INTRODUCTION

The term spinal muscular atrophy embraces a heterogeneous group of hereditary neuromuscular disorders caused by the loss of the lower motoneurones, leading to progressive muscle weakness and atrophy. The most common of these disorders is infantile and juvenile proximal spinal muscular atrophy (SMA), which has an incidence of 1/10,000 newborns and a carrier frequency of 1/50 individuals (1,2). Spinal muscular atrophy is the second most common fatal autosomal recessive disorder of children after cystic fibrosis. The disease is usually subdivided into three forms - 1, 2 and 3 - on the basis of age at onset and achieved milestones (3).

Before the era of molecular genetics, the diagnosis of SMA was based on clinical features, muscle biopsy and electrophysiological findings (4). In 1990, all three forms of SMA were mapped to band q 11.2-13.3 of chromosome 5, suggesting that they are allelic disorders (5,6). The next step was the identification of an SMA determining gene – \textit{SMN} (\textit{survival of motor neurone}) gene – existing in two copies (telomeric and centromeric) (7,8). About 95% of SMA patients show homozygous absence (deletion or gene conversion) of exon 7 or 7 and 8 of telomeric copies of the \textit{SMN}
gene, while the rest carry small intragenic mutations (9). These findings have led to greatly improved genetic counselling of SMA families.

CLINICAL ASPECTS

Spinal muscular atrophy presents a very variable picture as regards clinical severity and range of age at onset. The most common features are symmetrical muscle weakness and atrophy, hypotonia, finger tremor, absence of tendon reflexes, and sparing of the diaphragm, myocardium and extraocular muscles.

Childhood SMA is classified as follows:
– SMA 1 (severe form, Werdnig-Hoffmann disease); onset from birth to 6 months; children are never able to sit without support; survival up to 2-4 years;
– SMA 2 (intermediate form); onset before the age of 18 months; children are unable to stand without aid; 90% survive up to 10 years;
– SMA 3 (mild form, Kugelberg-Welander disease), onset after the age of 18 months; children develop the ability to stand and walk; normal life expectancy. This group is divided into 3a (onset < 3 years of age, becoming wheelchair-bound after 5-7 years from onset) and 3b (onset >3 years, maintaining ambulation over many years).

MOLECULAR PATHOGENESIS

As already mentioned, all three forms of the SMA have been mapped by linkage analysis to chromosome 5q11.2-13.3. Further characterisation of the SMA locus showed a chromosomal region (SMA critical region) that contains two inverted elements (500kb) (Fig. 1). Four duplicated genes have been identified in the SMA critical region: the SMN gene (7), the NAIP (neural apoptosis inhibitory protein) gene (10), the p44 (which encodes the subunit p44 of the transcriptional factor TFIH) gene (11) and the H4F5 gene (12). The SMA region also contains numerous repeated sequences, pseudogenes and retrotransposons, making this region unstable.

The SMN gene exists in two copies: telomeric (SMNtel, SMN1) and centromeric (SMNcen, SMN2). Both copies are composed of nine exons, encoding identical amino acid sequences. They differ in their exons by only two base pairs, one in exon 7 and one in exon 8 (7). Alternative splicing has been shown to occur in both copies. About 90% of the SMNtel transcripts are full length, the rest are missing exon 5. In contrast, only 20-30% of the centromeric copy-produced transcripts are full length, the remaining ones lack exon 5, 7 or both exons 5 and 7 (13). The total amount of full length transcripts and the

Fig. 1 - Genes of SMA region (5q13).
level of SMN protein in SMA patients depends on the \textit{SMN}^cen copy number (14).

About 95\% of SMA patients carry a homozygous deletion of exon 7 or 7 and 8 of the telomeric copy of the \textit{SMN} gene, the rest (2-5\%) show small intragenic mutations (7,15). Thirteen small intragenic mutations have been identified to date, most often in exons 6 and 7. Patients showing homozygous deletion of exon 7, but not exon 8, probably lose exon 7 in a conversion event, in which \textit{SMN}^tel is partly replaced by \textit{SMN}^cen. The conversion event seems to be associated with the mild phenotype, whereas deletion of the telomeric \textit{SMN} copy predominates in the severe form of the disease.

The absence of the centromeric copy is found in about 5\% of normal individuals. Interestingly, the homozygous deletion of exon 7 \textit{SMN}^cen was found more often (36\%) in adult-onset motor neuron disease than in the general population (16).

The rest of the SMA region genes probably modify the clinical severity of the disease. The \textit{H4F5} gene is situated very close to \textit{SMN}^tel and is deleted in over 90\% of SMA 1 patients (12). Therefore, it seems to be a good candidate as a modifier of the clinical severity. Two other genes (\textit{NAIP, p44}) do not seem to be critical in SMA pathogenesis, although they are deleted more frequently in the severe forms.

There is a significant correlation between \textit{SMN}^cen copy number and SMA phenotype.

A lower \textit{SMN}^cen copy number (1-3 copies) is usually connected with the severe phenotype (SMA 1), whereas few \textit{SMN}^cen copies result in the milder form of the disease (SMA 3) (17).

The SMA critical region contains large duplicated fragments, and also numerous repeated sequences, pseudogenes and retrotransposones. This dynamic character means that it is prone to rearrangements between the highly homologous elements, which result in duplications, deletions and gene conversions. Gene conversion is considered to be an event explaining the lack of exon 7, but not exon 8, of the \textit{SMN}^tel gene. The homozygous absence of the exon 7 \textit{SMN}^tel alone has been reported in 3\% to 28\% of SMA patients (7-9). In this mechanism, part of the centromeric \textit{SMN} gene is copied into its telomeric counterpart, resulting in a hybrid \textit{SMN} gene (18). The same gene conversion would result in an increased number of \textit{SMN}^cen copies and modify SMA phenotype. According to some reports, this phenomenon was only found in SMA 2 and 3 patients (19).

The level of SMN protein correlates with the clinical severity of the disease. The amount of SMN protein is dramatically reduced in SMA 1 (down to 5-20\%), whereas SMA 2 and 3 patients show less decreased (26-82\%) or normal SMN protein levels (14). This correlation, however, has proved to be much more complicated than thought, depending on many factors.

### PROTEINS

Both SMN copies are expressed in the active protein, (which has a molecular weight of 38 kD, and consists of 294 amino acids). The protein shows no homology with any other known protein. It is expressed at high levels in brain, kidney and liver, at moderate levels in skeletal and cardiac muscles and at low levels in lymphocytes and fibroblasts. The expression of SMN protein undergoes a marked decay during the postnatal period when compared to foetal development, suggesting that it plays an important role in intrauterine life.

The SMN protein is detectable in the cytosol and particularly in the nucleus. It is complexed with Sm proteins, SIP1 (SMN interacting protein 1) and many others (18,20,21), such as gemine 3, 4, and it probably controls snRNP biogenesis (18).

Interactions of SMN protein with RNA-binding protein and with anti-apoptotic protein Bcl-2 have been demonstrated, pointing to an anti-apoptotic role of SMN. It has also been shown that the SMN protein was associated and colocalised in the cytoplasm and in gems (see below) with a novel SMN-interactive protein 1. The SMN-interactive protein 1 complex is involved in the cytoplasmic assembly of small nuclear ri-
bonucleoproteins and in the nuclear import of the small nuclear ribonucleoprotein complex. These facts provide evidence of a likely role of the SMN protein in the metabolism of RNA.

SMN proteins target the nucleus in newly identified structures called “gems” (for gemini—twins of coiled bodies – the structure described by Cajal in 1903 as nucleolar accessory bodies). Gems are associated with coiled bodies and are probably related to RNA metabolism (21,22). Gems are absent in SMA patients, coiled bodies are present (23).

At the molecular level SMA can be regarded as a disorder resulting from an abnormal RNA metabolism connected with defect of SMN nuclear targeting and failure of correct gene expression in motoneurons and/or muscles.

Important data on the pathogenesis of SMA came from transgenic mice – the animal model of this disease.

One of existing models is that of Melki (24), which is closest to the intermediate type of SMA. Melki tried to define the primary target of the disease – motoneurons or muscle. At the onset of disease, she observed a normal number of motoneurons, some changes in muscle morphology and, later, motoneuron degeneration. She interpreted these findings (with use of neuronal enolase) as motoneuron primary affection, however there is some doubt over the possibility of a primary role for muscle in these events, a doubt shared, for example, by Braun et al. (25), Gendron and MacKenzie (26) and ourselves (27,28).

THE PENDING CLINICAL QUESTIONS

The clinician looking forward to new achievements in science is interested in understanding the correlation between phenotype and genotype. This is quite a complex problem in SMA where the genotypes are practically identical and phenotypes are very variable. The size of deletion, the number of copies, and the conversion event explain some, but not all, cases of intrafamilial variability and even the protein dosage effect is sometimes not sufficient to provide an explanation. There is evidence, for example, that intrafamilial variability is expressed in 30% of families regardless of the fact that all the affected members share an identical genotype. This means that other factors are involved in defining phenotype (29) – possibly other genes (discovered or still unknown) or environmental factors.

In the premolecular era the abnormalities characteristic of SMA were thought to be signs of immaturity, or of delayed maturation resulting from disturbed nerve-muscle interaction during development (28,30,31).

The molecular findings could probably explain immaturity confirmed by the experiments of Vrbova (32).

Clinicians are also interested in gender influence in SMA, which was reported by us (33) and confirmed by others (34). Novelli et al. (35) recently described a strong association between NAIP absence and severity of phenotype in SMA girls, but not in boys.

We hypothesised that predominance of males, particularly expressed in form 3b could be explained by a protective role of female hormones – but this has not been demonstrated to date. From the viewpoint of genetic counselling, it would be important to study this problem more carefully.

Finally, the goal of all investigations in the coming years will be to find some therapeutic means. The search can be based on events such as conversion or on promoting nuclear targeting of mutant SMN. We, in 2000, reached the following conclusion: “The question arose, why SMN2 was unable to fully compensate the loss of SMN1. The answer was provided only recently by Lorson et al. (36) who found that it was due to one nucleotide exchange – C → T at codon 280 – a change which dictates exon 7 alternative splicing and production of SMN2-defective mRNA which lacks exon 7. This was a very important step forward in the elucidation of SMA pathogenesis and a finding which according to the authors may be helpful in designing a gene therapy targeted at the repair of the SMN2 gene transcript” (37).
ADDENDUM

We would like to add, for illustrative purposes, some information on characteristics of SMA patients in Poland.

Since 1960, we have observed 1,017 SMA patients (Table I). The clinical diagnosis was genetically verified in 335 of them.

The genetically verified group was subdivided (males and females separately) into three forms: 1, 2, 3 (3a and 3b) on the basis of achieved milestones and age at onset (Table II). The frequency of SMA 1, 2, 3 was 40%, 20% and 40%, respectively. Interestingly, a predominance of males (about 60%) was observed in all three types, reaching 71% in SMA 3b.

The age at onset is very variable in our patients. The symptoms of the disease were observed just after birth in about 25%. In a few cases mothers noticed the cessation of previously active foetal movements. Our oldest patient became ill at the age of 34. In different forms of SMA (1, 2 and 3) absence of at least exon 7 of the telomeric SMN gene was detected in 92.2%, 98.5% and 90% respectively.

Generally, about 30% of affected sibships present varying disease severity, so individual cases could be of different clinical types. We observed phenotypic variability in 16 out of 47 affected sibships (usually coexistence of type 2 and 3).

We analysed the deletion size of the SMA region in 160 patients. Deletions of exons 7 and 8 of the telomeric SMN gene were detected in 63% of these SMA patients (Table III).

The absence of only exon 7 was found in more than 17% of the examined patients. Different frequency (3% - 28%) for only 7 exon mutation has been reported, depending on the ethnic origin. Interestingly, we recently analysed a group of SMA patients (mainly SMA 2 and 3) from north-west Poland and they presented only with mutations of both 7 and 8 exons.

The deletions of exons 5 and 6 of the NAIP gene were detected in ~20% (34% in SMA 1 and 8% in SMA 2) (Table III). None of the SMA 3 patients reveal a deletion in the NAIP gene.

We also analysed a group of 143 SMA relatives (parents or healthy sibs) and failed to find any asymptomatic individual with a homozygous deletion.
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