Recessive mutations in *PTHR1* cause contrasting skeletal dysplasias in Eiken and Blomstrand syndromes

Sabine Duchatelet¹, Elsebet Ostergaard², Dina Cortes³, Arnaud Lemainque⁴ and Cécile Julier¹,*

¹Genetics of Infectious and Autoimmune Diseases, Pasteur Institute, INSERM E102, 28 rue du docteur Roux, 75724 Paris Cedex 15, France, ²Department of Medical Genetics, The John F. Kennedy Institute, Gl. Landevej 7, 2600 Glostrup, Denmark, ³Department of Pediatrics, Glostrup University Hospital, Ndr. Ringvej 57, 2600 Glostrup, Denmark and ⁴Centre National de Génotypage, 2 rue Gaston Crémieux, CP 5721, 91057 Evry Cedex, France

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Eiken syndrome is a rare autosomal recessive skeletal dysplasia. We identified a truncation mutation in the C-terminal cytoplasmic tail of the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) type 1 receptor (*PTHR1*) gene as the cause of this syndrome. Eiken syndrome differs from Jansen and Blomstrand chondrodysplasia and from enchondromatosis, which are all syndromes caused by *PTHR1* mutations. Notably, the skeletal features are opposite to those in Blomstrand chondrodysplasia, which is caused by inactivating recessive mutations in *PTHR1*. To our knowledge, this is the first description of opposite manifestations resulting from distinct recessive mutations in the same gene.

INTRODUCTION

Eiken syndrome is a rare familial skeletal dysplasia which has been described in a unique consanguineous family, where it segregates as a recessive trait (1). It is characterized by multiple epiphyseal dysplasia, with extremely retarded ossification, principally of the epiphyses, pelvis, hands and feet, as well as by abnormal modeling of the bones in hands and feet, abnormal persistence of cartilage in the pelvis and mild growth retardation (1). On the basis of the genetic study of this original family, we report here that a truncation mutation in the C-terminal tail of the parathyroid hormone (PTH)/PTH-related peptide (PTHrP) type 1 receptor (*PTHR1*) gene is responsible for this syndrome.

RESULTS

We have studied the family originally described by Eiken, from which six individuals were available (Fig. 1). In addition to Eiken syndrome, one of the patients developed type 1 diabetes at 9 years of age. The association of multiple epiphyseal dysplasia and insulin-dependent diabetes in this case would strictly classify her as having Wolcott–Rallison syndrome (WRS) (2), although the later age at onset, the absence of a systematic association of skeletal dysplasia and diabetes in this family and the unique clinical features, particularly in the pelvis, hands and feet, make it clearly distinct. By linkage analysis and mutation screening, we excluded the implication of the pancreatic eIF2α kinase gene (*EIF2AK3*), which is responsible for WRS (3, 4), in this patient (data not shown).

Despite the limited size of this family (five informative individuals), the maximum expected LOD score, assuming full genetic information in the region of linkage, would reach a value of 3.3. We therefore performed a genome-wide scan using 400 microsatellite markers, and identified a single region of linkage, located on chromosome 3p, with a maximum multilocus LOD score of 3.2 (Fig. 1). As expected, the region of linkage is broad, ~50 cM, between markers D3S2338 and D3S1285. This region may contain in the order of 500 genes, and mutation screening of all these genes would not be practical. Using Mapviewer interface (http://www.ncbi.nlm.nih.gov/mapview), we found one gene in this region that has been previously implicated in some forms of chondrodysplasias: the *PTHR1* gene; this is a G protein-coupled receptor, which is involved in the regulation of chondrocyte proliferation and differentiation and plays a major role in bone development (5, 6). We therefore considered this gene as a good candidate for Eiken syndrome. We screened the totality of the coding region of the gene in two heterozygous
individuals (individuals 5 and 6) and their homozygous affected child (individual 1) and identified a C > T substitution in the last exon, resulting in a nonsense mutation ARG485STOP (Fig. 2A). We confirmed the cosegregation of this mutation in the homozygous status with Eiken syndrome in this family, both by sequencing (data not shown) and by a PCR–RFLP assay (Fig. 2B). Using the same assay, we confirmed the absence of this mutation in 160 Caucasian controls. The mutation is located in the C-terminal cytoplasmic tail of the protein, resulting in a variant truncated of its last 108 amino acids. This domain contains a cluster of serine residues which are phosphorylated upon ligand binding; it is able to bind to several proteins, including G protein receptor kinases and β-arrestin, and has been shown to be involved in the desensitization/internalization process of the receptor and in its regulation (5).

**DISCUSSION**

Mutations in PTHR1 have been reported in two types of skeletal dysplasias: metaphyseal dysplasia in Jansen chondrodysplasia, a dominant disorder resulting from constitutively activating mutations (7), and osteosclerosis and advanced skeletal maturation in Blomstrand chondrodysplasia, a recessive lethal disorder resulting from inactivating mutations (8). A mutation in PTHR1 has also been reported in enchondromatosis, a dominant disorder characterized by multiple cartilage tumors, frequently associated with skeletal deformity (9). In addition, PTHR1 knock-out mice exhibit a phenotype similar to Blomstrand syndrome (10). In contrast to Blomstrand chondrodysplasia patients, patients with Eiken syndrome have a severely delayed skeletal maturation, although both disorders are caused by recessive mutations in PTHR1. The phenotype in Eiken syndrome is more similar to Jansen chondrodysplasia, although the syndromes clearly differ by the mode of inheritance, specific skeletal features and calcium and phosphate concentrations; calcium and phosphate levels were found to be within normal range in Eiken patients (1), while Jansen patients have severe hypercalcemia and hypophosphatemia (6). In addition, the serum PTH level was found to be slightly elevated in Eiken patient 3, while it was normal in
patients with Jansen syndrome (6). Circulating levels of 1,25-(OH)₂VitD were normal in the Eiken patient, while the levels were found to be elevated in patients with Jansen syndrome (6). To our knowledge, this is the first description of opposite manifestations resulting from distinct recessive mutations in the same gene.

PTHR1 activates several signal transduction pathways, including adenyl cyclase (AC)/protein kinase A (PKA) and phospholipase C (PLC)/protein kinase C (PKC). Recent studies have shown opposite effects of these two pathways on chondrocyte differentiation, the former increasing the proliferation of chondrocytes and delaying their differentiation, opposite to the latter (6). This was evidenced in a knock-in mouse model expressing solely a mutant form of PTHR1 (DSEL) modified in the second intracellular loop, that activates AC/PKA normally, but not PLC/PKC; this mouse shows a recessive phenotype with delayed ossification, particularly for the 480STOP variant, while the PLC/PKC activity was unaltered (12). In addition, these truncated variants carrying truncations in the C-terminal cytoplasmic areas and normal calcium and phosphate levels (11). Despite the different nature and location of the mutations, these abnormalities are remarkably similar to the distinctive features observed in Eiken patients, who also have delayed ossification, principally of the hands and feet, abnormal development of some cartilage areas and normal calcium and phosphate levels.

Extended in vitro studies have been performed on PTHR1 variants carrying truncations in the C-terminal cytoplasmic tail. PTHR1 variants truncated at positions 480 and 513 showed a marked increase of the AC/PKA signaling activity, particularly for the 480STOP variant, while the PLC/PKC activity was unaltered (12). In addition, these truncated variants showed decreased expression, so that the net effect may be an unchanged AC/PKA activity and a decreased PLC/PKC activity in vitro (12), leading to similar overall consequences as the DSEL variant (13). We therefore propose that the expected unbalanced AC/PKA versus PLC/PKC activity caused by the Eiken mutation is responsible for a phenotype similar to the DSEL mouse. Alternatively, or in addition, part of the biological functions that are mediated by PTHR1 and altered in Eiken syndrome may occur in the cytoplasm or the nucleus, where PTHR1 has also been localized, and for which there is increasing evidence for a role in mediating biological effects (5,14). The C-terminal tail of PTHR1 is likely to be involved in these functions, because of its role in the receptor conformation, in the stabilization of some protein complexes and because of the presence of a predicted nuclear localization signal at positions 471–487 (15), which is disrupted in the ARG485STOP mutant.

One of the four Eiken syndrome patients (individual 3) developed type 1 diabetes at 9 years of age. This patient presented GAD autoantibodies at onset of diabetes, and had the high risk HLA (DRB1*03/DRB1*04) and insulin (INS-23HphI A/A) genotypes. The diabetes in Eiken syndrome therefore differs from that in typical WRS, which manifests early, usually before 6 months of age, is not autoimmune and is systematically associated with the specific epiphyseal dysplasia in patients with EIF2AK3 mutations (4). Interestingly, PTHRH was shown to mediate pancreatic β-cell growth (5), and we hypothesize that the PTHRII mutation in Eiken syndrome may be responsible for a reduced β-cell mass, which may increase the risk of diabetes in genetically predisposed individuals.

Despite the extreme rarity of Eiken syndrome (a small unique family), we were able to characterize the molecular defect underlying it. This is the fourth disease associated with mutations in the PTHRII gene, and our observation provides further insight into the multiple functions mediated by this receptor, which has important therapeutic potentials for many diseases, including osteoporosis and diabetes.

MATERIALS AND METHODS

Patients and family

We studied the family originally reported by Eiken et al. (1). Six individuals were available for study, four of whom were affected with this syndrome (Fig. 1). Individuals 1 and 4 belong to the original sibship of three affected children described in this previous report (cases 3 and 1, respectively), where they were still in childhood. Individual 2 was an affected cousin. A previous child from this couple (individuals 1 and 2), affected with the same syndrome, died earlier and samples were not available for study. Adult patients’ height was slightly decreased (153.5 and 154 cm for individuals 1 and 2, respectively), as was their child (individual 3) at 10 years of age (130.2 cm, ±1 SD). In the original article, all standard biochemical analyses were normal in all the patients examined, including calcium and phosphate levels. In addition to these, serum PTH level was measured in child 3 and found to be slightly elevated (63 ng/l; normal range: 10–50 ng/l); 1,25-(OH)₂VitD was normal in this patient. In addition to Eiken syndrome, individual 3 developed type 1 diabetes, with onset at 9 years of age. GAD autoantibodies were positive at onset of the diabetes. She was treated by subcutaneous insulin injections. All individuals participating in this study gave their informed consent.

Microsatellite genotyping

Genome scan was performed by semi-automated fluorescent genotyping, using 400 microsatellite markers (Linkage Mapping Set 2, Applied Biosystems), as described (http://www.cng.fr/fr/teams/microsatellite/index.html).

Mutation screening by sequencing of genomic DNA

Mutation screening was performed on genomic DNA from an affected individual (individual 1) and his unaffected parents (individuals 5 and 6) using primers shown in Table 1. Sequencing reactions were performed using big-dye terminator chemistry using standard protocols and run on an Applied Biosystems Sequencer ABI3700.

PCR–RFLP genotyping of the mutation

The region containing the mutation was first amplified from genomic DNA with primers used for sequencing (fragment 24, Table 1); a nested PCR was then performed with primers 5’-cactggcaactgacctacg-3’ and 5’-tgccagtgggtagg-3’. The forward primer includes a mismatched base in
order to create an artificial discriminative site. PCR fragments were then digested with BsaAI restriction yielding fragments of 148 bp (non-mutated allele) or 129 + 19 bp (mutated allele), which were resolved by agarose gel electrophoresis.

**Linkage analyses**

Parametric multic locus linkage analysis was performed using SIMWALK program (16).

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