RDCI, the vasoactive intestinal peptide receptor: a candidate gene for the features of Albright hereditary osteodystrophy associated with deletion of 2q37

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Abstract

Albright hereditary osteodystrophy (AHO) is an autosomal dominant disorder characterised by the presence of brachymetacarpal hypoplasia, short stature, obesity, and mental retardation. Variable biochemical changes may represent either pseudohypoparathyroidism (PHP) owing to resistance to parathormone (PTH) or pseudopseudohypoparathyroidism (PPHP) with no hormone resistance. In most cases of AHO, reduced levels of Gsα have been found and a number of deactivating mutations in the gene for Gsα located on chromosome 20q13 have been described. Recently a number of people with an AHO-like phenotype have been reported in whom a deletion of chromosomal region 2q37 has been found in the absence of biochemical abnormalities or a reduction in Gsα activity.

We present a further female patient with a cytogenetically visible deletion of 2q37, an AHO-like phenotype, and unusual biochemistry suggesting moderate PTH resistance. The vasoactive intestinal peptide receptor (RDCI) has recently been mapped to 2q37 and we propose that this is a candidate gene, hemizygosity of which affects signal transduction and leads to the AHO-like phenotype found in patients with 2q37 deletions.

Case report

This young woman was referred at the age of 18 years to the Clinical Genetics Department for assessment of her severe obesity and mental retardation. She was born to a 22 year old mother and 25 year old father. Her parents were healthy and non-consanguineous. The pregnancy was complicated by toxemia and she was delivered by caesarean section at term, weighing 2440 g. The mother had suffered from toxemia in a previous pregnancy, which had resulted in the delivery, by caesarean section at 32 weeks’ gestation, of a growth retarded male who had died at 4 days of age. There was a younger male sib who was well.

A squat was noted in this girl in the neonatal period, as were bilateral toe deformities, including short fourth metatarsals bilaterally, more pronounced on the right. At 2 months of age she was noted to be hypotonic and feeding poorly. She was unable to sit unsupported until 18 months of age and did not walk independently until 4 years of age. She had marked learning difficulties and attended a special school for children with learning difficulties. Until puberty (at the age of 13 years), her height was on the 25th centile and her weight was on the 90th centile. When assessed at the age of 18, her height was below the 3rd centile while her weight was significantly above the 97th centile. In addition to short stature and gross obesity, particularly involving the lower
I The four hands were relatively small and limbs, she was noted to have a round face and short fourth metatarsals bilaterally, as before. Her hands and feet were generally small and the fourth and fifth metacarpals of her hands were relatively short (figs 1, 2, 3, and 4).

CYTOGENETIC AND MOLECULAR CYTOGENETIC INVESTIGATIONS

Conventional G banded chromosome analysis of semisynchronised PHA stimulated lymphocytes showed the patient to have a female karyotype with a small terminal deletion of the long arm of chromosome 2 with a breakpoint at q37.1. Chromosome painting with a flow sorted chromosome 2 library (Cambio) showed the deleted chromosome 2 to be composed entirely of chromosome 2 material. No hybridisation of the chromosome 2 paint to any other chromosome was found. Parental chromosomes were normal. The patient’s karyotype was, therefore, 46,XX,del(2)(pter→q37.1):de novo.ish del(2)(wcp2+) (fig 5).

Cell lines have been established at the European Collection of Animal Cell Cultures, Porton Down: patient’s No CB 0320, mother’s No CB0319, father’s No CB0318.

MOLECULAR INVESTIGATIONS

DNA was obtained from the proband and both parents. The parental origin of the distal 2q deletion was determined by PCR amplification of polymorphic microsatellite repeat sequences located within the region encompassed by the deletion, at D2S125 and D2S140. Primer sequences have been published and are available on the Genome Data Base. The results were visualised using a 6% denaturing polyacrylamide gel followed by autoradiography. The results at loci D2S125 and D2S140 show that only paternal alleles have been inherited at these loci, consistent with a deletion of maternal origin (fig 6).

BIOCHEMICAL INVESTIGATIONS

At the age of 18 normal levels were reported for calcium, phosphate, 25-OH vitamin D, T4, TSH, LH, and FSH. Alkaline phosphatase was slightly raised at 109 IU/l (age related reference range 30-95 IU/l). The parathyroid hormone (PTH) level was raised at 17.5 pmol/l on one occasion and 9.9 pmol/l on another (normal level <7.3 pmol/l). A PTH infusion test based on previously published methodologies was undertaken: 200 NRC units of PTH were injected over five minutes. Blood and urine samples were collected as outlined in table 1. A less than twofold increase in plasma cAMP and less than fourfold increase in urinary cAMP is taken as evidence of PHP with hormone resistance. Our results showed a greater than threefold increase in cAMP in plasma and a greater than 12-fold increase in urinary cAMP. However, these results were in the low normal range as normal patients may exhibit a 10-fold increase in plasma cAMP and a 200-fold increase in urinary cAMP. These results, together with the raised PTH levels, would suggest a moderate PTH resistance or feed back mechanism in this patient.

Discussion

There are now 10 cases of de novo 2q37 deletions reported with an AHO-like phenotype not including the present case. These were seven females and two males ranging in age from 6½ years to 20 years of age and one case of unstated age and sex. PTH was assessed and reported to be normal in four of these cases and a PTH infusion test in one patient was reported to show a normal response. A diagnosis of PPHP was made in most cases on the basis of normal calcium and phosphate levels.

The possibility of imprinting has previously been suggested as a likely explanation for the variable occurrences of PHP and PPHP in familial instances of AHO. However, the parental origin of the 2q37 deletion was investigated in six of these cases and was shown to be maternal in five cases including the present case (fig 6) and paternal in one case. We have also investigated the parental origin of the
deletion in two previously reported cases from our laboratory in which the AHO phenotype has yet to be fully investigated. One case showed a maternal deletion and one had a paternal deletion (R S James, unpublished data). These results would seem to exclude any simple imprinting effect in this condition.
D2S125, the most telomeric 2q marker described. Molecular analysis confirms that the subject of this report has inherited only paternal copies of two distal microsatellite alleles including D2S125, confirming that the MRO is deleted in this case. These data suggest the presence of a candidate gene or genes in the 2q37.3 region which may be involved in hormone signal transduction. Haploinsufficiency of these genes may provide an alternative pathway for development of an AHO-like phenotype.

Vasoactive intestinal peptide (VIP) is a neuroendocrine mediator found in the central and peripheral nervous system. RDCI has recently been identified as the VIP receptor and has been cloned and mapped to 2q37. It is a member of the G protein coupled receptor family whose effect is to increase intracellular cAMP via activation of adenyl cyclase. VIP has been shown to be a potent activator of adenyl cyclase in many systems. In particular it has been shown that in vitro organ cultures VIP acts to enhance bone resorption via a cAMP dependent mechanism which is similar to that induced by PTH. In addition, raised levels of VIP are found in the rare non-beta islet cell tumours of the pancreas known as vipomas (Verner-Morrison syndrome) and associated with hypercalcaemia in 75% of patients. Raised levels of VIP resulting in increased stimulation of intracellular cAMP via the VIP receptor could, therefore, be the cause of the majority of hypercalcaemic vipoma patients in whom hypercalcaemia cannot be explained by concomitant parathyroid hyperplasia. In the case of deletion of 2q37 leading to hemizygosity of the VIP receptor, an equivalent but opposite effect on calcium metabolism may therefore provide an alternative aetiology for the AHO phenotype. In particular, a reduction of VIP receptor sites may lead to an increase in PTH via a feedback mechanism consistent with the moderately raised PTH levels in the subject of this report. The implication of this proposal is that VIP and its receptor are required for calcium homeostasis.

No syndrome has as yet been associated with a deficiency of VIP itself, which has been mapped to 6q26-27. It may be, however, that microndeletion or other mutations of this region are implicated in some cases of AHO that are not caused by either deletivating mutations in Gsα gene (GNAS1) in Albright hereditary osteodystrophy.