JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue

JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)

An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON HUTCHINSON GILFORD PROGERIA SYNDROME

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ABSTRACT: Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic disease that leads premature aging .Progeria cases occur in 1 per four million live births. Because of accelerated aging, a child in ten year will have similar condition that of 70-year old would have. Many children with progeria appear normal at birth and then progressively develop the characteristic features during

early childhood. Various methods for diagnosis are being developed and clinical trials on some drugs that may be used in treatment are being done. The goal of this study was to determine sign and symptoms, cause of HGPS and how to treat the occasional illness of progeria.

Keywords: Progeria, sign and symptom, pathophysiology, treatment.

INTRODUCTION

Hutchinson– Gilford Progeria Syndrome is a rare, autosomal dominant, fatal childhood disease first founded by Dr Jonathan Hutchinson and Dr. Hastings Gilford in 1886[1,2,3]. In 1886, Jonathan Hutchinson reported a case of a 3.5 year old boy who had the appearance of an old man[4] after that Hastings Guilford reported a second case with similar features[5] . The term Progeria is derived from the Greek word geras, meaning old age. This disorder has a very low incidence and occurs in one per four million live births[6]. Those born with Progeria typically live about thirteen years, but in some cases it can extend upto their late teens and early twenties[7,8]. Because of accelerated aging, a child in ten year will have similar condition that of 70-year old would have.

Progeria is associated with severe morbidity and mortality due to atherosclerosis of cerebral and coronary arteries, leading to premature death. This condition greatly increases the chances of having a heart attack or stroke at a young age .It also lead to poor growth, atrophy of muscle and skin, loss of subcutaneous fat, osteoporosis, arthritis, alopecia and occasionally tumors, cataracts, diabetes, and hyperlipidemia [9,10,11]. Also, kidneys, brain, adrenals, liver, testis and heart can be infected[12]. This condition does not affect intellectual development.

EPIDEMIOLOGY

HGPS is mainly affecting about one per four to eight million births[13]. Progeria Research Foundation database estimated that there are 200–250 children living with progeria in the worldwide. It can affects both sexes and all races. The male-to-female ratio is estimated as 1.5: 1. Generally the disease does not get inherited from parents to child because the victim dies before the age of reproduction. There were 20 cases

reported in Northern America, 16 cases in Southern America, 24 cases in Europe, four cases in Africa, and 18 cases in Asia.

PATHOPHYSIOLOGY

Mutations in the LMNA gene cause Hutchinson-Gilford progeria syndrome. The LMNA gene helps in making a protein called lamin A . A point mutation occur in prelamin A that results in a protein lacking 50 aminoacid near the C terminus that lead to formation of defective lamin A.

Lamin A contain twelve exon protein. Prelamin A get spliced from exon 10 to exon 11 then to exon 12[14]. It has CAAX as terminal amino acids. The C of the CAAX cysteine get farnesylation using a cytosolic enzyme farnesyltransferase. This farnesylated Prelamin A get attaches with Endoplasmic Reticulum. After the process of farnesylation the last three amino acids of Prelamin A get cleaved by an enzymes, a Zinc metalloprotein ZMPSTE24 and a prenyl protein endopeptidase RCE1[15,16]. After the removal of 3 terminal amino acids, farnesyl-cystein residue get methylated by an enzyme Isoprenylcystein Carboxy Methyl Transferase[17,18]. The last step is that the end 15 amino acids of Prelamin A along with farnesylcystein methyl ester are get released with the help of ZMPSTE24 and mature Lamin A is then released from endoplasmic reticulum into the cytosol which is no longer a membrane-bound, and carries out functions inside the nucleus[19,20]. This protein plays an important role in determining the shape of the nucleus within cells. It is an essential supporting component of the nuclear envelope, which is the membrane that surrounds the nucleus.

In case of diseased person there occur mutation in one allele of the LMNA gene ie, C-to-T substitution at nucleotide 1824 (1824C→T). The mutation does not change the amino acid of the corresponding codon in the messenger RNA,but it causes defective mRNA splicing by the activation of a cryptic splice donor in exon 11 which result in the synthesis of abnormal protein Progerin with a deficiency 50 amino acid. The defective splicing deletes the part of protein that is targeted by ZMPSTE24 at the release step[21]. This makes the Progerin to remain farnesylated and membrane bound. The Progerin formed enters the nucleus by diffusion from endoplasmic reticulum . The altered protein makes the nuclear envelope unstable and

damages the nucleus, making cells to die prematurely. Researchers are now working to determine how these changes lead to the characteristic features of Hutchinson-Gilford progeria syndrome.

SIGNS AND SYMPTOMS

Patients with this condition appear normal at birth, but by the age 1 or 2 years severe growth retardation occur. They are usually short and thin with an average height of 100 cm and an average weight of 12–15 kg or even less. The characteristic clinical findings of HGPS include abnormalities of the skin and hair in addition with characteristic facial features and skeletal abnormalities[22].

Skin and hair

The skin will be elastic , shiny, wrinkled with low cutaneous fat. The patient seems to be physically weak. When come in contact with bright sun light, hyper pigmentation and irritation occur in the skin . There will be complete loss of hair all over the body parts including scalp, eye lash and skin[23].

Musculoskeletal abnormalities

The limbs are lean with low muscular mass. There may be flexion of knee joint that make the patient walks a bit abnormally. The thoracic cage will look alike that of a pear shape. Face appears like aged person, with eyes and ears slightly bigger in size. Incisors teeth may fall at early age.

Other abnormalities

High pitched voice, Scars may be present all over the body, Weight to height ratio become low ,the nails may appear dystrophic. Sometimes complete loss of hearing may occur. The patient is more prone to osteoporosis that lead to fractures. There may be complete loss of appetite. Delayed teeth growth or loss of teeth . The prothrombin time is prolonged. Elevated platelet count has been seen. The serum level of phosphorus increases and calcium decreases.

These patients have the feelings similar to that of age-matched healthy persons. They express proper mood and affection[24]. Patients are aware of their different appearance as compared to others. They have tendency

to keep away from strangers. They show good social interaction with friends. Mental development is normal in this patients.

Morbidity and mortality occur as a result of atherosclerosis of the coronary and cerebrovascular arteries. Cardiovascular complications include myocardial infarction and congestive heart failure ,the most common reason for the death. Cerebrovascular complications include cerebrovascular infarction with hemiplegia, subdural hematoma, and seizures. The other reason for death may include marasmus, loss of mobility or weakness.

DIAGNOSIS

It is usually diagnosed during the second year of life [25]or later, when clinical features begin to notice. The diagnosis is done thorough clinical evaluation, characteristic physical findings, a careful patient history and diagnostic genetic testing[26].

●Specialized imaging tests are conducted to confirm certain skeletal abnormalities such as degenerative changes (osteolysis) of certain bones of the fingers (terminal phalanges),the hip socket (acetabulum). The loss of bones of fingers and toes are major abnormalities associated with progression of disease.

●Elevated levels of hyaluronic acid are seen in urine.

● Brain magnetic resonance angiography done to identify cerebrovascular complications.

●ECG and echocardiography should be done to identify coronary artery disease and congestive heart failure[27].

●The histological examination of skin lesions shows scleroderma.

●The radiological findings are also done in first or second year of patient's life, especially in the skull, thorax, long bones and phalanges. This helps us to identify diffuse osteopenia, acro-osteolysis of

phalanges, peripheral keys, vertebral bodies like "fish mouth", valgus hips, thinning of the skull diplois, thinning of the long bones and widening of metaphyses, vormiana ossicles, open cranial sutures and sources, marrow hypoplasia and facial sinuses, hypoplasia of the jaw, small chest cage[28,29].

TREATMENT

A wide spectrum of treatment has been developed to correct the defects in HGPS,

- Directly repair the disease -causing mutation
- Inhibit pre-mRNA abnormal splicing
- To alleviate toxicity of isoprenylated and methylated progerin
- Induce progerin clearance
- To allivate the noxious downstream effects associated with progerin accumulation[30]

Prelamin A Isoprenylation and methylation inhibitors

These include Lonafarnib, Zoledronate/Pravastatin, Monoaminopyrimidines and isoprenylcysteine carboxyl methyltransferase inhibitors. The abnormal splicing event that gives rise to progerin leads to the deletion of the ZMPSTE24 cleavage site used to remove the farnesylated carboxy terminus from prelamin A,lead to disruption of the nuclear scaffold upon progerin dimerization[31]. farnesyltransferase inhibitor (FTI) drugs that block farnesylation result in alleviating progerin production and toxicity. FTI reversibly bind to the farnesyltransferase CAAX binding site[32] result in blocking farnesylation of progerin lead to normal nuclear structure and resulted in reductions in nuclear blebbing. FTIs increases bone mineralization, gain in weight, lifespan get extended[33,34]and prevent cardiovascular[35].

A FTI called lonafarnib used for the treatment of cancer showed a modest improvement in weight gain, improvements in vascular stiffness including decreases in arterial pulse wave velocity,increases in skeletal rigidity. A combination of Zoledronate (N-BisPhosphonate) and Pravastatin (statin) (ZOPRA) helps in reduce prenylation and rescue HGPS cells and progeroid phenotypes of Zmpste24.Additionally, it prevent growth retardation, loss of weight, lipodystrophy, hair loss and bone defects[36]. But caution should be taken when long-term treatment FTIs because it can cause cardiomyopathy[37].

Monoaminopyrimidines a new modulators of farnesylation, result in improved phenotypes associated with HGPS[38]. This target both farnesyl pyrophosphate synthase and farnesyl transferase when given as a combination with FTIs and bisphosphonates.

Isoprenylcysteine carboxyl methyltransferase (ICMT) inhibitor, increases proliferation and delayed senescence in HGPS fibroblasts.Additionally increases body weight, normalized grip strength, prevente bone fractures and death of Zmpste24[39].

Autophagy-activating drugs:

These include Rapamycin, Sulforaphane, Retinoids and MG132. Rapamycin, an immunosuppressive agent promote progerin clearance with thwe help of autophagy, prevent the abnormal nuclear shape, delayed the cellular senescence of HGPS fibroblasts[40,41] and improves cardiac and skeletal muscle function and enhances survival [42]associated with increased body weight and fat.

Sulforaphane, an antioxidant formed from cruciferous vegetables enhance progerin clearance by autophagy[43]. Intermittent treatment using Sulforaphane and Lonafarnib rescued the HGPS cellular phenotype [44]. Retinoids alone or in a combination with rapamycin alleviate the amount of progerin and reverse aging defects .

MG132 helps in progerin degradation.It induces progerin nucleocytoplasmic translocation after a transition take place in the nucleolus, progerin clearance by macroautophagy . MG132 improves cellular HGPS phenotypes, reduces cellular senescence and enhances potentiality and proliferation in HGPS fibroblasts.

Downregulation of Prelamin A abnormal splicing

Thses include Antisense oligonucleotides, Metformin and MG132. Antisense oligonucleotides (AON) block the abnormal LMNA splicing site result in progerin productionThe combined administration of two AONs MmEx11 which target the exon 11 abnoraml splice site activated by the progeria mutation and MmEx10 geting the exon 10 splice site, in order to increases the action of the first AON by shifting splicing events towards lamin C production.

The antidiabetic drug Metformin decreases SRSF-1 (RNA-binding protein Serine/arginine-Rich Splicing Factor 1 was shown to favor this abnormal alternative splicing[45]) and progerin expression in mesenchymal stem cells derived from HGPS induced pluripotent stem cells (HGPS MSCs) and improve nuclear shape abnormalities and premature osteoblastic differentiation of HGPS MSCs[46].MG132 strongly reduces progerin production by downregulation of SRSF-1 and controlling prelamin A mRNA abnormal splicing.

Reduction of progerin downstream toxic effects

The altered downstream pathways due to progerin accumulation causes nuclear shape abnormalities,reactive oxygen species(ROS) generation, accumulation of oxidized proteins, mitochondrial dysfunction[47,48].These defects can be corrected using rho-associated protein kinase (ROCK) inhibitor,Methylene blue,N-Acetyl cysteine,Vitamin D.

ROCK inhibitor result in decreased ROS levels and reduce mitochondrial dysfunction along with a reduction in abnormal nuclear morphology and reduce the breakage of double-stranded DNA[49].

Methylene Blue stimulate mitochondrial function, improves the mitochondrial morphology and function, rescue premature aging phenotypes in HGPS cells and corrects misregulated gene expression[50].

1a,25-dihydroxyvitamin D3 the active hormonal form of vitamin D which improves HGPS phenotypes and nuclear morphological abnormalities, repair DNA defects and premature senescence.[51]

SUPPORTIVE TREATMENT

●A daily low - dose aspirin, help to prevent heart attacks and strokes[28,52].

●Statins which lower cholesterol or blood thinners with the aim of preventing blood clots[53].

●Use of growth hormone can help the increase of height and weight[52].

●The use of statins and bisphosphonates helps in reducing lipodystrophy and hair loss also improve bone defects[54]

●Physical and occupational therapy: It provide some relief to joint stiffness and hip problems and allow the child to remain active[28,29]

●Nutrini- Patients has loss of appetites so Nutrini provides all of the nutrients essential for well-being and health[55].

●Fluoride help in strengthening the tooth enamel and making it more resistant to tooth decay[56].

CONCLUSION

Hutchinson– Gilford Progeria syndrome is a disease which has made curiosity among many scientists. The research is being carried out worldwide to understand the underlying cause. Although the mutation in LMNA gene is responsible, the exact mechanism is not clear till now. There is no cure for HGPS but Currently Farnesyltransferase Inhibitors (FTIs) is being used as the potential drug treatment for the disease. The supportive therapies like Growth Hormone, Aspirins etc along with various measures to prevent the complications of the disease and this may helpful in prolonging the life.

REFERENCES

1. Hutchinson J, Lancet. 1886; 1: 923.
2. Rapini, Ronald P., Bolognia, Jean L., Jorizzo, Joseph L.. Dermatology 2007, 2, 1847.
3. Hutchinson J, Med. Press, 1937, 34, 194.
4. Hutchinson J. Congenital absence of hair and mammary glands with atrophic condition of the skin and its appendages in a boy whose mother had been almost totally bald from alopecia areata from the age of six. Medicochirurgical Transactions 1886;69:473–7
5. Guilford H. Progeria: a form of senilism. Practitioner 1904;73:188–217.
6. Hennekam RC, Am J Med Genet A. 2006; 140: 2603.
7. Ewell Steve Roach, Van S. Miller, Cambridge University Press. 2004; 36 :150
8. Kwang-Jen Hsiao, Advances in Clinical Chemistry, 1998; 33 :10
9. Sinha JK, Ghosh S, Raghunath M. Progeria: A rare genetic premature ageing disorder. Indian J Med Res. 2014;139(5):667-74
10. Domínguez-Gerpe L, Araújo-Vilar D. Prematurely aged children: molecular alterations leading to HutchinsonGilford progeria and Werner syndromes. Curr Aging Sci. 2008;1(3):202-12.
11. Hennekam RC. Hutchinson-Gilford progeria syndrome: review of the phenotype. Am J Med Genet A. 20061;140(23):2603-24.
12. Hutchinson-Gilford progeria syndrome. e-rheumatology. Accessed 14 August 2014. Available: http://www.erheumatology.gr/scientificarticles/syndromo-hutchinson-gilfordprogiriaTsiligiri et al.; BJMMR, 5(12): 1527-1533, 2015; Article no.BJMMR.2015.17
13. Pollex RL, Hegele RA. Hutchinson-Gilford progeria syndrome. Clin Genet. 2004;66(5):375–381
14. Capell BC, Collins FS, Nat Rev Genet. 2006; 7: 940-52
15. Young SG, Fong LG, Machaelis. J. Lipid Res. 2005; 46: 2531-2558.
16. Bergo MO, Gavino B, Ross J . Proc. Natl. Acad. Sci.2002; 99: 13049-13054
17. Zhang Y, Kausik S, Dai R et al. J. Biol. Chem. 1998; 273: 15030-15034.
18. Clarke S., Vogel JP, Deschenes RLS et al. Proc. Natl. Acad. Sci. 1988; 85: 4643-4647
19. Beck LA, Hosick TJ, Sinensky M, J. Cell Biol. 1990; 110: 1489-1499.
20. Kilic F, Dalton FB, Burrel SK S et al. J. Biol. Chem. 1997; 272: 5298-5304
21. Beck LA, Hosick TJ, Sinensky M, J. Cell Biol. 1990; 110: 1489-1499. [25] Kilic F, Dalton FB, Burrel SK S et al. J. Biol. Chem. 1997; 272: 5298-5304
22. Hutchinson J, Lancet. 1886; 1: 923

23. Pollex RL, Hegele RA. Hutchinson–Gilford progeria syndrome. Clin Genet. 2004;66(5):375–381.
24. Gordon LB, McCarten KM, GiobbieHurder a, et al. Pediatrics. 2007; 120(4): 824-33
25. Russo-Menna I, Arancibias C. The Hutchinson-Gilford Progeria Syndrome: a case report. Minerva Anestesiol. 2010;76(2):151-154.
26. Gordon, LB. The Premature Aging Syndrome Hutchinson-Gilford Progeria: Insights into Normal Aging in: Brocklehurst's Textbook of Geriatric Medicine and Gerontology, Seventh Edition. 2010:66-72
27. Olive M, et al. Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. Arterioscler Thromb Vasc Biol. 2010;30(11):2301-9.
28. Shah KN, Elston DM. Hutchinson-Gilford Progeria: Follow-up. Accessed 12 August 2014. Available: http://emedicine.medscape.com/article/1 117344-followup#showall
29. Xiong Z, Lu Y, Xue J, Luo S, Xu X, Zhang L, et al. Hutchinson-Gilford progeria syndrome accompanied by severe skeletal abnormalities in two Chinese siblings: two case reports. J Med Case Rep. 2013;7:63. doi: 10.1186/1752-1947-7-63.
30. Cau P, Navarro C, Harhouri K, et al. Nuclear matrix, nuclear envelope and premature aging syndromes in a translational research perspective. Seminars Cell Dev Biol. 2014;29:125–47.
31. Delbarre E, Tramier M, Coppey-Moisan M, et al. The truncated prelamin A in Hutchinson-Gilford progeria syndrome alters segregation of A-type and B-type lamin homopolymers. Hum Mol Genetics. 2006;15:1113–22
32. Basso AD, Kirschmeier P, Bishop WR. Lipid posttranslational modifications. Farnesyl transferase inhibitors. J Lipid Res. 2006;47:15–31
33. Yang SH, Meta M, Qiao X, et al. A farnesyltransferase inhibitor improves disease phenotypes in mice with a Hutchinson-Gilford progeria syndrome mutation. J Clin Invest. 2006;116:2115–21.
34. Yang SH, Andres DA, Spielmann HP, et al. Progerin elicits disease phenotypes of progeria in mice whether or not it is farnesylated. J Clin Invest. 2008;118:3291–300.
35. Capell BC, Olive M, Erdos MR, et al. A farnesyltransferase inhibitor prevents both the onset and late progression of cardiovascular disease in a progeria mouse model. Proc Natl Acad Sci U S A. 2008;105:15902–7.
36. Varela I, Pereira S, Ugalde AP, et al. Combined treatment with statins and aminobisphosphonates extends longevity in a mouse model of human premature aging. Nat Med. 2008;14:767–72.
37. Davies BS, Barnes RH, 2nd, Tu Y, et al. An accumulation of non-farnesylated prelamin A causes cardiomyopathy but not progeria. Hum Mol Genetics. 2010;19:2682–94.
38. Blondel S, Egesipe AL, Picardi P, et al. Drug screening on Hutchinson Gilford progeria pluripotent stem cells reveals aminopyrimidines as new modulators of farnesylation. Cell Death Dis. 2016;7:e2105.

39.] Ibrahim MX, Sayin VI, Akula MK, et al. Targeting isoprenylcysteine methylation ameliorates disease in a mouse model of progeria. Science. 2013;340:1330–3
40. Cao K, Graziotto JJ, Blair CD, et al. Rapamycin reverses cellular phenotypes and enhances mutant protein clearance in Hutchinson-Gilford progeria syndrome cells. Sci Transl Med. 2011;3:89ra58.
41. Graziotto JJ, Cao K, Collins FS, et al. Rapamycin activates autophagy in Hutchinson-Gilford progeria syndrome: implications for normal aging and agedependent neurodegenerative disorders. Autophagy. 2012;8:147–51
42. Ramos FJ, Chen SC, Garelick MG, Rapamycin reverses elevated mTORC1 signaling in Lamin A/Cdeficient mice, rescues cardiac and skeletal muscle function, and extends survival. Sci Translational Med. 2012;4:144ra103
43. Gabriel D, Roedl D, Gordon LB,. Sulforaphane enhances progerin clearance in Hutchinson-Gilford progeria fibroblasts. Aging Cell. 2015;14:78–91.
44. Gabriel D, Shafry DD, Gordon LB, . Intermittent treatment with farnesyltransferase inhibitor and sulforaphane improves cellular homeostasis in Hutchinson-Gilford progeria fibroblasts. Oncotarget. 2017;8:64809–26.
45. Gabriel D, Shafry DD, Gordon LB, . Intermittent treatment with farnesyltransferase inhibitor and sulforaphane improves cellular homeostasis in Hutchinson-Gilford progeria fibroblasts. Oncotarget. 2017;8:64809–26.
46. Egesipe AL, Blondel S, Cicero AL,. Metformin decreases progerin expression and alleviates pathological defects of Hutchinson-Gilford progeria syndrome cells. NPJ Aging Mechan Dis. 2016;2:16026
47. Viteri G, Chung YW, Stadtman ER. Effect of progerin on the accumulation of oxidized proteins in fibroblasts from Hutchinson Gilford progeria patients. Mech Ageing Dev. 2010;131:2–8
48. Richards SA, Muter J, Ritchie P, The accumulation of un-repairable DNA damage in laminopathy progeria fibroblasts is caused by ROS generation and is prevented by treatment with N-acetyl cysteine. Hum Mol Genetics. 2011;20:3997–4004
49. Kang HT, Park JT, Choi K,Chemical screening identifies ROCK as a target for recovering mitochondrial function in Hutchinson-Gilford progeria syndrome. Aging Cell. 2017;16:54
50. Xiong ZM, Choi JY, Wang K, . Methylene blue alleviates nuclear and mitochondrial abnormalities in progeria. Aging Cell. 2016;15:2
51. Kreienkamp R, Croke M, Neumann MA, . Vitamin D receptor signaling improves Hutchinson-Gilford progeria syndrome cellular phenotypes. Oncotarget. 2016;7:30018–31.

52. Gordon LB, Harten IA, Patti ME, Lichtenstein AH. Reduced adiponectin and HDL cholesterol without elevated Creactive protein: clues to the biology of premature atherosclerosis in HutchinsonGilford Progeria Syndrome. J Pediatr. 2005;146(3):336-41.
53. DeBusk FL. The Hutchinson-Guilford progeria syndrome. J Pediatr 1972;90:697–724
54. Hasty MF, Vann WF, Pediet. Dent. 1998; 10: 314-319
55. Gordon LB, Kleinman ME, Miller DT, Neuberg DS, Giobbie-Hurder A, GerhardHerman M, et al. Clinical trial of a farnesyltransferase inhibitor in children with Hutchinson-Gilford progeriasyndrome.ProcNatlAcadSci.USA.2012;109(41):1666671.doi:10.1073/pnas.1202529109. Epub 2012 Sep 24.
56. Sagelius H, Rosengardten Y, Schmidt E . J Med Genet. 2008; 45(12): 794-801