LETTER TO THE EDITOR

Structural and functional insights into γ-glutamyl carboxylase-factor IX interaction: implications for vitamin K-dependent bleeding disorders

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Supplemental Materials

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The Supplemental Data includes:

Supplemental Table 1

Supplemental Figures 1 to 2

Supplementary Table 1. List of the known pathogenic mutations within GGCX's critical regions.

FIX binding regions				
Region	Mutation site	Symptom	GGCX characterization	Carboxylation activity
Region 1	P80L[1-3]	VKCFD after trauma	Wild-type GGCX activity	FIX (98 ± 1%) MGP (99 ± 1%) BGP (78 ± 8%)
	R83W[3-5]	PXE-like and VKCFD	/	FIX (32 ± 6%) MGP (81 ± 3%) BGP (59 ± 6%) FII (23.1% at 1 μM K_1/38% at 10 μM K_1) FX (0.5% at1 μM K_1/43.3% at 10 μM K_1) PC (64.1% at 1 μM K_1/40.9% at 10 μM K_1)
	R83P[3, 5, 6]	VKCFD and facial dysmorphism	/	FIX (40 ± 4%) MGP (76 ± 2%) BGP (55 ± 6%) FII (0% at 1 μM K_1/29.2% at 10 μM K_1) FX (55.4% at1 μM K_1/85.1% at 10 μM K_1) PC (25.4% at 1 μM K_1/13% at 10 μM K_1)
Region 2	D153G[3, 5, 7]	VKCFD and Keutel syndrome	Reduced GGCX activity	FIX (66 ± 1%) MGP (39 ± 7%) BGP (32 ± 1%） FII (68.7% at 1 μM K_1/101% at10 μM K_1) FX (0.2% at1 μM K_1/66.8% at 10 μM K_1) PC (106.8% at1 μM K_1/44.2% at 10 μM K_1)
	W157R[3, 5, 6, 8]	VKCFD, midfacial hypoplasia, and chondrodysplasia punctata	Significantly reduced GGCX activity	FIX (49 ± 6%) MGP (34 ± 2%) BGP (12 ± 4%) FII (25.4% at 1 μM K_1/93.1% at 10 μM K_1) FX (13.1% at 1 μM K_1/97.2% at 10 μM K_1) PC (7.4% at 1 μM K_1/16.4% at 10 μM K_1)
Region 3	V255M[3, 5, 9]	PXE-like and VKCFD	Impaired carboxylation processivity	FIX (106 ± 7%) MGP (163 ± 14%) BGP (102 ± 2%) FII (0% at 1 μM K_1/119% at 10 μM K_1) FX (49.4% at 1 μM K_1/130.9% at 10 μM K_1) PC (144.1% at 1 μM K_1/87% at 10 μM K_1)

Region 4	F299S[5, 10]	PXE-like and VKCFD	/	FII (0% at 1 μM K_1/0% at 10 μM K_1) FX (0% at 1 μM K_1/0% at 10 μM K_1) PC (0% at 1 μM K_1/0% at 10 μM K_1)
	S300F[3, 5, 9]	PXE-like and VKCFD	Significantly reduced GGCX activity	FIX (8 ± 1%) MGP (21 ± 2%) BGP (19 ± 2%) FII (0% at 1 μM K_1/1% at 10 μM K_1) FX (0% at 1 μM K_1/0% at 10 μM K_1) PC (0% at 1 μM K_1/0.2% at 10 μM K_1)
Region 5	L394R[3, 5, 11-13]	VKCFD	Impaired binding for glutamate-containing substrates	FIX (38 ± 2%) MGP (72 ± 2%) BGP (64 ± 3%) FII (0% at 1 μM K_1/20.5% at 10 μM K_1) FX (0% at 1 μM K_1/49.3% at 10 μM K_1) PC (55.5% at 1 μM K_1/37.3% at 10 μM K_1)
	H404P[3, 5, 6, 14]	VKCFD	Impaired binding for glutamate-containing substrates	FIX (48 ± 2%) MGP (78 ± 3%) BGP (47 ± 3%) FII (0% at 1 μM K_1/8% at 10 μM K_1) FX (8% at 1 μM K_1/72.5% at 10 μM K_1) PC (0% at 1 μM K_1/0% at 10 μM K_1)
Region 7	R476C[3, 5, 10, 15]	PXE-like and VKCFD	/	FIX (94 ± 6%) MGP (183 ± 10%) BGP (82 ± 3%) FII (78.8%, 1 μM K_1/87.3%, 10 μM K_1) FX (12.1%, 1 μM K_1/12%, 10 μM K_1) PC (0%, 1 μM K_1/32.4%, 10 μM K_1)
	R476H[3, 5, 10, 15]	PXE-like and VKCFD	/	FIX (93 ± 1%) MGP (200 ± 12%) BGP (93 ± 4%) FII (0% at 1 μM K_1/113.5% at 10 μM K_1) FX (0% at 1 μM K_1/67.1% at 10 μM K_1) PC (95.9% at 1 μM K_1/57.4% at 10 μM K_1)
	R485P[3, 5, 14]	VKCFD and conradi-Hünermann-Happle syndrome	Reduced propeptide binding	FIX (59 ± 4%) MGP (37 ± 4%) BGP (33 ± 2%) FII (28% at 1 μM K_1/27.6% at 10 μM K_1) FX (57.2% at 1 μM K_1/117.9% at 10 μM K_1) PC (124.6% at 1 μM K_1/94.7% at 10 μM K_1)

Region 8	R513K[3, 16]	PXE-like and VKCFD	/	FIX (59 ± 3%) MGP (196 ± 6%) BGP (35 ± 5%)
	I532T[2, 3]	VKCFD after trauma	/	FIX (224 ± 10%) MGP (134 ± 3%) BGP (97 ± 17%)
	D534V[3, 6]	VKCFD, midfacial hypoplasia dystrophia, and microcephaly	/	FIX (49 ± 3%) MGP (43 ± 4%) BGP (38 ± 1%)
Region 9	G537A[3, 5, 10]	Clotting deficiency	/	FIX (61 ± 5%) MGP (94 ± 4%) BGP (79 ± 5%) FII (349.4% at 1 μM K_1/234.6% at 10 μM K_1) FX (25.7% at 1 μM K_1/79.5% at 10 μM K_1) PC (79% at 1 μM K_1/73.5% at 10 μM K_1)
	I553fs[17, 18]	VKCFD	Abolished GGCX activity	/
	G558R[3, 5, 10]	PXE-like and VKCFD	Reduced propeptide binding	FIX (19 ± 4%) MGP (13 ± 4%) BGP (28 ± 2%) FII (1.7% at 1 μM K_1/113.2% at 10 μM K_1) FX gla (0% at 1 μM K_1/0% at 10 μM K_1) PC (0% at 1 μM K_1/21.2% at 10 μM K_1)
Region 10	T591K[3, 8]	VKCFD	Abolished GGCX activity	FIX (19 ± 3%) MGP (9 ± 2%) BGP (6 ± 1%)
Region 14	R704X[3, 18, 19]	VKCFD	Minor effect on GGCX activity	FIX (50 ± 8%) MGP (46 ± 2%) BGP (5 ± 1%)
Region 15	S741fs[3, 20]	PXE-like and VKCFD	/	FIX (10 ± 1%) MGP (36 ± 3%) BGP (18 ± 5%)

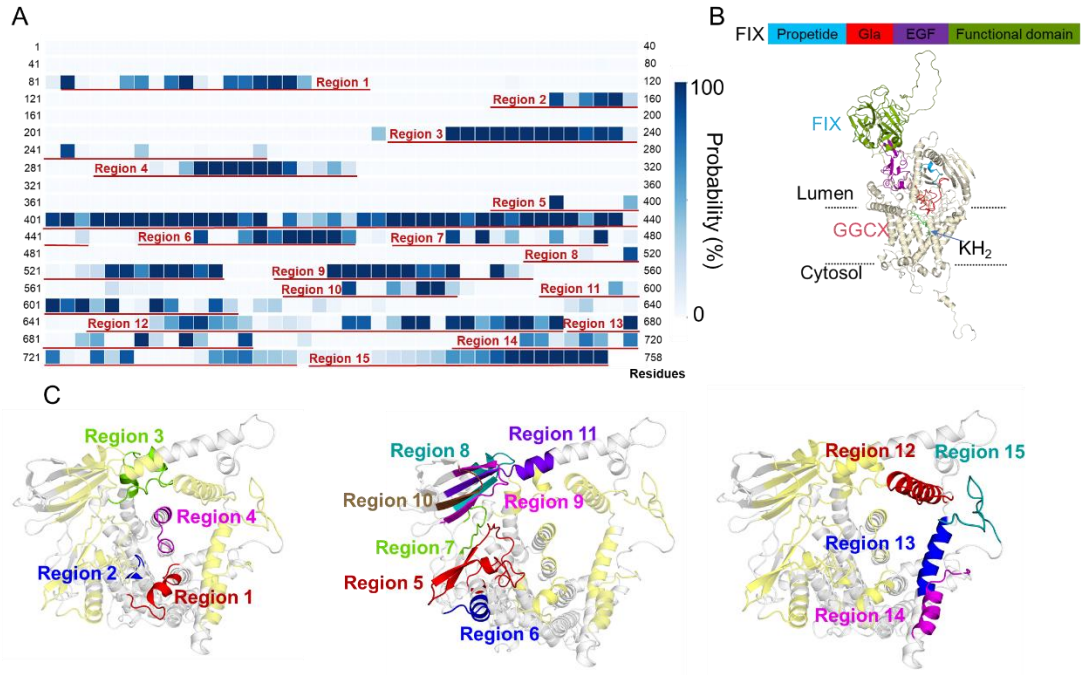
Reduced vitamin K binding regions

Region	Mutation site	symptom	GGCX characterization	Carboxylation activity
Region A	P80L[1-3]	VKCFD after trauma	Wild-type GGCX activity	FIX (98 ± 1%) MGP (99 ± 1%) BGP (78 ± 8%)

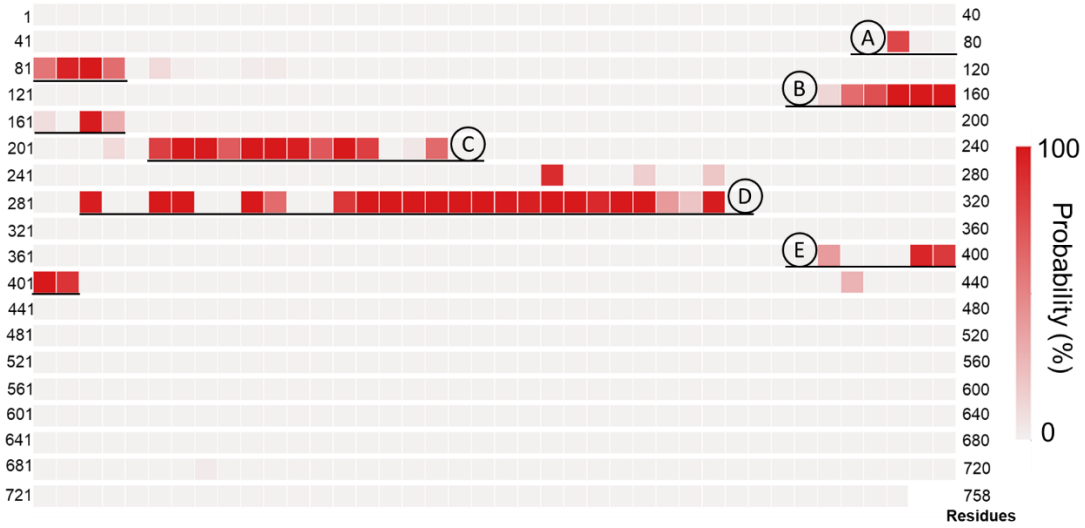
	R83W[3-5]	PXE-like and VKCFD	/	FIX (32 ± 6%) MGP (81 ± 3%) BGP (59 ± 6%) FII (23.1% at 1 μM K_1/38% at10 μM K_1) FX (0.5% at 1 μM K_1/43.3% at 10 μM K_1) PC (64.1% at 1 μM K_1/40.9% at 10 μM K_1)
	R83P[3, 5, 6]	VKCFD and facial dysmorphism	/	FIX (40 ± 4%) MGP (76 ± 2%) BGP (55 ± 6%) FII (0% at 1 μM K_1/29.2% at 10 μM K_1) FX (55.4% at 1 μM K_1/85.1% at 10 μM K_1) PC (25.4% at 1 μM K_1/13% at 10 μM K_1)
Region B	D153G[3, 5, 7]	VKCFD and Keutel syndrome	Reduced GGCX activity	FIX (66 ± 1%) MGP (39 ± 7%) BGP (32 ± 1%) FII (68.7% at 1 μM K_1/101% at 10 μM K_1) FX (0.2% at 1 μM K_1/66.8% at 10 μM K_1) PC (106.8% at 1 μM K_1/44.2% at 10 μM K_1)
	W157R[3, 5, 6, 8]	VKCFD, midfacial hypoplasia, and chondrodysplasia punctata	Significantly reduced GGCX activity	FIX (49 ± 6%) MGP (34 ± 2%) BGP (12 ± 4%) FII (25.4% at 1 μM K_1/93.1% at 10 μM K_1) FX (13.1% at 1 μM K_1/97.2% at 10 μM K_1) PC (7.4% at 1 μM K_1/16.4% at 10 μM K_1)
	M174R[2, 5, 7]	VKCFD	Abolished GGCX activity	FII (0% at 1 μM K_1/0% at 10 μM K_1) FX (0% at 1 μM K_1/0.3% at 10 μM K_1) PC (0% at 1 μM K_1/0% at 10 μM K_1)
Region C	R204C[3, 5, 6]	VKCFD and midfacial hypoplasia	/	FIX (52 ± 4%) MGP (20 ± 6%) BGP (20 ± 6%) FII (20.1% at 1 μM K_1/105.1% at 10 μM K_1) FX (41.9% at 1 μM K_1/120.8% at 10 μM K_1) PC (0% at 1 μM K_1/49.9% at 10 μM K_1)
Region D	S277C[3, 21]	VKCFD	/	FIX (87 ± 5%) MGP (96 ± 4%) BGP (50 ± 3%)
	S284P[3, 5, 6]	Atrial septal defect and	/	FIX (133 ± 3%) MGP (134 ± 11%) BGP (90 ± 6%)

		supra valvular pulmonary artery stenosis		FII (30.3% at 1 μM K_1/84.1% at 10 μM K_1) FX (55% at 1 μM K_1/123% at 10 μM K_1) PC (37.5% at 1 μM K_1/128.6% at 10 μM K_1)
	F299S[5, 10]	PXE-like and VKCFD	/	FII (0% at 1 μM K_1/0% at 10 μM K_1) FX (0% at 1 μM K_1/0% at 10 μM K_1) PC (0% at 1 μM K_1/0% at 10 μM K_1)
	S300F[3, 5, 9]	PXE-like and VKCFD	Significantly reduced GGCX activity	FIX (8 ± 1%) MGP (21 ± 2%) BGP (19 ± 2%) FII (0% at 1 μM K_1/1% at 10 μM K_1) FX (0% at 1 μM K_1/0% at 10 μM K_1) PC (0% at 1 μM K_1/0.2% at 10 μM K_1)
	W315X[3, 6, 18]	/	/	BGP (5 ±2%)
Region E	L394R[3, 5, 11-13]	/	/	FIX (38 ± 2%) MGP (72 ± 2%) BGP (64 ± 3%) FII (0% at 1 μM K_1/20.5% at 10 μM K_1) FX (0% at 1 μM K_1/49.3% at 10 μM K_1) PC (55.5% at 1 μM K_1/37.3% at 10 μM K_1)

Abbreviations: Protein C is represented as PC, matrix gla protein as MGP, bone gla protein as BGP, and the coagulation factors IX, X, and II are denoted as FIX, FX, and FII, respectively. Vitamin K1 is denoted as K_1. Pseudoxanthoma elasticum-like is denoted as PXE-like. Vitamin K-dependent coagulation factor deficiency is denoted as VKCFD. Pathogenic mutations located within ten residues of the designated critical region boundaries for GGCX are underlined. Numerically labeled regions correspond to residue clusters contacting proFIX, while alphabetically labeled regions denote those interacting with reduced vitamin K, according to the topological map of GGCX provided in Figure 1B.



Supplementary Figure 1. Visualization of 15 binding regions in GGCX**. A.** Contact probability distribution of GGCX residues with proFIX based on molecular dynamics simulation trajectories. Residue–residue contacts were defined using a 0.6 nm cutoff for the minimum distance between any heavy atoms, encompassing both backbone and side chain atoms. The scale bar denotes normalized probability values. **B**. Theoretical calculation-predicted model of proFIX bound to GGCX with KH_2. GGCX is depicted in light goldenrod yellow, while KH_2 is shown in green. **C**. Visualization of 15 binding regions in GGCX**.** Data are representative of three independent experiments.



Supplementary Figure 2. Contact probability distribution of GGCX residues with KH_2 based on molecular dynamics simulation trajectory. The color intensity corresponds to the binding strength. The scale bar denotes normalized probability values. Data are representative of three independent experiments.

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