Mandibuloacral Dysplasia Mutation Detection in Three Egyptian Families: A Report of a Novel Mutation

Khalda Amr[1], Mostafa Mostafa[2] and Ghada El-Kamah[3]

[1]Dept. of Molecular Genetics, Human Genetics & Genome Research Division, National Research Center (NRC), Cairo, Egypt.
[2] Dept. of Oro-Dental Genetics, Division of Oro-Dental Research, NRC, Cairo, Egypt.
[3]Dept. of Clinical Genetics, Human Genetics & Genome Research Division, NRC, Cairo, Egypt.
Khalda_nrc@yahoo.com

Abstract: Mandibuloacral dysplasia (MAD) is a rare autosomal recessive disorder characterized by mandibular and clavicular hypoplasia, acroosteolysis, delayed closure of the cranial suture and joint contractures. Mutations in *Lamin A/C* have been reported in patients with MAD. Laminopathesies refer to many disorders caused by defects in the nuclear lamina associated proteins. Lamins are integral structural components of the nuclear lamina hypothesized to be involved in numerous cellular processes. LMNA gene maps to chromosome 1q21.2 and encodes lamin A and lamin C through alternative splicing. We investigated three consanguineous Egyptian families having severe MAD disorder. Subsequently, direct sequencing of the coding region of the LMNA gene in patients and their parents revealed the identification of two homozygous missense mutations that replace a conserved residue: Arginine 527, a novel R527L mutation in one patient and R527C mutation in the other two patients.
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1. Introduction

A-type lamins (lamins A and C), encoded by the *LMNA* gene, are major protein constituents of the mammalian nuclear lamina, a complex structure that acts as a scaffold for protein complexes that regulate nuclear structure and functions (**Andrés and González, 2009**). LMNA, which maps to chromosome 1q21.2 and encodes lamin A and lamin C through alternative splicing of LMNA is responsible for many unrelated diseases with different affected organ systems attributed to lamin A/C mutations. These include Charcot-Marie-Tooth disease type 2B (**De Sandre-Giovannoli *et al.*, 2002**), forms of dilated cardiomyopathy (**Fatkin *et al.*, 1999**), both autosomal dominant and autosomal recessive forms of Emery-Dreifuss muscular dystrophy (**Raffaele Di Barletta *et al.*, 2000**), limb girdle muscular dystrophy type 1B (**Muchir *et al.*, 2000**), Dunnigan-type familial partial lipodystrophy (**Speckman *et al.*, 2000**) and Hutchinson-Gilford progeria (**Prabhavathi *et al.*, 2011**).

Patients with MAD type A (partial) lipodystrophy have mutations in *lamin A/C* gene. Mutation in the zinc metalloproteinase (*ZMPSTE24*), also involved in the proteolytic cleavage of pre lamin A to form mature lamin A, have been noted in patients with MAD and type B (generalized) lipodystrophy (**Agarwal *et al.*, 2003**).

Mandibuloacral dysplasia (MAD; MIM 248370) is a rare autosomal recessive disorder combining a characteristic facial appearance with acro-osteolysis and lipodystrophy. In the majority of MAD patients, Symptoms appear around the age of four years, mostly in the form of mandibular hypoplasia with subsequent teeth crowding, delayed cranial suture closure, dysplastic clavicles, acroosteolysis, and typical facial changes. Patients may also suffer; lipodystrophy and clinical features of metabolic syndromes as insulin resistance diabetes mellitus and hypertriglyceridemia (**Agarwal *et al.*, 2008**). MAD is considered a progeroid disorder, so far one mutation in LMNA has been associated with the disease, R527H (**Simha *et al.*, 2003**). A homozygous missense mutation (R527H) in *LMNA* gene was first reported in nine Italian MAD patients with type A pattern of lipodystrophy belonging to five consanguineous pedigrees (**Novelli *et al.*, 2002**). The R527 amino acid is located in the C-terminal domain common to lamin A and lamin C, which has an immunoglobulin-like three-dimensional structure. R527 is localized at the external surface of the domain, and thus R527H substitution would disrupt the surface structure of the protein, altering binding fundamental sites (**Dhe-Paganon *et al.*, 2002**). However, we report a fully expressed phenotype of MAD Egyptian patient with a novel homozygous mutation R527L in *LMNA* gene in addition to the previously reported R257C.

2. Patients and Methods

Four Egyptian patients descending from three families were included in our study. A written informed consent was obtained from the patients' parents. Genomic DNA was extracted from peripheral mononuclear cells of patients and their parents by QIAamp blood kits (Qiagen, Hilden, Germany). Direct

sequencing of the entire coding region and the surrounding intron-exon boundaries of the LMNA gene was conducted for the probands and their parents from each pedigree. Primers of all twelve coding exons were amplified according to the published sequence information (De **Sandre-Giovannoli *et al.*, 2002**). PCR reaction was carried out in a total volume of 50ml containing 150ng DNA using the following condition 95^0C for 5 min followed by 35 cycles of 95^0C for 50 sec, annealing for 50 sec, 72^0C for 50 sec and extension of 72^0C for 7 min. The PCR products were purified with a QIA quick PCR purification Kit (Qiagen). Cycle sequencing reactions were performed on column-purified PCR product using Big Dye terminator cycle sequencing Kit (Applied Biosystem, Foster City, CA). Both strands of each exon were sequenced and analyzed on ABI Prism 310 automated sequencer (Applied Biosystem).

3. Results:

Four Egyptian patients descending from three unrelated families, fulfilling the diagnostic criteria of MAD through full clinical, dental examination as well as molecular findings (Table1), were included in our study. Patients No. 3 & 4 are two sibs.

Table1: Molecular findings with full clinical and dental examination.

Patient No.	1	2	3	4
Sex	Male	Female	Female	Male
Age of onset in months	9	18	7	8
Consanguinity	+ve	+ve	+ve	
Progeroid facies. Fig.1	+	+	+	+
Hypoplastic mandible	+	+	+	+
Hypoplastic clavicle	+	+	+	+
Acroosteolysis Fig. 2	+	+	+	+
Stature	-3.5 SD	-3.66 SD	-2.42 SD	-2.7 SD
Oro-Dental findings	Both mandibular and maxillary hypoplasia with subsequent teeth crowding, microstomia, limitation of mouth opening and gingivitis. Radiographic examination showed hypoplastic condyle and accentuated antigonial angle of the mandible (Fig. 1&3)			
Metabolic disorders	-	-	-	-
Molecular diagnosis	Arginine527leucine	Arginine527cysteine		

Direct sequencing of the LMNA gene of the first patient revealed a novel missense mutation R257L resulting in substitution of Argenine by leucine (Fig. 4). A transition of G to T at the second base of codon 527 resulted in a missense mutation. While sequencing of the other three patients revealed a substitution of the first base (C > T) at codon 527 leading to the transversion of Arginine to cysteine R257C. All detected mutations were present in a homozygous state in our studied patients and in a heterozygous state in their parents (Fig.4).

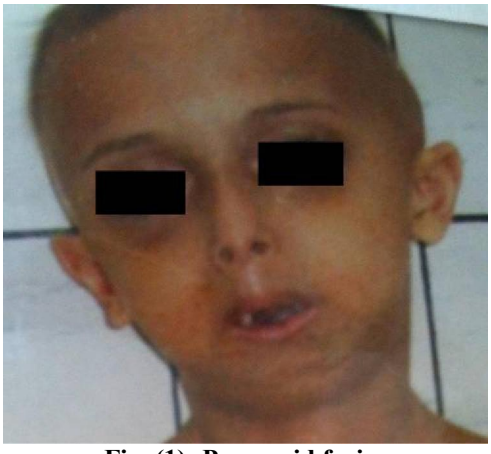

Fig. (1): Progeroid facies

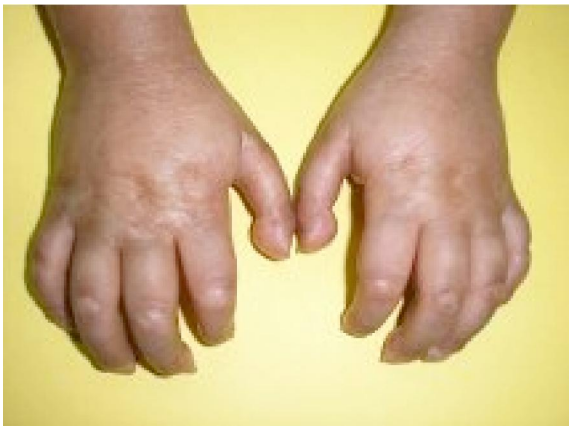

Fig. (2): Acro-osteolysis

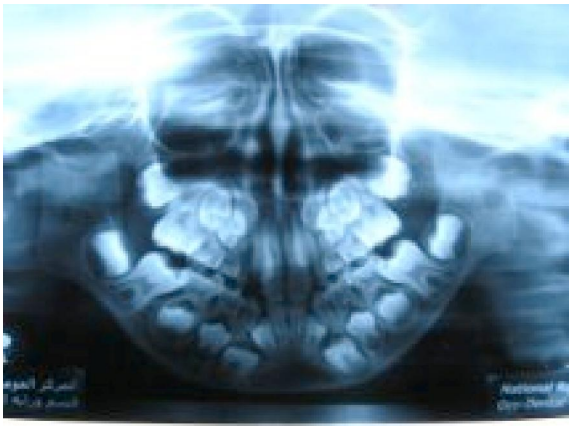

Fig.(3): Panoramic radiograph showing teeth crowding, hypoplastic condyle and accentuated antigonial angle

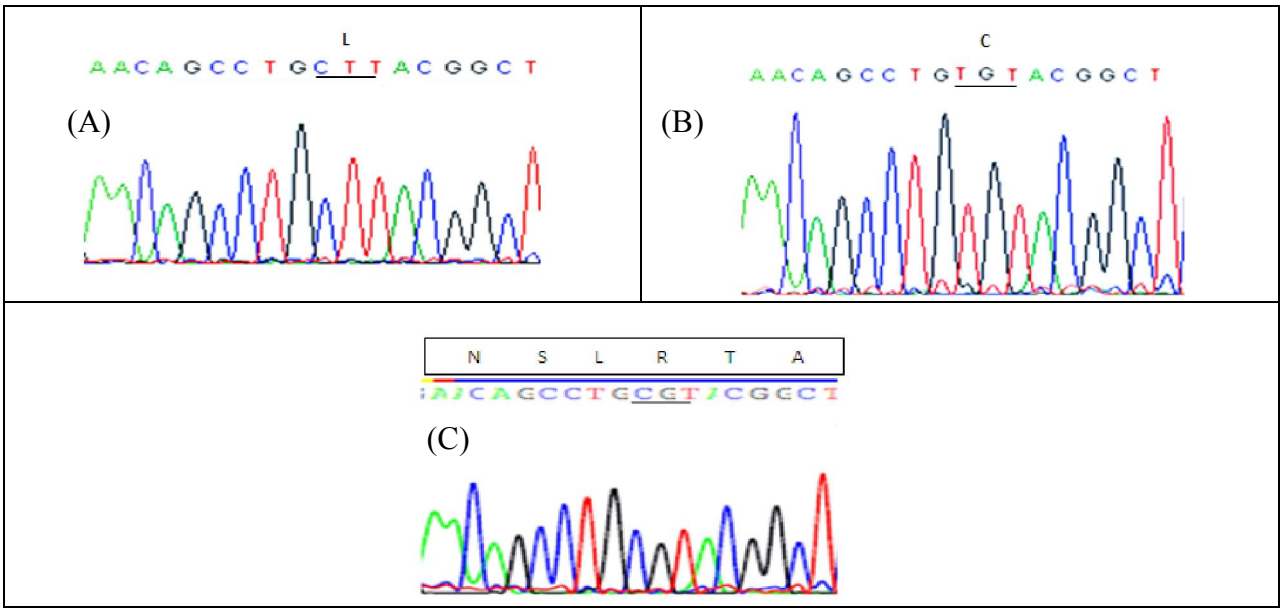

Fig.4 DNA sequence chromatograms of LMNA gene in 3 patients with MAD.

Figure (A) showing patient 1 with transition of G>T (R 527 L).

- **Figure (B) showing patient 2 and 3 with transition of C>T (R 527 C).**
- **Figure(C) showing the normal sequence.**

4. Discussion:

Laminopathies refers to different disorders with phenotypic variability caused by defects in the nuclear lamina associated proteins. We report the first study for four Egyptian patients with MAD syndrome. A novel homozygous mutation in codon 527, in which the Arginine is converted to Lucien in one patient–in addition to the previously reported, Arg 527 cys, in three patients (**Agarwal *et al.*, 2008**). It is highly likely that the Arg257leu missense mutation causes inactivation of LMNA protein; since the other previously reported mutation R257H is affecting the same amino acid in many MAD patients (**Novelli *et al.*, 2002**), suggesting that this amino acid plays a key role in the structure and function of the LMNA protein. Presumably, the substitution of a basic amino acid (Argenine) with a non- polar amino acid (cysteine) at codon **527** may alter the polarity of the protein, leading to conformational modifications of the protein.

So far, approximately 28 MAD patients have been reported to harbor homozygous or compound heterozygous missense mutations in the C-terminal of lamin A/C (**Novelli *et al.*, 2002; Cao and Hegele, 2003; Simha *et al.*, 2003; Plasilova *et al.*, 2004; Garg *et al.*, 2005; Van Esch *et al.*, 2006; Kosho *et al.*, 2007; Lombardi *et al.*, 2007; Agarwal *et al.*, 2008; Zirn *et al.*, 2008; Garavelli *et al.*, 2009; Madej-Pilarczyk *et al.*, 2009**). Nearly all of them have type A pattern of partial lipodystrophy. Our studied patients have a homozygous mutation in codon 527 and they all suffer manifestations of partial lipodystrophy together with all other clinical manifestations of MAD.

However, the age of onset among our studied patients was considerably younger than previous reports (**Agarwal *et al.*, 2008**). Only 6 MAD patients have been reported with either compound heterozygous or homozygous mutations in *ZMPSTE24* (**Agarwal *et al.*, 2003; Shackleton *et al.*, 2005; Agarwal *et al.*, 2006; Denecke *et al.*, 2006; Miyoshi *et al.*, 2008**).

Mutations in *LMNA* gene have been reported in 23 patients with MAD, which include homozygous Arg527His mutation in six Italian and two Hispanic pedigrees, homozygous Ala529Val mutation in two Turkish pedigrees, homozygous Ala529Thr mutation in a Japanese woman, homozygous Lys542Asn mutation in an Indian pedigree, and compound heterozygous Arg471Cys/Arg527Cys and Arg527His/Val440Met mutations in two Caucasian pedigrees (**Fatkin *et al.*, 1999; Muchir *et al.*, 2000; Raffaele Di Barletta *et al.*, 2000; Speckman *et al.*, 2000; De Sandre-Giovannoli *et al.*, 2002; Agarwal *et al.*, 2003;Cao and Hegele, 2003; Agarwal *et al.*, 2008; Andrés and González, 2009 ; Prabhavathi *et al.*, 2011**).

Our finding of a homozygous missense mutation, Arg527Cys LMNA mutation was firstly reported in a 7-yr-old girl German-Irish MAD patient descending from a consanguineous pedigree (**Agarwal *et al.*, 2008**). Subsequent subjects have been described with homozygous LMNA mutations causing R527C or A529V amino-acid substitutions (**Garg *et al.*, 2005; Agarwal *et al.*, 2008**). **Lombardi *et al.*, 2007**, also reported a compound heterozygous subject for the LMNA R527H and a V440M mutation with some features of mandibuloacral dysplasia, lack of muscle strength, and decreased muscle tone.

Interestingly, **Lloyd *et al.*, 2002**, have identified a binding site of lamin A for the adipocyte differentiation factor sterol-response element–binding protein 1 (SREBP1) between residues 227 and 487. This confirms the possibility that fat loss observed in FPLD and MAD, may be caused by reduced binding of the adipocyte-differentiation factor SREB to lamina (**Novelli *et al.*, 2002**). The polypeptide amino acids from 470 to 545 has been crystallized and shown to assume an Ig domain. The substitution of Arg to His at position 527 showed disruption of the salt bridge between Arg 527 and Glu at position 537. Similarly, the substitution of Arg 527 to Cys causes salt bridge disruption (**Agarwal *et al.*, 2008**). **Simha *et al.*, 2003**, have confirmed that only type A MAD is caused by the R527H mutation in LMNA and no mutations were detected in four families with type B MAD.

Conclusion:

In a first study of Egyptian MAD patients, we concluded that Egyptian mutation spectrum would belong to the LMNA gene (527 locus type) & not to *ZMPSTE24*. We also report a novel mutation (not previously reported according to our date search). We also concluded an earlier age of onset of the disease among Egyptian patients.

Corresponding author
Khalda Amr
Dept. of Molecular Genetics, Human Genetics & Genome Research Division, National Research Center (NRC), Cairo, Egypt.
Khalda_nrc@yahoo.com

References:

1. Agarwal AK, Fryns JP, Auchus RJ, Garg A., 2003. Zinc metalloproteinase, ZMPSTE24, is mutated in mandibuloacral dysplasia. *Hum Mol Genet*, 12:1995–2001.
2. Agarwal AK, Kazachkova I, Ten S, Garg A., 2008. Severe mandibuloacral dysplasia-associated lipodystrophy and progeria in a young girl with a novel homozygous Arg527Cys LMNA mutation. *J Clin Endocrinol Metab*, 93: 4617- 4623.
3. Agarwal AK. and Garg A., 2006. Genetic Basis of Lipodystrophies and Management of Metabolic Complications. *Annurev. Med*, 57.022605.114424.
4. Andrés V and González GM 2009. Role of A-type lamins in signaling, transcription, and chromatin organization. *J. Cell Biol*, 187: 7 947.
5. Cao H, Hegele RA., 2003. LMNA is mutated in Hutchinson-Gilford progeria (MIM 176670) but not in Wiedemann-Rautenstrauch progeroid syndrome (MIM 264090). *J Hum Genet*. 48:271–274.
6. De Sandre-Giovannoli A, Chaouch M, Kozlov S, Vallat JM, Tazir M, Kassouri N, Szepetowski P, Hammadouche T, Vandenberghe A, Stewart CL, Grid D, Levy J., 2002. Homozygous defects in LMNA, encoding lamin A/C nuclear-envelope proteins, cause autosomal recessive axonal neuropathy in human (Charcot-Marie- Tooth disorder type 2) and mouse. *American Journal of Human Genetic*,70:726-736.
7. Denecke J, Brune T, Feldhaus T, Robenek H, Kranz C, Auchus RJ, Agarwal AK Marquardt T., 2006. A homozygous ZMPSTE24 null mutation in combination with a heterozygous mutation in the LMNA gene causes Hutchinson-Gilford progeria syndrome (HGPS): insights into thepathophysiology of HGPS. *Human Mutation*, 27,6: 524–531.
8. Dhe-Paganon S, Werner ED, Chi YI, Shoelson SE., 2002. Structure of the globular tail of nuclear lamin. *J Biol Chem*,277:17381–17384. 821–4.
9. Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, Atherton J, Vidaillet HJ, Spudich S, De Girolami U, Seidman JG, Seidman CE, Muntoni F, Muehle G, Johnson W, McDonough B., 1999. Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conductionsystem disease. *N Engl J Med.*, 341:1715–24.
10. Garavelli L, D'Apice MR, Rivieri F, Bertoli M, Wischmeijer A, Gelmini C, De Nigris V, Albertini

E, Rosato S, Virdis R, Bacchini E, Dal Zotto R, Banchini G, Iughetti L, Bernasconi S, Superti-Furga A, Novelli G.,2009. Mandibuloacral dysplasia type A in childhood. *Am J Med Genet,* A., 149A(10):2258-2264.

11. Garg A, Cogulu O, Ozkinay F, Onay H, Agarwal AK., 2005. A novel homozygous Ala529Val LMNA mutation in Turkish patients with mandibuloacral dysplasia. *J Clin Endocrinol Metab*. 90:5259–5264.
12. Kosho T, Takahashi J, Momose T, Nakamura A, Sakurai A, Wada T, Yoshida K, Wakui K, Suzuki T, Kasuga K, Nishimura G, Kato H, Fukushima Y., 2007. Mandibuloacral dysplasia and a novel LMNA mutation in a woman with severe progressive skeletal changes. *Am J Med Genet*, A 143:2598–2603.
13. Lloyd DJ, Trembath RC and Shackleton S., 2002. A nove interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies. Hum. Mol. Genet, 11: 769–777.
14. Lombardi F, Gullotta F, Columbaro M, Filareto A, D'Adamo M, Vielle A, Guglielmi V, Nardone AM, Azzolini V, Grosso E, Lattanzi G, D'Apice MR, Masala S, Maraldi NM, Sbraccia P, Novelli G., 2007. Compound heterozygosity for mutations in LMNA in a patient with a myopathic and lipodystrophic mandibuloacral dysplasia type A phenotype. *J Clin Endocrinol Metab*. 92:4467–4471.
15. Madej-Pilarczyk A, Rosińska-Borkowska D, Rekawek J, Marchel M, Szaluś E, Jabłońska S, Hausmanowa-Petrusewicz I., 2009. Progeroid syndrome with scleroderma-like skin changes associated with homozygous R435C LMNA mutation. *Am J Med Gene,t* A.;149A(11):2387-2392.
16. McKusick VA. Online Mendelian Inheritance in Man (MIM). Available at: http://www.ncbi.nlm.nih.gov/omim. Accessed, 2012.
17. Miyoshi Y, Akagi M, Agarwal AK, Namba N, Kato-Nishimura K, Mohri I, Yamagata M, Nakajima S, Mushiake S, Shima M, Auchus RJ, Taniike M, Garg A, Ozono K .,2006. Severe mandibuloacral dysplasia caused by novel compound heterozygous ZMPSTE24 mutations in two Japanese siblings. *Clinical Genetics*, 73,6: 535–544.
18. Muchir A, Bonne G, van der Kooi AJ, van Meegen M, Baas F, Bolhuis PA, de Visser M, Schwartz K., 2000. Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). *Hum Mol Genet* 9:1453– 1459.
19. Novelli G, Muchir A, Sangiuolo F, Helbling-Leclerc A, D'Apice MR, Massart C, Capon F, Sbraccia P, Federici M, Lauro R, Tudisco C, Pallotta R, Scarano G, Dallapiccola B, Merlini L, Bonne G 2002. Mandibuloacral dysplasia is caused by a mutation in LMNA-encoding lamin A/C. *Am J Hum Genet*, 71:426–431.
20. Plasilova M, Chattopadhyay C, Pal P, Schaub NA, Buechner SA, Mueller H, Miny P, Ghosh A, Heinimann K .,2004. Homozygous missense mutation in the lamin A/C gene causes autosomal recessive Hutchinson-Gilford progeria syndrome. *J Med Genet*, 41:609–614.
21. Prabhavathi NB, Mastanaiah J, Sunil kumar MV., 2011. PROGERIA - A PREMATURE AGING IN CHILDRENS (REVIEW). *J, IJPRD*, 2.
22. Raffaele Di Barletta M, Ricci E, Galluzzi G, Tonali P, Mora M, Morandi L, Romorini A, Voit T, Orstavik KH, Merlini L, Trevisan C, Biancalana V, Housmanowa-Petrusewicz I, Bione S, Ricotti R, Schwartz K, Bonne G, Toniolo D 2000. Different mutations in the LMNA gene cause autosomal dominant and autosomal recessive Emery-Dreifuss muscular dystrophy. *Am J Hum Genet*,66:1407–12.
23. Shackleton S, SmallwoodDT, Clayton P,Wilson LC, Agarwal AK, Garg A, Trembath RC .,2005. Compound heterozygous ZMPSTE24 mutations reduce prelamin A processing and result in a severe progeroid phenotype. *J Med Genet*, 42: e36.
24. Simha V, Agarwal AK, Oral EA, Fryns J-P, Garg A., 2003. Genetic and phenotypic heterogeneity in patients with mandibuloacral dysplasia-associated lipodystrophy. *J Clin Endocrinol Metab*, 88:2.
25. Speckman RA, Garg A, Du F, Bennett L, Veile R, Arioglu E, Taylor SI, Lovett M, Bowcock AM., 2000. Mutational and haplotype analyses of families with familial partial lipodystrophy (Dunnigan variety) reveal recurrent missense mutations in the globular C-terminal domain of lamin A/C. *Hum Genet,* 66:1192–119.
26. Van Esch H, Agarwal AK, Debeer P, Fryns JP, Garg A., 2006. A homozygous mutation in the lamin A/C gene associated with a novel syndrome of arthropathy, tendinous calcinosis, and progeroid features. *J Clin Endocrinol Metab*. 91(2):517-21.
27. Zirn B, Kress W, Grimm T, Berthold LD, Neubauer B, Kuchelmeister K, Müller U, Hahn A, 2008. Association of homozygous LMNA mutation R471C with new phenotype: mandibuloacral dysplasia, progeria, and rigid spine muscular dystrophy. *American Journal of Medical Genetics*, 1054:1049 – 1054.

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