatal screening, regardless of the randomization group. There was neither a difference in rates of informed choice for women *accepting* prenatal screening according to the method of offering testing (23.7% in the combined visit group vs. 22.5% in the separate visit group), nor a difference for women *declining* prenatal screening according to the method of offering testing (20.0 vs. 20.8%).

There was no evidence to support the study hypothesis: prenatal screening requiring a combined visit did not lead to higher rates of informed choices among test acceptors. The authors discuss the possibility that the hypothesized relation between the method of conducting prenatal screening and informed choice does exist, but the present trial failed to identify it. This may be due to violations of the trial protocol, or to other biases. For example, midwives in the combined visit group may have influenced women to decline prenatal screening more strongly than midwives in the separate visit group. As the percentage of informed choice was very low, methods that increase knowledge and methods that increase attitude-behavior consistency need to be identified.

Comments

This trial of Dormandy et al. resulted in a low uptake rate of prenatal screening. This is noteworthy because other studies on the utilization of prenatal screening in the UK reported much higher percentages of women undergoing prenatal screening (e.g. Spencer et al. [1] found an uptake rate of over 90%). What factors cause these differences? Is there a relation with the setting of prenatal care, or the attitudes of health care professionals, or is it a reflection of the different values and preferences of pregnant women in the different studies? Interestingly, the uptake matches that in our recent study in the Netherlands [2], as both studies report that less than half of the pregnant women underwent prenatal screening.

Second, the overall rate of informed choice is low in both the routine visit setting and the separate visit setting. This is in accordance with findings of the systematic review of Green et al. [3]. It seems to be the international status quo that prenatal screening behavior in general cannot be considered to be based on informed choices. This is worrying because prenatal screening is precisely offered to facilitate informed decision making. The authors argue for the implementation of decision aids for prenatal screening, which might indeed be helpful in increasing the number of women making their own, knowledge-based and value-consistent choices. As the multidimensional measure of informed choice is currently coming into use to evaluate preg-