Iranian J Publ Health, Vol. 33, No. 2, pp.56-59, 2004

Computational Analysis of the Effect of *fbn1* Gene Mutations in the Marfan Syndrome

H Mohabatkar

Dept. of Biology, School of Sciences, Shiraz University, Shiraz, Iran

Abstract

Fibrillin is a large glycoprotein synthesized in the tissues involved in Marfan syndrome, and known to be involved in tissue elasticity. The syndrome is corresponded to *fbn1* gene and is characterized by cardiovascular, ocular, and skeletal abnormalities. N-terminus of fibrillin 1 binds to microfibril-associated glycoprotein 1 (MAGP-1) in a calcium-dependent manner. In this study, the amino acid sequence of fibrillin protein of a patient with Marfan syndrome (accession No. XM-034890) has been compared to the amino acid sequence of normal fibrillin (accession No. P-35555). In this patient, mutations causing a Gly (267) to Thr and Tyr (532) to Cys amino acids changes have been occurred. Method of Garnier was used to predict the secondary structure of the proteins and probable N-glycosylation sites were searched. Results of these analyses show no significant structural difference between the mutant and normal fibrillin proteins. Although in some cases characterization of the binding requirements has shown that a folded, secondary structure of fibrillin was necessary for binding, our results are in agreement with those findings that at least in some cases, fibrillin gene defects are not sole determinants of Marfan phenotype.

Keywords: *Marfan syndrome, Fibrillin, Computational analysis*, *Iran*

Introduction

Human fibrillin-1 is a 350-kDa glycoprotein found in 10-nm connective tissue microfibrils. The importance of fibrillin-1 in the maintenance of connective tissue architecture is emphasized by the linkage of the gene for fibrillin-1 to the Marfan syndrome, an autosomal dominant disease of connective tissue that occurs at a frequency of at least 1 in 10,000 in the population (1-3).

The presumed complete cDNA sequence (8.6 kb) has been recently determined (4, 5) and shows fibrillin-1 to be a modular protein comprising 47 EGF-like domains, 7 domains that have homology to transforming growth factor 1 binding protein (8-cysteine motif), 2 hybrid domains with features of both the transforming growth factor 1 binding protein motif and EGF-like domain motif, a proline-rich region, and 2 unique regions located at the predicted N and C terminus, respectively (6).

Pre-fibrillin consists of 2,871 amino acids which, excluding the signal peptide, are arranged into five structurally distinct regions. The largest of these regions comprises about 75% of the entire protein and consists of numerous repeated cysteine-rich sequences homologous to the peptide motifs of the epidermal growth factor (EGF) and transforming growth factor-beta binding protein (TGF-bp). Forty-three of the forty-six EGF-like repeats contain a calcium binding consensus sequence (EGF-CB) conceivably mediating protein-protein interactions. Fibrillin exhibits a few

***Corresponding author:** Tel: +98 711 2280916, Fax: +98 711 2280926, E-mail: h-mohabatkar@yahoo.com

additional cysteine-rich modules that are apparently unique to this macromolecule and may represent evolutionary variants of the EGF-CB and TGF-bp motifs. Almost all of the cysteine-rich repeats are encoded by single exons; consequently, the fibrillin gene is relatively large (approximately 110 kb) and highly fragmented (65 exons) (4).

Materials and Methods

Numbering of the human fibrillin-1 amino acid sequence to study the structure of fibrillin, I fetched two sequences of this protein were collected from the Swiss-Prot databank. The amino acid sequence of fibrillin protein of a Marfan syndrome patient (accession No. XM-034890) was compared to the amino acid sequence of a normal fibrillin (accession No. P-35555).

Theoretical analysis Garnier scales were used to study the structure of the proteins (7- 9). N-glycosylation sites were searched as Asn-X-Thr or Asn-X-Ser sequences, where X was any residue (10-12). The amino acid sequence of the proteins was read as a moving window of seven residues and their values corresponding to each of the scales taken here into consideration and the mean was plotted against the fourth residue of the window (13).

Results

In the patient, mutations causing a Gly (267) to Thr and Tyr (532) to Cys amino acids changes have been occurred.

The results of the computer-assisted secondary structure prediction for the normal fibrillin are shown in Table 1. These results show that 5 regions predicted to be α-helix and 10 regions predicted to β-sheet. According to this analysis 10.2%, 12.8% and 65.2% of the studied sequence were in the α-helix, β-sheet and tuns forms respectively. The same analysis was performed to study the mutated fibrillin. The result was very interesting, because it showed 100% similarity with that of the normal one.

Table 1: Regions of normal and mutated fibrillins predicted to be α-helix or β-sheet by computational analysis (the numbers refer to the number of amino acids as explained in Materials and Methods)

α–helices	β–sheets
1–9	10–18
27–42	80–85
273–289	187–190
375–384	208–213
571–579	327–332
	336–340
	391–396
	408–416
	425–435
	444–458

Discussion

The extracellular microfibril, 10-14 nm in diameter, performs a number of functions, including serving as the scaffolding for deposition of tropoelastin to form elastic fibers. A variety of proteins compose the structure of microfibrils, the most prominent of which is fibrillin-1, Which Fibrillin-1 is encoded by *fbn1* on human chromosome 15q21. Each fibrillin monomer contains a large number of epidermal growth factor-like motifs, most capable of binding calcium ions, and a few motifs resembling the binding protein for transforming growth factor beta. In vitro polymerization of fibrillin monomers produces 'beads on a string' structures that look on electron microscopy much like microfibrils purified from the extracellular matrices of a variety of tissues. Mutations in *fbn1* produce Marfan syndrome, a pleiotropic autosomal dominant connective tissue disorder with prominent manifestations in the skeleton, eye and cardiovascular system (14). Results of the previous studies indicate that mutations in the gene that encodes fibrillin are responsible for the Marfan syndrome in the majority of individuals (but not all) and that a

variety of mutations can produce the phenotype associated with the syndrome (15).

Besides, it was already shown that fibrillin defects at the protein level per se, are not specific for the Marfan syndrome, but that the drastically reduced fibrillin deposition, caused by a dominant-negative effect of abnormal fibrillin molecules, may be of prognostic and possibly diagnostic significance (16).

In this study, in the mutated fibrillin a glycine was changed to a threonine and a cysteine was changed to a tyrosine. Glycine is a smaller and more nonpolar amino acid than threonine.

Cysteine and tyrosine both are polar amino acids but the latter is an aromatic amino acid. None of these mutations have changed the structure of the protein severely and the number and percentage of predicted α-helix and β -sheet remained the same. These results confirmed those findings that at least in some cases, fibrillin gene defects are not the sole determinant of Marfan syndrome (17).

Acknowledgements

Support to this study by Shiraz University is gratefully acknowledged.

References

1. Lee B, Godfrey M, Vitale E, Hori H, Mattei MG et al (1991). Linkage of Marfan syndrome and a phynotypically related disorder to two different fibrillin genes. *Nature,* 352: 330-34.
2. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY et al (1991). Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin genes. *Nature,* 352: 337-39.
3. Maslen CL, Corson GM, Maddox BK, Glanville RW, Sakai LY (1991). Partial sequence of a candidate gene for the Marfan syndrome. *Nature,* 352: 334-37.
4. Pereira L, D'Alessio M, Ramirez F, Lynch JR, Sykes B, Pangilinan T, Bonadio J (1993). Genomic organization of the sequence coding for fibrillin, the defective gene product in Marfan syndrome. *Hum Mol Genet,* 2: 961-68.
5. Corson GM, Chalberg SC, Dietz HC, Charbonneau NL, Sakai LS (1993). Fibrillin binds calcium and is coded by cDNA that reveal a multidomain structure and alternativelyspliced exones at the 5' end. *Genomics,* 17: 476-84.
6. Handford P, Downing A, Rao Z, Hewett D, Sykes B, Kielty C (1995). The calcium binding properties and molecular organization of epidermal growth factor-like domains in human fibrillin-1. *J Biol Chem,* 270: 6751-56.
7. Garnier J, Osguthorpe DJ, Robson B (1978). Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J Mol Biol,* 120: 97-120.
8. Garnier J, Gibrat J-F, Robson B (1996). GOR method for predicting protein secondary structure from amino acid sequence. *Methods Enzymol*,266:450-553.
9. Kloczkowski A, Ting KL, Jernigan RL, Garnier J (2002). Combining the GOR V algorithm with evolutionary information for protein secondary structure prediction from amino acid sequence. *Proteins*, 49: 154-66.
10. Bause A (1983). Structural requirements of N-glycosylation of proteins, Studies with proline peptides as conformational probes. *Biochem J*, 209: 331-36.
11. Mellquist JL, Kasturi L, Spitalnik L, Shakin-Eshleman SH (1998). The amino acid folowing an Asn-X-Ser/ Thr sequon is an important determinant of N-linked core glycosylation efficiency. *Biochemistry*, 38: 6733-37.
12. Hubbard SC, Ivatt RJ (1981). Synthesis and processing of asparagine-linked oligosaccharides. *Annu Rev Biochem,* 50: 555-83.
13. Carmenes RS, Freije JP, Molina MM, Martin JM (1989). predict7, a program

for protein structure prediction. *Biochem Biophys Res Commun,* 159: 687-93.

14. Dietz HC, Pyeritz RE (1995). Mutations in the human gene for fibrillin-1 (FBN1) in the Marfan syndrome and related disorders. *Hum Mol Genet,* 4: 1799-1809.
15. Milewicz DM, Pyeritz RE, Crawford ES, Byers PH (1992). Marfan syndrome: defective synthesis, secretion, and extracellular matrix formation of fibrillin by cultured dermal fibroblasts. *J Clin Invest,* 89: 79-86.
16. Aoyama T, Francke U, Gasner C, Furthmayr H (1995). Fibrillin abnormalities and prognosis in Marfan syndrome and related disorders. *Am J Med Genet,* 28:169-76.
17. Dietz HC, Pyeritz RE, Puffenbreger EG, Kendzior RJ Jr, Corson GM et al (1992). Marfan phenotype variability in a family segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin gene. *J Clin Invest*, 89: 1674-80.