Missense mutations of conserved glycine residues in fibrillin-1 highlight a potential subtype of cb-EGF-like domains

Philippe Khau van Kien, David Baux, Nathalie Pallares-Ruiz, Corinne Baudoin, Aurélie Plancke, Nicolas Chassaing, Patrick Collignon, Valérie Drouin-Garraud, Alain Hovnanian, Dominique Martin-Coignard, et al.

Missense Mutations of Conserved Glycine Residues in Fibrillin-1 Highlight a Potential Subtype of cb-EGF-like Domains

Philippe Khau Van Kien[1], David Baux[1], Nathalie Pallares-Ruiz[1], Corinne Baudoin[1], Aurélie Plancke[1], Nicolas Chassaing[2,3,4], Patrick Collignon[5], Valérie Drouin-Garraud[6], Alain Hovnanian[2,3,4], Dominique Martin-Coignard[7], Gwenaëlle Collod-Béroud[8,9], Christophe Béroud[1,8,9], Anne-Françoise Roux[1], and Mireille Claustres[1,8,9]

[1]CHU Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de Génétique Moléculaire, Montpellier, F-34000 France; [2]CHU Toulouse, Hôpital Purpan, Service de Génétique Médicale, Toulouse, F-31300, France; [3]INSERM, U563, Centre de Physiopathologie de Toulouse Purpan, Toulouse, F-31300, France; [4]Université Toulouse III Paul-Sabatier, Toulouse, F-31400, France; [5]CHU Marseille, Hôpital d'Enfants de la Timone, Département de Génétique Médicale, Marseille F-13000, France; [6]CHU Rouen, Département de Génétique, Rouen, France; [7]CH le Mans, Service de Génétique Médicale, le Mans, F-72000, France; [8]INSERM, U827, Montpellier, F-34000 France; [9]Université Montpellier1, UFR Médecine, Montpellier, F-34000 France

*Correspondence to Philippe Khau Van Kien, MD-PhD, CHU Montpellier, Laboratoire de Génétique Moléculaire, UFR Médecine site Nord UPM/IURC, 640 Avenue du doyen Gaston GIRAUD, 34295 MONTPELLIER CEDEX 5, FRANCE. Phone: +33467415360; Fax: +33467415365; E-mail: p-khau_van_kien@chu-montpellier.fr

ABSTRACT: In six index cases/families referred for Marfan syndrome (MFS) molecular diagnosis, we identified six novel mutations in the *FBN1* gene: c.1753G>C (p.Gly585Arg), c.2456G>A (p.Gly819Glu), c.4981G>A (p.Gly1661Arg), c.5339G>A (p.Gly1780Glu), c.6418G>A (p.Gly2140Arg) and c.6419G>A (p.Gly2140Glu). These variants, predicted to result in Glycine substitutions are located at the third position of a 4 amino acids loop-region of calcium-binding Epidermal Growth Factor-like (cb-EGF) fibrillin-1 domains #5, #9, #24, #25 and #32. Familial segregation studies showing cosegregation with MFS manifestations or *de novo* inheritance in addition to *in silico* analyses (conservation, 3D modeling) suggest evidence for a crucial role of the respective Glycine positions. Extending these analyses to all Glycine residue at position 3 of this 4 residues loop in fibrillin-1 cb-EGF with the UMD predictor tool and alignment of 2038 available related sequences strongly support a steric strain that only allows Glycine or even Alanine residues for domain structure maintenance and for the fibrillin functions. Our data compared with those of the literature strongly suggest the existence of a cb-EGF domain subtype with implications for related diseases.

KEY WORDS: cb-EGF-like domain, Marfan Syndrome, Fibrillin 1 (*FBN1*) gene, fibrillinopathies

INTRODUCTION

Human fibrillin-1, the protein involved in Marfan syndrome (MFS; MIM#154700) is characterized by a specific modular organization with structural and non-structural functions in the extracellular matrix. The components of this organization consist in repeated cysteine rich structural modules that include: multiple copies of an epidermal growth factor-like (EGF) module of which 43/47 match with the consensus sequence of class I calcium-binding EGF (cb-EGF) (Downing, et al., 1996), TB (or 8-cysteine) or hybrid modules. About 60% of the reported mutations of the *FBN1* gene are missense. More than 75% of them are located in the cb-EGF modules of fibrillin-1 and involve mostly their obligatory residues (in particular, cysteine residues implicated in disulfide bond formation or other residues implicated in calcium-binding and/or intra- intermolecular interactions) (Collod-Beroud, et al., 2003; Downing, et al., 1996). Interpretation of the remaining missense mutations can be very challenging and requires additional arguments (familial segregation, epidemiological studies, functional data…) to assess their causality and thus, complicates the outcome of genetic testing with uninformative results. Reciprocally, the observation of deleterious mutations can help to identify additional key positions and therefore to assess clinical significance of missense variants.

To date, among 228 non-related index cases/families referred and analyzed in our laboratory for MFS molecular diagnosis, we have identified six missense mutations in the *FBN1* gene in six index cases, which were predicted to result in Glycine substitutions sharing an identical position in their corresponding cb-EGF domain (1.3% of tested chromosomes). This position corresponds to the p.Gly1127Ser mutation that has been intensively studied elsewhere (Francke, et al., 1995; Smallridge, et al., 2003; Whiteman, et al., 1998; Whiteman, et al., 2001). We provide here arguments to generalize previous findings concerning the p.Gly1127Ser mutation and demonstrate a crucial structural role for Glycine (or Alanine) at this position in class I cb-EGF domains.

PATIENTS AND METHODS

Patients

Pedigrees and main clinical data are presented in Fig. 1A and 1B. All the patients were of French Caucasian origin.

Case 1 / pedigree MFS 00021

A 39-year-old female, who was treated since 26 years of age with beta-blockers for a paroxysmal supraventricular tachycardia was referred for a MFS diagnosis (-1 Ghent criteria) because of a suggestive familial history of MFS and a bilateral ectopia lentis operated on at the age of 29 years. She also presented significant striae, positive wrist and thumb signs. A computed tomography (CT) of the hips and the lombosacral region revealed that she had a protrusio acetabulae. The rest of the investigation was normal. Her father and her brother (Fig. 1A) died from a type A aortic dissection, respectively at the age of 59 and 34 years. Both were reported to be of tall stature (194 and 202cm, respectively) with suggestive evidence of skeletal (arachnodactyly and dolichostenomelia) and ocular (strong myopia, which complicate in blindness in the father) involvements. There was no other familial antecedent.

Case 2 / pedigree MFS 00055

A 15-year-old female from healthy parents was referred for a MFS diagnosis (-1 Ghent criteria) after a comprehensive Ghent criteria assessment that identified a skeletal system involvement (scoliosis >20°, wrist and thumb signs, flexum at the elbows, characteristic face, high arched palate with crowding of teeth) and a major dural criterion on MRI. She had no familial history of MFS.

Case 3 / pedigree MFS 00126

A 60-year-old male was referred to confirm the diagnosis as well as for familial screening (Ghent criteria fulfilled). He had a familial history of MFS, a bilateral ectopia lentis operated on at the age of 16 years and 3 spontaneous pneumothorax events. Ghent criteria evaluation revealed that he also presented a scoliosis of more

than 20°, significant pes planus and a high arched palate with crowding of teeth. CT of the lombosacral region also showed a significant meningocele on S3. Ectopia lentis and other suggestive MFS manifestations were also reported in his son, sisters and mother (Fig. 1A and 1B).

Case 4 / pedigree MFS 00231

A 59-year-old father and his 14-year-old son were both referred to confirm the diagnosis (Ghent criteria fulfilled). They showed a similar score for Ghent criteria: a major criterion for the skeletal system (reduced upper to lower segment ratio, wrist and thumb signs, reduced extension at the elbows, protrusio acetabulae, moderate pectus excavatum, joint hypermobility and a high arched palate with crowding of teeth), a major criterion for the cardiovascular system (type A aortic dissection at 40 years of age for the father and aortic root dilatation for the son) and recurrent herniae. There were no other evident MFS manifestations in the family (notably in the parents, who died from a breast cancer and Alzheimer disease at the age of 52 and 80 years, respectively for the mother and the father).

Case 5 / pedigree MFS 00249

A 41-year-old female with no familial history of MFS was referred for a MFS diagnosis (-1 Ghent criteria). Ghent criteria assessment identified a major criterion for the skeletal system (pectus carinatum, scoliosis >20°, wrist and thumb signs, protrusio acetabulae, high arched palate with crowding of teeth), a strong bilateral myopia (>6.5 diopters), a mitral insufficiency with a mild valve prolapse and significant striae.

Case 6 / pedigree MFS 00405

A 12-year-old female with a familial history of MFS manifestations including ectopia lentis (in the father and in an aunt) was referred for a MFS diagnosis (-1 Ghent criteria): major ocular criterion (ectopia lentis) and skeletal system involvement (wrist and thumb signs, high arched palate with crowding of teeth, joint hypermobility). Her paternal grandmother died suddenly at the age of 58 years. She was operated on heart and ascending aorta at the age of 34 and 48 years.

FBN1 mutation screening in probands and family study

Genomic DNA or blood samples from patients were sent to the “Laboratoire de Génétique Moléculaire, CHU Montpellier” from other French centers. DNA was harvested from peripheral blood using various methods. Mutation screening allowing the scanning of the 65 *FBN1* exons and flanking regions including the splice sites up to the branching regions was performed in probands with a robot-assisted and single condition PCR/direct sequencing strategy as described elsewhere (Tjeldhorn, et al., 2006; Stheneur, et al., 2009). For several amplicons, at least one of the two primers was newly designed. Mutations were validated with two independent methods (performed from an independent dilution of the DNA and with other primers (Comeglio, et al., 2001; Sakai, et al., 2006; Howarth, et al., 2007)): 1- bidirectional sequencing and 2- dHPLC (performed on a WAVE DNA fragment analysis system (Transgenomic, Elancourt, France) for the c.1753G>C, c.2456G>A, c.4981G>A, c.6418G>A and the c.6419G>A mutations) or High-Resolution Melting analysis (HRM) (performed on a LightCycler 480 (Roche Diagnostics Corporation) for the c.5339G>A mutation). Both methods were then applied in proband relatives to establish familial segregation of each variant and in 95 French Caucasian controls. In all familial cases, haplotype analysis were performed in view to check for the good concordance of the samples origin and to complete Ghent criteria assessment using at least 8 microsatellites markers at the *FBN1* locus (Judge, et al., 2001). Detailed protocols are available upon request.

Mutation numbering refers to the *FBN1* cDNA GenBank reference sequence: NM_000138.3, with the A of the ATG translation initiation codon as nucleotide +1 (www.hgvs.org/mutnomen).

Conservation patterns and *in silico* predictions

Orthologs of the *FBN1* gene were identified with the NCBI and EnsEMBL websites (http://www.ncbi.nlm.nih.gov/ and http://www.ensembl.org/) and sequences were aligned with the human fibrillin 1 precursor reference sequence accession NP_000129.2 using ClustalW (Chenna, et al., 2003). Jalview Alignment Editor (Clamp, et al., 2004), and homemade Perl (http://www.perl.org/) scripts were used to visualize and analyze

the respective alignments. The UMD-predictor tool (Frederic, et al., 2009) was used to assess the impact at both RNA and protein level for all possible single nucleotide substitutions of the 31 fibrillin-1 Glycine codons that correspond to the third amino acid position of all the fibrillin-1 cb-EGF modules identified with a 4 amino acid loops between obligatory Cysteine residues 2 and 3. This includes the six missense mutations reported here and the 6 previously described (see further).

3D Modeling of the fibrillin-1 Glycine substitution effects

Supramolecular structure and NMR data available in the Protein Data Bank (http://www.rcsb.org/, PDB ID:1EMN) (Downing, et al., 1996) was used for the cb-EGF#32 domain (p.Gly2140Arg and p.Gly2140Glu). For p.Gly585Arg, and p.Gly1661Arg, located in the cb-EGF#5 and #24 domains of the fibrillin-1, respectively, a homology model was created based on PDB template 1LMJ (40 and 45% sequence identity, respectively)(Smallridge, et al., 2003). PDB template 1UZP (40% and 54% sequence identity with cb-EGF-like domain #9 and #25, respectively)(Lee, et al., 2004) was used to build the model to analyze p.Gly819Glu and p.Gly1780Glu. Template search was performed with Domain Fishing 1.0 (Contreras-Moreira and Bates, 2002) and Modeller program (version 8.2, http://salilab.org/modeller/, (Eswar, et al., 2007)) was used for homology modeling. The quality of the resulting structures was assessed using the evaluation tools available on the CBS web site (http://bioserv.cbs.cnrs.fr/). Mutagenesis and visualization were performed with PyMOL (http://pymol.sourceforge.net).

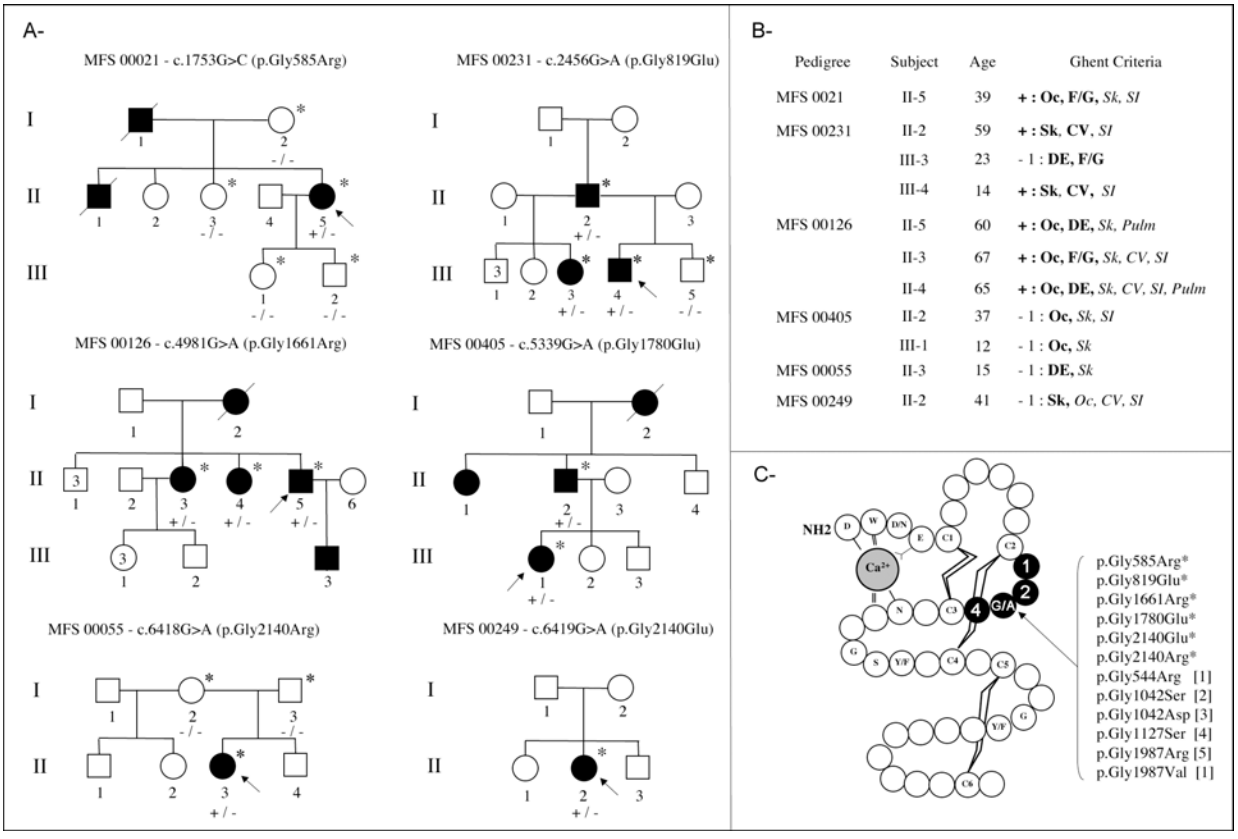

Figure 1. Clinical data and familial investigation of the six Glycine missense mutations suggesting a class I cb-EGF subtype. (A)- Affected pedigrees with familial segregation of each Glycine mutation. Pedigree/Generation/subject number and status concerning Marfan (MFS) manifestations in each family member are indicated. Squares, male subjects; circles, female. Affected and unaffected subjects are represented by solid and open symbols, respectively. Slashes denote deceased. Subjects with samples available for this study are indicated with an asterisk. Presence and absence of the Glycine missense mutation is represented by +/- or -/-, respectively. (B)- Main clinical data and Ghent criteria evaluation for MFS in affected subjects. +: criteria are fulfilled (*i.e.* the diagnosis of MFS is established); -1: a criterion is missing before testing to fulfill the criteria

(incomplete MFS). Skeletal, Ocular, Cardio-Vascular, Dural, Skin and Integuments, Pulmonary and Familial/Genetic systems are indicated in the following order with bold characters if considered as a major criterion or in italics if the system is involved (minor criterion): Sk, Oc, CV, DE, SI, Pulm and F/G. (C)- Class I cb-EGF subtype suggested by conservation pattern and UMD-predictor analysis (adapted from (Handford, et al., 1991)). The Glycine missense mutations reported here (asterisk) or elsewhere: [1], (Stheneur, et al., 2009); [2], (Attanasio, et al., 2008); [3], (Sakai, et al., 2006); [4], (Francke, et al., 1995) [5], (Comeglio, et al., 2001); involve a Glycine residue located at position 3 of a loop region composed of four residues (solid symbols) between obligatory Cysteine residues 2 and 3.

RESULTS

Mutation identification and segregation studies

Six missense mutations were identified in the 6 unrelated French index cases, in whom no other mutation suspected to be deleterious was identified (Fig. 1). The c.1753G>C (p.Gly585Arg), c.2456G>A (p.Gly819Glu), c.4981G>A (p.Gly1661Arg), c.5339G>A (p.Gly1780Glu), c.6418G>A (p.Gly2140Arg) and c.6419G>A (p.Gly2140Glu) mutations are, to date, not reported in the literature, in the currently updating UMD-*FBN1* online database (Collod-Beroud, et al., 2003) and were absent in 190 ethnically-matched control chromosomes. Data concerning MFS diagnosis criteria assessments (Ghent criteria) in positive tested relatives, familial investigations and mutation analyses are given in Fig. 1. With the exception of pedigree MFS 00249 for which no familial sample was available, each mutation cosegregated with manifestations of the disease in pedigrees MFS 00021, MFS 00126, MFS 00231 and MFS 00405. In pedigree MFS 00055, the c.6418G>A (p.Gly2140Arg) mutation was shown to arise *de novo*, although a possible germinal mosaicism in the parents could not be ruled out. Results of the haplotype study were concordant with the origin of the samples, data of the pedigrees and disease status.

Conservation patterns and *in silico* predictions

These missense mutations involved a conserved Glycine residue, located in a loop-region between obligatory Cysteine residues 2 and 3, that is present in 31/43 cb-EGF-like modules in fibrillin-1. Alignment of these 43 modules revealed two subtypes: one with a loop involving 4 residues (32 sequences including a Glycine (n=31) or an Alanine (n=1) at position 3 in cbEGF#42) and a second (the 11 remaining sequences) involving either a shorter or longer loop (*i.e.* cbEGF#15, #18-19, #26, #34-35, #38-41, #43). Orthologous alignments of fibrillin-1 in 19 different species (including *Homo sapiens*) revealed a similar pattern on a set of 833 sequences matching class I cb-EGF domains. A Glycine is at position 3 in 592/611 (97%) loops involving 4 residues. The only alternative residue is Alanine (100%, *i.e.* 19/611-592), the second smallest amino-acid after Glycine. A comparison of the respective distribution of Glycine and Alanine (excluding Glycine) from the position 1 (Glycine 115/611=19%, Fischer's exact test: $P<0.0001$ and Alanine 42/611-115=8%, $P<0.0001$)), from the position 2 (Glycine 120/611=20%, $P<0.0001$ and Alanine 5/611-120=1%, $P<0.0001$) and position 4 (Glycine 0/611=0%, $P<0.0001$ and Alanine 9/611-0=1.5%, $P<0.0001$) showed that both residues are specific of the third position. Amino-acid sequence alignments of fibrillin-2 and fibrillin-3 confirmed these findings. Finally, we assessed an NCBI alignment of 10421 cb-EGF sequences identified with the PFAM website (http://pfam.sanger.ac.uk, for PF07645). Only 8050/10421 contained the obligatory 6 Cysteines of the module and were taken into account. Of these, 2006 showed a loop composed of 4 residues between Cysteines 2 and 3. Glycine (1713/2006) and Alanine (143/2006) are found in position 3 of these loops in 85 and 7%, respectively (each alignment text file is available on request). The high conservation pattern of this Glycine position was also confirmed by the UMD-predictor analysis. Outpout of this analysis (Supp. Table S1) showed that all potential Glycine substitutions are predicted to be "pathogenous" except for the Alanine residues for which depending on the cb-EGF module predictions and scores were ranging from "probably polymorphism" to "probably pathogenous" or "pathogenous" and from 59 to 82/100, respectively.

All these data suggest an important role for the domain maintenance of this position and a subtype of the fibrillin class I cb-EGF described by Downing et al (Downing, et al., 1996), Fig. 1.

3D Modeling of the fibrillin-1 Glycine substitution effects

The possible effect of these Glycine substitutions on the structure of their corresponding modules was assessed by *in silico* 3D Modeling analysis (Fig. 2). The Ramachadran plot obtained for the Glycine residue1661 showed

that the Phi-Psi angles are impossible to adopt for an Arginine residue. Therefore, the conformation of the mutant domain is likely to be strongly altered by the presence of this mutation. For the Glycine residues 585, 819, 1780 and 2140, the predicted change to Arginine or Glutamic Acid involves the same mechanism. Indeed, in each case, the mutant residue is predicted to induce steric clashes with its surroundings. The p.Arg585 breaks a loop involving p.Lys612, p.Lys599 and p.Pro600. Thus, the structure of the cb-EGF-like#5 domain is likely to be strongly affected to retain the volume of the Arginine residue (*i.e.:* van der Waals volume of Glycine is 48Å^3 compared to 148Å^3 for Arginine). The p.Arg2140 and p.Glu2140 mutants are predicted to interfere with a crucial structural component of the cb-EGF-like domains, a disulfide bridge involving p.Cys2153-p.Cys2164, and p.Cys2137-p.Cys2151, respectively (Fig. 2). A similar effect is predicted with p.Gly819Glu and p.Gly1780Glu, which are likely to disrupt respectively p.Cys832-p.Cys845 and p.Cys1806-p.Cys1793 as well as p.Cys1777-p.Cys1791 covalent bonds (data available on request).

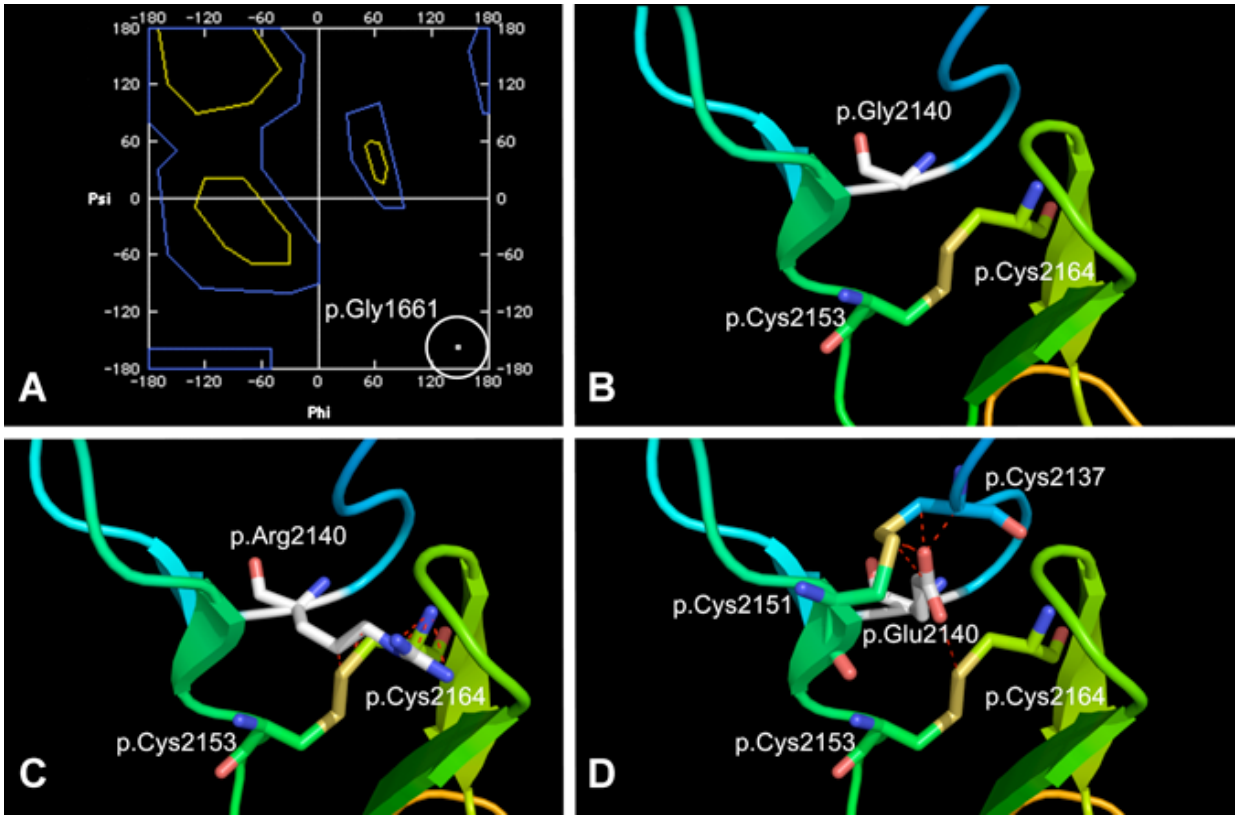

Figure 2. Prediction of the impact of mutations on cb-EGF-like domains. (A)- Ramachandran plot of predicted p.Gly1661: Phi and Psi angles are not accessible to other amino-acids. Plot drawn with Swiss-Pdb Viewer (http://spdbv.vital-it.ch/). (B)- prediction of p.Gly2140 position in the domain cb-EGF-like#32. (C)- According to PyMOL (http://pymol.sourceforge.net/), the most probable rotamer for p.Arg2140 would interfere with a disulfide bond involving p.Cys2153 and p.Cys2164. (D)- A similar mechanism is predicted with the mutant p.Glu2140. (C ; D)- Red dots indicate too short interatomic distances between residues. Figures showing predictions of p.Gly585Arg, p.Gly819Glu and p.Gly1780Glu effects in cb-EGF#5, #9, #25 are available upon request.

DISCUSSION

To date, we have sequenced the 65 *FBN1* exons and flanking regions in 228 non-related patients referred for molecular diagnosis of Marfan syndrome. We have identified in 6 of them (1.3 % of tested chromosomes) a single nucleotide substitution predicting a missense mutation of Glycine residues as the only unclassified and potentially deleterious variant. These mutations were absent in 190 ethnically-matched control chromosomes. They cosegregated with manifestations of the disease within the pedigrees or occured *de novo*. Study of conservation patterns showed that the corresponding Glycine position is highly conserved in orthologous, paralogous and also in

cb-EGF like modules sequences that include a 4 amino-acids loop between Cysteine residues 2 and 3. These results were also sustained by the UMD-predictor (Frederic, et al., 2009) analysis that in addition showed no argument for an impact on splicing. Interestingly, the alternative residue in the alignments and for which the predictions made by UMD-predictor were variable at this position was Alanine (Supp. Table S1). Alanine is the second smallest amino-acid after Glycine. This suggests that the third position of the loop could have particular local strains with steric exclusion for larger residues. Thus, we studied the possible effect of these Glycine substitutions on the structure of their corresponding modules by *in silico* 3D Modeling analysis and we bring arguments for a key position in terms of steric hindrance. All this results suggest a potential cb-EGF type 1 subtype (Fig. 1) with potential implication for related diseases.

To our knowledge, six missense mutations in the *FBN1* gene implicating a Glycine residue in such a position (p.Gly544Arg, p.Gly1042Ser, p.Gly1042Asp, p.Gly1127Ser, p.Gly1987Arg, p.Gly1987Val in cb-EGF#4, #11, #13, #30, respectively), in MFS or incomplete MFS subjects have been described and support our arguments (Attanasio, et al., 2008; Collod-Beroud, et al., 2003; Comeglio, et al., 2001; Francke, et al., 1995; Sakai, et al., 2006; Stheneur, et al., 2009). Only the p.Gly1127Ser mutation has been exhaustively studied: it was shown to result in a local cb-EGF like#13 domain folding disruption, a normal synthesis but a reduced fibrillin deposition into the extracellular matrix and proposed to cause disease via an extracellular dominant negative effect (Francke, et al., 1995; Whiteman, et al., 1998; Whiteman and Handford, 2003; Whiteman, et al., 2001). In their studies, Whiteman et al. (Whiteman, et al., 1998; Whiteman, et al., 2001) have previously noted that the p.Gly1127 position in cb-EGF like#13 was highly conserved and located in a turn of a two stranded antiparallel beta-sheet. However, neither Glycine nor Alanine were reported as mandatory at this position in the cb-EGF type I model (Downing, et al., 1996), thereby allowing to class variants and to facilitate the molecular diagnosis workflow of related diseases. This could be explained because of a variable composition in terms of type and number of amino acids in the loop between Cysteine residues 2 and 3. Here, the subtype of cb-EGF type I model, suggested by alignments of 8050 sequences (Fig. 1) and highlighted by 6 related missense mutations with some genetics/independent arguments for pathogenicity, shows how the findings previously made for p.Gly1127Ser could be extended. The p.Gly1127Ser mutation, associated with a mild effect on protein folding, was reported in 10 related-patients with a moderate phenotype (Francke, et al., 1995). None of the patients reported here as well as those described elsewhere when sufficient data are available to assess severity of the phenotype (Attanasio, et al., 2008; Comeglio, et al., 2001; Sakai, et al., 2006) have a severe and a typical MFS phenotype, suggesting that these *FBN1* gene mutation class could be encountered in incomplete or atypical MFS. It is difficult to conclude definitely on the precise genotype/phenotype correlations associated with this mutation class. First, because of the marked intra- and inter-familial variability in MFS manifestations and age of onset of the symptoms, that is notably illustrated in pedigree MFS 00231 (c.2456G>A (p.Gly819Glu), Fig. 1). The father (subject II-2), who suffered from a type A aortic dissection at 40 years of age, fulfilled the Ghent criteria clinically. His 14-years-old affected son (subject III-3) displayed a similar phenotype and had an aortic root dilatation unlike her 23-year-old daughter (subject III-2), which also inherited the mutation. Following a comprehensive Ghent criteria assessment, she only presented a high arched palate with crowding of teeth, a suggestive MFS facial appearance, a wrist sign without thumb sign, a strong myopia and a dural ectasia on MRI (not fulfilling Ghent criteria). Considering that she is adult (but also that she may develop an aortic complication), the penetrance of the c.2456G>A mutation towards MFS is incomplete suggesting the importance of modifying factors. Second, we could not extend the clinical and molecular investigations because of a lack of participation of the relatives to fully assess the phenotypes associated with this class of mutation. However, from our study and as reported before we claim that this singular missense mutation class affecting Glycine residues located at the third position of this cb-EGF-like subtype (if resulting in a substitution other than Alanine) should be at least considered as a strong risk factor for a wide range of MFS manifestations, including aortic complications. Aortic dissection and sudden death are highly prevalent within families reported with such missense mutations. To our opinion, the medical management and follow-up should not differ from classic MFS.

We have shown a key residue-position in a potential subtype of class I cb-EGF like module. Both, Glycine and Alanine residues appear mandatory for the structure maintenance and therefore for the fibrillin-1 functions. These findings have implications for genetic testing in MFS, other fibrillinopathies and in other cb-EGF-like related-disorders.

ACKNOWLEDGMENTS

We gratefully acknowledge the clinical research team of the CHU of Montpellier, notably J. Dinet and S. Plagnol for their support. Corinne Baudoin, Aurélie Plancke and part of this work were funded through the « Programme de Soutien aux Techniques Innovantes Coûteuses 2006 » from the French Ministry of Health.

REFERENCES

Attanasio M, Lapini I, Evangelisti L, Lucarini L, Giusti B, Porciani M, Fattori R, Anichini C, Abbate R, Gensini G, Pepe G. 2008. FBN1 mutation screening of patients with Marfan syndrome and related disorders: detection of 46 novel FBN1 mutations. Clin Genet 74(1):39-46.

Chenna R, Sugawara H, Koike T, Lopez R, Gibson TJ, Higgins DG, Thompson JD. 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 31(13):3497-500.

Clamp M, Cuff J, Searle SM, Barton GJ. 2004. The Jalview Java alignment editor. Bioinformatics 20(3):426-7.

Collod-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, Child A, Comeglio P, De Paepe A, Hyland JC, Holman K, Kaitila I, Loeys B, Matyas G, Nuytinck L, Peltonen L, Rantamaki T, Robinson P, Steinmann B, Junien C, Beroud C, Boileau C. 2003. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. Hum Mutat 22(3):199-208.

Comeglio P, Evans AL, Brice GW, Child AH. 2001. Detection of six novel FBN1 mutations in British patients affected by Marfan syndrome. Hum Mutat 18(3):251.

Contreras-Moreira B, Bates PA. 2002. Domain fishing: a first step in protein comparative modelling. Bioinformatics 18(8):1141-2.

Downing AK, Knott V, Werner JM, Cardy CM, Campbell ID, Handford PA. 1996. Solution structure of a pair of calcium-binding epidermal growth factor-like domains: implications for the Marfan syndrome and other genetic disorders. Cell 85(4):597-605.

Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, Pieper U, Sali A. 2007. Comparative protein structure modeling using MODELLER. Curr Protoc Protein Sci Chapter 2:Unit 2 9.

Francke U, Berg MA, Tynan K, Brenn T, Liu W, Aoyama T, Gasner C, Miller DC, Furthmayr H. 1995. A Gly1127Ser mutation in an EGF-like domain of the fibrillin-1 gene is a risk factor for ascending aortic aneurysm and dissection. Am J Hum Genet 56(6):1287-96.

Frederic MY, Lalande M, Boileau C, Hamroun D, Claustres M, Beroud C, Collod-Beroud G. 2009. UMD-predictor, a new prediction tool for nucleotide substitution pathogenicity - application to four genes: FBN1, FBN2, TGFBR1 and TGFBR2. Hum Mutat in press.

Handford PA, Mayhew M, Baron M, Winship PR, Campbell ID, Brownlee GG. 1991. Key residues involved in calcium-binding motifs in EGF-like domains. Nature 351(6322):164-7.

Howarth R, Yearwood C, Harvey JF. 2007. Application of dHPLC for mutation detection of the fibrillin-1 gene for the diagnosis of Marfan syndrome in a National Health Service Laboratory. Genet Test 11(2):146-52.

Judge DP, Biery NJ, Dietz HC. 2001. Characterization of microsatellite markers flanking FBN1: utility in the diagnostic evaluation for Marfan syndrome. Am J Med Genet 99(1):39-47.

Lee SS, Knott V, Jovanovic J, Harlos K, Grimes JM, Choulier L, Mardon HJ, Stuart DI, Handford PA. 2004. Structure of the integrin binding fragment from fibrillin-1 gives new insights into microfibril organization. Structure 12(4):717-29.

Sakai H, Visser R, Ikegawa S, Ito E, Numabe H, Watanabe Y, Mikami H, Kondoh T, Kitoh H, Sugiyama R, Okamoto N, Ogata T, Fodde R, Mizuno S, Takamura K, Egashira M, Sasaki N, Watanabe S, Nishimaki S, Takada F, Nagai T, Okada Y, Aoka Y, Yasuda K, Iwasa M, Kogaki S, Harada N, Mizuguchi T, Matsumoto N. 2006. Comprehensive genetic analysis of relevant four genes in 49 patients with Marfan syndrome or Marfan-related phenotypes. Am J Med Genet A 140(16):1719-25.

Smallridge RS, Whiteman P, Werner JM, Campbell ID, Handford PA, Downing AK. 2003. Solution structure and dynamics of a calcium binding epidermal growth factor-like domain pair from the neonatal region of human fibrillin-1. J Biol Chem 278(14):12199-206.

Stheneur C, Collod-Beroud G, Faivre L, Buyck JF, Gouya L, Le Parc JM, Moura B, Muti C, Grandchamp B, Sultan G, Claustres M, Aegerter P, Chevallier B, Jondeau G, Boileau C. 2009. Identification of the minimal combination of clinical features in probands for efficient mutation detection in the FBN1 gene. Eur J Hum Genet.

Tjeldhorn L, Rand-Hendriksen S, Gervin K, Brandal K, Inderhaug E, Geiran O, Paus B. 2006. Rapid and efficient FBN1 mutation detection using automated sample preparation and direct sequencing as the primary strategy. Genet Test 10(4):258-64.

Whiteman P, Downing AK, Smallridge R, Winship PR, Handford PA. 1998. A Gly --> Ser change causes defective folding in vitro of calcium-binding epidermal growth factor-like domains from factor IX and fibrillin-1. J Biol Chem 273(14):7807-13.

Whiteman P, Handford PA. 2003. Defective secretion of recombinant fragments of fibrillin-1: implications of protein misfolding for the pathogenesis of Marfan syndrome and related disorders. Hum Mol Genet 12(7):727-37.

Whiteman P, Smallridge RS, Knott V, Cordle JJ, Downing AK, Handford PA. 2001. A G1127S change in calcium-binding epidermal growth factor-like domain 13 of human fibrillin-1 causes short range conformational effects. J Biol Chem 276(20):17156-62.