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Molecular and in silico analyses validates pathogenicity of homozygous mutations in the *NPR2* gene underlying variable phenotypes of *Acromesomelic dysplasia*, type Maroteaux

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ABSTRACT

Homozygous and/or heterozygous loss of function mutations in the natriuretic peptide receptor B (*NPR2*) have been reported in causing acromesomelic dysplasia, type Maroteaux with variable clinical features and idiopathic short stature with nonspecific skeletal deformities. On the other hand, gain of function mutations in the same gene result in overgrowth disorder suggesting that NPR2 and its ligand, natriuretic peptide precursor C (CNP), are the key players of endochondral bone growth. However, the precise mechanism behind phenotypic variability of the *NPR2* mutations is not fully understood so far.

In the present study, three consanguineous families of Pakistani origin (A, B, C) with variable phenotypes of acromesomelic dysplasia, type Maroteaux were evaluated at clinical and molecular levels. Linkage analysis followed by Sanger sequencing of the *NPR2* gene revealed three homozygous mutations including p.(Leu314 Arg), p.(Arg371*), and p.(Arg1032*) in family A, B and C, respectively. In silico structural and functional analyses substantiated that a novel missense mutation [p.(Leu314 Arg)] in family A allosterically affects binding of NPR2 homodimer to its ligand (CNP) which ultimately results in defective guanylate cyclase activity. A nonsense mutation [p.(Arg371*)] in family B entirely removed the transmembrane domain, protein kinase domain and guanylate cyclase domains of the NPR2 resulting in abolishing its guanylate cyclase activity. Another novel mutation [p.(Arg1032*)], found in family C, deteriorated the guanylate cyclase domain of the protein and probably plundered its guanylate cyclase activity. These results suggest that guanylate cyclase activity is the most critical function of the NPR2 and phenotypic severity of the NPR2 mutations is proportional to the reduction in its guanylate cyclase activity.

1. Introduction

The gene *NPR2* encodes natriuretic peptide receptor B (NPR2) which contains four functional domains: an extracellular ligand-binding domain, a single membrane-spanning region, an intracellular protein kinase homology domain and a carboxyl-terminal guanylyl cyclase catalytic domain (van den Akker, 2001; Dickey et al., 2016). This protein is primary receptor for C-type natriuretic peptide (CNP) (Koller and Goeddel, 1992). Physiologically active NPR2 protein is a homodimer that produces cytoplasmic cyclic GMP from GTP on binding its

Abbreviations: AMDM, *Acromesomelic dysplasia*, Maroteaux type; ECD, extracellular domain; GC, guanylate cyclase; CNP, C-type natriuretic peptide; PDB, protein databank; PDF, probability density function; DOPE, discrete optimized protein energy; MD, molecular dynamics; HsNPR2, *Homo + apiens* natriuretic peptide receptor 2; DS, discovery studio; VMD, visualization of molecular dynamics; RnNPR1, *Rattus norvegicus* natriuretic peptide receptor 1; CrCYG12, *Chlamydomonas reinhardtii* guanylate cyclase; ECD^{wt}, wild type extracellular domain; ECD^{mt}, mutant extracellular domain; GC^{wt}, wild type guanylate cyclase; GC^{mt}, mutant guanylate cyclase

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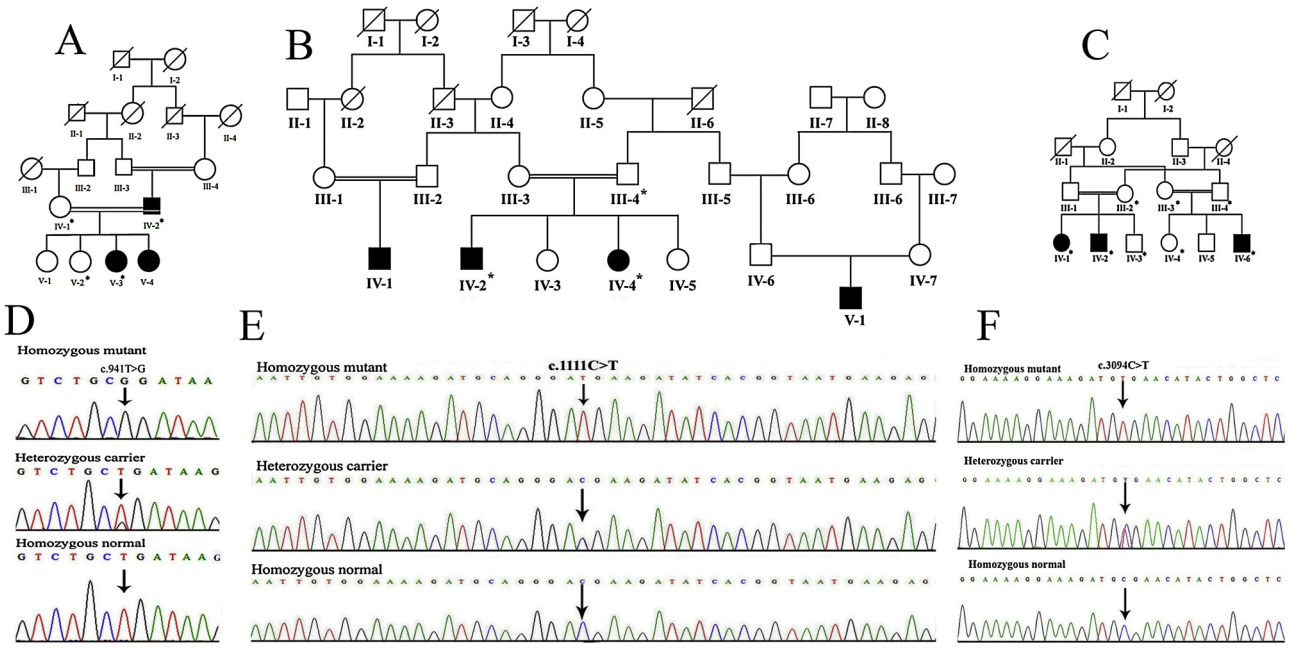

Fig. 1. Pedigrees and genetic information of the families. Pedigree of family A containing three affected individuals (III-2, V-3, V-4) with AMDM. The '*' sign is used for the members whose samples were incorporated in this study (A). Pedigree of family B demonstrating segregation of autosomal recessive AMDM in a five generation family (B). Pedigree drawing of family C having three affected individuals in the fourth generation (C). Partial DNA sequence *NPR2* gene exhibiting novel missense mutation [c.941 T > G; p.(Leu314 Arg)] identified in family A (D). DNA sequencing chromatograms of *NPR2* showing nonsense mutation [c.1111C > T; p.(Arg371*)] identified in family B (E). Sequencing upshots demonstrating novel stop gain mutation [c.3094c > T; p.(Arg1032*)] in the *NPR2* identified in family C (F).

Table 1
Clinical features of affected individuals in family A, family B and family C.

Clinical features	Affected individuals in family A			Affected individuals in family B			Affected individuals in family C		
	IV-2*	V-3*	V-4	IV-1*	IV-2*	IV-4*	IV-1*	IV-2*	IV-6*
Sex	M	F	F	M	M	F	F	M	M
Age in years	38	13	9	13	10	17	30	26	21
Height in cm	137.25	121.95	101.66	106.25	99.52	111.76	112.00	121.92	119.38
Arms span	130.52	114.85	96.30	101.20	95.88	105.95	107.00	112.90	111.00
Disproportionate Short stature	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Normal head circumference	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Prominent forehead	- -	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +
Short and broad nose	- -	- -	- -	- -	- -	+ +	+ +	+ +	+ +
Superiorly curved clavicles	N/A	N/A	N/A	N/A	N/A	N/A	+ +	+ +	+ +
Pectus carinatum	- -	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +
Pectus excavatum	- -	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +
Increased lumbar lordosis	- -	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +
Lower thoracic kyphosis	- -	- -	- -	- -	- -	- -	+ +	+ +	+ +
Gibbus deformity	- -	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +
Joint laxity	- -	- -	- -	+ +	+ +	+ +	+ +	+ +	+ +
Acromesomelia	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Bowed forearms	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Limited elbow extension	- +	- +	- +	+ +	+ +	+ +	+ +	+ +	+ +
Short tubular bones	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Bowed radius	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Short, broad fingers	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Small orbiculated hands	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Short toes	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Large halluces	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Short, broad phalanges	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Loose, redundant skin on fingers	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Short and dampened nails	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Normal intelligence	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +

Legends: Roman numerals represent affected individuals in the pedigrees of family A, family B and family C. M stands for male and F for female. N/A means not assessed while - - represent absence and + + represent presence of a feature while - + signifies an unconvinced feature.

extracellular ligand, CNP (Lincoln and Cornwell, 1993). Guanylyl cyclase activity of the natriuretic peptide (NPR2) receptor is boosted up by binding to its ligand (CNP) to enhance the synthesis of cGMP that activates type II cGMP-dependent protein kinase (Vasques et al., 2014). In this way, CNP/NPR2 cascade acts locally as a positive regulator of endochondral ossification via prompting cartilage homeostasis,

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