Rapid Communication

Identification of the first deletion in the LRP5 gene in a patient with Autosomal Dominant Osteopetrosis type I

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A B S T R A C T
In the last decade, the low-density lipoprotein receptor-related protein 5 (LRP5) gene, coding for a coreceptor in the canonical Wnt signalling pathway, has been shown to play an important role in regulating bone mass and to be involved in the pathogenesis of several bone disorders. Here we describe a patient who presented with a clinical picture of Autosomal Dominant Osteopetrosis type I (ADO I), in whom we could identify the first deletion in the LRP5 gene causing increased bone mass. This mutation caused the in-frame deletion of two amino acids in the fourth blade of the first propeller of the protein, namely the highly conserved glycine at position 171 and the following glutamate residue. In vitro studies suggested that the pathogenic effect of this novel mutation could be due to a decreased inhibition of Wnt signalling by the antagonistic proteins sclerostin and Dickkopf-1, encoded respectively by the SOST and DKK1 genes, in the presence of mutated LRP5. Our results highlight an increasing molecular heterogeneity in LRP5-related bone diseases.

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Introduction

At the end of the eighties, Bollerslev and Andersen reviewed a large group of patients affected by Autosomal Dominant Osteopetrosis (ADO) and, on the basis of radiological and biochemical findings, suggested that two different types of ADO existed [1]. More recently this clinical observation was supported by the results of molecular investigations in patients, which showed that monoallelic defects in low-density lipoprotein receptor-related protein 5 (LRP5) gene caused human ADO I, in which the long bones and the skull are mainly affected, while mutations in a single allele of the chloride channel 7 (ClCN7) gene were responsible for ADO II, in which an increased rate of bone fractures is reported. Both LRP5 and ClCN7 proteins are involved in signalling pathways or cellular processes which are crucial in bone metabolism as demonstrated by the range of bone diseases arising from different mutations in either encoding gene. In particular, biallelic loss of function mutations in LRP5 are responsible for the autosomal recessive osteopetrosis pseudoglioma syndrome (OPPG; MIM 259770) [2–4]; on the contrary, monoallelic mutations in LRP5, initially thought to lead to a gain of function of the protein product, cause a range of phenotypes inherited in an autosomal dominant way and characterised by increased bone density. These are endosteal hyperostosis (MIM 144750), osteosclerosis (MIM 144750), dominant osteopetrosis (MIM 607634), van Buchem disease type 2 (VBC2; MIM 607636) and high bone mass syndrome (HBM; MIM 601884) [4–9]. In addition, studies in different populations have suggested that LRP5 could be a susceptibility gene for osteoporosis and fracture risk [10,11].

The specific clinical picture is strongly related to the LRPS domain affected by the mutation. So far, the LRP5 mutations reported to have an activating effect on Wnt signalling are all missense mutations and clustered in the first β-propeller domain of the protein. Biochemical studies showed that their effect is likely due to a reduced inhibition of the canonical Wnt pathway by sclerostin and Dickkopf-1 [12–17].

Here we report for the first time the identification and biochemical characterization of a mutation in the LRP5 gene leading to a two amino acid deletion in the protein, found in a patient affected by ADO I.

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Materials and methods

Case report

The patient herein described is a 56 year old woman of Caucasian origin, presenting with an ADO I phenotype. The diagnosis was made on the basis of radiological examinations, performed at menopause due to generalized bone pain, which she had been suffering from for many years. Increased bone density mainly involved skull base, mandible and legs.

No fractures were reported. At 16 years of age, she experienced complete and sudden blindness of the left eye, whose origin was not investigated. At 50 years of age, she had an infection of the right ear and subsequently monolateral impairment of the hearing capacity arose. At 55 years of age, ophthalmological and audiometric examinations demonstrated reduction of the visual capacity also of the right eye and worsening of the auditory problems. A CT scan performed after diagnosis showed a generalised thickening of the skull (Fig. 1) and restriction of both optical and auditory canals; in addition the patient referred frequent headaches.

Biochemical studies revealed normal values for serum calcium, phosphorus, 1,25(OH)2D3 and bone-specific alkaline phosphatase (ALP), while PTH was slightly increased.

Fig. 1. Axial view of a CT scan of our patient. A generalized thickening of the skull is evident.

Surgery and mutation analysis

DNA sample from the patient was obtained after receiving informed consent. Investigation has been approved by the Local Ethic Committee. Genomic DNA was extracted from PBL by standard techniques; mutation analysis of the LRP5 gene (AF283320) was performed as previously described [2].

Expression constructs and in vitro mutagenesis

The deletion found in the proband (g.69547_69552delGGTGAG) was introduced in untagged full-length human WT LRP5 construct (obtained from Dr. Matthew Warman, Howard Hughes Medical Institute, Orthopaedic Research Laboratories, Boston, MA; [2]) using the QuickChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) with forward primer 5′-CTGGACAGACTGAGGCCCCCCGATTG-3′ and reverse primer 5′-CAATCCGGGGCCTCACGTGTCGAC-3′.

The inserted sequence was verified for the presence of the mutation and absence of PCR errors by DNA sequencing.

A mouse Wnt1-V5 expression construct was provided by Dr. Bart Williams (Van Andel Research Institute, Grand Rapids, MI), a mouse mesd-2 expression construct was provided by Dr. Bernadette Holdener (State University of New York, Stony Brook, NY), a human DKK1-FLAG expression construct was provided by Dr. Sergei Sokol (Mount Sinai School of Medicine, New York, NY), a mouse amino terminal HA-tagged Sost (HA-mSost) expression construct was obtained from Dr. Matt Warman (Howard Hughes Medical Institute, Orthopaedic Research Laboratories, USA) and Dr. Bert Vogelstein (Johns Hopkins University School of Medicine, Baltimore, MD) kindly provided the Topflash Wnt reporter construct (pGL3-OT). The Renilla Luciferase construct prL-TK was purchased from Promega Corporation (Madison, WI) [9].

Cell culture, transfection and luciferase reporter assays

HEK293T and Saos-2 cells were grown in DMEM supplemented with 10% v/v FBS (Invitrogen, San Diego, CA). Twenty-four hours prior to transfection, cells were plated at 1.25 × 105 cells/well in 24-well plates. Cells were transfected using Fugene 6 (Roche Applied Science, Indianapolis, IN) according to the manufacturer's instructions. Transfection and luciferase reporter assay were performed as previously described [9].

Statistical analysis

Data are expressed as mean values ± SD. Comparison between two measurements for a single experiment was performed using a Student’s t-test. Values of p < 0.05 were considered significant. Statistical tests were provided by the SPSS 15.0 software package (IBM Corporation, Somers, NY).

Results

Mutation analysis

Direct sequencing of the region of interest in the LRP5 gene revealed the presence of an in-frame deletion of six nucleotides (g.69547_69552delGGTGAG; c.511_516delGGTGAG) in exon 3 in one allele, corresponding to two amino acid residues (p.Gly171_Glu172-del), while the other allele was normal (Fig. 2A). As has been reported for the other high bone mass–causing mutations, this newly identified one is also located in the first β-propeller domain of the protein, in its amino terminal, extracellular portion. It involves the glycine at position 171, which is highly conserved throughout evolution and between LRP5 and its homologue LRP6, and has been extensively studied. Interestingly, two missense mutations have been already reported at this very same position: a p.Gly171_Val, found in a family including phenotypically normal individuals with extremely dense bones [6] and in another kindred with other clinical features: torus palatinus and wide, deep mandible, in addition to increased bone density [5]; and a p.Gly171_Arg in a Belgian classical ADO I family [7].

Functional evaluation

To evaluate the functional effect of this new mutation, wild-type (WT) and mutant (Mut) LRP5 proteins were expressed independently either in the Saos-2 human osteosarcoma cell line or in HEK293T cells along with a luciferase reporter construct.

Co-transfection of LRP5 (either WT or Mut) with Wnt1 resulted in an identical increase in Wnt signalling (Fig. 2B). However, decreased...
inhibition was observed for the mutant LRP5 after co-transfection with either SOST or DKK1 or a combination of both. Similar results were obtained in HEK293T cells (data not shown). This suggested that the in vivo bone phenotype was caused by a decreased ability of the mutant protein to interact with these two inhibitory molecules.

Discussion

In the last two decades, an increasing amount of genetic data has proposed that the canonical Wnt signalling has been reported to regulate key checkpoints in the development of many tissues, and among them, the immune system. Overall, this disease could be considered as a gain of function for its inhibitory ligands. The canonical Wnt signalling is known to play an important role in haematopoiesis, and rarely present visual deficits, while these defects can be very evident in some ADO II patients. Interestingly, our patient did show a complete and abrupt occurrence of blindness at early age, although the exact cause could not be documented at that time (more than 40 years ago). All these findings confirm the original observation of Bollerslev and Andersen [1] that ADO I and ADO II are two distinct entities both from a clinical and molecular point of view.

The identification of a new type of mutation in the LRP5 gene in our patient highlights for the first time the presence of a molecular heterogeneity in LRP5-related diseases characterised by high bone density, which adds to the well-known clinical heterogeneity arising from mutations in this gene.

Conflict of interest

All authors declare no conflicts of interest.

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