Zentrum für Kardiovaskuläre Genetik und Gendiagnostik
Das Genetikzentrum der Stiftung für Menschen mit seltenen Krankheiten

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New Insights into Fibrillinopathies and Pitfalls of Variant Filtering in the Current Genomics Era

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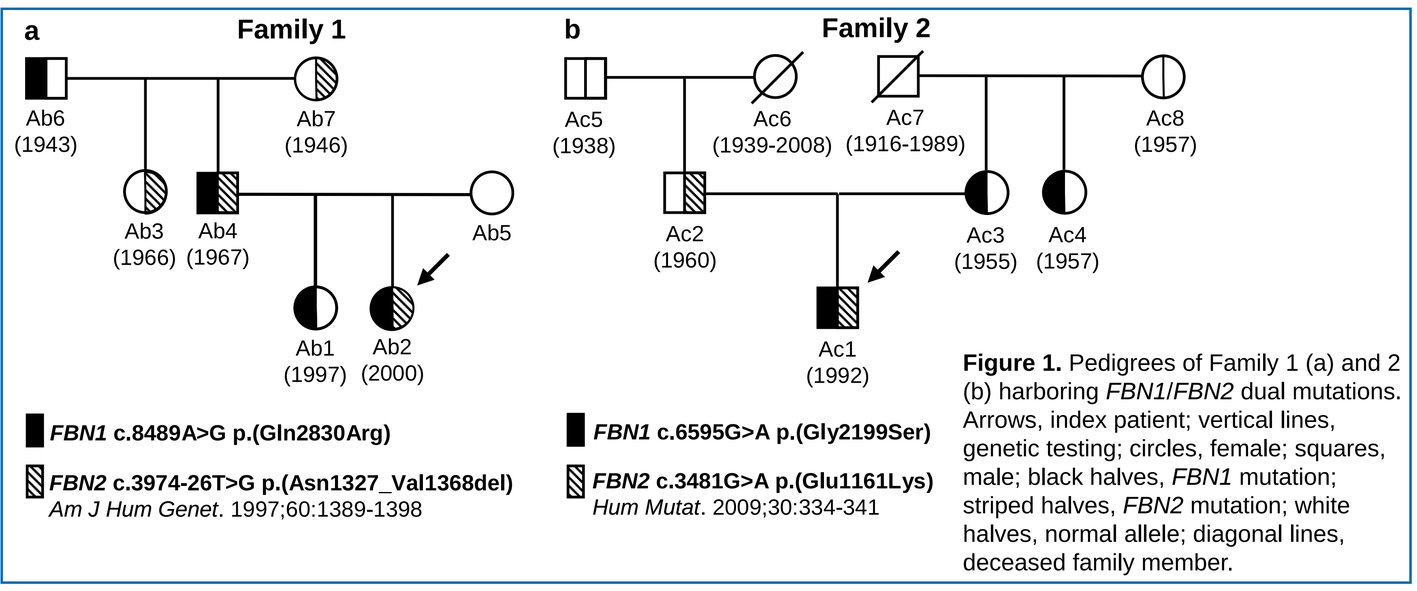
Conclusions

- ***FBN1/FBN2* dual mutations cause a mixed phenotype of MFS and CCA, thereby increasing or hiding certain clinical features of these fibrillinopathies.**
- **We show the presence and assess the frequency of disease-affected individuals in apparently healthy reference databases, demonstrating a pitfall in NGS variant interpretation.**
- **gnomAD-based prevalence estimates are 6:10,000 for MFS and 3:10,000 for CCA.**

Introduction: Fibrillinopathies such as *FBN1*-related Marfan syndrome (MFS) and *FBN2*-related congenital contractural arachnodactyly (CCA) are characterized by a wide and overlapping range of clinical signs with neonatal to adult onset. Although the underlying molecular etiology of MFS and CCA has been known since 1991 and 1995, respectively, their prevalence, co-occurrence, and genetic modifiers have only been estimated or are unknown. Here we address these issues by assessing the frequency of pathogenic *FBN1* and *FBN2* sequence variants in the largest Swiss database of MFS genomes as well as in the Genome Aggregation Database (gnomAD).



***FBN1* c.8489A>G p.(Gln2830Arg)**
***FBN2* c.3974-26T>G p.(Asn1327_Val1368del)**
Am J Hum Genet. 1997;60:1389-1398

***FBN1* c.6595G>A p.(Gly2199Ser)**
***FBN2* c.3481G>A p.(Glu1161Lys)**
Hum Mutat. 2009;30:334-341

Figure 1. Pedigrees of Family 1 (a) and 2 (b) harboring *FBN1*/*FBN2* dual mutations. Arrows, index patient; vertical lines, genetic testing; circles, female; squares, male; black halves, *FBN1* mutation; striped halves, *FBN2* mutation; white halves, normal allele; diagonal lines, deceased family member.

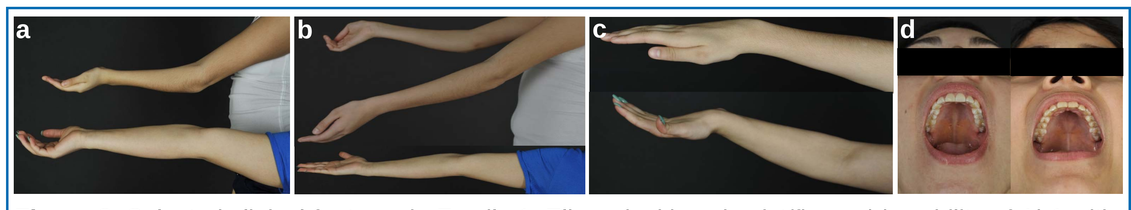


Figure 2. Selected clinical features in Family 1. Elbow (a, b) and wrist/finger (c) mobility of Ab1 with *FBN1* mutation (bottom) and Ab2 with *FBN1*/*FBN2* dual mutations (top), (d) narrow-arched palate of Ab1 (left) and high-arched palate of Ab2 (right).

Table 1. Selected clinical features observed in Familiy 1 and 2 harboring mutations in *FBN1* and *FBN2*.

	Family 1					Family 2		
Clinical features	**Ab6**	**Ab1**	**Ab2**	**Ab4**	**Ab3**	**Ac3**	**Ac1**	**Ac2**
Year of birth / Sex	1943 / M	1997 / F	2000 / F	1967 / M	1966 / F	1955 / F	1992 / M	1960 / M
Affected gene	*FBN1*	*FBN1*	*FBN1*/*FBN2*	*FBN1*/*FBN2*	*FBN2*	*FBN1*	*FBN1*/*FBN2*	*FBN2*
Elbow hyperextension	–	+	–	–	–	+	(+)	–
Aortic dilation (cm)	n/a	–	–	+ (4.9)	–	–	Ectasia (3.9)	–
Contractures								
Fingers	–	–	+	+	+	–	–	+
Elbow	+	–	+	+	–	–	–	–
Scoliosis	(+)	–	+	–	(+)	(+)	+	–

+, feature present; (+), feature mildly present; –, feature absent; F, female; M, male; n/a, information not available.

Table 2. *A priori* pathogenic *FBN1* and *FBN2* sequence variants in gnomAD.

	FBN1 **exons 23-34**	***FBN1*** **all exons**	***FBN2*** **exons 23-34**	***FBN2*** **all exons**
Nonsense / frameshift (i)	2	**8**	5	**29 (27)**
Splicing (ii)	0	**26 (10)**	**0**	68 (26)
In-frame indels (iii)	1	**9 (6)**	**0**	25 (7)
Disulfide bonds (iv)	1	**8**	**4**	36 (31)
Calcium binding (iv)	4	**25 (13)**	**6 (5)**	49 (23)
HGMD / ClinVar (v)[a]	1	**4 (3)**	**0**	0
All variants (i)-(v)	9:138,632 0.65:10,000	**80:138,632** **5.77:10,000**	**39:138,632** **2.81:10,000**	207:138,632 14.93:10,000

Numbers in parentheses indicate the number of unique sequence variants in gnomAD, while numbers outside parentheses indicate total number of mutated alleles in gnomAD. Blue letters indicate the sequence variants considered for the prevalence calculations; in *FBN1* all sequence variants in all exons were considered, in *FBN2* only sequence variants in exons 23-34 as well as nonsense and frameshift variants in all exons were considered.
[a]Only sequence variants not already included in other categories were counted.
gnomAD, Genome Aggregation Consortium; HGMD, Human Gene Mutation Database; indel, small insertion/deletion.

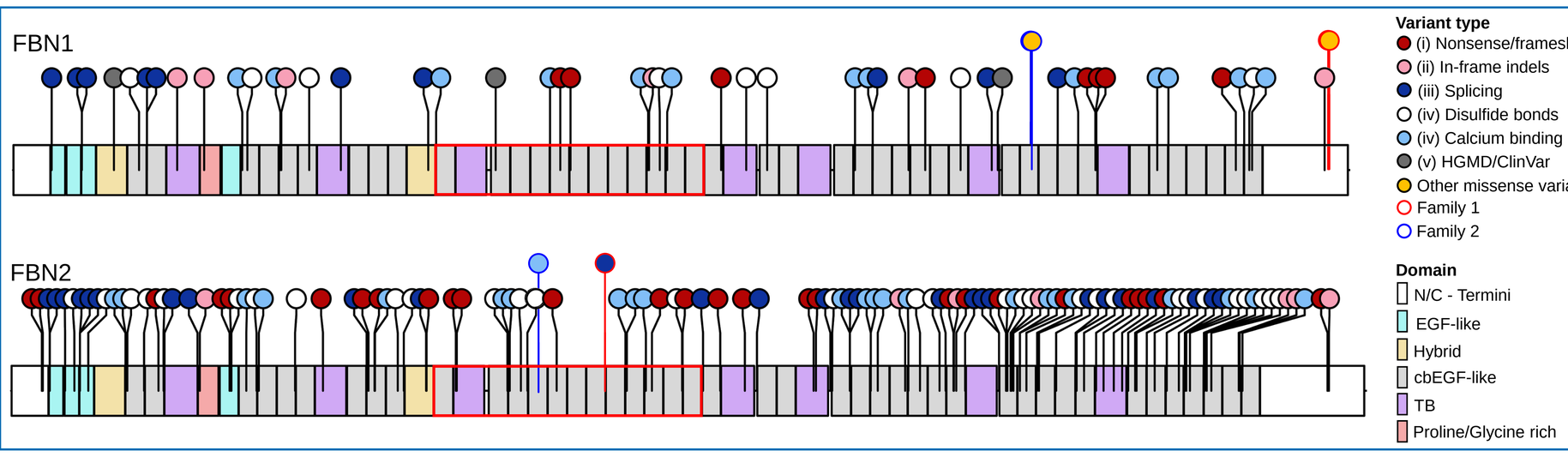


Figure 3. *FBN1* and *FBN2* mutations in gnomAD as well as *FBN1*/*FBN2* dual mutations in Family 1 and 2.
Lollipops show the type and position of sequence variants in relation to protein domain structure. Red boxes indicate the severe/neonatal region in *FBN1* (exons 23-34) and the comparable CCA-causing region in *FBN2* (exons 23-34).
cbEGF, calcium-binding epithelial growth factor; EGF, epithelial growth factor; HGMD, Human Gene Mutation Database; indel, insertion/deletion; TB, transforming growth factor β binding.
Protein domains were obtained from umd.be/FBN1 and umd.be/FBN2. Lollipop diagrams were generated using the R package "trackViewer", available from: bioconductor.org/packages/release/bioc/html/trackViewer.html

Results: We detected two families harboring *FBN1*/*FBN2* dual mutations resulting in modified MFS/CCA phenotypes (Figure 1, Table 1). In dual mutation carries, certain clinical features of fibrillinopathies such as aortic dilation (Ab4 and Ac1) and scoliosis (Ab2 and Ac1) are exaggerated, while for other clinical features, although inconsistently, the effect of one of the mutations appears to dominate. For example, in Family 1 the dual mutation carriers (Ab2 and Ab4) show CCA-associated finger and elbow contractures (Figure 2), whereas in Family 2 the dual mutation carrier (Ac1) shows MFS-related elbow hyperextension but no CCA-specific contractures. Moreover, we show the presence of clearly pathogenic *FBN1* and *FBN2* sequence variants in the apparently healthy reference cohort gnomAD, allowing the calculation of gnomAD-based minimal prevalence estimates of 6:10,000 and 3:10,000 for MFS and CCA, respectively (Figure 3, Table 2).

Materials and Methods: Whole genomes (60×PCR-free WGS, PE150) of ~450 Swiss patients with MFS or rare (aortic) disorders were screened for sequence variants in *FBN1*, *FBN2*, and other related genes. Variant confirmations and segregation analyses were performed using Sanger sequencing. Moreover, we queried gnomAD for different types of *a priori* pathogenic sequence variants in *FBN1* and in the CCA-associated exons 23-34 in *FBN2* (Table 2) and thereby calculated conservative prevalence estimates for MFS and CCA.

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