Unequal expression of *NF1* alleles

Sir — After the cloning of the neurofibromatosis type 1 (NF1) gene in 1990[1–3], numerous groups began to search for causative mutations. Although several hundred patients were included in studies carried out by the members of the NNFF International NF1 Genetic Analysis Consortium, only 54 mutations have so far been detected worldwide. This frustratingly low efficiency is usually explained by the fact that with the possible exception of mutations in exon 31 (ref. 4), no real mutational hot spot seems to exist within the coding region of this very complex gene. *NF1* spans approximately 350 kilobase (kb) of genomic DNA organized in 52 exons, encoding a large 13 kb mRNA with a coding region of 9 kb. However, the promotor is not well characterized, and the 3' untranslated region has not been cloned so far. Putative mutations within these structures will not have a direct effect on the NF1 protein but they may affect the expression of the mutant allele or the stability of the mutant messengers and may therefore lead to an unequal representation of wild type and mutant messengers in cells of NF1-patients.

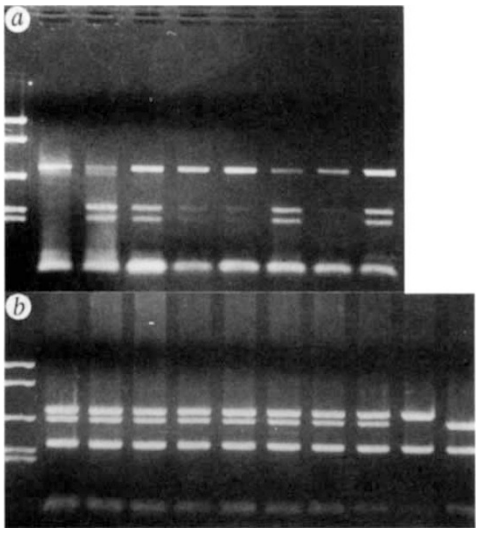

Fig. 1 RT-PCR analysis of *NF1* transcripts heterozygous for an *Rsa*I polymorphism. *Rsa*I-cut PCR-products were derived from cDNA (*a*) or genomic DNA (*b*) of NF1-patients: NF58a, NF56, NF48, NF106, NF112, NF71a, NF113, NF120 (lane 2–9). Lanes 10 and 11 (*b*), probands homozygous for allele 2 (lane 10) and for allele 1 (lane 11). On the DNA level, allele 2 corresponds to a 500 bp fragment and allele 1 to a 450 bp fragment. On the cDNA level, allele 2 corresponds to a 532 bp fragment and allele 1 equally to a 290 and 242 bp fragment. Lane 1 (*a*, *b*): Size marker 9ZBD (1121, 822, 457, 271 and 235 bp). Ratios of densitometric band intensities (arbitrary units), A2 (532 bp fragment) to A1 (sum of 290 bp and 242 bp fragments), for the 8 *NF1* samples in (*a*) lanes 2–9, were as follows: 247, 0.39, 0.80, 1.51, 2.69, 0.43, 1.74 and 0.83.

We detected recently a polymorphic *Rsa*I restriction site in exon 5 of the NF1 gene[5]. The polymorphic site is caused by a silent mutation in codon 234 and therefore does not affect the protein product. In patients heterozygous for the polymorphism, the relative abundance of mRNAs transcribed from both alleles can be evaluated.

From fibroblast-like cells of eight heterozygous patients, we isolated total RNA, amplified the corresponding region of *NF1* by RT-PCR, cut the products with *Rsa*I and analysed the cDNA fragments on agarose gels. In six cases, we found notable differences in the band intensities of the PCR products derived from the two allelic messages (Fig. 1*a*, lanes 2, 3, 5–8), even in cells thought not to be primarily affected by the defect. In two patients (Fig. 1*a*, lanes 4, 9), as well as in four unaffected probands (data not shown), we found apparently equal band intensities from the two alleles. As a control, we examined *Rsa*I-cut PCR products from DNA of the eight patients (Fig. 1*b*). The experiments were repeated several times using different RNA-preparations and reverse transcription assays and always yielded reproducible results. The results obtained by visual estimation could be confirmed by densitometric measurements of the RT-PCR banding patterns (see legend).

As disproportionately low amounts of mRNA from mutant alleles in patients heterozygous for stop mutations has repeatedly been observed[6–10] we examined the relationship of the RT-PCR patterns to the kind of mutations affecting the patients' NF1 genes. This was possible with patients NF10 and NF25 whose mutations (deletion of exon 3 and nonsense mutation in codon 1538, respectively) enabled the distinction of the two alleles on the RT-PCR level without using the polymorphic site, and with patient NF71a (lane 7 in Fig. 1), affected by a stop-mutation in exon 28 (ref. 11). Surprisingly, one of the stop mutations (NF25) did not affect the allelic RT-PCR band intensities, while the other one (NF71a) reduced the amount of message from one allele. The deletion (NF10), which is not expected to affect the reading frame, does not affect the relative amounts of the allelic RT-PCR fragments.

To find out whether the wild-type or the mutant allele appears to produce a reduced mRNA level, we took advantage of the informative cosegregation of one of the polymorphic alleles with NF1 in the families of patients NF71a, NF112, and NF58a. In these three cases, the message of the mutant allele seemed to be reduced. The mutant *NF1* allele passed through either the paternal (NF71a[12] and NF112) or maternal (NF58a) germ line, hence imprinting is not the cause of its disproportionately low expression.

In summary, six of eight NF1 patients carry a class of mutations that result in reduced amounts of mRNA from one of the *NF1* alleles. These mutations may affect regulatory sequences in the 5'- or the 3'-region of *NF1* or they may exert a detrimental effect on RNA processing and/or mRNA stability[8]. The proposal that stop mutations in non-terminal exons interfere with RNA processing is not, however, corroborated by our results: fibroblast-like cells from one patient with a stop-codon in the centre of *NF1* exhibited apparently equal expression of the two alleles.

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