Diagnostic and Advisory Service for Rare Neuromuscular Diseases

National Referral Centre for Congenital Muscular Dystrophies and Myopathies

Funded through the National Commissioning Group (NCG).

Supplementary information for the referral of fibroblasts cultures for collagen VI studies

The data we have collected enables us to interpret with confidence the results of the analysis of collagen VI secretion in fibroblast cultures derived from Ullrich Congenital Muscular Dystrophy patients, but always in the context of the clinical features and the muscle pathology. We have optimized the procedure in fibroblasts from Ullrich patients with various degrees of severity and different modes of inheritance (dominant or recessive mutations).

Collagen VI secretion by fibroblasts was abnormal in all the molecularly confirmed UCMD cases we have studied so far. In addition, in all the cases where a muscle biopsy was available, collagen VI immunolabelling was also reduced at the basal lamina. On the contrary, immunolabelling of the skin biopsy appeared normal in most cases (See Jimenez-Mallebrera et al., 2006, Neuromuscular Disorders 16 : 571-82).

When should analysis of collagen VI in fibroblasts be considered in UCMD?
This analysis can be of help as a tool to confirm the immunocytochemical findings on a muscle biopsy, especially if the reduction in the basal lamina is subtle. It can also be considered as an alternative source of diagnostic material in cases where a muscle biopsy is not available/adequate and not likely to become available.
Involvement of the COL6A genes must be eventually confirmed at the molecular level given that a secondary reduction in collagen VI labelling and in collagen VI deposition *in vitro* have been reported. Molecular analysis of the COL6A genes is now available – see NCG referral form for information.

Collagen VI analysis in fibroblast from patients with Bethlem myopathy.
Only a few patients with molecularly confirmed Bethlem myopathy have been studied with this technique worldwide and while abnormalities in collagen VI production have been reported in some cases, the full range of abnormalities and the sensitivity of this technique in the milder Bethlem patients are currently not fully known. In our experience, abnormalities can be detected in some Bethlem cases but not in others. For this reason and given that we are commissioned for Congenital Muscular Dystrophies we will not accept Bethlem fibroblasts routinely.

How to use the NCG service for Collagen VI analysis in cultured fibroblasts.

1. In the first place please send us detailed clinical information (this can be a recent summary letter) and expand on the information requested in the NCG pre-referral form in particular; maximum functional achievement, age at walking and ambulation status at present, laxity and contractures and skin changes. If a muscle MRI is available, please include a copy. If you have a clinical photograph of the patient, this might also be helpful. Also include the muscle biopsy report if available.
2. If you suspect Ullrich CMD in the first instance please send us the frozen muscle (if available) to perform collagen VI and perlecan immunolabelling.
3. Once the collagen VI immunolabelling results are available and the clinical information have been reviewed, we will write to you and we would let you know if we consider it is necessary to perform the collagen VI studies in cultured fibroblasts or not.
4. When forwarding cultured fibroblasts, please provide passage number, age of patient at time of biopsy and a certificate of clearance from mycoplasma contamination

Samples that arrive without the necessary information will be frozen and stored until this information is forthcoming.