1268-LBP

Genetic causes of disproportional short stature identified by whole exome sequencing

Mariana F. A. Funari[1], Gabriela A. Vasques[1,2], Antonio M. Lerario[2], Bruna Lucheze Freire[1], Sueli Mieko Oba Shinjo[3], Suely Kazue Nagahashi Marie[3], Ivo J. P. Arnhold[1] and Alexander A. L. Jorge[1,2]

[1] Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular LIM-42, Disciplina de Endocrinologia;
[2] Unidade de Endocrinologia Genética, Laboratório de Endocrinologia Celular e Molecular LIM-25, Disciplina de Endocrinologia;
[3] Laboratório de Biologia Molecular e Celular LIM-15, Departamento de Neurologia;
Faculdade de Medicina, Universidade de São Paulo, São Paulo, Brasil
(e-mail: alexj@usp.br)

Disclosure: There is no conflict of interest in this study.

Introduction

Disproportional short stature (DSS) is the most frequent clinical presentation of skeletal dysplasias, which are a heterogeneous group of more than 450 disorders of bone. Skeletal survey is important to establish the diagnosis and to guide the genetic test, but has several limitations, especially in mild and atypical cases.

Objectives

To identify the genetic aetiology of disproportional short stature by exome sequencing (WES).

Methods

DNA	Library Prep	WES	FASTQ	VCF	Sanger
6 patients and available relatives: 6 affected 5 unaffected	Agilent Sure Select XT All exonsV5	Illumina HiSeq2500 V3 and V4 (2x100)	Mean coverage: 170x	Freebayes ANNOVAR	Confirmation and segregation

Our analysis focused on functional variants absent in controls (ExAC, ESP6500, 1000Genomes and 1,218 alleles from healthy ethnic matched individuals) that segregate in the families.

Results

The mean coverage of the captured regions was 170x (99.6% of target region with more than 10x). Each patient had an average of 64,490 allelic variants. All pedigrees suggested an autosomal dominant pattern of inheritance.

We identified a causative variant predicted as pathogenic in 3 patients:

- **Case 1** with height SDS of -2.0 has a novel heterozygous mutation in *NPR2* gene (c.2905G>C/p.Val969Leu) (Figure 1 and Table 1). Heterozygous mutations in *NPR2* are a cause of short stature without a distinct phenotype[1].
- **Case 2** with height SDS of -4.5 has a heterozygous mutation in *FBN1* gene (c.5183C>T/p.Ala1728Val) (Figure 2 and Table 2). Mutations in *FBN1* were associates with geleophysic and acromicric dysplasias, but this patient lacks some of the cardinal features of these conditions[2].
- **Case 3** with height SDS of -2.5 and bilateral osteonecrosis of the femoral epiphysis has a heterozygous mutation in *COL2A1* gene (c.1852G>A/p.Gly618Ser) (Figure 3 and Table 3). Mutations in *COL2A1* cause several skeletal disorders with highly variable phenotype[3].

Case 1

Familial disproportional short stature with postnatal onset
- Appearance of muscular hypertrophy
- Good response to rhGH therapy

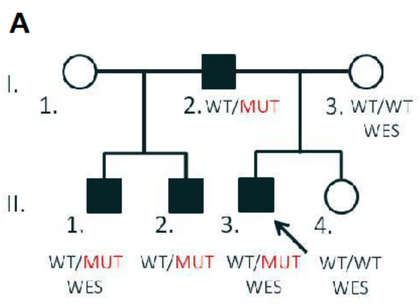
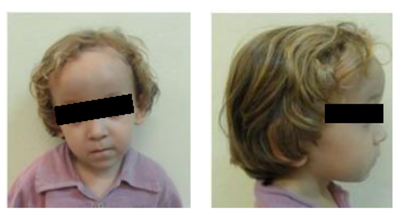


Table 1: Anthropometric measurements of family 1

	I.2	I.3	II.1	II.2	II.3	II.4
Age (y)	adult	adult	adult	adult	5.5	3.8
Height (cm)	157.3	164	153.8	162.7	95.7	96.2
Height SDS	-2.6		-3.1	-1.8	-3.2	-0.7
SH:H SDS	+1.3		+2.2	+1.3	+2.0	+0.8

SDS: standard deviation score; SH:H: sitting height: height ratio



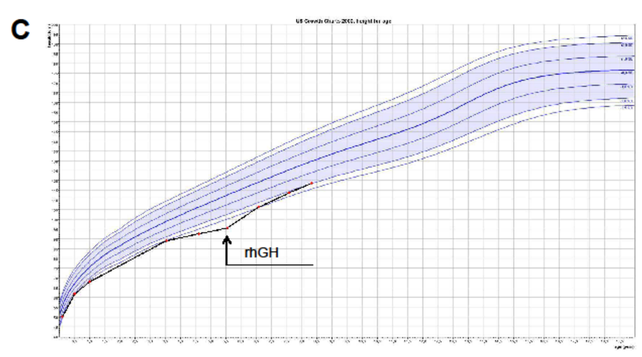


Figure 1. Family pedigree (A). The arrow indicates the proband. Affected subjects are indicated by black symbols, whereas individuals with normal stature are indicated by open symbols. The genotype (WT, wild type allele; MUT, mutant allele) for each family member are as indicated. WES indicate the individuals submitted to whole exome sequencing. The proband (II.3) (B). Growth chart of the proband (C). Flowchart of the WES study (D). The numbers indicate the allelic variants after each filtering process.

Case 2

Familial disproportional short stature with *genu varum*
- Mild acromelia (hands SDS -4.5)
- Poor response to rhGH therapy

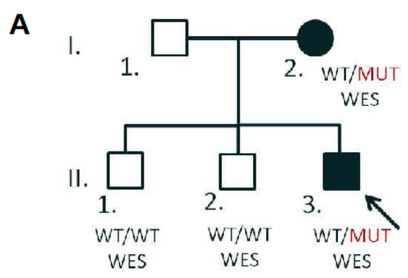


Table 2: Anthropometric measurements of the family 2

	I.1	I.2	II.1	II.2	II.3
Adult height (cm)	168	131.2	170	174	144.4
Height SDS	-1.0	-5.1	-0.7	-0.1	-4.5
SH:H SDS		+4.0			+5.2

SDS: standard deviation score; SH:H: sitting height: height ratio

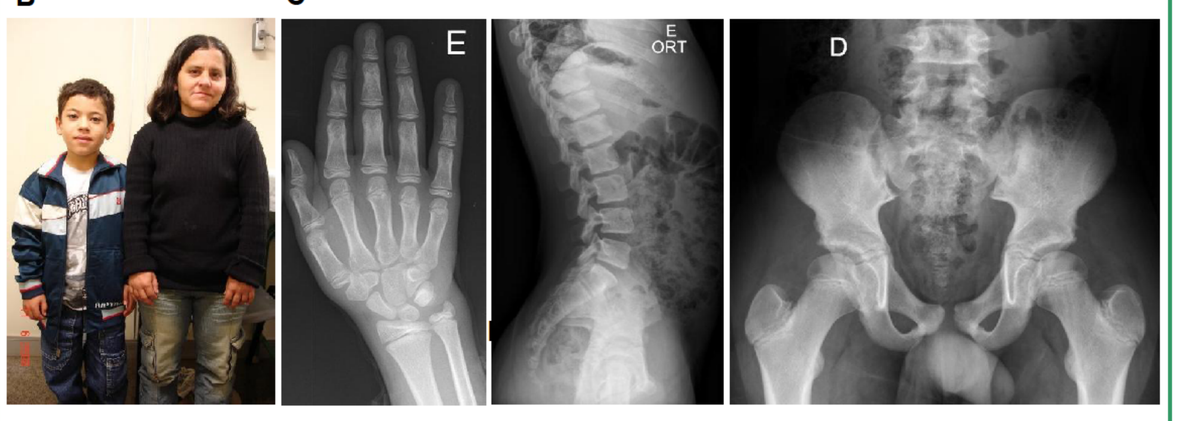


Figure 2. Family pedigree (A). The arrow indicates the proband. Affected subjects are indicated by black symbols, whereas individuals with normal stature are indicated by open symbols. The genotype (WT, wild type allele; MUT, mutant allele) for each family member are as indicated. WES indicate the individuals submitted to whole exome sequencing. The proband (II.3) and his mother (I.2) (B). Radiographic images of the proband (C). Flowchart of the WES study (D). The numbers indicate the allelic variants after each filtering process.

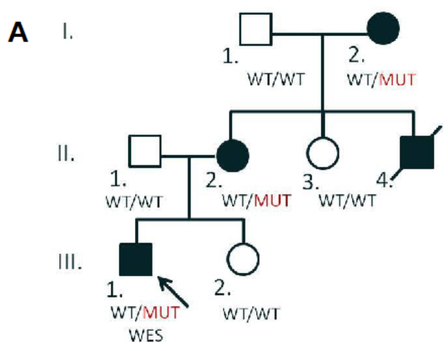
Case 3

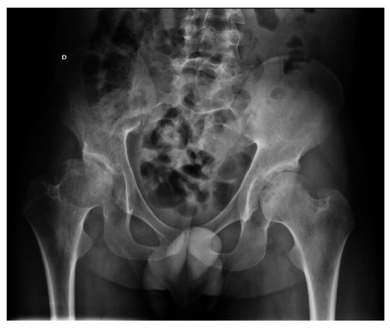
Familial short stature with a mild skeletal dysplasia
Index case:
- Low birth length (41 cm; SDS -4.8) with normal birth weight (2.9 kg; SDS -1.2)
- Mild skeletal dysplasia: bilateral osteonecrosis of the femoral epiphysis

Table 3: Anthropometric measurements of the family 3

	II.2	II.3	II.4	III.1	III.2
Adult height (cm)	133	155	<150	158	152
Height SDS	-4.9	-1.2	<-3.8	-2.5	-1.7

SDS: standard deviation score





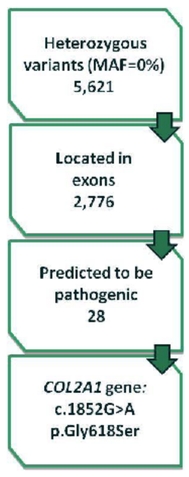


Figure 3. Family pedigree (A). The arrow indicates the proband. Affected subjects are indicated by black symbols, whereas individuals with normal stature are indicated by open symbols. The genotype (WT, wild type allele; MUT, mutant allele) for each family member are as indicated. WES indicate the individual submitted to whole exome sequencing. Radiographic image of the proband (B). Flowchart of the WES study (C). The numbers indicate the allelic variants after each filtering process.

Conclusions

We identified 3 heterozygous mutations in 3 different genes that explain the disproportional short stature phenotype observed in our patients. Because of the mild and unspecific phenotype, only a genomic approach allowed the identification of the aetiology of short stature in these patients.

References

1. Vasques, G. A. *et al*. Heterozygous mutations in natriuretic peptide receptor-B (NPR2) gene as a cause of short stature in patients initially classified as idiopathic short stature. J Clin Endocrinol and Metab, 2013; 98(10).
2. Le Goff, C. *et al*. Mutations in the TGFβ binding- protein-like domain 5 of FBN1 are responsible for acromicric and geleophysic dysplasias. Am J Hum Genet, 2011; 89(1).
3. Kannu, P. *et al*. Clinical phenotypes associated with type II collagen mutations. J Paediatr Child Health, 2012; 48(2).

ESPE 2015 1268--LBP
Growth
Mariana Funari
DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: 54th Annual Meeting 1-3 October ESPE 2015 BARCELONA European Society for Paediatric Endocrinology
PosterSessionOnline.com