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1 **Clinical and mutation type analysis from an international series of** 2 **198 probands with a pathogenic *FBN1* exons 24-32 mutation**

3
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35 ABSTRACT

36 Mutations in the *FBNI* gene cause Marfan syndrome (MFS) and a wide range of
37 overlapping phenotypes. The severe end of the spectrum is represented by neonatal MFS, the
38 vast majority of probands carrying a mutation within exons 24-32. We previously showed that
39 a mutation in exons 24-32 is predictive of a severe cardiovascular phenotype even in non-
40 neonatal cases, and that mutations leading to premature truncation codons are under-
41 represented in this region. To describe patients carrying a mutation in this so-called
42 “neonatal” region, we studied the clinical and molecular characteristics of 198 probands with
43 a mutation in exons 24-32 from a series of 1013 probands with a *FBNI* mutation (20%).
44 When comparing patients with mutations leading to a premature termination codon within
45 exons 24-32 to patients with an in-frame mutation within the same region, a significantly
46 higher probability of developing ectopia lentis and mitral insufficiency were found in the
47 second group. Patients with a premature termination codon within exons 24-32 rarely
48 displayed a neonatal or severe MFS presentation. We also found a higher probability of
49 neonatal presentations associated with exon 25 mutations, as well as a higher probability of
50 cardiovascular manifestations. A high phenotypic heterogeneity could be described for
51 recurrent mutations, ranging from neonatal to classical MFS phenotype. In conclusion, even if
52 the exon 24-32 location appears as a major cause of the severity of the phenotype in patients
53 with a mutation in this region, other factors such as the type of mutation or modifier genes
54 might also be relevant.

55 INTRODUCTION

56 Marfan syndrome (MFS; MIM 154700) is a connective tissue disorder, with autosomal
57 dominant inheritance and a prevalence of 1/5000-10000 individuals¹. The cardinal features of
58 MFS involve the ocular, cardiovascular and skeletal systems². Neonatal MFS is considered as
59 the severe end of the MFS phenotype, and most cases are sporadic. Rare homozygote forms
60 and a few compound heterozygote patients born to parents each displaying or not a MFS
61 phenotype, have been reported³⁻⁴.

62 While the known mutations of *FBNI* are spread over the entire gene, the mutations
63 causing neonatal MFS seem to cluster in a specific region from exons 24 to 32⁵⁻⁷. Besides
64 neonatal MFS, atypically severe phenotypes also cluster in exons 24-32⁸. This region includes
65 the central longest stretch of 12 cbEGF repeats that is thought to form a rigid rod-like
66 structure which may be important for microfibril assembly. Previously, we showed that
67 mutations in exons 24-32 were associated with a more severe phenotype than mutations
68 located in other exons of the gene, including younger age at diagnosis of type I
69 fibrillinopathy, higher probability of ectopia lentis, ascending aortic dilatation, aortic surgery,
70 mitral valve abnormalities, scoliosis and shorter survival⁹. The majority of these results were
71 replicated even when neonatal cases were excluded, leading to the conclusion that exon 24-32
72 mutations define a high-risk group for cardiac manifestations, associated with severe
73 prognosis at all ages⁹. We also showed an under-representation of nonsense mutations and an
74 over-representation of missense mutations in this region, when compared to other exons of the
75 gene. Here, we focus on the clinical and molecular characterization of patients with a
76 mutation in the so-called exon 24-32 “neonatal region”, out of a series of 1013 probands with
77 MFS or type I fibrillinopathy carrying a pathogenic *FBNI* mutation.

78

79 PATIENTS, MATERIALS AND METHODS

80 Out of a series of 1013 probands carrying a pathogenic *FBNI* mutation recruited for a
81 genotype-phenotype correlation study^{9,10}, we extracted a subgroup of 198 probands with a
82 mutation in exons 24-32 in order to better describe their clinical and molecular characteristics.
83 Patients were recruited to this study during the period 1995-2005 via the framework of the
84 Universal Mutation Database-*FBNI*^{11,12} (UMD-*FBNI*; <http://www.umd.be>), or were referred
85 by specialised Marfan syndrome clinics in their respective countries. Patients originated from
86 38 countries in five continents. The required clinical information included a range of
87 qualitative and quantitative clinical parameters, including age of diagnosis, presence or
88 absence of clinical features including cardiac, ophthalmological, skeletal, cutaneous,
89 pulmonary and dural manifestations of the Ghent nosology¹³. The ages at diagnosis and at
90 surgery for aortic dilatation, mitral valve prolapse and regurgitation, ectopia lentis and
91 scoliosis were also collected. Patients were classified as “*neonatal MFS*” when characteristic
92 features of MFS including severe valvular anomalies by 4 weeks of age; “*severe MFS*” when
93 presenting with positive Ghent criteria including the presence of ascending aortic dilatation
94 before 10 years of age; “*classical MFS*” when Ghent criteria were positive in the remaining
95 patients; “*incomplete MFS*” when Ghent criteria were negative in adulthood; and “*probable*
96 *MFS*” when Ghent criteria were negative and follow-up was limited to childhood.

97 The pathogenic nature of a putative mutation was assessed using recognized criteria.
98 In brief, all nonsense mutations, all deletions or insertions (in or out of frame) were
99 considered pathogenic; for all splice mutations the wild-type and mutant strength values of
100 the splice sites were compared using genetic algorithms^{12,14,15} and only mutations displaying
101 significant deviation from the normal were included. Missense mutations were considered
102 pathogenic when at least one of the following features was found: *i*) de novo missense
103 mutation, *ii*) missense mutation substituting or creating a cysteine, *iii*) missense mutation
104 involving a consensus calcium-binding residue¹⁶, *iv*) substitution of glycines implicated in

105 correct domain-domain packing¹⁷, v) intrafamilial segregation of a missense mutation
106 involving a conserved amino acid. For other missense mutations not displaying one of the
107 above features, additional data provided by SIFT^{18,19}, BLOSUM-62²⁰ and biochemical value
108 (<http://www.biochem218.stanford.edu/Projects%202001/Yu.pdf>) were gathered and analysed
109 using a new UMD tool²¹ (Collod-Beroud, personal communication).

110 The phenotypes and the genotypes of the overall cohort of patients are described
111 elsewhere^{9,10}. Here, we focus on the clinical and molecular characteristics of patients with a
112 mutation in exons 24-32. We took advantage of this large series to study the MFS
113 presentation types associated with these mutations, the distribution of mutations in this region,
114 and the genotype-phenotype correlations.

115 Since the prevalence of many features of MFS increases with age, and since our study
116 included patients with different lengths of follow-up, we performed a time-to-event analysis
117 technique in order to estimate a reliable cumulative probability of observation of the different
118 manifestations of MFS. This technique could be applied for the following events: diagnosis of
119 MFS or type I fibrillinopathy, scoliosis, ectopia lentis, aortic dilatation or dissection, mitral
120 abnormalities, as well as surgery for these different manifestations for which the age at
121 diagnosis was systematically collected. In all time-to-event analyses, the baseline date (time
122 zero) was the date of birth. The time-to-event diagnosis was defined as the interval between
123 the baseline date and the date of event observation. Subjects who did not manifest the studied
124 event during the follow-up course were censored at their last follow-up. Subjects for whom
125 the age at diagnosis of a specific manifestation was not available were excluded from these
126 analyses. The Kaplan-Meier method²² was used to estimate the cumulative probabilities of
127 clinical manifestations of the disease at 10, 25 and 40 years of age in order to describe the
128 evolution of clinical features over time. Differences among the different types of mutation
129 groups (different locations or type of mutations) were tested using the non-parametric log-

130 rank test. For the other features (skeletal features other than scoliosis, skin, lung and dural
131 involvement), for which the ages at diagnosis were not collected, age at last follow-up was the
132 only information available concerning the time of clinical features observation. In order to
133 indirectly take into account the length of patient follow-up even in this situation, we adjusted
134 all comparisons of MFS manifestation proportions for the ages at last follow-up, categorized
135 into 10-year age groups. These adjusted comparisons were performed using the Mantel-
136 Haenszel test²³. We compared the phenotypic data for each exon of the region with the others.
137 To study the effect of mutation types, we compared patients with a premature termination
138 codon to patients with an in-frame mutation and patients with missense mutations involving a
139 cysteine vs other missense mutations. We also searched if the position of the substituted
140 cystein influenced the phenotype by comparing clinical data of patient with a mutation
141 affecting the first disulfide bond with patients with a mutation in the second or third disulfide
142 bond and conversely. To study the effect of the position of the affected EGF-like domain
143 relative to the TGFBP-like domain, we compared the phenotype of patients with a missense
144 mutation in exons 25 and 26 encoding EGF-like domains 11 and 12 (located near the TGFBP-
145 like domain) to the phenotype of patients with a missense mutation in exons 27 to 32
146 encoding EGF-like domains 13 to 18.

147 SASTM software version 9.2 (SAS Institute Inc., Cary, NC) and Stata software version
148 9 (Stata Corp, College Station, TX) were used for all statistical analyses. In order to take into
149 account multiple testing, only p-values of less than 0.001 were considered significant.

150

151 RESULTS

152 The genotype/phenotype correlation study in exons 24-32 versus other exons has been
153 reported elsewhere⁹. The MFS presentation type of patients with a mutation in exons 24-32 is
154 summarized in Figure 1. An over-representation of neonatal and severe MFS and an under-

155 representation of classical MFS were noted when compared to the overall series⁹.
156 Accordingly, a high percentage of sporadic cases were found (69%).

157 Twenty percent of the *FBNI* mutations in the overall series were found in the exon 24-
158 32 region (n=198), indicating a clustering of mutations in this region as only 14.5% was
159 expected based on the length of genomic sequence of the gene. Figure 2 shows the number of
160 mutations by exon, from exon 24 to exon 32, and, although results were non significant, the
161 clustering of mutations can be mainly explained by an excess of mutations in exons 25 and
162 27. An unequal distribution regarding the type of MFS presentation was found between exons
163 of the studied region, with severe phenotypes most likely to be associated with mutations in
164 exons 25, 26, 29, 31 and 32. Conversely, neonatal MFS was under-represented in patients
165 with a mutation in exons 24, 27, 28 and 30 (Table 1). When comparing the probability of the
166 different clinical features for one individual exon compared to the other exons of the region,
167 significant results were found only for patients carrying a mutation in exon 25. Indeed, a
168 younger age at diagnosis of MFS or type I fibrillinopathy, a higher probability of ascending
169 aortic dilatation, mitral regurgitation, valvular surgery and scoliosis, as well as a lower chance
170 of survival, were all found when compared to patients with a mutation within other exons of
171 the exon 24-32 region (Figure 3). These results can be explained at least in part by a higher
172 frequency of patients with neonatal MFS in exon 25 (57%, Table 1, $p < 0.001$).

173 The majority of mutations was in-frame and predicted to result in an altered protein
174 (79%), while 21% were predicted to result in a premature termination codon (PTC). Within
175 the 139 missense mutations, 75 involved a cysteine (54%). Twenty-five mutations affected
176 the first disulfide bond, 11 mutations the second disulfide bond and 23 mutations the third
177 disulfide bond. Fifty-two patients had a mutation in the EGF-like domains 11 or 12, and 96 in
178 the EGF-like domains 13 to 17. Figure 4 presents the distribution of types of mutations,
179 depending on the severity of the clinical presentation. In particular, PTCs were under-

180 represented in patients with severe phenotypes and an absence of nonsense mutations, while
181 missense mutations were over-represented. We questioned whether the type of mutation
182 within the exon 24-32 region could lead to a differing clinical phenotype. Some significant
183 results were found when patients with an exon 24-32 PTC mutation were compared with
184 patients with an exons 24-32 missense mutation (Figure 5). Indeed, the cumulative probability
185 of mitral insufficiency diagnosed before or at 25 years was 54% (99.9%-CI=39%-69%) in
186 patients with a missense mutation in exons 24-32, compared to 20% (99.9%-CI=5%-53%) in
187 patients with a PTC mutation in the same region (log-rank test $p=0.001$). Similarly, the
188 cumulative probability of ectopia lentis diagnosed before or at 25 years was 61% (99.9%-
189 CI=45%-77%) in patients with a missense mutation in exons 24-32, compared to 31%
190 (99.9%-CI=11%-63%) in patients with a PTC mutation in the same region (log-rank test
191 $p=0.0009$). Conversely, a higher frequency of pectus deformity was found in patients with a
192 PTC mutation in exons 24-32 when compared to patients with a missense mutation in the
193 same region (83% versus 54%, MH test $p=0.001$). No significant results were found for the
194 other clinical features of the MFS spectrum. A tendency towards a higher probability of
195 ascending aortic dilatation and a younger age at diagnosis was noted with missense mutations,
196 although these associations were only marginally significant ($p=0.0218$ and $p=0.0278$,
197 respectively) (Figure 5). When comparing patients with missense mutations involving a
198 cysteine to other missense mutations, significant results were found for ectopia lentis. Indeed,
199 the cumulative probability of ectopia lentis diagnosed before or at 25 years was 76% (99.9%-
200 CI=60%-89%) in patients with a missense mutation involving a cysteine in exons 24-32,
201 compared to 41% (99.9%-CI=25%-63%) in patients with another missense mutation in the
202 same region (log-rank test $p=0.0001$). A tendency towards a higher probability of ascending
203 aortic dilatation was noted in patients with missense mutations involving a cysteine, although
204 this association was only marginally significant ($p=0.0022$) (Figure 5). No significant

205 differences were found when comparing clinical data of patients with a mutation affecting the
206 first, second or third disulfide bond but the numbers were small. Significant differences were
207 found when comparing the clinical phenotype of patients carrying a missense mutation in
208 exons 25 and 26 encoding EGF-like domains 11 and 12 located near the TGF β -like domain
209 with patients carrying a missense mutation in exons 27 to 32 encoding EGF-like domains 13
210 to 18. Indeed, patients with a missense mutation affecting EGF-like domains 11 or 12 (n=52)
211 have a shorter survival, a younger age at diagnosis, a higher risk of presenting a neonatal
212 presentation, a higher risk of developing ascending aortic dilatation and a higher risk of
213 developing mitral insufficiency than patients with a missense mutation affecting EGF-like
214 domains 13 to 17(p<0.001).

215 Twenty-four mutations were recurrent. Table 2 shows the MFS presentation types in
216 these recurrent mutations. Interestingly, some recurrent mutations lead to a similar phenotype,
217 while others lead to different presentations.

218

219 DISCUSSION

220 Here, we further delineate the clinical and molecular characteristics of the so-called
221 “neonatal exon 24-32 region” from the data of a large series in which the phenotype of 1013
222 probands with MFS and other type I fibrillinopathies were collected. We confirm that the
223 region encompassing exons 24 to 32 is associated with more severe phenotypes than the other
224 exons of the gene. Indeed, a third of the patients with a mutation within this region had
225 neonatal or severe MFS, as compared to 6% in the other regions⁹.

226 We previously showed that the presence of a mutation in exons 24-32 was predictive
227 of a severe cardiovascular phenotype even in non-neonatal phenotypes⁹, but it is unknown if
228 the location of the mutation is the only cause of the phenotypic severity. Genotype-phenotype
229 correlation analyses can be complicated by the fact that both the location and the type of a

230 mutation are critical in producing a severe phenotype and these data are often studied
231 independently. For this reason, we looked for clinical differences between patients with
232 different mutation types within this region. A higher probability of mitral regurgitation and
233 ectopia lentis, as well as a lower frequency of pectus deformity were found in patients with a
234 missense mutation within this region when compared to patients with a PTC mutation. Also, a
235 higher probability of ectopia lentis was found in patients with a missense mutation involving a
236 cysteine within this region when compared to patients with other missense mutations. These
237 results were highly superposable to those obtained for all the regions of the *FBNI* gene⁹,
238 showing that, beside the predominant role of the location of the mutations within the exon 24-
239 32 region, the type of mutation is also important.

240 We previously showed that the distribution of the mutation types in exons 24-32 is
241 different from the distribution found in other exons of the gene. Indeed, mutations leading to
242 PTC are under-represented, contrasting with an over-representation of in-frame mutations⁹.
243 Here, we show that PTC mutations are under-represented in the severe MFS phenotype. In
244 particular, nonsense mutations have never been described in association with neonatal and
245 severe MFS presentations. In contrast, in the overall cohort, we showed that patients with an
246 *FBNI* PTC mutation had a more severe skeletal and skin phenotype than patients with an in-
247 frame mutation⁹. Therefore, it is not known whether the absence of nonsense mutations in the
248 neonatal and severe phenotypes, as well as the under-representation of PTC mutations in these
249 phenotypes, could be explained by early lethality or by a milder effect on phenotype of PTC
250 mutations within this region. In searching for differences in various clinical system
251 involvements between PTC and in-frame mutations within this region, there were no
252 emerging clues for this region regarding the dominant negative versus haploinsufficiency
253 pathogenesis models and no evidence to support a differential mechanism for the phenotypic
254 and genotypic differences within the exon 24-32 region and other regions of the gene. Recent

255 data has highlighted the complexity of the pathogenicity of *FBNI* mutations, with some
256 mutations acting as dominant negative, and others as haploinsufficiency secondary to
257 different effects on trafficking²⁴⁻²⁷. However mutation data accumulated by diagnostic
258 laboratories worldwide is generally not associated with mRNA and protein studies. Therefore
259 no data are available to assess the true effect of PTC mutations and whether they are
260 submitted to nonsense-mediated RNA decay or they give rise to truncated peptides of various
261 sizes. Until more information is available, the true impact of PTC mutations on microfibril
262 formation can only be speculated.

263 The clustering of mutations with an excess of mutations in exons 24-32 has been
264 postulated before^{8,28}. This hypothesis is confirmed in this study and might explain the high
265 proportion of sporadic cases. The same clustering of mutations in exons 24-34 of the *FBN2*
266 gene in patients with congenital contractural arachnodactyly (OMIM 121050)^{7,29-30} is in favor
267 of a critical role of this region in both fibrillin-1 and fibrillin-2. The domains encoded by
268 exons 25-36 in fibrillin-1 are found midway through the protein and constitute the longest
269 stretch of EGF-like domains in the protein. Exon 24 encodes an eight-cysteine domain found
270 immediately amino-terminal to this stretch of EGF-like domains. Schrijver et al.³¹ reported
271 that the position of an affected EGF-like domain relative to an eight-cysteine domain could be
272 related to the severity of the phenotype. In keeping with this report, we queried for possible
273 differences in clinical presentation in patients carrying a missense mutation in exons 25 and
274 26, versus exons 27 to 32. We found a significantly more severe presentation in the patients
275 with mutations in exons 25 and 26 that encode EGF-like domains 11 and 12. Furthermore,
276 exon 25 was associated with a higher frequency of neonatal presentations and a higher
277 probability of ascending aortic dilatation, than mutations in other exons within this region.
278 This exon encodes EGF-like domain 11 which is immediately downstream from the eight-
279 cysteine domain. Interestingly, this relative location is conserved between fibrillin-1 and

280 transforming growth factor β 1 binding protein (LTBP)³². LTBP plays a role in the assembly
281 and secretion of TGF β 1 and is thought to target TGF β 1 to particular extracellular matrix sites,
282 thus controlling the production and structure of the extracellular matrix, along with affecting
283 cell growth, morphology and differentiation³³⁻³⁴. The homology of fibrillin-1 and LTBP raises
284 the possibility that disruption of the extracellular targeting of the action of TGF β 1 during
285 development underpins the more severe phenotype. Alternatively, mutations in this region of
286 fibrillin-1 may be more disruptive to microfibril formation. Although mutations throughout
287 the *FBNI* gene have been shown to disrupt fibrillin-1 incorporation into microfibrils, exons
288 24-32 may encode a region of fibrillin-1 with a unique function in the multimerization of the
289 protein into stable microfibrils. In contrast to microfibrils formed by classic MFS fibroblasts,
290 the fibrils formed by neonatal MFS show not only an apparent decrease in fibrillin
291 accumulation, but are also short, fragmented and frayed³⁵. Therefore, alterations in this region
292 of the protein may have a significant and specific effect on microfibril formation, implying a
293 unique role of this region in microfibril formation.

294 Finally, the study of recurrent mutations was of interest. The majority of these
295 recurrent mutations were only found in two instances. Three mutations were represented in 5
296 instances or more. While the c.3302A>G mutation was generally associated with the classical
297 MFS, the c.3037G>A mutation led to different phenotypes, ranging from neonatal to classical
298 MFS. Five mutations responsible for a neonatal MFS phenotype in some patients were also
299 found in other patients with another MFS type of presentation (c.3037G>A, c.3143T>C,
300 c.3202T>C, c.3217G>A and c.3976T>C). These data give further emphasis to the clinical
301 variability in *FBNI* mutations and strongly argue for the role of modifier genes or the
302 existence of a digenic mechanism to explain neonatal MFS.

303 In conclusion, even if the exon 24-32 location of mutations appears as a major cause
304 of the severity of the phenotype in patients harboring a mutation in this region, other factors

305 such as the type of mutation or modifier genes might also be involved. These data could be
306 helpful in understanding the role of the central region of the *FBNI* gene in disease
307 pathogenicity.

308

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318 LEGENDS TO FIGURES

319

320 Figure 1: Type of presentation of MFS in patients with a *FBNI* mutation in exons 24-32 (N =
321 198)

322

323 Figure 2: Number of mutations in the “exon 24-32 region” for each exon (black), as compared
324 to the number of mutations expected from the genomic sequence of the gene (grey), N = 198

325

326 Figure 3: Kaplan Meier analyses for various clinical features in patients with a mutation in
327 exon 25 as compared to patients with a mutation in other exons of the “24-32 region”.

328 *A: Age at diagnosis of type I fibrillinopathy in exon 25 mutations versus mutations in other*
329 *exons of the “24-32 region”.*

330 79% of patients with a mutation in exon 25 (solid line) were diagnosed by 10 years (99.9%-
331 CI=51%-98%) of age versus 46% (99.9%-CI=35%-60%) of patients with a mutation in other
332 exons of the “24-32 region” (broken line) (log-rank test $p < 0.0001$)

333 *B: Survival of patients with exon 25 mutations versus mutations in other exons of the 24-32*
334 *region.*

335 46% of patients with mutations within exons 25 (solid line) were alive at 10 years (99.9%-
336 CI=13%-75%) compared to 90% (99.9%-CI=80%-96%) of patients with a mutation in other
337 exons of the 24-32 region (broken line) (log-rank test $p < 0.0001$)

338 *C: Probability of ascending aortic dilatation in exon 25 mutations versus mutations in other*
339 *exons of the “exon 24-32 region”.*

340 The cumulative probability of ascending aortic dilatation before or at 10 years was 67%
341 (99.9%-CI=44%-88%) in patients with mutations within exon 25 (solid line) compared to

342 39% (99.9%-CI=30%-49%) in patients with a mutation in other exons of the “24-32 region”
343 (broken line) ($p=0.0001$).

344 *D: Probability of mitral regurgitation in exon 25 mutations versus mutations in other exons of*
345 *the “exon 24-32 region”.*

346 The cumulative probability of mitral regurgitation before or at 10 years was 59% (99.9%-
347 CI=35%-84%) in patients with mutations within exon 25 (solid line) compared to 30%
348 (99.9%-CI=22%-40%) in patients with a mutation in other exons of the “24-32 region”
349 (broken line) ($p<0.0001$).

350

351 Figure 4: Distribution of types of mutations within the exon 24-32 region depending on the
352 clinical presentation (N=191) (7 splicing mutations could not be classified as in-frame or out
353 of frame)

354

355 Figure 5: Kaplan Meier analyses for various clinical features in patients with a missense
356 mutation in exons 24-32 compared to patients with a PTC mutation in the same region.

357 *A: Probability of mitral insufficiency in missense mutations in exons 24-32 versus PTC*
358 *mutations in the same region.*

359 The cumulative probability of mitral insufficiency diagnosed before or at 25 years was 54%
360 (99.9%-CI=39%-69%) in patients with a missense mutation in exons 24-32 (solid line)
361 compared to 20% (99.9%-CI=5%-53%) in patients with a PTC mutation in the same region
362 (broken line) ($p=0.001$).

363 *B: Probability of ascending aortic dilatation in missense mutations in exons 24-32 versus*
364 *PTC mutations in the same region.*

365 The cumulative probability of ascending aortic dilatation before or at 25 years was 74%
366 (99.9%-CI=60%-86%) in patients with a missense mutation in exons 24-32 (solid line)

367 compared to 40% (99.9%-CI=18%-70%) in patients with a PTC mutation in the same region
368 (broken line), but these results were not significant because the curves join together with
369 follow-up ($p=0.0218$).

370 *C: Probability of ectopia lentis in missense mutations in exons 24-32 versus PTC mutations in*
371 *the same region.*

372 The cumulative probability of ectopia lentis diagnosed before or at 25 years was 61% (99.9%-
373 CI=45%-77%) in patients with a missense mutation in exons 24-32 (solid line) compared to
374 31% (99.9%-CI=11%-63%) in patients with a PTC mutation in the same region (broken line)
375 ($p=0.0009$).

376 *D: Age at diagnosis of type I fibrillinopathy in missense mutations in exons 24-32 versus PTC*
377 *mutations in the same region.*

378 50% of patients with a missense mutation in exons 24-32 (solid line) were diagnosed at 6
379 years (IQR [0.7;18]) of age versus 21 years (IQR [11;32]) of age in patients with a PTC
380 mutation in the same region (broken line), but results of the log-rank test were not significant
381 because the curves join together with follow-up ($p=0.0278$)

382 *E. Probability of ascending aortic dilatation in missense mutations involving a cysteine in*
383 *exons 24-32 versus other missense mutations in the same region.*

384 The cumulative probability of ascending aortic dilatation before or at 25 years was 83%
385 (99.9%-CI=70%-92%) in patients with a missense mutation involving a cysteine in exons 24-
386 32 (solid line) compared to 62% (99.9%-CI=45%-78%) in patients with another missense
387 mutation in the same region (broken line), but these results were only marginally significant
388 ($p=0.0022$).

389 *F: Probability of ectopia lentis in missense mutations involving a cysteine in exons 24-32*
390 *versus other missense mutations in the same region.*

391 The cumulative probability of ectopia lentis diagnosed before or at 25 years was 76% (99.9%-
392 CI=60%-89%) in patients with a missense mutation involving a cysteine in exons 24-32 (solid
393 line) compared to 41% (99.9%-CI=25%-63%) in patients with another missense mutation in
394 the same region (broken line) (p=0.0001).

395 REFERENCES

- 396 1 Pyeritz RE. Marfan syndrome: current and future clinical and genetic management of cardiovascular
397 manifestations. *Semin Thorac Cardiovasc Surg* 1993; **5**:11-16.
- 398 2 Judge DP, Dietz HC. Marfan's syndrome. *Lancet* 2005; **366**:1965-1976.
- 399 3 Karttunen L, Raghunath M, Lonnqvist L, Peltonen L. Compound-heterozygous Marfan patient: two
400 defective fibrillin alleles result in a lethal phenotype. *Am J Hum Genet* 1994; **55**:1083-1091.
- 401 4 De Vries BBA, Pals G, Odink R, Hamel BCJ. Homozygosity for a *FBNI* missense mutation: clinical
402 and molecular evidence for recessive Marfan syndrome. *Eur J Hum Genet* 2007; **15**:930-935.
- 403 5 Milewicz DM, Duvic M. Severe neonatal Marfan syndrome resulting from a de novo 3-bp insertion
404 into the fibrillin gene on chromosome 15. *Am J Hum Genet* 1994; **54**:447-453.
- 405 6 Kainulainen K, Karttunen L, Puhakka L, Sakai L, Peltonen L. Mutations in the fibrillin gene
406 responsible for dominant ectopia lentis and neonatal Marfan syndrome. *Nat Genet* 1994; **6**:64-69.
- 407 7 Putnam EA, Cho M, Zinn AB, Towbin JA, Byers PH, Milewicz DM. Delineation of the Marfan
408 phenotype associated with mutations in exons 23-32 of the *FBNI* gene. *Am J Med Genet* 1996;
409 **62**:233-242.
- 410 8 Tiecke F, Katzke S, Booms P, Robinson PN, Neumann L, Godfrey M, Mathews KR, Scheuner M,
411 Hinkel GK, Brenner RE, Hovels-Gurich HH, Hagemeyer C, Fuchs J, Skovby F, Rosenberg T.
412 Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and genotype-
413 phenotype correlations in *FBNI* exons 24-40. *Eur J Hum Genet* 2001;**9**:13-21.
- 414 9 Faivre L, Collod-Beroud G, Loeys BL, Child A, Binquet C, Gautier E, Callewaert B, Arbustini E,
415 Mayer K, Arslan-Kirchner M, Kiotsekoglou A, Comeglio P, Marziliano N, Dietz HC, Halliday D,
416 Beroud C, Bonithon-Kopp C, Claustres M, Muti C, Plauchu H, Robinson PN, Adès LC, Biggin
417 A, Benetts B, Brett M, Holman KJ, De Baecker J, Coucke P, Francke U, De Paepe A, Jondeau G,
418 Boileau C. Effect of mutation type and location on clinical outcome in 1013 probands with
419 Marfan syndrome or related phenotypes with *FBNI* mutations : an international study. *Am J Hum*
420 *Genet* 2007; **81**:454-466
- 421 10 Faivre L, Collod-Beroud G, Child A, Callewaert B, Loeys BL, Binquet C, Gautier E, Arbustini E,
422 Mayer K, Arslan-Kirchner M, Stheneur C, Kiotsekoglou A, Comeglio P, Marziliano N, Halliday
423 D, Beroud C, Bonithon-Kopp C, Claustres M, Plauchu H, Robinson PN, Adès L, De Backer J,
424 Coucke P, Francke U, De Paepe A, Boileau C, Jondeau G. Contribution of molecular analyses in
425 diagnosing Marfan syndrome and type I fibrillinopathies: an international study of 1009
426 probands. *J Med Genet* 2008;**45**:384-90.
- 427 11 Collod-Beroud G, Le Bourdelles S, Adès L, Ala-Kokko L, Booms P, Boxer M, Child A, Comeglio
428 P, De Paepe A, Hyland JC, Holman K, Kaitila I, Loeys B, Matyas G, Nuytinck L, Peltonen L,
429 Rantamaki T, Robinson P, Steinmann B, Junien C, Beroud C, Boileau C. Update of the UMD-

- 430 FBN1 mutation database and creation of an FBN1 polymorphism database. *Hum Mutat* 2003;
431 22:199-208.
- 432 12 Beroud C, Hamroun D, Collod-Beroud G, Boileau C, Soussi T, Claustres M. UMD (Universal
433 Mutation Database): 2005 update. *Hum Mutat* 2005;26: 184-91.
- 434 13 De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for
435 the Marfan syndrome. *Am J Med Genet* 1996; 62:417-426.
- 436 14 Dietz HC, Pyeritz RE. Mutations in the human gene for fibrillin-1 (FBN1) in the Marfan syndrome
437 and related disorders. *Hum Mol Genet* 1995;4:1799-1809.
- 438 15 Shapiro MB, Senapathy P. "RNA splice junctions of different classes of eukaryotes: sequence
439 statistics and functional implications in gene expression". *Nucleic Acids Res* 1987;15:7155-7174.
- 440 16 Senapathy P, Shapiro MB, Harris NL. Splice junctions, branch point sites, and exons: sequence
441 statistics, identification, and applications to genome project. *Methods Enzymol* 1990;183:252-78
- 442 17 Downing A, Knott V, Werner J, Cardy C, Campbell ID, Handford PA. Solution structure of a pair
443 of calcium-binding epidermal growth factor-like domains: implications for the Marfan syndrome
444 and other genetic disorders. *Cell* 1996;85:597-605.
- 445 18 Ng PC, Henikoff S. "Predicting deleterious amino acid substitutions". *Genome Res* 2001;11:863-
446 874.
- 447 19 Ng PC, Henikoff S. "SIFT: Predicting amino acid changes that affect protein function". *Nucleic
448 Acids Res* 2003;31:3812-3814.
- 449 20 Henikoff S, Henikoff JG. "Amino acid substitution matrices from protein blocks". *Proc Natl Acad
450 Sci USA* 1992;89:10915-10919.
- 451 21 Frédéric M, Hamroun D, Claustres M, Boileau C, Bérout C, Collod-Bérout G. A New prediction
452 tool for missense mutation pathogenicity; the example of the UMD-FBN1 mutation database. In
453 preparation.
- 454 22 Kaplan E, Meier P. Non parametric estimation from incomplete observations. *J Am Stat Assoc*
455 1958; 53:457-481.
- 456 23 Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of
457 disease. *J Natl Cancer Inst* 1959; 22: 719-748.
- 458 24 Whiteman P, Handford PA. Effective secretion of recombinant fragments of fibrillin-1:
459 implications of protein misfolding for the pathogenesis of Marfan syndrome and related
460 disorders. *Hum Mol Genet* 2003; 12:727-737.
- 461 25 Eldadah ZA, Brenn T, Furthmayr H, Dietz HC. Expression of a mutant human fibrillin allele upon
462 a normal human or murine genetic background recapitulates a Marfan cellular phenotype. *J Clin
463 Invest* 1995; 95:874-880.
- 464 26 Judge DP, Biery NJ, Keene DR, Geubtner J, Myers L, Huso DL, Sakai LY, Dietz HC. Evidence for
465 a critical contribution of haploinsufficiency in the complex pathogenesis of Marfan syndrome. *J
466 Clin Invest* 2004; 114:172-181.

- 467 27 Mátyás G, Alonso S, Patrignani A, Marti M, Arnold E, Magyar I, Henggeler C, Carrel T,
468 Steinmann B, Berger W. Large genomic fibrillin-1 (*FBNI*) gene deletions provide evidence for
469 true haploinsufficiency in Marfan syndrome. *Hum Genet* 2007;**122**:23-32
- 470 28 Robinson PN, Booms P, Katzke S, Ladewig M, Neumann L, Palz M, Pregla R, Tiecke F,
471 Rosenberg T. Mutations of *FBNI* and genotype-phenotype correlations in Marfan syndrome and
472 related fibrillinopathies. *Hum Mutat* 2002; **20**:153-161.
- 473 29 Park ES, Putnam EA, Chitayat D, Child A, Milewicz DM. Clustering of *FBN2* mutations in
474 patients with congenital contractural arachnodactyly indicates an important role of the domains
475 encoded by exons 24 through 34 during human development. *Am J Med Genet* 1998;**78**:350-355.
- 476 30 Frederic M, Monino C, Marschall C, Hamroun D, Faivre L, Jondeau G, Klein HG, Neumann L,
477 Gautier E, Binquet C, Maslen C, Godfrey M, Gupta P, Milewicz D, Boileau C, Claustres M,
478 Béroud C, Collod-Béroud G. *FBN2* gene: New mutations, Locus Specific DataBase (UMD-
479 *FBN2*) and genotype-phenotype correlations. *Hum Mut*, in press.
- 480 31 Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U. Cysteine substitutions in epidermal growth
481 factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. *Am J*
482 *Hum Genet* 1999;**65**:1007-20.
- 483 32 Kanzaki T, Olofsson A, Moren A, Wernstedt C, Hellman U, Miyazono K, Claesson-Welsh L,
484 Heldin CH. GF-beta 1 binding protein: a component of the large latent complex of TGF-beta 1
485 with multiple repeat sequences. *Cell* 1990; **61**:1051-1061.
- 486 33 Taipale J, Miyazono K, Heldin CH, Keski-Oja J. Latent transforming growth factor-beta 1
487 associates to fibroblast extracellular matrix via latent TGF-beta binding protein.
488 *J Cell Biol* 1994; **124**:171-181.
- 489 34 Kingsley DM. The TGF-beta superfamily: new members, new receptors, and new genetic tests of
490 function in different organisms. *Genes Dev* 1994; **8**:133-146.
- 491 35 Comeglio P, Johnson P, Arno G, Brice G, Evans A, Aragon-Martin J, da Silva FP, Kiotsekoglou A,
492 Child A. The importance of mutation detection in Marfan syndrome and Marfan-related
493 disorders: report of 193 *FBNI* mutations. *Hum Mutat* 2007;**28**: 928.
- 494 36 Loeys B, Nuytinck L, Delvaux I, De Bie S, De Paepe A. Genotype and phenotype analysis of 171
495 patients referred for molecular study of the fibrillin-1 gene *FBNI* because of suspected Marfan
496 syndrome. *Arch Intern Med* 2001;**161**:2447-54.
- 497 37 Rommel K, Karck M, Haverich A, von Kodolitsch Y, Rybczynski M, Muller G, Singh KK,
498 Schmidtke J, Arslan-Kirchner M. Identification of 29 novel and nine recurrent fibrillin-1 (*FBNI*)
499 mutations and genotype-phenotype correlations in 76 patients with Marfan syndrome. *Hum Mutat*
500 2005;**26**:529-39.
- 501 38 Karttunen L, Ukkonen T, Kainulainen K, Syvänen A-C, Peltonen L. Two novel Fibrillin-1
502 mutations resulting in premature termination codons but in different mutant transcript levels and
503 clinical phenotype. *Hum Mutat* 1998; **Suppl 1**:S34-37.

- 504 39 Nijbroek G, Sood S, McIntosh I, Francomano CA, Bull E, Pereira L, Ramirez F, Pyeritz RE, Dietz
505 HC. Fifteen novel *FBN1* mutations causing Marfan syndrome detected by heteroduplex analysis
506 of genomic amplicons. *Am J Hum Genet* 1995;**57**:8-21.
- 507 40 Liu WO, Oefner PJ, Qian C, Odom RS, Francke U. Denaturing HPLC-identified novel *FBN1*
508 mutations, polymorphisms, and sequence variants in Marfan syndrome and related connective
509 tissue disorders. *Genet Test* 1997-98;**1**:237-42.
- 510 41 Lo IF, Wong RM, Lam FW, Tong TM, Lam ST. Missense mutations of the fibrillin-1 gene in two
511 Chinese patients with severe Marfan syndrome. *Chin Med J* 2001;**114**:473-476.
- 512 42 Biggin A, Holman K, Brett M, Bennetts B, Ades L. Detection of thirty novel *FBN1* mutations in
513 patients with Marfan syndrome or a related fibrillinopathy. *Hum Mutat* 2004; **23**: 99.
- 514 43 Ng DK, Chau KW, Black C, Thomas TM, Boxer M. Neonatal Marfan syndrome: a case report. *J*
515 *Paediatr Child Health* 1999;**35**:321-323.
- 516 44 Lonqvist L, Child A, Kainulainen K, Davidson R, Puhakka L, Peltonen L. A novel mutation of the
517 fibrillin gene causing ectopia lentis. *Genomics* 1994;**19**:573-576.
- 518 45 Jacobs AM, Toudjarska I, Racine A, Tsipouras P, Kilpatrick MW, Shanske A. A recurring *FBN1*
519 gene mutation in neonatal Marfan syndrome. *Arch Pediatr Adolesc Med* 2002;**156**: 1081-5.
- 520 46 Loeys B, De Backer J, Van Acker P, Wettinck K, Pals G, Nuytinck L, Coucke P, De Paepe A.
521 Comprehensive molecular screening of the *FBN1* gene favors locus homogeneity of classical
522 Marfan syndrome. *Hum Mutat* 2004;**24**:140-146.
- 523 47 Rommel K, Karck M, Haverich A, Schmidtke J, Arslan-Kirchner M. Mutation screening of the
524 fibrillin-1 (*FBN1*) gene in 76 unrelated patients with Marfan syndrome or Marfanoid features
525 leads to the identification of 11 novel and three previously reported mutations. *Hum Mutat*
526 2002;**20**:406-407.
- 527 48 Arbustini E, Grasso M, Ansaldi S, Malattia C, Pilotto A, Porcu E, Disabella E, Marziliano N,
528 Pisani A, Lanzarini L, Mannarino S, Larizza D, Mosconi M, Antoniazzi E, Zoia MC, Meloni G,
529 Magrassi L, Brega A, Bedeschi MF, Torrente I, Mari F, Tavazzi L. Identification of sixty-two
530 novel and twelve known *FBN1* mutations in eighty-one unrelated probands with Marfan
531 syndrome and other fibrillinopathies. *Hum Mutat* 2005; **26**: 494.
- 532 49 Tynan K, Comeau K, Pearson M, Wilgenbus P, Levitt D, Gasner C, Berg MA, Miller DC, Francke
533 U. Mutation screening of complete fibrillin-1 coding sequence: report of five new mutations,
534 including two in 8-cysteine domains. *Hum Molec Genet* 1993;**2**:1813-1821.
- 535 50 Dietz H, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, Puffenberger EG, Hamosh
536 A, Nanthakumar EJ, Curristin SM, Stetten G, Meyers DA, Francomano CA. Marfan syndrome
537 caused by a recurrent de novo missense mutation in the fibrillin gene. *Nature* 1991;**352**: 337-339.
- 538 51 Milewicz DM, Michael K, Fisher N, Coselli JS, Markello T, Biddinger A. Fibrillin-1 (*FBN1*)
539 mutations in patients with thoracic aortic aneurysms. *Circulation* 1996;**94**:2708-11.

- 540 52 Hewett DR, Lynch JR, Child A, Sykes BC. A new missense mutation of fibrillin in a patient with
541 Marfan syndrome. *J Med Genet* 1994;**31**:338-9.
- 542 53 Sood S, Eldadah ZA, Krause WL, McIntosh I, Dietz HC. Mutation in fibrillin-1 and the Marfanoid-
543 craniosynostosis (Shprintzen-Goldberg) syndrome. *Nat Genet* 1996;**12**:209-11.