

**Fig. 2** Molecular analysis of *HOXA11*. **a**, Sequencing autoradiograph from an affected individual (mutant) shows a single nucleotide deletion (arrow) in exon 2 compared with a normal control. **b**, Amino-acid sequence encoded by exon 2 of *HOXA11* demonstrates the location of the mutation and expected consequences. This region encodes the tri-helical homeodomain. The point mutation identified in our patients occurs at a highly conserved asparagine residue (arrow) in the third helix, a region that is vital for DNA binding. Compared with normal sequence, this deletion (nucleotide underlined) results in premature termination codon and truncation of the remaining 22 aa of the *HOXA11* protein.

human non-neoplastic haematologic disorder, and only the third HOX gene implicated in a human syndrome<sup>6,7</sup>. Although current data indicate a correlation between the point mutation and abnormal skeletal development, evidence positively linking the two is required. Studies of Hox genes have largely involved the use of null mutants, involving loss of function rather than an alteration of function. Mouse models of hypodactyly (*Hd*) and synpolydactyly (*spdh*), carrying mutations of *Hoxa13* and *Hoxd13*, respectively, reinforce the usefulness of examining non-null mutants to further our understanding of homeobox regulation<sup>14,15</sup>. Studies of

this new *HOXA11* mutation will advance our knowledge of Hox gene function, and determine their roles in bone morphogenesis and early haematopoietic lineage commitment and proliferation.

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## A region on chromosome 3 is linked to dizygotic twinning

A chromosome 3 region containing the gene (*PPARG*) encoding peroxisome proliferator activated receptor (*PPAR* $\gamma$ ) may be related to dizygotic twinning. Linkage in 181 dizygotic twin pairs yielded a 6.93 lod score. The *PPARG* C→T substitution allele was far less common in monozygotic twins than in dizygotic twins. Dizygotic heterozygosity was less than expected and transmission disequilibrium test (TDT) analysis gave further evidence for association.

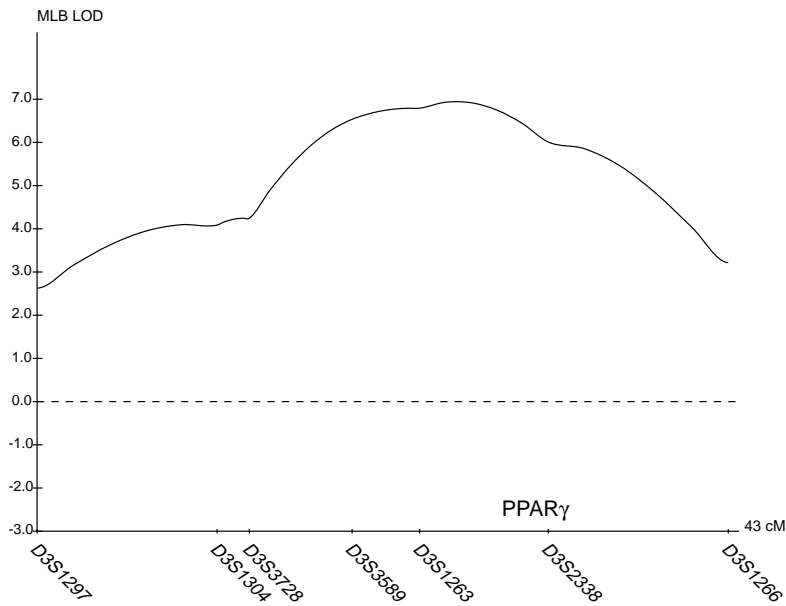
We initially studied 222 pairs of normal young monozygotic (122) and dizygotic (100 pairs) twins in an investigation of *PPARG* as a candidate quantitative trait locus for cholesterol levels<sup>1</sup>. We analysed *D3S1297*, *D3S1304*, *D3S3728*, *D3S3589*, *D3S1263*, *D3S2338* and *D3S1266*, as well as the silent C→T substitution. Heterozygosity for this polymorphism was much lower than expected, causing us to hypothesize

that *PPARG* is linked to dizygotic twinning. A linkage analysis in dizygotic twins (phenotype: being a dizygotic twin) whose parents were available for analysis yielded a multipoint 3.49 lod score. Mean sharing of alleles ( $\pi$ ) was 0.64 (expected 0.50). After approval, we recruited 116 nontwin sibpairs from Berlin, 50 unselected dizygotic twin pairs from Finland and 31 unselected dizygotic twin pairs from Poland. The nontwin sibpairs did not deviate from mendelian transmission. The 181 dizygotic twin pairs gave a 6.93 lod score ( $\pi=0.65$ ; Fig. 1). The C→T substitution allele was lower in monozygotic twins (0.09) and parents (0.13) than in dizygotic twins (0.19). Genotype-based tests were significant for monozygotic compared with dizygotic twins ( $P<0.001$ ) and for parents compared with dizygotic twins ( $P<0.05$ ). Allele frequencies of dizygotic twin mothers and fathers were not different (0.15 versus

0.12). In dizygotic twins, the C→T substitution was not in Hardy–Weinberg equilibrium (CC, 72%; CT, 18%; TT, 10%); homozygosity was over-represented. In monozygotic twins and parents of dizygotic twins, Hardy–Weinberg proportions were maintained.

The Pro12Ala *PPARG* polymorphism in exon B obeyed Hardy–Weinberg equilibrium. We compared allelic frequencies between dizygotic twins, monozygotic twins and the general population for five randomly selected microsatellites and other biallelic polymorphisms. We found a significant difference in dizygotic twins compared with monozygotic twins and the general population for only the C→T polymorphism in *PPARG*. We next carried out TDT tests in dizygotic twins whose parents were informative for analysis. Haplotype analysis was significant when both *PPARG* polymorphisms were studied, even when only one sibling was selected randomly from each pair. Single polymorphism TDTs were not significant.

Our evidence implicating the chromosome 3 region is supported by four



**Fig. 1** The multipoint lod scores in all dizygotic twin subjects for the markers tested are shown, with *D3S3608* showing the maximum lod score of 6.93.

observations. We have evidence from model-free linkage analysis. We have a positive allelic association. Hardy–Weinberg proportions for the intragenic *PPARG* polymorphism were not maintained in dizygotic twins. We have evidence from a haplotype-based TDT analysis with both the biallelic Pro12Ala marker in exon B and the C→T substitution in exon 6. These two polymorphisms are not in tight linkage disequilibrium. Exon B is present in the *PPARG* splice variant *PPARγ2*, which is present in white fat, whereas exon 6 is present in both<sup>2</sup>.

Multiple births require both multiple conceptions and intrauterine survival of both twins. ‘Vanishing twins’ are well known, with about 40% of spontaneous dizygotic twin pregnancies resulting in singleton births<sup>3</sup>. About 30% of all multiple pregnancies after assisted reproduction result in a singleton birth<sup>4</sup>, and this effect is not explained by environmental factors<sup>5</sup>. Dizygous twinning has long been suspected to have a genetic basis<sup>6</sup>. Weinberg suggested that hereditary twinning is transmitted through the female line, applies only to dizy-

gotic twins and is probably recessive<sup>7</sup>. In our study, the C→T substitution allele was greater in dizygotic twins compared with their mothers and fathers, and monozygotic twins, making a maternal effect less likely<sup>8</sup>. Evidence from Mormon records supports a recessive inheritance<sup>9</sup>. We found that the C→T polymorphism was not in Hardy–Weinberg equilibrium in dizygotic twins with an over-representation of homozygous individuals. This phenomenon has been described<sup>10</sup> as indicative of a heterogeneous recessive model.

A tenfold increase in the twinning rate has been recorded in a small village in southern Brazil<sup>10</sup>. High rates for twinning have also been recorded on the archipelago of Åland and Aboland, in southwest Finland<sup>12</sup>. Lummaa *et al.*<sup>13</sup> used Finnish church records in a case-control design and provided evidence that differences in twinning frequencies in isolated populations may be maintained by natural selection. Life-history models predict that resource levels favour the evolution of increased reproductive output.

There are many genes in this segment of chromosome 3. We believe that *PPARG* is a candidate for dizygotic twinning. *PPARγ* influences insulin-related effects, lipid metabolism and body mass index, which are all important to the growth process. The two splice variants, only one of which contains the Pro12Ala polymorphism, may explain the fact that we observed a positive association only with the C→T polymorphism. The role of *PPARG* as a ‘thrifty’ gene seems to be established<sup>14</sup>. Intra-uterine selection may be responsible for the Hardy–Weinberg deviation and we may in fact be dealing with a gene involved in the intrauterine survival of dizygotic twins.

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