Mutations in the follicle-stimulating hormone receptor and familial dizygotic twinning

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Concentrations of follicle-stimulating hormone (FSH) have an important role in multiple ovulation. An association has been reported between mutations in the FSH receptor (FSHR) in a family with increased twinning frequency. We sequenced the transmembrane region of FSHR (located on chromosome 2) in 21 unrelated mothers of dizygotic twins and found no differences to the published sequence. A linkage study of 183 sister pairs and trios, in which all sisters had given birth to spontaneous dizygotic twins, excluded linkage to this region of chromosome 2. We conclude that mutations in FSHR are not a common cause of familial dizygotic twinning.

Genetic predisposition is an important factor that determines natural dizygotic twinning. The probability of a subsequent twin pregnancy is increased four-fold in mothers of twins, and the risk of having dizygotic twins is roughly doubled for women whose mother or sister has dizygotic twins.[1,2] We ascertained Australian and New Zealand families with two or more sisters who had each given birth to spontaneous dizygotic twins.[3] The study protocol was reviewed and approved by the Bancroft Centre Research Ethics Committee and every participant gave informed consent. Mothers were asked about fertility treatments and all such cases were excluded. Twins were confirmed as dizygotic on the basis of difference in sex, obvious phenotypic differences in colouring or appearance, or genetic-marker analysis.

Gain-of-function mutations in the follicle-stimulating hormone receptor (FSHR) could increase the incidence of dizygotic twinning. Two mutations in FSHR that were in linkage disequilibrium have been reported,[4] and a woman who was homozygous for the mutations gave birth to two sets of twins. Exon 10 of FSHR encodes the entire transmembrane region of the gene. We sequenced this region of exon 10 in 21 unrelated mothers of dizygotic twins from our sister-pair families with the highest twin incidence. In six of these families three sisters each had dizygotic twins, and in ten there were sister pairs with twins and a set of twins in at least one other generation. In one family, two sisters each had two sets of twins (figure 1). Genomic DNA samples were amplified with primers that flanked the transmembrane region (5′-GACTATGACTTATGCAATGAA-3′ and 5′-CTCTGGGAGCTGAAGAGCAGT-3′) and DNA polymerase (VENT, New England Bio Lab; Beverly, MA, USA). The PCR product on both strands was sequenced with Sequenase by the dideoxy-chain-termination method (US Biochemical; Cleveland, OH, USA). In all 21 mothers the exon 10 sequence was identical to the published sequence and no mutations were detected.

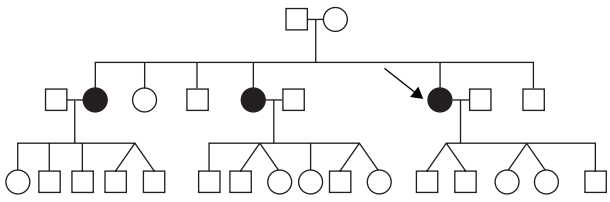

Figure 1: **Pedigree for one of the cases showing three sisters, all with dizygotic twins**
Arrow=transmembrane region of the follicle-stimulating hormone receptor was sequenced for this individual.

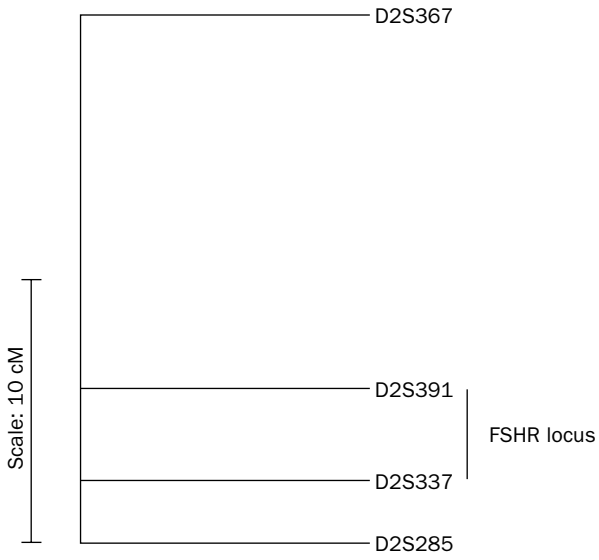

Figure 2: **Linkage map of the follicle-stimulating hormone receptor locus and flanking markers that were typed in the genome scan**
cM=centimorgan.

A genome scan was done in 383 mothers of dizygotic twins from 166 sister pairs and 17 sister trios ascertained as before. Genomic DNA samples were typed for 25 highly polymorphic microsatellite markers across chromosome 2,[3] which included flanking markers within 10 centimorgans of FSHR (figure 2). Markers were typed by standard methods and products separated on denaturing polyacrylamide gels on ABI 377 automated DNA sequencers. Neither non-parametric methods nor models for common recessive or dominant inheritance of a gene for twinning[1,2] showed linkage to the region containing FSHR.

Bulmer[1] suggested that a single recessive locus (allele frequency 50%) could explain variation in twinning. Under this model, with allele frequencies similar to those reported for variants in the FSHR,[4,5] we excluded linkage in the region between D2S391 and D2S337 that contains FSHR with a lod score of −8.

We searched for activating mutations in the transmembrane region of FSHR. We sequenced a fragment of exon 10 that included the entire transmembrane region of the receptor, but not portions of exon 10 that contain two linked mutations in the extracellular domain (Thr307Ala) and intracellular domain (Asn680Ser). These mutations reduce sensitivity of the receptor.[5] We detected no mutations in the 21 women previously described.

The two linked mutations in the receptor are common in white populations and affect ovarian response to FSH.[5] Perez Mayorga and colleagues[5] have shown that women homozygous for the serine residue at position 680 have raised basal FSH concentrations and reduced sensitivity to exogenous FSH. The index case, who Al-Hendy and colleagues[4] described, was homozygous for the serine variant at position 680. Despite the reduced sensitivity of this variant form of FSHR, it could still play some part in twinning frequency. However, the region of DNA that contains FSHR was not linked to dizygotic twinning in our families. We conclude that the mutations in FSHR do not explain familial aggregation of twinning in the population we have studied, which is largely descended from British and Irish people.

This work was supported by grants from the Cooperative Research Centre for the Discovery of Genes for Common Human Disease, the Australian NH, and MRC (900499 and 950995).

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Velopharyngeal incompetence and chromosome 22q11 deletion

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Chromosome 22q11 deletion gives rise to various phenotypes, including cardiac malformations, velopharyngeal abnormalities, absent thymus, and neurological defects. We assessed, in a prospective study, chromosome 22q11 deletion in 50 of 144 patients with velopharyngeal incompetence in the absence of overt clefting. 18 (12·5% of the whole cohort and 36% of patients tested for the deletion) had the 22q11 deletion. This frequency differs from an estimated population prevalence of 0·025% and suggests a need for screening for the 22q11 deletion in these patients.

Interstitial deletion within chromosome 22q11 is thought to be one of the main causes of genetic syndromes, with a frequency estimated as high as one in 4000 live births.[1] This deletion gives rise to various phenotypes, including cardiac malformations, velopharyngeal abnormalities, absent thymus, and neurological defects. Raymond and colleagues [2] showed a 22q11 deletion in 1% of fetuses with cardiac abnormalities and in 10% of those with conotruncal (anterosuperior portion of the right ventricle and adjacent pulmonary artery) heart defects. This deletion has also been shown in 5% of children with congenital heart defects.[3] Tests for the 22q11 deletion are recommended for patients with conotruncal heart defects. However, guidelines for deletion tests on patients with velopharyngeal abnormalities are not clear. We are aware of no reported deletion frequency. However, after the probes for this region became available, an initial retrospective frequency for the deletion was high in patients with full velocardiofacial syndrome.[3] In a European collaborative study of 496 patients with the 22q11 deletion, 14·5% had either an overt cleft palate or submucous cleft, and 32% had velopharyngeal incompetence.[4] Additionally, Zori and colleagues[5] noted that six (38%) of 16 patients with velopharyngeal incompetence had this deletion.

We assessed the deletion prospectively in a group of patients with velopharyngeal incompetence, without overt clefting, referred to the plastic surgery unit at the Queen Victoria Hospital, East Grinstead, UK. From May, 1995, to May, 1999, 144 patients were referred for velopharyngeal incompetence. Of these patients 50 (35%, median age 8 years [range 4-26]) presented with two or more specific features (table) and were referred for deletion tests. We analysed data by the non-parametic Wilcoxon's rank sum test or Fisher's exact test for contingency tables.

18 (36% [95% CI 22·9%–50·8%]) of these patients had 22q deletions. Median age of the patients with and without deletions did not differ significantly (p=0·86). Information on characteristics was incomplete for some patients and some totals are not equal. For most of these characteristics, there were no significant differences between the deleted group and the normal group, although learning difficulties and problems with infant feeding had borderline significance. Characteristic facial features (broad nasal bridge with narrow tip and nostrils, slant to the eyes characteristic of Down's syndrome with narrow palpebral fissures and an appearance of ptosis, flattened malar prominences, and flat midface), and a bland aspect, which suggests a neurological involvement, were strong indicators of 22q11 deletion (p<0·001). Fewer patients had submucous clefting in the deleted group than in the non-deleted group, although this difference was of borderline significance (table). Many of these patients had been screened by other health professionals who had not referred them for genetic testing, possibly because of the absence of clefting.

Early diagnosis of patients with 22q11 deletions is important for clinical management and for the patient's family because up to 28% of these deletions may have been inherited.[4] Our data suggest that the frequency of 22q11 deletions might be high in patients with velopharyngeal incompetence and we recommend that these patients are screened.

	Deleted group		Non-deleted group		p
	Present (%)	Absent	Present (%)	Absent	
Speech and language delay*	16 (94%)	1 (6%)	23 (77%)	7 (23%)	0·228
Learning difficulties†	13 (81%)	3 (19%)	13 (50%)	13 (50%)	0·056
Infant feeding: regurgitation or slow	14 (93%)	1 (7%)	16 (64%)	9 (36%)	0·060
Snoring	9 (82%)	2 (18%)	11 (92%)	1 (8%)	0·590
Mouth breather	6 (86%)	1 (14%)	9 (82%)	2 (18%)	1·000
History of adenoidectomy	3 (25%)	9 (75%)	8 (38%)	13 (62%)	0·703
History of tonsillectomy	1 (8%)	11 (92%)	4 (19%)	17 (81%)	0·630
Grommets inserted	7 (58%)	5 (42%)	7 (67%)	14 (67%)	0·273
Family history	2 (13%)	13 (87%)	2 (8%)	22 (92%)	0·631
Cardiac anomalies	5 (28%)	13 (72%)	4 (13%)	28 (87%)	0·253
Typical facial appearance	15 (94%)	1 (6%)	1 (3%)	31 (97%)	<0·001
Submucous cleft	1 (6%)	17 (94%)	9 (28%)	23 (72%)	0·073

*Assessed by a cleft palate specialist speech and language therapist with standard protocols. †Not formally assessed.

Clinical features in velopharyngeal incompetence patients with and without 22q11 deletion

We thank Cathryn Lewis for statistical analysis.

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