MEETING REPORT
INTERNATIONAL SMA CONSORTIUM MEETING

(26–28 June 1992, Bonn, Germany)

The fourth meeting of the International SMA Consortium was held in Bonn, Germany on 26–28 June 1992, under the auspices of ENMC. The meeting was attended by most of the major groups actively involved with the quest for the SMA gene. Several important advances were made: (1) all the laboratories attending the meeting signed an agreement of collaboration which defined authorship on publications, operational procedures, exchange of DNA between groups, availability of probes and the collaborative use of YAC DNA; (2) the diagnostic criteria for SMA were further defined and extensive discussion of SMA variants was carried out as described below; (3) preliminary discussions were carried out regarding the advisability and form of a uniform clinical database; (4) there was strong unanimous support for exploring the organization and implementation of therapeutic trials which will be the primary subjects for discussion at the next meeting to be held in Boston.

REPORT OF CLINICAL DATABASE DEVELOPMENT (KLAUS ZERRES)

The main object of this session was to discuss the value of a standardized clinical database in SMA. It was generally accepted that detailed documentation of the clinical features in combination with the results of diagnostic investigations is worthwhile for future genotype-phenotype studies and as a basis for the first therapeutic trials. Furthermore, a documentation system was regarded as very useful for the inclusion and exclusion of patients in DNA studies, as members of the Consortium agreed that many DNA samples would have been excluded from linkage studies if the clinical picture had been better defined.

The documentation system of the German study on classification and genetics of the spinal muscular atrophies, was introduced by S. Rudnik and K. Zerres. The case report forms were provided. Suggestions for a common database combining diagnostic, clinical and pedigree information of the different study groups were made.

J. M. Cobben presented preliminary results and diagnostic documentation of the Dutch SMA study which is designed for DNA studies. A central point of discussion was the problem of diagnostic evaluation and how to deal with insufficient data.

The closing discussion documented a considerable demand for a collaborative clinical documentation system.

REPORT ON GENETIC MAPPING (KAY E. DAVIES)

This meeting was to prove a landmark in the search for the SMA gene. Progress on markers for prenatal prediction was described and a consortium was formed to accelerate the cloning of the gene sequence. All groups presenting linkage data agreed that the most probable location for the SMA gene was between MAPIB and D5S125 a genetic distance of 2–4 cM. Microsatellites are available for both of these loci, thus providing a rapid PCR based test for the clinical geneticists. All the recombinant families in the world were presented with their typings. Incredibly, all the data suggested a single locus indicating that SMA is probably genetically homogeneous if the strict clinical criteria are adhered to. It is credit to the clinical collaborators that the molecular geneticists should have been able to refine the position of the gene to 1–3 Mb so rapidly.

A consortium was formed between the investigators to exchange DNA from the recombinant families. This will greatly accelerate the identification of the mutant sequence and made this meeting particularly memorable.

The somatic cell hybrid panel from John
MacPherson was still very helpful in ordering new markers relative to each other. Gilliam's group described the isolation of a new marker, JK348, which maps between DSS125 and MAP1B and hence in the candidate region. No evidence of linkage disequilibrium has yet been observed for any of the loci.

The genetic data localize the gene within a 1–3 cM region. The estimate of a physical distance of 2–3 Mb from fluorescent in situ hybridization (FISH) and pulsed field gel studies would be consistent with this.

The linkage data mapping all three forms of SMA were reviewed. It was agreed that the most probable location of the SMA gene was between DSS6 and DSS112 and that this information could be used for prenatal diagnosis. It was proposed that recombinant families should be identified in each of the main research centres so that these might be exchanged at a later date. The difficulties raised by the possibility of heterogeneity were discussed. The groups had differing views on this: Gilliam's group had evidence for heterogeneity, whereas this had not yet been confirmed in other families. This question may well remain unresolved until the cloning of the gene.

The mapping of MAP1B in the region close to DSS115 provided the exciting possibility that this was the mutant locus. However, the linkage analysis did not support this, but supported a rather more proximal location. However, microsatellites developed for this locus, in addition to those developed at DSS115, will provide excellent markers for prenatal diagnosis.

Mapping data using pulsed field gel electrophoresis and in situ hybridization suggest that the region between DSS6 and DSS112 might be several megabases. The isolation of new markers to provide start points for YAC walking was therefore desirable.

John MacPherson presented his reduced hybrids which covered the candidate region of chromosome 5. He volunteered to map any new sequence in this mapping panel so that sequences from the different laboratories could be directly compared. The hybrid map was consistent with 3–4 Mb between DSS6 and DSS112.

The general feeling was that progress had been reasonably rapid since the localization of the gene. Techniques such as Alu–PCR in reduced hybrids, coincident cloning and microdissection were discussed as possible fruitful strategies to isolate closer markers in the candidate region.

REPORT OF PHYSICAL MAPPING (BRUNHILDE WIRTH)

John MacPherson presented an irradiation hybrid map on which he put all the probes around the SMA gene available at the moment. On these results there was no resolution possible between the loci MAP1B and DSS112. It was possible to localize the new probe JK348 from Gilliam et al. between MAP1B and DSS125 which seems to be concordant with the results from genetic linkage studies.

Gilliam et al. isolated YACs from the CEPH YAC library with JK348 and MAP1B but they did not get an overlap between these YACs. They estimated the distance between these loci of at least 1.0–1.25 Mb, based on PFGE maps with KspI.

Wirth et al. isolated 13 YACs (ICRF–YAC library) from the region around the SMA gene by using the markers pM4, EF(TG/AG) n, JK53 and p105–153. These YACs are covering at least 2.6 Mb of the region. The MAP1B gene, DSS112 and JK53CA1/2 are all included in five YACs, not further than 350 kb from each other. The loci DSS125 and DSS39 are also included in the same YAC not further than 350 kb from each other.

Melki et al. screened the CEPH- and MEGABASE-YAC library and they got seven YACs with EF (TG/AG) and 114ye7 (the largest one of 1800 kb), which are overlapping in part. They do not yet know the orientation of these two probes and they did not get any recombinant on linkage studies.

Speer et al. presented a PFGE map by using full and partial digests with SfiI. No common fragments with JK53 and an end fragment of a YAC isolated with EF (TG/A) G were detected. On the partial restricted PFGE map a common band between these markers seems to light up at about 2.4 Mb.

K. E. Davies presented results from their in situ hybridization studies on high resolution spread chromosomes. They estimated the distance between DSS6 and DSS125 2–3X to be larger than that between DSS125 and DSS112.

"VARIANTS" OF INFANTILE SMA (JAAKKO IGNATIUS)

Several patients have been described in the literature designated as infantile SMA, but with associated “atypical features” such as cerebellar
hypoplasia, pontocerebellar or cerebellotralamospinal degeneration, multiple long bone fractures at birth, diaphragmatic paralysis with early respiratory failure and congenital heart defects. Most of these patients also had arthrogryposis. Opinions exist as to whether these patients represent separate clinical entities different from SMA5q. A literature review of these cases was presented followed by additional case reports by Consortium members. The discussions of these cases are summarized as follows.

**Anterior horn cell disease with pontocerebellar hypoplasia**

This condition might be mistaken for SMA I because of the profound flaccidity at birth, tongue fasciculations and findings consistent with anterior horn cell drop-out in EMG and muscle biopsy. However, unlike severe SMA, these infants are not alert, there may be signs of upper motor neuron involvement (brisk reflexes, jerky eye movements, pathological EEG), multiple joint contractures are frequent, and at post-mortem there is cerebellar atrophy and involvement of the pons, medulla and spinal cord. Although this condition may be heterogeneous, evidence suggests that this is a separate autosomal recessive disorder. Based on linkage analysis data obtained in one family (presented by V. Dubowitz) this condition was not linked to 5q.

**Anterior horn cell disease with congenital fractures**

This entity (OMIM No. 271225) is characterized by multiple congenital, metaphyseal or epiphysial long bone fractures associated with large joint and digital contractures. It may include two separate conditions. In some families polyhydramanios, intrauterine growth retardation (IUGR), hypomineralized bones, and dysmorphic features are present. The pedigrees are consistent with autosomal recessive (AR) inheritance. In some families the pregnancy is uneventful, birth weight is normal and dysmorphic features are not prominent. Pedigrees consistent with X-linked recessive inheritance have been published. The clinical pattern appears consistent among affected sibs which suggests that this is a separate entity. Linkage analysis has been performed in one family [1]. This disorder also appears to be unlinked to 5q.

**Anterior horn cell disease with early respiratory insufficiency**

The presenting symptom is acute respiratory distress at birth or during the first weeks of life. Generalized muscle weakness may not be evident before disease onset. Distal joint contractures are common. The distribution of muscle weakness may be atypical (often bilateral wrist drop). Eventration or abnormal motion of the diaphragm is seen by X-ray/fluoroscopy. Findings in EMG, muscle biopsy and post-mortem have been similar to SMA. Only a few familial cases have been reported, but the clinical pattern appears consistent among sibs, and it is likely that this condition is hereditary. No DNA studies have been reported. A sibship with early respiratory insufficiency was presented by G. Morgan.

**Anterior horn cell disease with congenital heart defects (S. Rudnik)**

Congenital heart defects are common. They occur in 1% of births. Among these septal defects are the most common. The incidence of VSDIS 2.5–5/1000 and ASD 1/1000 live births. Thus a child presenting with both SMA and heart defect may represent a coincidence. Congenital heart defect (most often ASD) associated with SMA has been proposed as a distinct entity. This association has been observed among sibs. If carefully examined, however, these patients appear to have additional atypical features such as arthrogryposis, respiratory distress, bone fractures and at post-mortem arrhinencephaly or partial corpus callosum hypoplasia. Thus some may actually belong with entities described above. A heart defect associated with SMA should prompt a search for additional atypical clinical features. On the other hand, cardiac investigation should be done in SMA patients to clarify this entity. Linkage analysis has only been performed in one family (J. Melki) and no recombinants were found.

**Anterior horn cell disease associated with arthrogryposis**

When carefully studied, most patients described in the literature as having “SMA and arthrogryposis” appear to represent entities described above. Arthrogryposis associated with anterior horn cell disease but without other organ pathology seems to be very rare. These cases have almost invariably been
sporadic. In some patients the disease has not been progressive and these may represent neurogenic arthrogryposis multiplex. Arthrogryposis as an exclusion criterion is justified at present, especially for linkage studies and prenatal testing.

**SMA of non-recessive inheritance**

At present, there is no good evidence for X-chromosomal inheritance in childhood SMA. The patients described in the literature as X-linked severe SMA also had arthrogryposis and bone fractures. Several patients suffering from mild X-linked SMA (“SMA of adolescent onset with hypertrophied calf muscles” etc.) have later been re-examined and defined as Becker muscular dystrophy.

Autosomal dominant inheritance cannot be excluded in some families with childhood-onset SMA. Based on segregation analysis data, some patients classified as SMA II or SMA III may represent new dominant mutations. There are also some rare pedigrees (e.g. those presented by K. Zerres) where the child born to a SMA patient (without a family history of the disease) has also been affected. Clinically, these patients are indistinguishable from those suffering from the autosomal recessive SMA5q. This possibility—as well as the proposed genetic complexity at the SMA locus—has important implications for prenatal diagnosis particularly as regards to the milder forms of SMA.

**Differential diagnosis of SMA**

Several congenital myopathies may mimic SMA (reviewed by V. Dubowitz). However, SMA infants have very weak intercostals associated with relative sparing of the diaphragm. As a result they have the highly characteristic thorax deformity and “abdominal type of breathing which often allows diagnosis. In many myopathies there is also facial weakness. Muscle biopsy is an important tool to differentiate these conditions. Congenital hypomyelination neuropathy may also mimic early onset severe SMA. This entity is rare, and most cases described have been sporadic. The muscle biopsy may show grouped atrophy like SMA. The differential diagnosis is based on nerve conduction velocities which are extremely slow (<10 m s⁻¹). Hexosaminidase efficiency may rarely produce a clinical picture resembling juvenile SMA. The adult type of hexosaminidase A deficiency is very rare. Most patients have been of Ashkenazi Jewish origin. Signs of cerebellar dysfunction (particularly speech difficulties) are highly characteristic for this disorder, and in those cases with onset before 10 yr of age, dysarthria has been invariably present. Cerebellar symptoms may appear years after clinical onset. The level of hex A in serum and most tissues of these patients is very low.

**THERAPEUTIC STRATEGIES (ARTHUR BURGUES)**

The therapy session covered two topics: cell transplantation of motor neurons into the spinal cord and delivery of genes to motor neurons. K. Sieradzan presented data showing the feasibility of transplanting motor neuron cells into the spinal cord. She demonstrated that neurons grew out of the spinal cord and innervated muscle. A. Burghes spoke on the injection of plasmids into the sciatic nerve. He showed that gene constructs can be delivered to motor neurons via retrograde axonal transport. It may be possible to increase the efficiency of the system through the use of virus vectors in the near future. The session ended with a discussion concerning the neurotrophic growth factor CNTF. This has recently been shown to benefit mouse models of motor neuron disease and trials are beginning in amyotrophic lateral sclerosis in humans. A debate ensued regarding how to measure a beneficial response. It was concluded that a future meeting to define these parameters would be useful.

**PRENATAL TESTING (T. C. GILLIAM)**

Recent linkage of SMA to polymorphic markers on chromosome 5q has allowed the introduction of prenatal diagnosis. During the past year approximately 112 families were admitted to a protocol. All families were screened to determine whether or not they met the international criteria for the diagnosis of SMA (T. Munsat). DNA testing was carried out by Integrated Genetics of Framingham, MA (B. Handelin). Of the 112 families submitted, 102 had complete medical information available. Seventy five of the families were confirmed as SMA type I and seven confirmed as SMA type II. In 20 families (19.6%) the diagnosis could not be confirmed. Seven had arthrogryposis multiplex, three arthrogryposis multiplex with other dysmorphic features, four did not have a
biopsy or autopsy and six had atypical clinical courses. Of the 112 families, 59 were found to be informative, 20 did not have the diagnosis confirmed and 14 samples are pending. Of the 56 informative families, 26 prenatal samples were available from 24 families. Analysis revealed five affected, nine carriers, four non-carriers, three recombinations and the others pending. Of the 59 families, 21 have gone on to prenatal diagnosis. The early demise of many affected individuals necessitated alternative sources of DNA when blood was not available. Thus, of the 83 samples submitted from affected individuals, only 23 (31.3%) were blood. The remainder included stored DNA (23), frozen cell lines (8) and tissue from muscle biopsies or autopsies (26). To date 59 families have completed informativeness testing with 5 polymorphic markers. Seventy seven percent of parents were found to be informative for flanking markers and of these prenatal diagnosis was carried out by CVS (18) and amniocentesis (3) which has led to results of 5 affected, 8 carriers and 4 non-carrier predictions. In addition, four recombination events involving the region of the SMA gene were observed at prenatal diagnosis which prevented a definitive prediction. One case of recombination led to a prediction of either carrier or noncarrier. The accuracy of prenatal prediction, not involving a recombination was calculated to be 88–99%. This was based on a conservative estimate of 10% incidence of non-allelic genetic heterogeneity.

**Muscle weakness**

I. Muscle weakness of the trunk and limbs (proximal limb muscles more than distal; lower limbs weaker than upper limbs).

I. Symmetrical.

E. Weakness of extra-ocular muscles, diaphragm and the myocardium, with marked facial weakness.

C. Wasting is often not conspicuous in SMA type I since there is a tendency for compensatory proliferation of subcutaneous tissues.

**Other associated features**

I. Fasciculations and tremor.

C. Fasciculations of the tongue are not a striking feature in SMA type I, but are observed in 70% of the patients with SMA type II.

Fasciculations of the limbs are apparent in about half of the patients with SMA type III.

Tremor of the hands is frequently observed in SMA types II and III.

E. Sensory disturbances.

C. In SMA type I, there may be some limitation of abduction of the hips or of full extension of the knees or elbows.

E. Involvement of other neurologic systems or organs, i.e. hearing or vision.

**Course**

I. In SMA type I and II there is an arrest of development of motor milestone:

- children with SMA type I are never able to sit without support;
- children with SMA type II are unable to stand or walk without aid;
- patients with SMA type III have developed the ability to stand and walk.

I. Course.

In SMA type I death is usually <2 yr of age.

In SMA type II death is usually above the age of 2 yr.

In SMA type III death is in adulthood.

C. These criteria are arbitrary and proposed with the full understanding that there will be certain patients who do not clearly fit any one category.
Ancillary Investigations

Biochemistry

E. Serum CK activity > 10 × the upper limit of normal.
E. Dystrophin deficiency.
E. Hexosaminidase deficiency.
C. Biochemical analysis of dystrophin or hexosaminidase should only be considered if other tests such as serum CK activity or muscle biopsy are equivocal.

Electrophysiology

I. • Abnormal spontaneous activity, i.e. fibrillations, positive sharp waves, fasciculations;
• increased mean duration and amplitude of motor unit action potentials.
E. Reduction of motor nerve conduction velocities < 70% of lower limit of normal.
E. Abnormal sensory nerve action potentials.

Histopathology

I. • Groups of atrophic fibres of both types;
• hypertrophic fibres of one type, usually type I;
• type grouping (chronic cases).
C. If a biopsy is nondiagnostic and the clinical picture is characteristic, rebiopsy should be considered.

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