Supplementary Data

A Guideline for the Diagnosis of Pediatric Mitochondrial Diseases: The Value of Muscle and Skin Biopsies in the Genetics Era

Practical Aspects Concerning Muscle Biopsy

Wherever possible, the clinically affected tissue should be selected for further investigations, due to possible tissue specificity of mitochondrial defects. A biopsy should provide material for morphological, biochemical and occasionally also for molecular-genetic investigations.

The muscle biopsy should be performed in accordance with the examining laboratory. Special note should be taken of the following: Sampling point (e.g., musculus vastus lateralis), suitable anesthesia (especially in the case of local anesthetics), amount of pure muscle tissue. Depending on the intended analyses a minimum of 20 to 100 mg will be required, which equals approximately the size between a grain of rice and a coffee bean.

Collection Technique

On principle, there are two possible collection methods: needle biopsy and open biopsy. Different centers might have their own preference with regard to the method used. In principal both techniques are adequate, open biopsy might provide a larger amount of tissue and is more favorable in case of degenerated muscle.

Muscle Preservation for Biochemical Tests

Function of the mitochondrial energy metabolism can be investigated most extensively from unfrozen biopsy tissue, since mitochondrial membranes are still intact. A muscle biopsy can be shipped with a same-day courier in a special transport buffer to the analyzing laboratory using water-ice refrigeration during transport. Material intended solely for measuring the respiratory chain enzymes and pyruvate dehydrogenase complex should be frozen immediately, preferably in liquid nitrogen in a native state, and then stored in liquid nitrogen or at –80°C until being shipped on dry ice. If possible, it would be desirable to produce a myoblast and fibroblast culture in parallel to the muscle biopsy.

Other tissues for biopsy are e.g., liver, cardiac muscle, kidney, depending on clinical symptoms. The investigating laboratory should be consulted first, if there are reference values available for these tissues. Furthermore the function of mitochondrial enzymes can be investigated in skin fibroblasts and leucocytes; however, in ~50% of mitochondrial gene defects, the severity of the defect is not sufficient to be identified by enzyme analysis in cells.

Selection of Samples to be Preserved Post Mortem

On principle, if a defect in the mitochondrial energy metabolism is suspected the same principles apply for post mortem regarding the selection of samples to be preserved: clinically affected tissue must, if possible, be obtained for subsequent diagnostics. If possible, samples should be collected 1–2 hours post mortem and preserved as described. A skin biopsy should always be taken and a fibroblast culture should be cultivated.