the possible effects of shared ancestry and exercise on the clinical expression of a specific mutation, especially in subpopulation groups with known founder effects.

Although these examples of divergent phenotypic expression in kindred 101a and the twins in pedigree 138 are based on small numbers, they lend support to the notion that HCM is not a simple monogenic disorder and that both genetic and environmental factors are modifiers of the disease phenotype. A strategy followed in studies of disease phenotypes with multifactorial aetiology is to reduce the complexity of analysis by investigating genetically homogeneous subjects. The presence of the founder *MYH7* A797T mutation suggests that the families harbouring it share a degree of common ancestry. We therefore propose that the presence of this HCM causing mutation with incomplete penetrance, in a substantial group of related people, provides an opportunity to investigate the role of additional factors involved in the development of the disease phenotype. Only when these factors are known will the puzzling variability in the clinical expression which is a feature of HCM mutations, and the true pathophysiology of this disease, be understood.

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Germline and somatic mosaicism in achondroplasia

EDITOR—We describe a sib recurrence in achondroplasia with parents of normal stature. Both affected offspring carry the same causal mutation (G1138C) in the fibroblast growth factor receptor 3 (*FGFR3*) gene. Despite having no clinical features of achondroplasia, a proportion of the mother's peripheral blood leucocytes also contained the mutant *FGFR3* allele. We conclude she is a germline and somatic mosaic for achondroplasia and that both children have inherited the condition from her. To our knowledge, this is the first confirmed case of germline mosaicism in achondroplasia.

Achondroplasia is the commonest form of short limbed dwarfism (birth incidence estimated at between 1:10 000 and 1:70 000)[1] and is transmitted as an autosomal dominant trait. As is often the case among dominant traits, a high proportion of cases are new mutations but achondroplasia is unusual in that the great majority are caused by one of two mutations at the same nucleotide in the transmembrane domain of the *FGFR3* gene (G1138A transition and G1138C transversion).[1] In common with other *FGFR3* mutations which cause skeletal dysplasia, the pathogenic effect of the achondroplasia mutations is thought to be altered mitogenesis and/or differentiation owing to constitutive activation of the receptor.[2] There is a marked paternal age effect in achondroplasia and it has recently been shown that new mutations in achondroplasia are almost exclusively of paternal origin.[3] We received

peripheral blood DNA from a family with two children with achondroplasia; both parents were of normal stature. They had a total of four children of whom the second and fourth were affected. The mother was 27 years of age and the father 53 years at the birth of their second affected child. Our first thoughts in this case, taking into account the age of the father, were that the affected sibs were the result of two independent new mutations in the paternal germline, as would be expected to occur by chance as a very rare event.

To determine the *FGFR3* mutation(s) in the affected offspring, blood was collected from the affected children and from both parents. DNA was extracted and exon 10 of the *FGFR3* gene was amplified and products were digested with the restriction enzymes *Bfm*I and *Msp*I. The G1138A transition creates a restriction site for *Bfm*I whereas the G1138C transversion creates a restriction site for *Msp*I. Analysis showed that both children were heterozygous for the rare G1138C transversion. The father did not have the mutation in his blood but, surprisingly, the mother did. However, the ratio of the G1138C allele compared to the wild type allele was less than the 1:1, which would be expected for a straightforward heterozygote (fig 1). The relative proportion of the G1138C allele in the mother's blood leucocytes was determined using primer extension[4] (fig 1) followed by densitometry. The proportion of the mutant allele in the mother was found to be 28%. She has a height of 169 cm, span of 171 cm, upper segment/lower segment 0.09, left hand 17.6 cm, and head circumference of 58 cm. Apart from her slightly larger head size and mild obesity her appearance is normal.

We conclude that, despite her normal appearance, the mother is a germline and somatic mosaic for the G1138C mutation and both her affected children have inherited the mutant allele from her. Given the mother's relatively high proportion of mutant alleles, her lack of phenotypic expression is surprising; a hypochondroplasia-like phenotype, which is less severe than achondroplasia, might have been expected. The most likely explanation for this is the tissue specific distribution of the mosaicism, although the mutant allele is present in 28% of her peripheral blood leucocytes it may be at lower levels in her chondrocytes.

Germline and somatic mosaicism are both reasonably common features of genetic disorders. For example, in Duchenne muscular dystrophy and osteogenesis imperfecta, 15% and 6% of cases, respectively, inherit the condition from a detectably mosaic parent.[5] Germline mosaicism results from a mutation in gamete precursors which then continue to divide, whereas combined germline and somatic mosaicism arises when the mutation occurs very early in development before the germline and somatic lineages have separated. As achondroplasia is a common condition which arises from a highly mutable nucleotide, high frequencies of mosaicism might have been expected. Surprisingly, the frequency of germline mosaicism as evidenced by sib recurrence is very low. A few cases of recurrence have been reported,[5 6] but so infrequently that it has been calculated that they could be accounted for by independent mutations alone. Clinical reports of somatic mosaicism in achondroplasia are also extremely rare.[7] For some reason, somatic and germline mosaics occur much more rarely in achondroplasia than in many other dominant traits. One possibility is that for reasons as yet unclear, *FGFR3* nucleotide 1138 is only hypermutable in the male germline. Alternatively, there could be somatic selection against cells carrying the mutant allele. Interestingly, Apert syndrome, which is mainly caused by either of two point mutations in *FGFR2*, also seems to have low levels of germline mosaicism.[5] This apparent low incidence of somatic mutation is at variance with recent
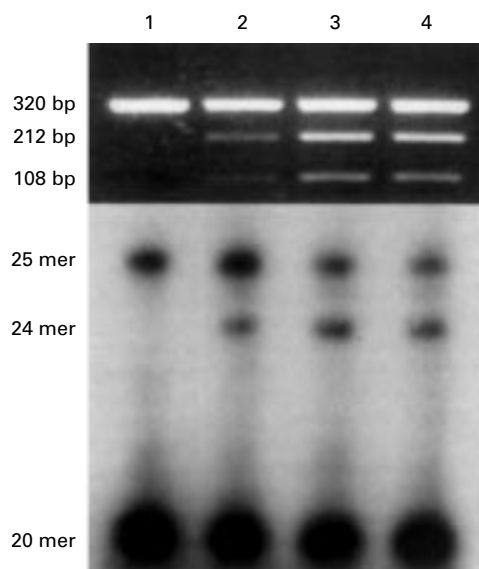
Figure 1   (Upper panel). Digestion of 320 bp FGFR3 genomic PCR product with MspI. Lanes 1, father; 2, mother; 3, first affected child; 4, second affected child. Primers used were 5'-GGAGATCTTGTGCACGGTGG-3' and 5'-GCGCGTGCTGAGGTTCTGAG-3'. (Lower panel) Primer extension was carried out with primer 5'-GATGAACAGGAAGAAGCCCA-3' (which binds 4 bp downstream of nucleotide 1138) using methods described in Loughlin et al.[4] The primer was end labelled with γ³²P dATP and the extension mix contained dA, dT, dC, and ddG. The 20mer primer was extended by 4 bp for the achondroplastic G1138C allele and by 5 bp for the wild type allele as shown below (added nucleotides are shown underlined, nucleotide 1138 is shown in bold).

*Wild type template: 5'-C**G**GGGTGGGCTTCTTCCTGTTCATC-3'*
Extended primer
(25mer):   3'-ddGCCCCACCCGAAGAAGGACAAGTAG-5'
*G1138C template: 5'-C**C**GGGTGGGCTTCTTCCTGTTCATC-3'*
Extended primer
(24mer):   3'-ddGCCCACCCGAAGAAGGACAAGTAG-5'
The products were then separated by electrophoresis through a 15% denaturing polyacrylamide gel. The relative intensity of the 24mer and 25mer products was used to calculate the proportion of achondroplastic to wild type allele. Lane order and PCR primers as above.

findings that somatic activating mutations of *FGFR3* are relatively common in multiple myeloma[8] and carcinomas.[9] However, all the *FGFR3* mutations so far identified in these malignant neoplasms are identical to activating mutations that cause thanatophoric dysplasia. The greater severity of this phenotype in comparison to achondroplasia is thought to be a reflection of the more strongly activating nature of the thanatophoric dysplasia mutations.[10] That only these highly activating *FGFR3* mutations have so far been found in neoplasms may suggest that the achondroplasia mutations, when they occur in somatic cells, do not activate the receptor to a level that it becomes oncogenic.

This is the first confirmed report of germline and somatic mosaicism for an achondroplasia mutation. *FGFR3* nucleotide 1138 appears to be highly mutable in the male germline, but somatic mutations resulting in mosaicism are rare. The reasons for this discrepancy are unknown but are clearly of importance to the understanding of mutagenesis. The observation that the mother has a normal appearance, despite a high proportion of the achondroplastic allele in her somatic tissues, exemplifies the fine balance that the fibroblast growth factors play in morphological determination.

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Achondroplasia with the *FGFR3* 1138g→a (G380R) mutation in two sibs sharing a 4p haplotype derived from their unaffected father

EDITOR—The study of achondroplasia, the most frequent skeletal dysplasia in man, has contributed several important insights into both developmental biology and human genetics, such as the recognition of the paternal age effect for dominant mutations,[1][2] the first indication of the importance of FGFR molecules in growth and development,[3] and the identification of the nucleotide with the highest mutation rate known so far in man, nucleotide 1138 of the *FGFR3* gene.[4] Most cases of achondroplasia are associated with the g→a transition at nucleotide 1138 of *FGFR3*.[4]

In spite of the frequency of achondroplasia, the birth of two or more children with achondroplasia to unaffected parents is surprisingly rare, with only a few examples published.[5][6] One instance of half sibs with achondroplasia born to the same father has been reported.[7] In contrast, observations of achondroplasia in people more distantly related are relatively more common.[8–10] Thus, it is uncertain whether instances of achondroplasia in sibs born to unaffected parents are caused by somatic mosaicism (as suggested by the observation of three affected sibs from normal parents[6][7]) or by independent chance events.[8] Undoubtedly, somatic or germinal mosaicism for achondroplasia must be orders of magnitude rarer than for osteogenesis imperfecta or other dominant conditions.[11]

Approximately, 90% of cases of achondroplasia are caused by de novo mutations, and all de novo achondroplasia mutations studied so far were found to have occurred on paternal chromosomes.[12] We observed achondroplasia with characteristic clinical and radiographic signs in a brother and sister born to parents of normal stature, lacking any clinical sign of either hypochondroplasia or achondroplasia, aged 28 years (mother) and 25 years (father) at the time of birth of the first child (fig 1). The family agreed to have the molecular mechanism of recurrence investigated and consented to venepuncture and buccal smears. Both children were heterozygous (in leucocyte DNA) for the g1138a (G380R) *FGFR3* mutation, while that mutation was not found in parental leucocyte or buccal smear DNA by either SSCP analysis or direct sequencing of PCR products. This made parental somatic mosaicism unlikely.

Figure 1   Clinical appearance of the brother (right) and sister (left) with achondroplasia aged 12 and 10 years, respectively.

To investigate the origin of the mutation shared by the two affected sibs, inheritance of VNTR alleles on chromosome 4p was studied (fig 2). The affected children had two different maternal haplotypes but shared a paternal 4p haplotype encompassing the *FGFR3* locus. As the *FGFR3* g1138a mutation occurs exclusively on paternal chromosomes, and the affected children had two different maternal 4p haplotypes, the most likely explanations for these findings would be either two independent mutational events occurring by chance on the same paternal haplotype, or mosaicism at the spermatogonial level (before meiosis I) in the father. Paternal sperm was not available and the hypothesis of gonadal mosaicism could not be further substantiated.

We conclude that recurrence of achondroplasia in this family was associated with de novo mutational event(s) occurring in the paternal germline, as is the case in sporadic cases,[12] but could not distinguish between paternal gonadal mosaicism or the chance occurrence of two independent mutation events.[8] The apparent rarity of