New possibilities for prenatal diagnosis of muscular dystrophies: forced myogenesis with an adenoviral MyoD-vector

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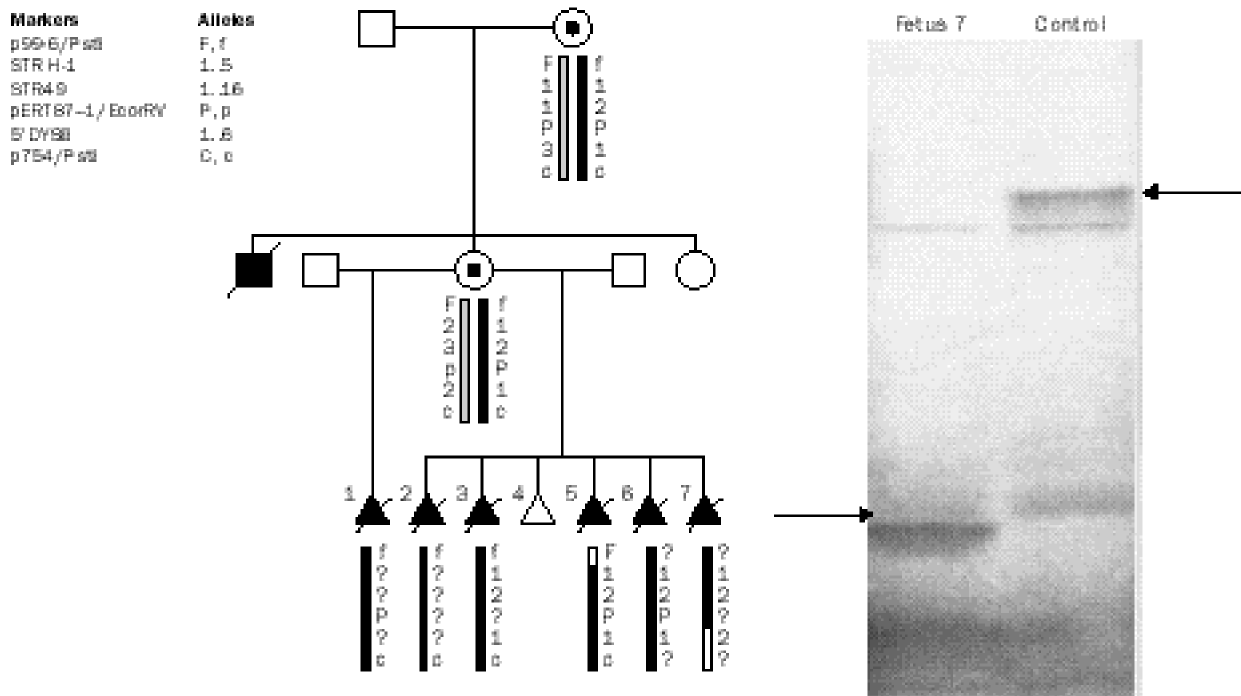
In some requests for prenatal diagnosis of neuromuscular disorders, such as Duchenne muscular dystrophy (DMD), the analysis yields an unsatisfactory risk estimate and in some cases a conclusive answer is not possible at all. Such results derive from the fact that the actual mutation is unknown and thus indirect tests have to be performed. These tests are problematic when no patient material is available or when recombinations hamper haplotype analysis.

MyoD-induced myogenesis allows the study of muscle proteins in non-muscle cells[1,2] and we have successfully applied this method for diagnosis of difficult cases of DMD.[2] Until now, however, forced myogenesis has been done using a retroviral vector, leading to a very low transduction efficiency of slow-growing cells—such as human skin fibroblasts, amniocytes, or chorionic villi cells—and thus requiring a selection step lengthening the procedure to 4–6 weeks. This has so far precluded application of forced myogenesis in prenatal diagnosis. We have now tested an adenoviral myoD-vector[3] on some 15 samples of different origin (fibroblasts, amniocytes, and chorionic villi cells). Transduction efficiency was up to 95% of the cells, with chorionic villi cells transducing best. Moreover, in a short time, myo-differentiation of a large fraction of the cells was achieved; 50–80% of the cells expressed desmin and 1–25% dystrophin (using the retroviral system these figures were optimally 25% and 1% respectively). Transduction efficiency and myogenic differentiation towards dystrophin-positive staining varied between samples but was sufficient to draw conclusions. False-negative results, no dystrophin staining due to unsuccessful myo-differentiation, were excluded by staining against other proteins expressed late during myodifferentation, namely titin or embryonic myosin. Furthermore, conclusions were verified by a mutation scan at the RNA-level.

Because the overall time needed for the analysis could be reduced to two weeks, we were able to apply the adenoviral system in a prenatal diagnosis of an exceptional DMD family (figure). In this Belgian family, the proband was deceased, the risk haplotype was unknown, and no mutation had been found. The first two pregnancies were terminated because the male fetuses carried the grand maternal haplotype. Dystrophin detection was done on a muscle biopsy taken from the aborted second fetus. No dystrophin could be detected, and so the affected haplotype was identified and confirmed the carrier status of the mother. The third, fifth, and sixth pregnancies were again male, carrying the affected haplotype, the fourth pregnancy ended in a miscarriage. A request for prenatal diagnosis came in for the seventh fetus. The fetus was male and haplotype analysis revealed a recombination in the gene (between markers 5'-DYSII and STR49). Since the mutation was still unknown, the risk status could not be determined. In an attempt to resolve this complex case, we did a forced myogenesis on chorionic villi cells. Dystrophin staining using central rod antibody MANDYS109[4] was negative, whereas other muscle-differentiation markers were positive (desmin and titin). We concluded that the fetus was affected and the parents opted once again for abortion. To verify the affected status, the raised dystrophin expression[2] was used as a tool to do RNA-based mutation analysis. Total RNA was isolated from the differentiated cells and a protein truncation test[5] showed a translation-terminating mutation (figure) in exon 54, a 4 bp AAAT-duplication (8116^8117ins8113–8116). The



Prenatal diagnosis by forced myogenesis in a DMD family

Haplotypes known are indicated (black=chromosome carrying the mutation). On the right is the analysis of dystrophin cDNA fragment 4EF[2] by the protein truncation test, showing a mutation causing premature translation termination. The truncated (left) and normal (right) translation products are shown by arrows. Sequence analysis showed a 4 bp duplication of bases 8113–8116 (AAAT).

mutation was confirmed on genomic DNA, which provides a direct and 100% conclusive test for future pregnancies in this family.

The use of an adenoviral myoD-vector greatly improved forced myogenesis; it increased the transduction efficacy, raised the number of myo-differentiated cells, and reduced the time needed for the analysis to 2 weeks. The system provides a direct functional test and improves the prospects to clarify hitherto unresolvable diagnostic cases of DMD and other neuromuscular disorders. The improvements obtained now facilitate application of forced myogenesis in prenatal diagnosis.

1 Sancho S, Mongini T, Tanji K, et al. Analysis of dystrophin expressison after activation of myogenesis in amniocytes, chorionic-villus cells, and fibroblasts. *N Engl J Med* 1993; **329:** 915–20.

2 Roest PAM, Van Der Tuijn AC, Ginjaar HB, et al. Application of in vitro myo-differentiation of non-nuscle cells to enhance gene expression and facilitate analysis of muscle proteins. *Neuromusc Disord* 1996; **6:** 195–202.

3 Murry CE, Kay MA, Bartosek T, Hauschka SD, Schwartz SM. Muscle differentiation during repair of myocardial necrosis in rats via gene transfer with MyoD. *J Clin Invest* 1996; **98:** 2209–17.

4 Thi Man N, Morris GE. Use of epitope libraries to identify exon-specific monoclonal antibodies for characterization of altered dystrophin in muscular dystrophy. *Am J Hum Genet* 1993; **52:** 1057–66.

5 Roest PAM, Roberts RG, Sugino S, Van Ommen GJB, DenDunnen JT. Protein truncation test (PTT) for rapid detection of translation-terminating mutations. *Hum Mol Genet* 1993; **2:** 1719–21.

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Brother and sister with different vasculitides

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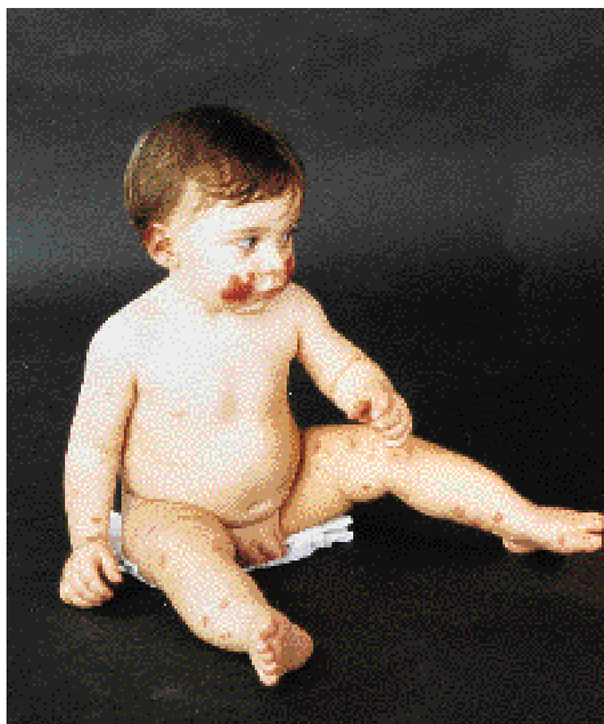
A brother and sister came to our observation at the ages of 3·6 years and 16 months. 20 days before admission, both had pharyngitis treated with amoxicillin for 10 days. 10 days before admission, niflumic acid was given to the girl for rhinitis. 2 days later, a purpuric rash appeared on her legs and she had abdominal pain. Henoch-Schönlein purpura (HSP) was diagnosed. Three days later, her brother had a cough and rhinitis and was given ketoprofen. The next day he developed a widespread rash (figure) consistent with the diagnosis of acute haemorrhagic oedema of childhood (AHE). Neither joint effusions nor abdominal symptoms were present. White blood cell and platelet counts, complement concentrations, anti-ASO titre, ANA, and ANCA, were normal in both children. Culture of pharyngeal exudate and faecal occult blood were also normal. Urinalyses were negative. IgM and IgG antibodies to influenza (A, B subtypes) and parainfluenzae viruses, adenovirus, *Myocoplasma pneumoniae*, cytomegalovirus, coxsackie, echovirus, Epstein Barr virus, parvovirus, and herpes simplex 6 were negative in both children. Cutaneous manifestation spontaneously disappeared in 2 weeks in the boy and in 3 weeks in the girl.

AHE (or Finkelstein's disease) is a leukocytoclastic vasculitis reported in children under 24 months of age. Despite its dramatic onset, the course of the disease is benign. Although AHE was described in *JAMA* in 1913,[1] this entity has been forgotten by the English-language



Acute haemorrhagic oedema of childhood

literature for almost 80 years, and its further description has been left to non-English literature.[2] English-language authors began to consider AHE as a variant HSP as classically reported by Allen.[3] However, during the past decade, AHE has appeared in a growing number of English-language reports[4] which challenge the unifying concept, and clinical criteria for AHE have been proposed.[5] Moreover, the greater deposition of IgM than IgA immune complexes at direct immunofluorescence study of skin biopsies of patients with AHE has differentiated AHE from HSP.[2,4]

Our report of simultaneous appearance of HSP and AHE in siblings seems to support the idea that they are variants of the same clinical entity. Whatever the triggering agent, it is likely that both siblings had a common immunopathological response, characterised by a different phenotype because of their different ages and stage of maturation of their immune systems. It seems improbable that the different phenotypes observed were related to the different non-steroidal anti-inflammatory agents given. Skin biopsies were not done on these children for understandable reasons.

We suggest the debate on the putative distinction between AHE and HSP returns to 1960: "Thus this disease (HSP) in younger children is milder, of shorter duration with fewer recurrences, and with significantly fewer renal and gastrointestinal manifestations".[3]

1 Show IM. Purpura urticaria and angioneurotic edema of the hands and feet in a nursing baby. *JAMA* 1913; **61:** 18–19.

2 Legrain V, Lejean S, Taieb A, Guillard JM, Battin J, Maleville J. Infantile acute hemorrhagic edema of the skin: study of ten cases. *J Am Acad Dermatol* 1991; **24:** 17–22.

3 Allen DM, Diamond LK, Howell DA, Anaphylactoid purpura in children. *Am J Dis Child* 1960; **99:** 833–54.

4 Gattorno M, Picco P, Gambini C, et al. Erythema multiforme-like manifestations and arthritis in a 3-year-old child with leukocytoclastic vasculitis. *Clin Exp Rhem* 1997; **15:** 329–32.

5 Krause I, Lazarov A, Rachmel A, et al. Acute haemorrhagic oedema of infancy, a benign variant of leucocytoclastic vasculitis. *Acta Pediatr* 1996; **85:** 114–17.

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