De Novo Rearrangements Found in 2% of Index Patients with Spinal Muscular Atrophy: Mutational Mechanisms, Parental Origin, Mutation Rate, and Implications for Genetic Counseling

Brunhilde Wirth,[1] Thorsten Schmidt,[1] Eric Hahnen,[1] Sabine Rudnik-Schöneborn,[1] Michael Krawczak,[2] Bertram Müller-Myhsok,[3] Jutta Schönling[1], and Klaus Zerres[1]

[1]Institute of Human Genetics, Bonn; [2]Institute of Medical Genetics, University of Wales College of Medicine, Heath Park, Cardiff; and [3]Bernhard Nocht Institute for Tropical Medicine, Hamburg

Summary

Spinal muscular atrophy (SMA) is a relatively common autosomal recessive neuromuscular disorder. We have identified de novo rearrangements in 7 (~2%) index patients from 340 informative SMA families. In each, the rearrangements resulted in the absence of the telomeric copy of the survival motor neuron (SMN) gene (telSMN), in two cases accompanied by the loss of the neuronal apoptosis–inhibitory protein gene . Haplotype analysis revealed unequal recombination in four cases, with loss of markers Ag1-CA and C212, which are near the 5′ ends of the *SMN* genes. In one case, an interchromosomal rearrangement involving both the SMN genes and a regrouping of Ag1-CA and C212 alleles must have occurred, suggesting either interchromosomal gene conversion or double recombination. In two cases, no such rearrangement was observed, but loss of telSMN plus Ag1-CA and C212 alleles in one case suggested intrachromosomal deletion or gene conversion. In six of the seven cases, the de novo rearrangement had occurred during paternal meiosis. Direct detection of de novo SMA mutations by molecular genetic means has allowed us to estimate for the first time the mutation rate for a recessive disorder in humans. The sex-averaged rate of 1.1×10^{-4}, arrived at in a proband-based approach, compares well with the rate of 0.9×10^{-4} expected under a mutation-selection equilibrium for SMA. These findings have important implications for genetic counseling and prenatal diagnosis in that they emphasize the relevance of indirect genotype analysis in combination with direct SMN-gene deletion testing in SMA families.

Introduction

Proximal spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disorder leading to weakness and atrophy of the voluntary muscles. Because of the large phenotypic variability of SMA, patients have been classified into three types (I–III), depending on achieved motor milestones and age at onset (International SMA Consortium 1992; Zerres and Rudnik-Schöneborn 1995). Five years after the gene for all three SMA types had been mapped to 5q11.2-q13.3 (Brzustowicz et al. 1990; Gilliam et al. 1990; Melki et al. 1990*a*, 1990*b*), two candidate genes were reported: the survival motor neuron (SMN) gene (Lefebvre et al. 1995) and the neuronal apoptosis–inhibitory protein (NAIP) gene (Roy et al. 1995).

The SMN gene is present in two highly homologous copies—a telomeric (telSMN) copy and a centromeric (cenSMN) copy—which differ only by a small number of nucleotide substitutions (Lefebvre et al. 1995; Bürglen et al. 1996; Brahe et al. 1996; Hahnen and Wirth 1996). Irrespective of clinical severity, >90% of SMA patients lack exon 7, or both exon 7 and exon 8, of telSMN (Cobben et al. 1995; Hahnen et al. 1995; Lefebvre et al. 1995; Rodrigues et al. 1995). In patients with homozygous absence of telSMN exon 7 only, the remaining exon 8 was consistently shown to be part of a hybrid SMN gene, providing evidence for unequal recombination or gene conversion as the major molecular mechanisms underlying the disease (Hahnen et al. 1996; van der Steege et al. 1996; DiDonato et al. 1997). Although occasional homozygous deletions of telSMN in unaffected individuals may argue against a causative role of telSMN in the pathology of SMA (Cobben et al. 1995; Hahnen et al. 1995; Wang et al. 1996), several intragenic mutations identified in nondeleted SMA patients support the view that telSMN is an SMA-determining gene (Bussaglia et al. 1995; Lefebvre et al. 1995; Brahe et al. 1996; Parsons et al. 1996; Hahnen et al. 1997; Talbot et al. 1997).

The NAIP gene is present in one functional copy and

several truncated pseudogenes (Roy et al. 1995). Homozygous deletion of exons 5 and 6 of the NAIP gene has been observed in ~50% of type I SMA patients and in <20% of type II and type III SMA patients but also in 1%–2% of unaffected individuals (Cobben et al. 1995; Hahnen et al. 1995; Roy et al. 1995).

The SMA critical region consists of a duplicated and inverted element of ~500 kb, comprising several morphic marker loci in addition to the SMN, NAIP, and other genes and pseudogenes (Burghes et al. 1994; DiDonato et al. 1994; Melki et al. 1994; Lefebvre et al. 1995; Roy et al. 1995; Bürglen et al. 1997). Two polymorphic multicopy markers, Ag1-CA (C272) and C212, are localized close to the 5′ ends of the SMN genes (DiDonato et al. 1994; Melki et al. 1994; Bürglen et al. 1996). The copy number of these markers has been shown to be reduced in patients with severe SMA (DiDonato et al. 1994; Melki et al. 1994; Wirth et al. 1995a).

A small number of de novo rearrangements involving telSMN, Ag1-CA, and C212 has been reported. In most cases, the patients exhibited loss of telSMN and of Ag1-CA and C212 alleles when compared with an unaffected sib haploidentical for single-copy markers flanking the SMA critical region (Melki et al. 1994; Capon et al. 1995; Rodrigues et al. 1995; Wirth et al. 1995a; Raclin et al. 1997).

The present study is the first to report a larger number of de novo rearrangements in SMA patients, thus allowing inference as to the underlying mutational mechanisms, determination of the parental origin of mutation, assessment of the implications for prenatal diagnosis and genetic counseling, and estimation of the mutation rate, μ, for SMA.

Patients and Methods

Family Ascertainment

All patients fulfilled the diagnostic criteria defined by the International SMA Consortium (1992; also see Zerres and Rudnik-Schöneborn 1995). Clinical diagnosis was confirmed by homozygous absence of at least exon 7 of telSMN. On this basis, we have included family A even though the patient in that family presented with features excluding SMA, such as distal joint contractures and cerebral atrophy. De novo rearrangements in families F and G have been described elsewhere (Wirth et al. 1995a). Informed consent was obtained from all subjects prior to sampling.

The following criteria were defined for the inclusion of an SMA family in the present study: (1) the index patient, both parents, and at least one further sib had to be available for DNA analysis; (2) SMA patients had to be homozygous for the absence of at least exon 7 of telSMN; and (3) marker analysis including Ag1-CA and

C212 had to be informative, so as to allow unambiguous reconstruction of haplotypes.

Molecular Analysis

Molecular analysis of SMA candidate genes SMN (Lefebvre et al. 1995) and NAIP (Roy et al. 1995) was performed as described by Hahnen et al. (1995); that of markers Ag1-CA (D5S1556) (DiDonato et al. 1994) and C212 (D5F149S1/S2) (Melki et al. 1994) was performed as described by Wirth et al. (1995a); and that of flanking single-copy markers (listed from centromere to telomere) EF13/14 (D5S680), JK348 (D5S435), AFM265wf5 (D5S629), A31 (D5S823), 2AE9.1 (D5S557), AFM281yh9 (D5S637), MITI105 (D5S351), RB110/111 (5′MAP1B), and RB104/106 (3′MAP1B) was performed as described by Wirth et al. (1994, 1995b). Haplotypes for multicopy markers Ag1-CA and C212 were reconstructed as described by Wirth et al. (1995a).

Estimation of μ

When family history regarding the presence or absence of disease is not taken into consideration, the probability of a healthy couple having a child with an autosomal recessive disorder is approximately

$$1/4 \times [2q(1-q)]^2 + 2 \times 1/2 \times \mu \times 2q(1-q)$$
$$\times (1-q)^2 + \mu^2 \times [(1-q)^2]^2 \ , \quad (1)$$

in which q is the population frequency of the disease allele(s). The three terms in formula (1) correspond to none, one, or two of the mating partners being heterozygous for the disease allele, respectively. Thus, the proportion of cases in which an affected proband carries a new mutation, r, equals the ratio of the second term over the total sum. Since μ^2 is usually small and $1 - q$ approximates unity, this ratio can be simplified to

$$r = 2\mu/(q + 2\mu) \ . \quad (2)$$

μ can thus be estimated from a given estimate, r^*, of r, via

$$\mu^* = r^*q(2 - 2r^*) \ , \quad (3)$$

if q is known. The quantity μ^* is a maximum-likelihood estimate of μ even if r is estimated from a sample in which some families have two or more affected children (which families will then almost always carry two inherited lesions). This is because the conditional probability of any realistic number of affected sibs, given the proband, is virtually independent of μ if μ is small. It has to be ensured, however, that only one patient (i.e., the proband) is counted in multiplex families when the denominator of r^* is determined.

Results

Identification of De Novo Rearrangements

The SMN gene and the polymorphic multicopy markers Ag1-CA and C212 were analyzed, for various scientific and clinical reasons, in >400 SMA families. From 351 families, DNA of the parents and at least two children (including the SMN-deleted index patient) was available. Of these 351, a total of 340 SMA families (211 type I, 78 type II, and 51 type III) fulfilled the above-mentioned criteria (see Patients and Methods) and were included in the present study. In seven of these families, the only affected individual was found to carry a de novo rearrangement involving telSMN. Five of the patients were type I SMA, one was type II, and one was type III. Pedigrees and reconstructed haplotypes are presented in figure 1; and the major findings are summarized in table 1.

All seven patients showed homozygous lack of telSMN exons 7 and 8; only two patients were also homozygous for deletion of NAIP exons 5 and 6. Crucial evidence for a de novo rearrangement was provided by haplotype analysis with polymorphic multicopy markers Ag1-CA and C212. In six cases (families A–F), various alleles of these markers were involved in the de novo rearrangement.

In families A, B, and F, the patient showed homozygous deletion of telSMN exons 7 and 8, as well as loss of one Ag1-CA and one C212 allele, compared with the paternal haplotype. In families A and B, the patients (both type I SMA) were also lacking NAIP exons 5 and 6. In family D, the patient exhibited a large scale-deletion comprising both paternal Ag1-CA and C212 alleles, in addition to telSMN. In family C, a loss of only one Ag1-CA allele and the absence of telSMN were identified in the patient. In family G, the patient did not display any rearrangement involving Ag1-CA and C212 but lacked telSMN, when compared with his haploidentical brother. In family E, the patient showed a rearrangement that resulted not in a loss but in a regrouping of paternal Ag1-CA and C212 alleles, in addition to the loss of telSMN. In the case of Ag1-CA, allele 114 was replaced by allele 104; in the case of C212, allele 22 was replaced by allele 29. Since the two healthy sibs of this patient inherited the same maternal chromosome as the patient but had different paternal chromosomes, the father did not seem to be a carrier of SMA.

Mutational Mechanisms of De Novo Rearrangement

While haplotype analysis using markers Ag1-CA and C212, together with SMN deletion testing, allowed the identification of de novo rearrangements, only the inclusion of additional single-copy flanking markers enabled us to disclose the most likely underlying mechanisms.

Haplotype analysis with flanking markers revealed unequal crossing-over between parental chromosomes in four cases (families A–D), resulting in the absence of telSMN, of various marker alleles, and, in two cases, of NAIP. In the remaining three cases (families E–G), no recombination involving markers flanking the SMA region could be observed. Haplotype analysis in family F therefore suggests intrachromosomal deletion comprising AG1-CA/C212 alleles and telSMN, whereas in family G we cannot distinguish between intrachromosomal deletion and gene conversion. In family E, double recombination or interchromosomal gene conversion during paternal meiosis resulted in the interchromosomal rearrangement of Ag1-CA and C212 alleles and the loss of telSMN.

Parental Origin of De Novo Rearrangements

The paternal origin of the de novo rearrangements could be determined in six cases (A–F). In family G, none of the markers was involved in the rearrangement, so the parental origin could not be identified. The average paternal age at birth of the index patient was 27.5 years (range 23–34 years), which compares well to the average in the general population.

Interestingly, in all four cases with unequal crossing-over, the paternal haplotypes show an unbalanced copy number, at least for Ag1-CA: in families A, B, and D, the paternal chromosomes carry one and two Ag1-CA alleles (genotype 1,2); in family C, the paternal chromosomes carry 1 and 3 alleles (genotype 1,3); and, in family F, in which an intrachromosomal deletion is the most probable mutational mechanism, marker C212 carries two and three copies on the paternal chromosomes (genotype 2,3).

Estimation of μ in SMA

In 7 (2%) of 340 SMA families, the index patient was found to carry a de novo rearrangement. Since it is known that mutations other than homozygous absence of telSMN are rare in SMA patients, this proportion represents a good estimate of the parameter r, as defined in formula (2). If it is assumed that $r = 7/340 = .021$ and that $q = 1/100$, then formula (3) yields an estimate of $\mu = 1.1 \times 10^{-4}$ for SMA. As can be inferred from figure 2, this is close to the μ expected from mutation-selection equilibrium, $\mu = (1 - f) \times q^2$, for a broad range of fitness values, f. Adopting the upper (.038) and lower (.012) 95% confidence limits for the parameter r results in confidence limits for μ are 1.9×10^{-4} and 6.1×10^{-5}, respectively. For these estimates to concur with their equilibrium expectation, however, q would
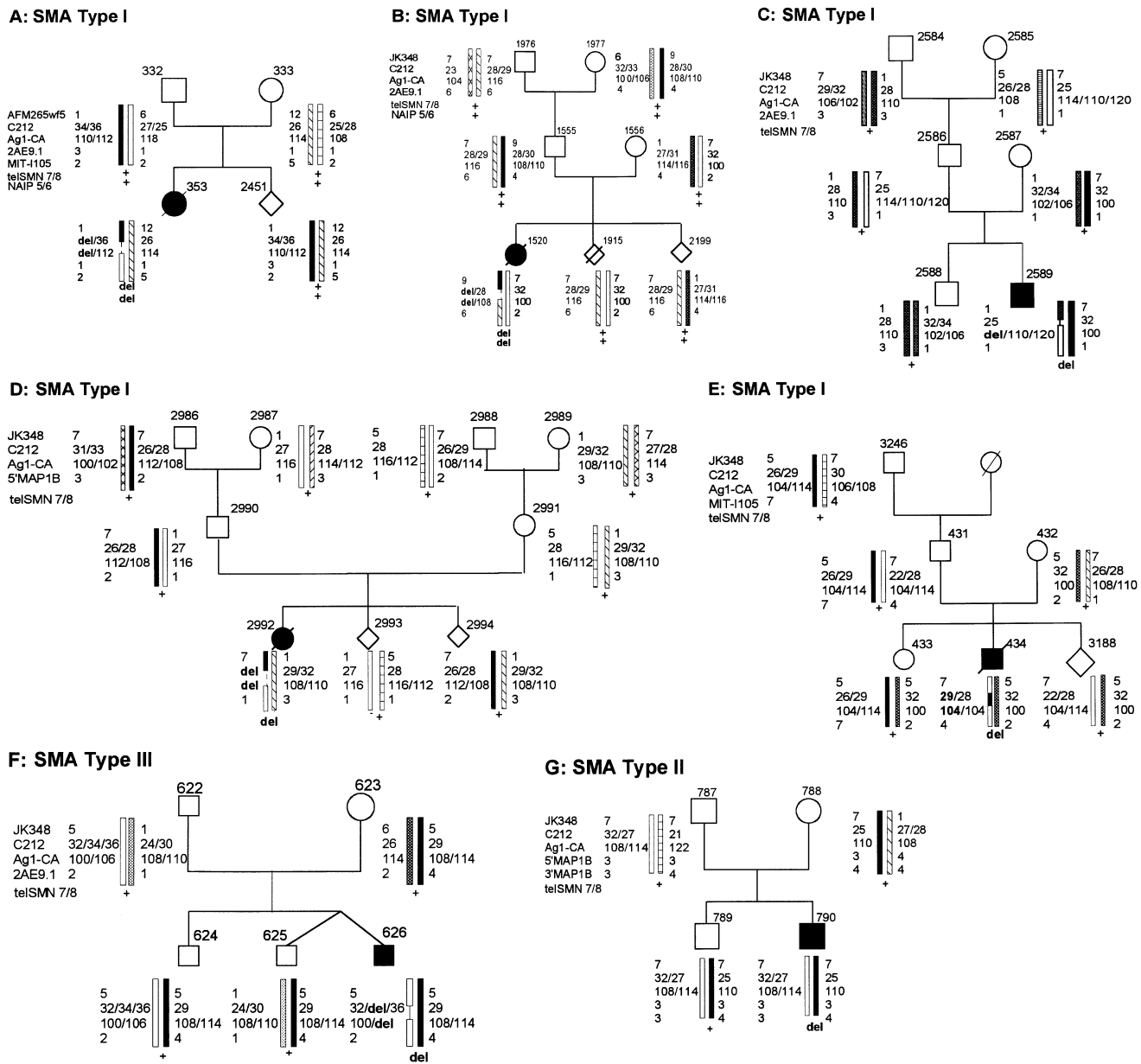
Figure 1 Pedigrees of SMA families with de novo rearrangements. Haplotype analysis of markers Ag1-CA and C212 and of informative single-copy markers is included. In pedigree G, the most likely haplotype is shown.

have to be either two times larger or two times smaller than that suggested by the 1/10,000 observed incidence of SMA (see fig. 2).

Since six of the seven de novo mutations were unequivocally of paternal origin, adoption of a sex-averaged μ for SMA may seem questionable. Thus, if sex-specific μ values were allowed in formulas (1), (2), and (3), the estimate for males would approximate twice the sex-averaged figure whereas that for females would be estimated as zero. Breakdown of the analysis by disease

type, on the other hand, is problematic for a recessive disease such as SMA, since estimation of μ, either by our direct approach or indirectly, when mutation-selection disequilibrium is assumed, would depend on q. For SMA, however, this entity is not yet well defined. A particular allele causing a particular disease type in the homozygous state may well cause a different type when it occurs in the compound-heterozygous state with another, say, milder allele. Nevertheless, since r for SMA I families (5/211) is literally identical to that noted for

Table 1

Characterization of Seven De Novo Rearrangements in Patients with SMA

Family	SMA Type	telSMN, exons 7 and 8	NAIP, exons 5 and 6	Ag1-CA and C212	Recombination?	Parent of Origin	Paternal Age at Birth of Patient (years)	Mutational Mechanism
A	I	Deleted	Deleted	Loss of one Ag1-CA copy and one C212 copy	Yes	Father	24	Unequal recombination
B	I	Deleted	Deleted	Loss of one Ag1-CA copy and one C212 copy	Yes	Father	25	Unequal recombination
C	I	Deleted	Present	Loss of one Ag1-CA copy	Yes	Father	34	Unequal recombination
D	I	Deleted	Present	Loss of two Ag1-CA copies and two C212 copies	Yes	Father	27	Unequal recombination
E	I	Deleted	Present	Regrouping of one Ag1-CA and one C212 copy	Yes	Father	30	Interchromosomal gene conversion or double recombination
F	III	Deleted	Present	Loss of one Ag1-CA copy and one C212 copy	No	Father	30	Intrachromosomal deletion
G	II	Deleted	Present	No marker rearrangement	No	Unknown	23	Gene conversion or intrachromosomal deletion

all SMA types combined (7/340), there is as yet no evidence for a significant difference in the rate at which mutations associated with either SMA type are generated.
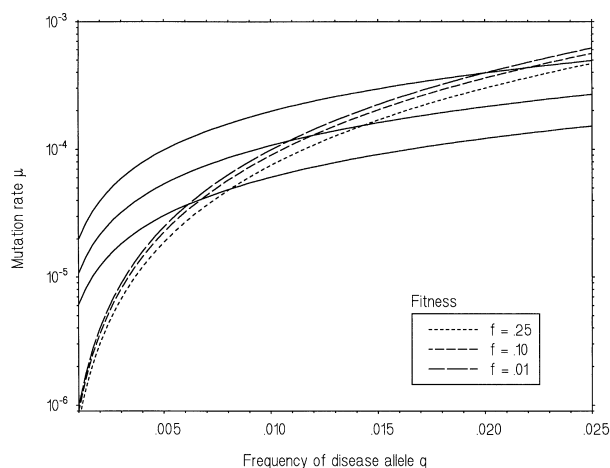

Figure 2   Relationship between μ and q, for SMA. The upper and lower continuous curves demarcate the 95% confidence intervals for the estimate of μ emerging, at a given value of q, from the observation that 7/340 SMA probands carry a de novo mutation in the heterozygous state. The central continuous curved line depicts the maximum-likelihood estimate of μ. Broken lines represent the mutation-selection equilibrium ensuing for various f for SMA.

Discussion

Identification of De Novo Rearrangements

Although the identification of de novo mutations for an autosomal dominant inherited disorder is straightforward via sporadic cases, there is no way in which recessive inherited disorders can be used to determine, on the basis of family history, which patients carry a de novo mutation. This is the first report of a sufficiently large number of SMA families in which the parents and at least two children have been analyzed for both the multicopy markers Ag1-CA and C212 and the SMN gene. Analysis of at least these four individuals is a prerequisite for identification of de novo mutations in a given family. If no sibs of the index patient are available, the haplotype analysis of grandparents may help to disclose de novo mutations. The few de novo mutations described so far have not been found in the course of systematic studies of a large number of SMA families, and, therefore, the ensuing relative mutation frequencies are not comparable (Melki et al. 1994; Capon et al. 1995; Rodrigues et al. 1995; Wirth et al. 1995a; Raclin et al. 1997).

We found seven de novo rearrangements comprising telSMN and the immediately surrounding region in 340 SMA families. Two haplotype constellations were helpful in revealing de novo rearrangements: (1) families with haploidentical affected and unaffected sibs and (2) fam-

ilies with a crossing-over between markers closely flanking the SMA region.

In a previous study, we observed that ~15% of type I SMA and ~6% of type II SMA patients had inherited a large-scale deletion from one parent, which implies that, in these families, one SMA chromosome was lacking Ag1-CA and C212 alleles (Wirth et al. 1995a). Some authors have erroneously mistaken such lesions for de novo deletions (Melki et al. 1994; Capon et al. 1996).

McAndrew et al. (1997) recently published a quantitative analysis of the telomeric and centromeric SMN genes of SMA patients, SMA carriers, and controls. Although their method might be useful for identification of de novo mutations in many cases, the authors themselves reported a carrier (76 individuals were tested) who had two affected children with deletions but who nevertheless carried two telSMN copies. Since two independent de novo mutations were deemed unlikely, the most parsimonious explanation for this finding was that the two telSMN copies were on the same chromosome. This phenomenon has also been observed in 3% controls. Therefore, quantitative SMN-gene analysis cannot replace a qualitative analysis using multicopy markers and the SMN gene. However, quantitative analysis might help to determine the parental origin of de novo mutations that involve the SMN gene only.

Mutational Mechanisms of De Novo Rearrangement in SMA Patients

Three mechanisms seem to play a crucial role in the occurrence of de novo mutations in SMA: (1) unequal crossing-over between homologous chromosomes, (2) intrachromosomal deletion, and (3) gene conversion. Recently, the same mechanisms have been suggested as the mechanism for the generation of hybrid SMN genes (Hahnen et al. 1996).

Because of its duplicated structure, including SMN and NAIP homologues, the SMA region seems to be prone to unequal rearrangement or gene conversion. Findings in a number of other genes indeed suggest that repetitive regions are susceptible to these types of lesions (for review, see Cooper et al. 1995).

Interestingly, unequal crossing-over involving the SMA region seems to be associated with an unbalanced genomic structure, as reflected by the copy number of Ag1-CA on homologous chromosomes (families A–D). In view of the localization of Ag1-CA alleles within the promoter region of the SMN genes, this marker might roughly reflect the number of repeated elements including the SMN genes. Thus, differences in Ag1-CA copy number on both chromosomes could represent differences in physical structure that might predispose to de novo rearrangements.

In those families in which the de novo rearrangement resulted in a deletion (families A–D and F), the different number of lost marker alleles and genes suggests different deletion sizes. It appears that unequal recombinations causing larger deletions are associated with more severe phenotypes, whereas intrachromosomal deletions and gene conversions are associated with milder SMA. Our data are thus consistent with a recent genotype-phenotype study in which pulsed-field gel electrophoresis has provided evidence that gene conversion of telSMN into cenSMN predominates in patients with mild SMA (Campbell et al. 1997). An increased number of cenSMN copies was also found in a number of mild SMA cases by quantitative analysis (Burghes 1997; McAndrew et al. 1997). We may thus summarize that, whereas severe SMA mutations are more likely to be deletions, mild SMA mutations are mainly due to gene conversion.

Parental Origin

In six of seven rearrangements we were able to determine the paternal origin of the de novo mutation. Melki et al. (1994) also reported a de novo telSMN deletion of paternal origin. However, since no flanking markers were tested, the underlying mechanism could not be disclosed. The de novo deletion presented by Rodrigues et al. (1995) resembles that in family G in that the parental origin could not be determined. Raclin et al. (1997) described a case in which the most likely mutational mechanism was similar to that in family F (Wirth et al. 1995a; present paper), but the mutation that they reported was of maternal origin. This is the only maternal de novo mutation described so far.

A bias toward a paternal origin of new mutations has been inferred for some autosomal dominant disorders, such as retinoblastoma, neurofibromatosis, Wilms tumor, and Apert syndrome (for review, see Chandley 1989, 1991; Moloney et al. 1996). In these conditions, de novo mutations are thought to originate mainly during spermatogenesis, involving mitotic replication errors, and a paternal age effect has been documented for many of them (for review, see Vogel and Motulsky 1996). However, since deletion resulted from meiotic unequal chromatid exchange in most of our cases, no paternal-age effect was to be expected. Indeed, the average paternal age at birth of a patient carrying a de novo mutation was 27.5 years (range 23–34 years), which concurs with the normal range.

The SMA region shows some similarity to chromosome region 17p11.2, which contains a 1.5-Mb fragment flanked by repeated elements. This region is known to undergo unequal recombination with consequent deletion or duplication, causing either hereditary neuropathy with liability to pressure palsies or Charcot-Marie-Tooth disease type 1A (CMT1A). For both diseases, de novo mutations also occur mainly during spermatogen-

esis (Palau et al. 1993; Blair et al. 1996; Pentao et al. 1997), although the recombination frequency in this particular region is higher in females than in males. Maternal de novo mutations seem to be very rare, since only two cases have been reported so far (Blair et al. 1996; LeGuern et al. 1996). Two possible explanations have been proposed to explain this sex difference: either misalignment of repeated sequences is more frequent during male meiosis, or paternally derived duplicated chromosomes are more stable than maternal ones (Palau et al. 1993). The proportion of de novo duplications in CMT1A is estimated to be ~10% (Blair et al. 1996).

Estimation of μ in SMA

Our large study of de novo mutations in SMA patients has allowed us to estimate μ for SMA directly on the basis of molecular-genetic findings. To our knowledge, this represents the first attempt to estimate the μ for an autosomal recessive disorder in humans. The result shows that μ for SMA corresponds to that expected from the high incidence of the disease under mutation-selection equilibrium. The incidence for recessive disorders can be substantially influenced by factors such as fitness, reproductive disadvantages, and genetic drift (Vogel and Motulsky 1996). However, since our direct estimate of $\mu = 1.1 \times 10^{-4}$ fits its theoretical expectation, the data reported herein provide no evidence that such confounding factors are seriously important in SMA.

When calculating μ, we deliberately avoided breaking down the analysis by SMA type. μ values refer to changes from "normal" to clearly defined "pathological" states, implying that, of two identical changes, either both are scored or neither is. At present, however, nothing is known about the phenotypic consequences of "severe" mutations in heterozygous combination with "mild" alleles. The severe allele might dominate the mild one, or vice versa. We have shown, for example, that the most frequent haplotype in hybrid genes (100/106 Ag1-CA and 31/33 C212) causes a mild phenotype in the homozygous state but can lead to all three types of SMA in heterozygotes (Hahnen et al. 1996). Even more striking was the report, by Rudnik-Schöneborn et al. (1996), of two SMN-deleted sibs who had inherited the same maternal, but different paternal, SMA chromosomes. Whereas their father was himself suffering type III SMA and one son presented with type I SMA, the other, elder son was asymptomatic. In any case, since r for SMA I families (5/211 families) is literally identical to that noted for all SMA types combined (7/340 families), there is as yet no evidence for a significant difference in the rate at which mutations associated with either SMA type are generated.

In two cases, we postulated gene conversion as being the most likely mutational mechanism. Gene conversions may either be confined to the SMN gene or include the multicopy markers. In the latter case, one multicopy marker allele will be duplicated whereas the other should be missing, compared with the parental haplotype; mutations of the former type, by contrast, can be identified only if exons 7 and 8 are involved. This implies that a small number of de novo gene conversions may not be recognizable. We are aware that our estimate of μ is based on a single population frequency, q, disregarding mutation type, but, in light of the results discussed above, no strong effect of gene conversions and deletions being combined in that parameter is to be expected. If a large proportion of de novo gene conversions had been overlooked, however, our comparatively high estimate of μ would still represent an underestimate.

Another factor that could have biased our direct estimate of the μ is the fact that ascertainment of families was based on informativity. However, the two multicopy markers together yield almost 100% informativity, and the proportion of families in which unambiguous construction of haplotypes was not feasible was low (3%). Therefore, ascertainment bias is unlikely to represent a serious source of error here.

Our finding of de novo mutations in 2% of index SMA patients most likely represents a lower limit, and the same is likely to hold for the emerging μ, 1.1×10^{-4}. μ values of similarly high order have so far only been found for some dominant disorders (Vogel and Motulsky 1996). However, although a dominant mutation causing a severe phenotype is rapidly eliminated from the population, the frequency of a recessive mutation usually increases comparatively slowly until mutation-selection equilibrium is reached. Therefore, despite the correspondence noted here between the direct and indirect μ estimates for SMA, a general prior assumption of mutation-selection disequilibrium is not meaningful for recessive disorders.

In cystic fibrosis (CF), the most frequent autosomal recessive disorder, with a heterozygosity frequency of ~4%, only two de novo mutations (White et al. 1991; Cremonesi et al. 1996) have been described in some 30,000 CF families tested (frequency <0.01%), and the most frequent CF mutation, ΔF508, is likely to be of unique origin. It has been suggested that individuals heterozygous for CF are more resistant to cholera, causing a heterozygote advantage during epidemics of cholera (Gabriel et al. 1994). Thus, the high incidence of CF is not a consequence of a particularly high μ.

Furthermore, whereas the mutations seen in CF are usually small (few-base-pair deletions, duplications, or single-base-pair substitutions), mutations in SMA are caused by complex genomic rearrangements. Mutations caused by different mechanisms, however, can also be assumed to occur at different frequencies.

Allelic association has been observed in SMA (Burghes

et al. 1994; DiDonato et al. 1994; Wirth et al. 1995*a*), which seems to contradict the high μ. One might hypothesize, however, that certain chromosomes are predisposed to de novo rearrangements or that particular marker haplotypes are associated with a mild course of disease.

An alternative method for direct determination of the μ would have been single-sperm analysis of the SMN gene. However, our results suggest that the diploid paternal genotype may be critical for the occurrence of de novo mutations. Therefore, it would have been necessary to analyze a large number of sperm donors with different genotypes and to compare the results with each other. Furthermore, since the accuracy of the typing of several markers in a single sperm is questionable, it would have been impossible to obtain reliable information as to the mechanism underlying any mutation observed.

Implications for Prenatal Diagnosis

Most of the de novo rearrangements presented here were identified through prenatal diagnosis in SMA families. We would therefore like to emphasize the importance of indirect haplotype analysis in combination with direct SMN deletion testing, for the molecular diagnosis of SMA. Haplotype analysis including the index patient, the parents, and sibs, using the polymorphic multicopy markers Ag1-CA and C212, can disclose the occurrence of de novo rearrangements, can help to exclude maternal contamination in cases of prenatal diagnosis, and allows one to determine the heterozygote status.

The detection of a de novo rearrangement resulting in the loss of the telSMN gene in an SMA family indicates a recurrence risk reduced from 25% to a substantially lower percentage, the only risk in this situation coming from recurrent de novo mutation or germ-line mosaicism.

Acknowledgments

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