Four frameshift mutations in neurofibromatosis type 1 caused by small insertions

Steven D Colman, Corinne R Abernathy, Vu T Ho, Margaret R Wallace

Abstract
We have been using heteroduplex analysis to assay individual exons within the NF1 gene in an effort to identify disease causing constitutional mutations in neurofibromatosis type 1 patients. Here we report the identification and characterisation of four insertional NF1 frameshift mutations in an analysis of exons 28-39 in a set of 78 patients. These include three 1 base pair insertions and one 2 base pair insertion. Three of these mutations can be attributed to replication slippage errors, while the mechanism behind the fourth may be related to formation of secondary structure during replication. It may be of significance that a majority of the previously reported small insertions in NF1 also lie within exons 28-39.

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Neurofibromatosis type 1 (NF1), an autosomal dominant disease with a prevalence estimated at 1 in 3000, is characterised by café au lait spots (CAL), cutaneous neurofibromas, axillary freckling, and Lisch nodules (hamartomas) of the iris.1 The NF1 gene, which is approximately 350 kb in length, comprises 59 exons.2 The large gene size and the fact that the majority of patients appear to have unique mutations has made the search for NF1 mutations difficult. The most recent distribution of the NF1 Genetic Analysis Consortium newsletter lists 194 characterised mutations, of which 21 (11%) are small insertions. These mutations do not cluster at a single site. Only two of these insertions have occurred in unrelated patients; 62% of those reported do, however, fall within exons 28-39.

Materials and methods
PATIENT SAMPLES
Seventy eight unrelated NF1 patients (all meeting accepted diagnostic criteria) were studied. All patient samples were obtained under IRB approval with the patient’s consent. Patient 1 is a white female with typical NF1 features and acute lymphoblastic leukaemia (ALL). Her mother also has NF1 but is not reported to have any signs of ALL or any other cancer. No other family information is available. Patient 2 is a white female who is a new mutation patient with multiple neurofibromas, including plexiform neurofibromas, and pseu-darthrosis of the tibia which necessitated amputation at the age of 9 years. Patient 3 is a new mutation white male with CAL, plexiform orbit neurofibroma, multiple cutaneous neurofibromas, axillary freckling, optic glioma, high blood pressure, learning disabilities, hypotelorism, and bitemporal narrowing. Patient 4 is a new mutation white female with CAL, multiple neurofibromas, axillary freckling, macrocephaly, and learning disabilities.

RESULTS AND DISCUSSION
Four frameshift insertions were detected and characterised. (1) Patient 4 has an A inserted into exon 37 (6791insA, where 6791 is the base in the cDNA sequence just before the insertion, I being the first base in the translation start site). This same mutation has been more recently reported in an unrelated patient by Upadhyaya et al., who postulate that this region of exon 37 may be hypermutable. There is no obvious similarity in phenotype between these two patients. (2) Patient 3 has a C insertion in exon 36 (6709insC). (3) Patient 2 has an AA dinucleotide insertion in exon 29 (5289insAA), which was secondarily confirmed by restriction analysis based on the formation of a de novo Alul site (data not shown). (4) Patient 1 has an A insertion in exon 28 (4873insA). The heteroduplex pattern for each of these mutations is shown in fig 1. These mutations are all predicted to result in truncated neurofibromin.
Figure 1  NF1 mutations identified by heteroduplex analysis. (A) This aberrant pattern was caused by a single base insertion in exon 29 from patient 2. (B) This pattern corresponded to a two base insertion in exon 29 from patient 4. (C) The extra band in lane 1 is the result of a 1 bp insertion in exon 36 of patient 3. Lanes 2 and 3 contain samples from NF1 patients apparently unaffected at this locus. (D) The extra band in lane 3 is caused by a 1 bp insertion in exon 28 of patient 1. Lanes 1 and 2 contain samples from NF1 patients who are apparently unaffected at this locus.

In general, small insertions occur far less frequently than small deletions or single base changes.\(^7\) In accordance, small insertions in NF1 have been less frequently reported than small deletions and substitutions; small insertions reported to the NNPF consortium account for 21 of 194 NF1 mutations (11%), while small deletions and substitutions respectively account for 19% and 34% of those reported. We detected four small insertions in 78 DNA samples after analysing exons 28-39. Our continued analysis of 66 of these patients has detected several deletions and substitutions scattered throughout the NF1 gene, but we have found no additional insertions.\(^7\) Thirteen of 21 small insertions previously reported to the NNPF consortium are also located within exons 28-39. Therefore, with the inclusion of our report, 17 of 25 (68%) small insertions detected lie within exons 28-39. It cannot be determined at this time whether this putative “clustering” is significant or merely an artefact owing to bias of ascertainment. As the NF1 research community continues to identify insertions in NF1, this relationship will become clear.

Patient 1 is the second NF1 patient we have encountered who has both a frameshift mutation in exon 28 and ALL.\(^7\) NF1 patients are known to be at increased risk for leukaemia (particularly juvenile chronic myelogenous leukaemia and ALL to a lesser degree); however, mutations have not been reported for any other NF1 patients with ALL. Thus, the significance of our finding is unclear. The relevance of this association is further complicated by the fact that, although patient 1’s mother also has NF1, she has not developed ALL. It may be of note that exon 28 is the first NF1 exon upstream from the two lymphoid specific genes (EVI12A and EVI12B) transcribed from the opposite strand of intron 27b. The function of these genes is not known, but it is conceivable that there is some relationship between NF1 mutations in exon 28 and regulation of these genes.

Three of these mutations (the single base insertions) could be attributed to slippage during replication. In each case, the extra base is inserted next to an identical base (that is, insertion of an A next to an A). The independent recurrence of 679insA suggests that there might be a driving force behind insertions at specific sites. Insertions can also be mediated by secondary structure. The two base AA insertion seen in exon 29 of patient 2 occurs in the middle of a 40 bp stretch that is 75% A+T. This sequence could provide an opportunity for secondary structure formation and subsequent mispairing during replication.

It has been suggested that NF1 mutations, like frameshift mutations in many other genes, can result in reduced levels of mutant transcripts relative to normal ones.\(^7\) However, there have also been reports of small mutations which do not have this effect.\(^7\) \(^7\) ASO analysis of leucocyte RNA from patient 2 detected approximately equal levels of message from both the normal and mutant NF1 alleles (fig 2), thus reinforcing the concept that the effect of small frameshift insertions on transcript level is variable in NF1.

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