larger amounts of SEB are repeatedly administered than with a single dose (data not shown). This supports the model that antigen re-stimulation of T cells under the influence of IL-2 will cause apoptosis. The responding T cells are not completely ablated, suggesting that some might escape death and become memory cells. How does IL-2, which is well known as a T-cell mitogen, paradoxically program the same cell type for apoptosis? One possibility is that IL-2 serves only to drive T cells into the division cycle that may predispose thymocytes and $\gamma\delta$ T cells to death[27,28]. If so, then any successful immune response could predispose mature $\alpha\beta$ T cells to apoptosis. Alternatively, IL-2 could provide a qualitatively or quantitatively distinct signal that entrains apoptosis to antigen-receptor stimulation. It will be important in either case, in evaluating IL-2 as a therapeutic agent in humans, to consider its unexpected ability to predispose T cells to apoptosis. □

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A distinct *Hox* code for the branchial region of the vertebrate head

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THE branchial region of the vertebrate head forms through complex interactions involving rhombomeric segments, neural crest and branchial arches[1]. It is thought that aspects of their patterning mechanisms are linked[2] and involve *Hox-2* genes, whose overlapping and spatially restricted expression domains represent a combinatorial code for generating regional diversity[3–5]. Vertebrates possess four Hox clusters of *Antennapedia* class homeobox genes, related to each other by duplication and divergence from a common ancestral complex[3,6–8]. In consequence, at equivalent positions in different clusters there are highly related genes known as subfamilies or paralogous groups. As *Hox-2* genes cannot fully account for patterning individual rhombomeres, we investigated whether offsets in expression limits of paralogous genes could account for the generation of regional diversity. We report here that, with the exception of the *labial* subfamily, paralogues show identical expression limits in rhombomeres, cranial ganglia and branchial arches, providing a combinatorial *Hox* code for the branchial region that seems to be different in organization to that of the trunk.

We isolated and identified two new genes, *Hox-1.11* and *Hox-4.9* for this study. Extensive screening both in mice and in humans suggests that there are only 12 members in the four 3′ *Hox* subfamilies, and we have analysed the expression of 11 of these in mouse embryos. All genes in a given subfamily, with the exception of *labial*, are expressed with the same rhombomere boundary (Fig. 1). The *Dfd* subfamily map to r6/r7 (Fig. 1*a–c*), the *Zen/pb* genes to r4/r5 (Fig. 1*d–f*), and the *pb* subfamily to r2/r3 (Fig. 1*g, h*). The boundaries between genes in *Hox-1* and *Hox-4* also vary with a two-segment periodicity, and there is no evidence for an offset in expression limits between members of a subfamily. But subfamily members can show distinct differences within their domains of expression. For example, *Hox-1.5* and *Hox-2.7* both show identical patterns of expression, with higher levels in r5 and lower levels posteriorly (Fig. 1*d, e*), whereas *Hox-4.1* shows a pattern complementary to this in the same overall domains (Fig. 1*f*). At this stage all members of the *labial* subfamily show significant differences between each other both in sites and in spatial domains of expression. At 9.5 days of development only *Hox-2.9* (refs 4, 9, 10) is expressed in the hindbrain (Fig. 1*l*). An equivalent section hybridized with *Hox-1.6* (Fig. 1*j*) shows no expression in the rhombomeres and cranial ganglia. *Hox-4.9* also shows no specific localization in the neural tube (Fig. 1*k*), but is expressed in patches of surface ectoderm overlying the hindbrain.

We have extended these comparisons to other tissues in the head, and show that members of a subfamily also have identical spatial restrictions in neural crest derivatives and branchial arches. This is illustrated for two subfamilies in Fig. 2. In the *Pb* subfamily, *Hox-1.11* and *Hox-2.8* hybridize to the vii/viii ganglion complex (Figs 2*f* and 1*g, h*) and show expression in the mesenchyme of the second and third branchial arches (Fig. 2*e, d*). In the *Zen/Pb* subfamily, *Hox-1.5* (Figs 1*e* and 2*b, c*) and *Hox-2.7* (Fig. 2*a*) show expression in the third and fourth branchial arches, their overlying surface ectoderm and the ix/x ganglion complex. Previous work and our own data suggest that there is only limited expression of the *labial* genes in neural crest derivatives[5,10–12].

The patterns of expression in rhombomeres and branchial arches at 9.5 days are a result of modulations of patterns established at earlier stages of development. In a one-somite embryo, *Hox-2.8* has a restricted domain of expression that respects the presumptive r2/r3 boundary (Fig. 3*b*), as defined by the early expression domain of *Krox 20* shown in a near adjacent section (Fig. 3*c*). It also seems to have a similar expression domain in a presomitic embryo (Fig. 3*a*). This early boundary is identical to that at later stages, and shows that *Hox-2.8* expression is established before overt rhombomere formation in presomite embryos. Previous work has shown that members of the *Zen/Pb* and *Dfd* subfamilies of *Hox-2* are not at their final anterior limits at this stage, but have been established by 8.5 days of development (8–12 somites)[4]. We think that the differences

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between these three subfamilies, summarized in Fig. 4*a*, reflect a temporal progression in the establishment of rhombomeric expression boundaries.

The *labial* subfamily is also different in that the later patterns of expression (described above) are dramatically different from earlier stages (Fig. 4*a*). At 8 days of development *Hox-1.6* and *Hox-2.9* are expressed in a continuous domain up to the presumptive r3/r4 boundary[11]. But at a similar stage of development (the same one-somite embryo as Fig. 3*b*, *c*) *Hox-4.9*, the third member of the family, is only expressed in the most posterior regions of the embryo (Fig. 3*d*). In contrast to other subfamilies, these early *labial* patterns are not maintained; in later stages only *Hox*-2.9 shows expression in the hindbrain and a limited subset of cranial neural crest[5,10] (Figs 1*i–k* and 4*a*, *b*).

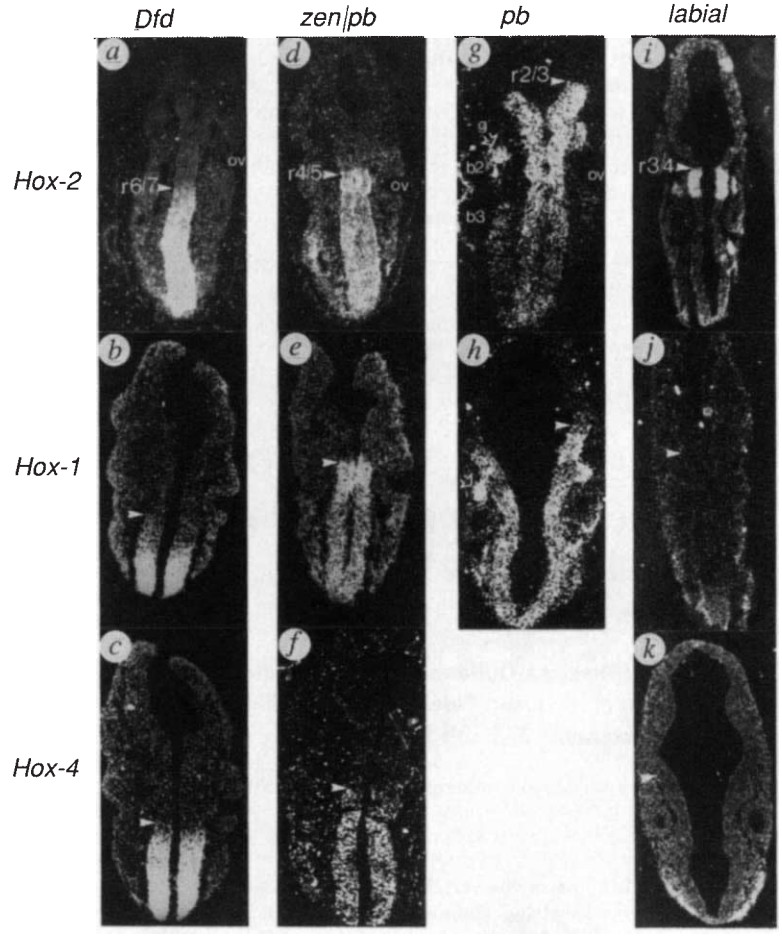

FIG. 1 Expression of genes of the *Dfd, zen/pb, pb* and *labial* subfamilies in the 9.5-day mouse embryo hindbrain. The *Dfd* group consists of *Hox-2.6* (*a*), *Hox-1.4* (*b*), *Hox-4.2* (*c*) and *Hox-3.5* (not shown). The *Zen/Pb* group consists of *Hox-2.7* (*d*), *Hox-1.5* (*e*) and *Hox-4.1* (*f*). The *Pb* group consists of only two members, *Hox-2.8* (*g*) and *Hox-1.11* (*h*). The *labial* group consists of *Hox-2.9* (*l*), *Hox-1.6* (*j*) and *Hox-4.9* (*k*). ov, Otic vesicle; r6/7, boundary between rhombomeres 6 and 7; b2 and b3, second and third branchial arches; *g*, vii/viii ganglion. In a subgroup the white arrowheads indicate the same rhombomere boundary on each section. The vii/viii ganglion complex is indicated by a white open arrow. Members of a subfamily are expressed in the same rhombomeres, and expression limits of subfamilies differ from each other by two rhombomeres, with the exception of *labial*. There are differences between subfamily members in amounts of expression in rhombomeres. *Hox-1.4* is expressed in posterior regions of r7 (*b*), but expression decreases to background amounts at the r6/r7 boundary. *Hox-2.6* is expressed up to this boundary (*a*), but expression is much weaker in the anterior portion of the rhombomere. *Hox-4.2* is expressed with a smaller decrease at the r6/r7 boundary (*c*). *Hox-4.1* has highest amounts of expression in rhombomeres posterior to r6, and a lower amount of expression distinct from background in r5 (*f*). *Hox-2.7* (*d*) and *Hox-1.5* (*e*) show complementary amounts of expression in the same rhombomeres as *Hox-4.1*. *Hox-2.8* has highest amounts of expression in r3, r4 and r5 (*g*), whereas *Hox-1.11* has highest amounts in r3 and r5 (*h*). *In situ* hybridization was as described[35]. Eight probes were as previously described: *Hox-2.6, 2.7, 2.8* and *2.9* (ref. 4); *Hox-1.4* and *-2.4* (refs 13, 36, *Hox-1.5* (ref. 27); *Hox-1.6* (ref. 37). *Hox-4.1* was isolated by polymerase chain reaction according to the protocol of Frohman and Martin[38], using oligonucleotide sequences derived from the EMBO database. In mouse only one gene related to *pb* is known, *Hox-2.8*. In human, however, there is also an equivalent in *HOX-1, HOX-1K* (ref. 39). Sequence comparison revealed little DNA sequence homology between *Hox-2.8* and *HOX-1K* (data not shown)[39], and northern analysis revealed specific hybridization of *HOX-1K* probe to mouse RNA, at a size distinct from *Hox-2.8* transcripts (data not shown). Human probes are able to hybridize specifically to their mouse equivalents under high-stringency *in situ* conditions, and *HOX-1K* probes recognize a distinct band in mouse genomic DNA whose size is different from that containing *Hox-2.8*. A *Hox-4.9* complementary DNA was isolated from an 8.5-day mouse embryo library, using a probe from the human *HOX-4G* Gene[39]. Sequence was determined enzymatically using dideoxy nucleotides, and shows greater similarity to human *HOX-4G* than to other *labial* family gnes. The peptide sequence of the homeobox is: PSAIRTNFSTKQLTELEKEFHFNKYLTRARRIEIANCLQLNDTQVKIWFQNRRMKQ-KKRER (single-letter amino-acid code).

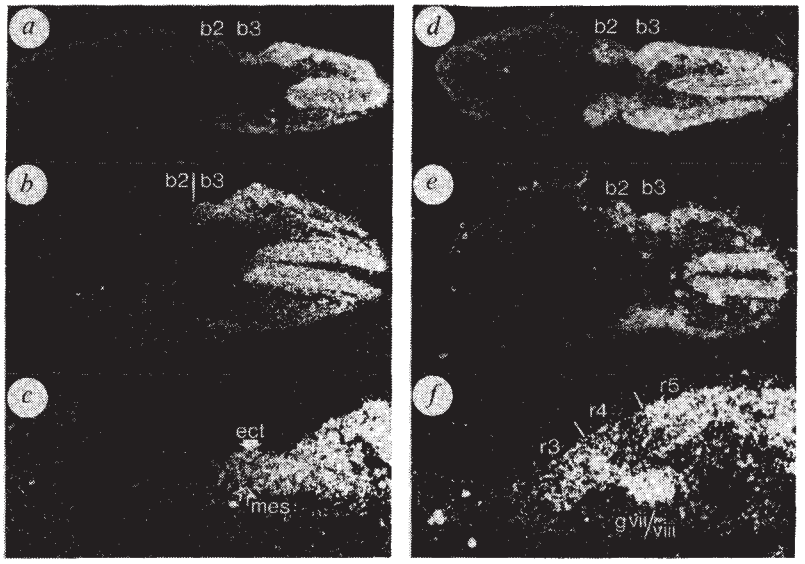

FIG. 2 Expression of genes of the *Dfd, zen*-like and *pb* subfamilies in the branchial arches of a 9.5-day mouse embryo. *a, Hox-2.7*; *b, c, Hox-1.5*; *d, Hox-2.8*; *e, f, Hox-1.11*. *Hox-2.7* and *Hox-1.5* are expressed in the mesenchyme (mes) of the third and posterior branchial arches, and not in b1 and b2. *c*, Detail of *b*, showing that *Hox-1.5* has the same restrictions of expression in branchial arch ectoderm (ect) as *Hox-2.7*. *Hox-2.8* (*d*) and *Hox-1.11* (*e*) are expressed in the second and posterior branchial arches, but not in the first. *f*, Detail of *e*, showing *1K* expression in the vii/viii ganglion complex and the expression in the neural tube. In contrast to *Hox-2.8*, which shows strong expression in rhombomeres 3, 4 and 5, *1K* shows strongest expression in r3 and r5, with similar levels in r4 to more posterior parts of the hindbrain. Probes as in Fig. 1.

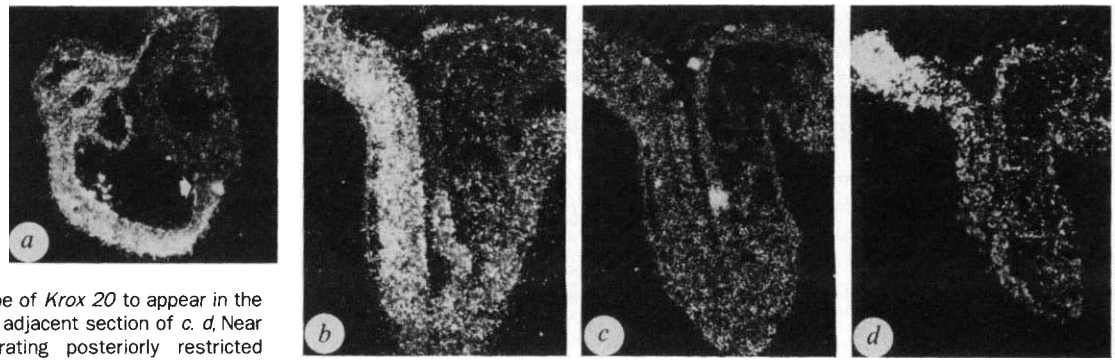

FIG. 3 Expression of *Hox* genes in 8-day mouse embryos. *a, b, Hox-2.8*; *c, Krox* 20; *d, Hox-4.9*. *a*, Sagittal section of a 0-somite embryo, with an anterior limit indicated by a white arrow. This shows a similar localization to the one-somite embryo shown in *b*, where the anterior expression limit corresponds to the first stripe of *Krox 20* to appear in the hindbrain, shown in the near adjacent section of *c*. *d*, Near adjacent section demonstrating posteriorly restricted expression of *Hox-4.9*. Probes as for Fig. 1.

Our results show that, with the exception of *labial*, the *Hox* subfamilies show very similar spatial and temporal expression patterns during critical phases of head morphogenesis (summarized in Fig. 4). This suggests conservation of regulatory elements between *Hox* clusters necessary to generate these expression domains. The high degree of similarity in protein sequence[13,14] and gene expression in a subgroup[15] could imply early functional compensation or redundancy. On the basis of dorso-ventral distributions[16] and differences between subfamily members in the same domains of expression[15] (Fig. 1), we think that subfamily members are uniquely required for the development of specific tissues. Neural crest migrates from specific rhombomeres[5,17] and carries a form of prepatterning, which not only enables it to specify a particular structural pattern, but also enables it to instruct surrounding mesodermal and ectodermal tissue[1]. The *Hox* patterns support the idea that by specifying rhombomere identity, patterning information can be transferred to the branchial arches through neural crest[5,18,19]. These patterns define a combinatorial *Hox* code for the generation of regional diversity in the branchial region, and make predictions of the effects of altering *Hox* gene expression on mouse development. A combinatorial *Hox* code is also important in the patterning of the limb[20-22] and paraxial mesoderm of the trunk[7,23,24].

Direct support for these predictions comes from mice homozygous for null alleles of *Hox*-1.5 (ref. 25) and *Hox-1.6* (ref. 26). *Hox-1.5* mutants show defects in tissues derived from mesenchymal cranial neural crest, yet have normal rhombomeres and cranial ganglia[25], despite expression in these tissues (Figs 1*a* and 2*b*)[27]. By contrast, *Hox-1.6* mutants have defects concentrated in cranial ganglia in the r4/r7 region[26]. These phenotypes support the suggestion that specific subfamily members are required for patterning particular subsets of cranial neural crest.

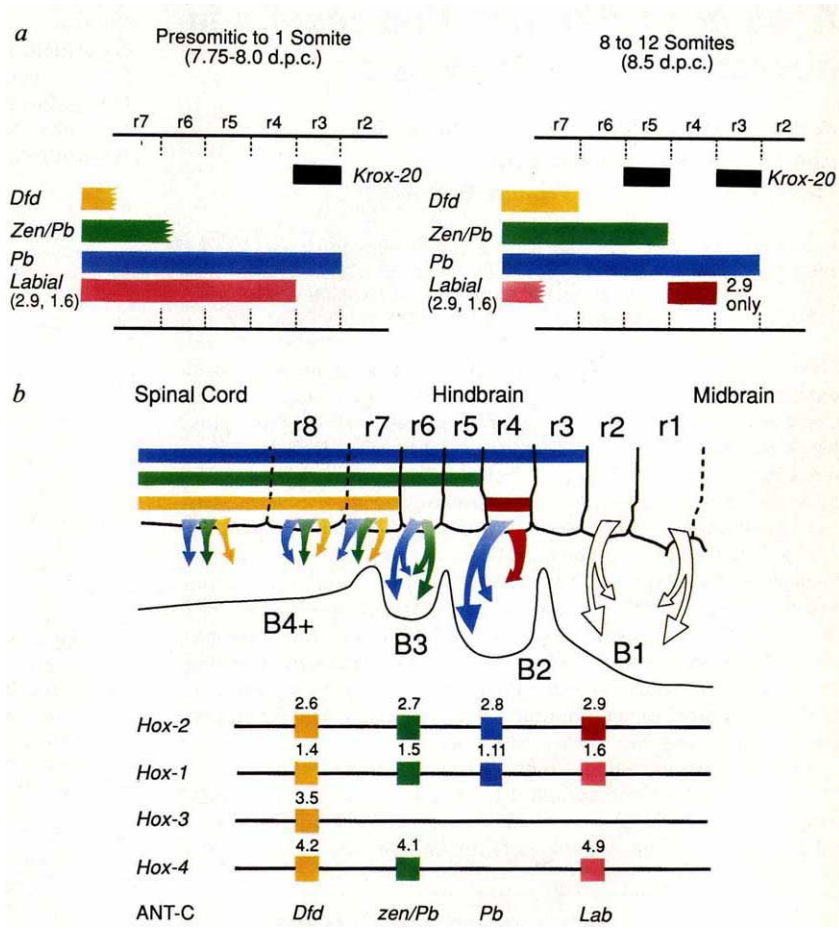

FIG. 4 Summary of the establishment of the *Hox* code in the head and its transmission to the branchial apparatus by migrating neural crest. The genes that correspond to the subfamily expression domains indicated are shown at the base of *b* in the same colours. There is evidence from characterization of human *HOX* clusters that there are no *Hox* genes 3′ of *Hox-3.5* (ref. 39). The early domain of *Hox-1.6* and *Hox-2.9* is indicated in pink, and the later domain of *Hox-2.9* is indicated in red. We have no evidence for *Hox-4.9* expression in the hindbrain at any stage. *a*, Establishment of expression before the appearance of morphologically indentifiable rhombomeres. The presumptive boundaries are indicated with dotted lines. At 8 days the *pb* family of genes and *Hox-1.6* and *Hox-2.9* of the *labial* family[11] have reached anterior expression boundaries that show the same relationship to the rhombomere 3 *Krox 20* stripe as they do when overt rhombomere morphology appears. At this stage genes of the *Dfd* and *zen/pb* groups have not yet reached their anterior limits[4] indicated by the broken anterior limits, consistent with the idea of temporal collinearity of establishing expression with position of a gene in a cluster. At 8.5 days when two *Krox 20* stripes are emerging, the differences in expression between *Hox-2.9* and *Hox-1.6* are apparent[11]. The posterior domains of both genes are receding caudally, whereas only *Hox-2.9* shows an expression domain restricted to r4. Genes of the other 3′ subfamilies are now all expressed with the same relationships to each other and *Krox 20* as at later stages. At this stage neural crest migration is occurring. *b*, Expression pattern after neural crest migration is complete, when distinct rhombomeres are apparent. *Krox 20* is expressed at this stage, but its expression domain is omitted for clarity. The migration of neural crest, indicated by coloured arrows, has transferred a combinatorial code of *Hox* subfamily expression to the cranial ganglia and branchial arches. The short red arrow indicates that *Hox-2.9* expression is confined to the ganglionic crest.

In the *Hox-1.5* and *-1.6* mutants there was no evidence for transformations of structures, in contrast to the transformations of cervical vertebrae seen when *Hox-1.1* is ectopically expressed in somites[24]. Because the development of the head involves interactions between rhombomeres, neural crest, surface ectoderm, paraxial mesoderm and pharyngeal endoderm, the alterations of *Hox* expression in a subset of these might not generate a simple transformation, particularly as the role of *Hox* genes in patterning the mesoderm and endoderm is not clear. Furthermore, removal of one *Hox* gene may alter expression of multiple *Hox* genes and other components of the head specification network.

The *Hox* code of the branchial region is different from that of the trunk, where anterior boundaries of subfamily members can be offset from each other. There are many morphological grounds for believing that the head and trunk have distinct developmental mechanisms[1,18,28,29], which we believe has resulted in the use of the same genes in different ways in the two contexts. *Antennapedia* class *Hox* genes are not expressed in more anterior parts of the head, which must therefore employ other genes[30–32] and patterning mechanisms[28]. In an analogous way the patterning of anterior parts of the head in *Drosophila* is also thought to involve molecular mechanisms independent of *Antennapedia* class genes[33,34]. □

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A *de novo Alu* insertion results in neurofibromatosis type 1

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NEUROFIBROMATOSIS type 1 (NF1) is a common autosomal dominant disorder with a high mutation rate and variable expression, characterized by neurofibromas, *café-au-lait* spots, Lisch nodules of the iris, and less frequent features including bone deformities and learning disabilities[1]. The recently cloned *NF1* gene encodes a transcript of 13 kilobases from a ubiquitously expressed locus on chromosome 17 (refs. 2–4). Most NF1 patients are expected to have unique mutations, but only a few have so far been characterized, restricting genetic and functional information and the design of DNA diagnostics. We report an unusual *NF1* mutation, that of a *de novo Alu* repetitive element insertion into an intron, which results in deletion of the downstream exon during splicing and consequently shifts the reading frame. This previously undescribed mechanism of mutation indicates that *Alu* retrotransposition is an ongoing process in the human germ line.

The 31-year-old male patient (D.D.) exhibits several features of NF1, including one cutaneous neurofibroma, axillary freckling, Lisch nodules, cervical nerve root tumours, and macrocephaly. *Café-au-lait* spots are not present. His parents show no signs of NF1, and DNA fingerprinting analysis found no evidence of nonpaternity. Part of the *NF1* complementary DNA detected an abnormal Southern blot pattern in the patient's DNA after digestion with several restriction enzymes[2]. This was consistent with a small insertion (300–500 basepairs (bp)) in a 3.8-kilobase (kb) *Eco*RI fragment which contains six *NF1* exons

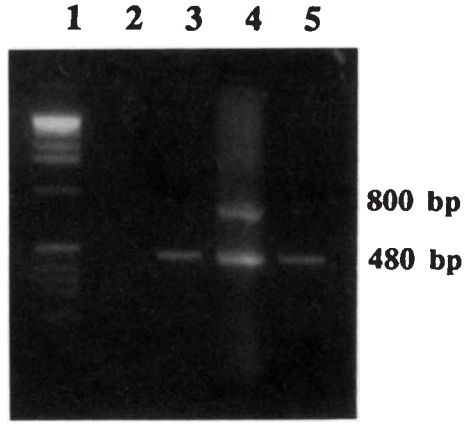

FIG. 1 Ethidium bromide staining of a 1.0% agarose gel demonstrates the insertion in the exon 6 PCR product. Lane 1 contains the BRL 1-kb ladder, lane 2 contains a water (negative) control, lanes 3 and 5 are products from the patient's father and mother, respectively. and the patient's PCR products are shown in lane 4. All show the normal fragment of 480 bp, but the patient also has an abnormal fragment of ~800 bp. DNA from both the patient's leukocytes and from an established lymphoblastoid line gave the same result (data not shown).

METHODS. Genomic DNA from the patient and his parents was extracted as described[2]. Genomic DNA (100–500 ng) was amplified using the exon 6 primers already described[3], with 35 cycles (each cycle entailed 1 min each at 94°C for denaturation, 65°C for annealing, and 72°C for extension) using standard buffers and reagents recommended by Cetus. One-tenth of each PCR reaction was loaded per lane.

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