Screening of genes involved in cAMP-mediated signalling in a large Italian series of patients affected with Albright hereditary osteodystrophy and/or Pseudohypoparathyroidism

Elli FM, Bordogna P, deSanctis L, Spada A, Mantovani G

Dep. of Clinical Sciences and Community Health, University of Milan, Endocrinology and Diabetology Unit, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico

INTRODUCTION

Pseudohypoparathyroidism (PHP) is a heterogeneous group of rare genetic metabolic disorders due to molecular defects at the GNAS locus, that encodes also for the α-subunit of the stimulatory G protein (Gsα), causing end-organ resistance to the actions of PTH. The classification of the different subtypes of PHP is based on the presence of specific somatic and developmental abnormalities, referred to as Albright hereditary osteodystrophy (AHO), and the resistance to other hormones acting via GPCRs.

Recently, mutations in genes encoding proteins crucial for cAMP-mediated signalling different from Gsα and deletions of chromosome 2q37.2 have been detected in a small subset of patients with PHP with no GNAS defects, showing a phenotypic overlap with Acrodystosis (ACRDYS) and brachydactyly-mental retardation syndrome (BDMR), also called AHO-like syndrome. Despite the high detection rate of genetic and epigenetic defects by currently available molecular approaches, about 30% of PHP patients still lack a molecular diagnosis, hence the need to screen patients negative for GNAS genetic or epigenetic defects also for chromosomal regions and genes associated to diseases that undergo differential diagnosis with PHP.

MATERIALS & METHODS

In this study, we screened by Sanger sequencing and multiplex ligand-dependent probe amplification (MLPA) our series of AHO/PHP patients negative for GNAS locus genetic and imprinting defects (sporadic or genetic-based), for the presence of mutations at PRKAR1A gene (n=79), PDE4D gene (n=18), as well as for deletions affecting the subtelomeric chromosome region 2q37 (n=89).

Sanger sequencing

Genomic DNA was extracted by Nucleon BACC2 genomic DNA purification kit from peripheral blood leukocytes of both patients and parents (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer’s instructions. PRKAR1A and PDE4D exons and flanking intronic sequences were amplified by PCR and direct sequencing of amplicons was performed using the AmpliTaq BigDye Terminator kit and 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The mutation nomenclature follows the guidelines indicated by Human Genome Variation Society (HGVS).

Multiplex Ligation-dependent Probe Amplification assay (MLPA)

Dosage of allele segments was performed by MLPA using the SALSA MLPA P264 Human Telomere-9 probemix (MRCHolland, Amsterdam, The Netherlands). The protocol was implemented following the manufacturer’s recommendations. MLPA PCR products were separated by capillary electrophoresis using ABI3130xl Genetic Analyzers (Perkin-Elmer Corp.) with an internal size standard GeneScan 500LZ (Applied Biosystems, Foster City, CA). Data analysis was performed using GeneMapper software (Applied Biosystems, Foster City, CA) and Coffalyser v9.4 (MRCHolland, Amsterdam, The Netherlands).

RESULTS

After the screening of our series of patients with a clinical diagnosis of PHP/AHO and no GNAS locus genetic and/or epigenetic defects, we detected 3 novel missense mutations (Fig.1) and 1 novel silent mutation at the PRKAR1A gene, 2 rare intrinsic variants at the PDE4D gene, with no apparent pathogenetic significance, and 4 heterozygous deletions of 2q37 (Fig.2), overlapping with previously described rearrangements affecting this subtelomeric region.

In silico analysis predicted a pathological effect for PRKAR1A genetic defects found in our patients, as they cause the substitution of highly conserved amino acid residues located in the cAMP binding domains (1 in the CBD-A and 2 in the CBD-B). Although most of the known PRKAR1A mutations alter the CBD-B, our finding strengthen the previous observation that also mutations affecting the CBD-A may be associated to Acrodystosis (Nagasaki et al. J Clin Endocrinol Metab 2012; 97: 1800-13). Interestingly, the patient with the de novo mutation Thr209Ala had an AHO phenotype with brachydactyly but no hormone resistances, while patients with Asp269Gly and Phe292Lue mutations showed a PHP-α phenotype (AHO + hormone resistances), so we confirm the variability of the clinical phenotype caused by PRKAR1A mutations.

Albeit the different extension of 2q37 deletions found in our cases (ranging from ~2.85-Mb up to ~4.71-Mb), patients share a smallest region of overlapping (SRO) that holds several genes (i.e. HDAC, GPC1, HDLBP, STK25) proposed as causative for the BDMR phenotype. Notably, 3 of 4 patients displayed also mild PTH resistance, a feature described only in one AHO-like patient (Power et al. J Med Genet 1997; 34: 287-290), but we exclude RCD1 as candidate gene as its location is upstream the deleted region found in our series.

CONCLUSIONS

Our data further confirm the molecular and clinical overlap among these disorders and highlight the complexity in performing an accurate diagnosis of PHP, as well as the pivotal role of the cAMP pathway in the development of the AHO phenotype.