

Table S1 Demographic and clinical characteristics of participants

No.	Age at diagnosis	Sex	Group	Comorbidities	Fusion levels	Classification*
1	11	M	KFS	Torticollis	C2-3	I
2	35	F	KFS	Basilar invagination, Torticollis	C1-6	III
3	24	M	KFS	None	C2-4, C5-T1	II
4	13	F	KFS	Hypogonadism	C2-5	III
5	49	M	KFS	Cervical spondylosis	C4-5	I
6	8	M	KFS	None	C6-7	I
7	12	F	KFS	Syringomyelia, Ventricular hypertrophy	C6-7	I
8	16	F	KFS	None	C2-6	III
9	21	M	CS	Kyphosis	L1-2	II
10	14	M	CS	None	T4-5	III
11	47	F	CS	Diastematomyelia, Tethered cord, Kyphosis	T12-L1	III
12	11	M	CS	None	T5-8	III
13	3	F	CS	None	T11-12	II
14	20	M	Control	Right ankle fracture	None	None
15	21	F	Control	Lumbar disc herniation	None	None
16	25	F	Control	None	None	None
17	30	M	Control	Synovitis	None	None
18	26	M	Control	Hyperuricemia	None	None
19	10	M	Control	None	None	None
20	13	F	Control	Obstructive sleep apnea syndrome, Obesity	None	None

*, KFS patients were evaluated based on following classification criteria: Type I, a single congenitally fused cervical segment; Type II, multiple noncontiguous congenitally fused cervical segments; Type III, multiple contiguous congenitally fused cervical segments. CS patients were evaluated based on following classification criteria. Type I, failure of segmentation; Type II, defects of formation; Type III, mixed form with both segmentation defect and failure of formation. KFS, Klippel-Feil syndrome; CS, congenital scoliosis.

Table S2 Identification of 49 differentially expressed proteins in serum between KFS patients and control participants

Protein Accessions	Protein Descriptions	t-test P value	Ratio (KFS/Control)
P01861	Immunoglobulin heavy constant gamma 4	0.005	0.134
A0A087WSY4	Immunoglobulin heavy variable 4-30-2	0.025	0.295
A0A0G2JRK6	Uncharacterized protein	0.029	0.320
A0A0B4J1V2	Immunoglobulin heavy variable 2-26	0.044	0.368
P15814	Immunoglobulin lambda-like polypeptide 1	0.012	0.369
A0A087WSY6	Immunoglobulin kappa variable 3D-15	0.042	0.370
P01877	Immunoglobulin heavy constant alpha 2	0.015	0.371
A0A0C4DH67	Immunoglobulin kappa variable 1-8	0.007	0.383
P42785	Lysosomal Pro-X carboxypeptidase	0.006	0.399
A0A075B7B8	Immunoglobulin heavy variable 3/OR16-12	0.023	0.406
A0A0C4DH72	Immunoglobulin kappa variable 1-6	0.002	0.410
A0A075B6H7	Immunoglobulin kappa variable 3-7	0.006	0.427
A0A2Q2TTZ9	Immunoglobulin kappa variable 1-33	0.028	0.431
P01619	Immunoglobulin kappa variable 3-20	0.037	0.433
P01876	Immunoglobulin heavy constant alpha 1	0.015	0.435
P01834	Immunoglobulin kappa constant	0.012	0.438
B9A064	Immunoglobulin lambda-like polypeptide 5	0.020	0.474
P0DOY2;P0DOY3	Immunoglobulin lambda constant	0.016	0.476
A0A075B6K4	Immunoglobulin lambda variable 3-10	0.043	0.478
P01714	Immunoglobulin lambda variable 3-19	0.005	0.488
P01718	Immunoglobulin lambda variable 3-27	0.040	0.497
P01624	Immunoglobulin kappa variable 3-15	0.033	0.498
P01599	Immunoglobulin kappa variable 1-17	0.015	0.501
Q06830	Peroxiredoxin-1	0.019	0.506
P01857	Immunoglobulin heavy constant gamma 1	0.035	0.511
A0A0C4DH25	Immunoglobulin kappa variable 3D-20	0.003	0.513
P01860	Immunoglobulin heavy constant gamma 3	0.043	0.517
P05543	Thyroxine-binding globulin	0.004	1.424
Q86U17	Serpin A11	0.048	1.557
P01008	Antithrombin-III	0.006	1.560
P04004	Vitronectin	0.045	1.576
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0.007	1.581
P49747	Cartilage oligomeric matrix protein	0.014	1.591
P08253	72 kDa type IV collagenase	0.008	1.610
Q99784	Noelin	0.005	1.627
Q13790	Apolipoprotein F	0.045	1.663
Q16853	Membrane primary amine oxidase	0.001	1.670
H7BYX6	Neural cell adhesion molecule 1	0.009	1.687
P02656	Apolipoprotein C-III	0.007	1.795
P33151	Cadherin-5	0.002	1.810
Q6YHK3	CD109 antigen	0.007	1.811
Q9H4G4	Golgi-associated plant pathogenesis-related protein 1	0.015	2.039
P55290	Cadherin-13	0.0004	2.079
Q08380	Galectin-3-binding protein	0.007	2.088
P23471	Receptor-type tyrosine-protein phosphatase zeta	0.012	2.230
P12830	Cadherin-1	0.028	2.255
P06727	Apolipoprotein A-IV	0.019	2.450
Q14520	Hyaluronan-binding protein 2	0.039	2.648
P05067	Amyloid-beta precursor protein	0.008	4.871

Table S3 Identification of 192 differentially expressed proteins in serum between KFS patients and CS patients

Protein accessions	Protein descriptions	t-test P value	Ratio (KFS/CS)
Q15942	Zyxin	0.000	0.165
P0DJ19	Serum amyloid A-2 protein	0.011	0.168
P0DJ18	Serum amyloid A-1 protein	0.006	0.168
P02741	C-reactive protein	0.024	0.209
Q9Y279	V-set and immunoglobulin domain-containing protein 4	0.012	0.215
P04040	Catalase	0.000	0.217
P07738	Bisphosphoglycerate mutase	0.002	0.217
P68871	Hemoglobin subunit beta	0.022	0.232
Q02818	Nucleobindin-1	0.000	0.237
Q06830	Peroxioredoxin-1	0.001	0.244
P13647	Keratin, type II cytoskeletal 5	0.006	0.260
P01009	Alpha-1-antitrypsin	0.001	0.265
Q13228	Methanethiol oxidase	0.017	0.268
P05089	Arginase-1	0.007	0.271
Q99497	Protein/nucleic acid deglycase DJ-1	0.002	0.273
Q86Y23	Hornerin	0.005	0.276
P60174	Triosephosphate isomerase	0.000	0.290
P69905	Hemoglobin subunit alpha	0.038	0.290
P31944	Caspase-14	0.005	0.293
P60709;P63261	Actin, cytoplasmic	0.000	0.298
P07911	Uromodulin	0.013	0.300
P35527	Keratin, type I cytoskeletal 9	0.002	0.304
P02750	Leucine-rich alpha-2-glycoprotein	0.000	0.304
P00995	Serine protease inhibitor Kazal-type1	0.007	0.305
P50552	Vasodilator-stimulated phosphoprotein	0.012	0.306
Q92954	Proteoglycan 4	0.002	0.308
Q9H299	SH3 domain-binding glutamic acid-rich-like protein 3	0.004	0.311
P63104	14-3-3 protein zeta/delta	0.001	0.316
P08571	Monocyte differentiation antigen CD14	0.002	0.321
Q00151	PDZ and LIM domain protein 1	0.014	0.322
Q99941	Cyclic AMP-dependent transcription factor ATF-6 beta	0.004	0.328
P29401	Transketolase	0.010	0.329
Q8NBJ4	Golgi membrane protein 1	0.000	0.330
P22392;Q32Q12	NME1-NME2 readthrough; Nucleoside diphosphate kinase	0.000	0.332
P62937	Peptidyl-prolyl cis-trans isomerase A	0.004	0.332
Q6E0U4	Dermokine	0.001	0.333
Q9BUN1	Protein MENT	0.011	0.339
H3BTN5	Pyruvate kinase (Fragment)	0.014	0.342
Q9ULI3	Protein HEG homolog 1	0.002	0.344
O75368	SH3 domain-binding glutamic acid-rich-like protein	0.028	0.348
P08572	Collagen alpha-2(IV) chain	0.020	0.356
P24666	Low molecular weight phosphotyrosine protein phosphatase	0.000	0.363
P32942	Intercellular adhesion molecule 3	0.001	0.365
P30086	Phosphatidylethanolamine-binding protein 1	0.006	0.372
P10586	Receptor-type tyrosine-protein phosphatase F	0.003	0.377
Q7Z3B1	Neuronal growth regulator 1	0.000	0.379
Q13332	Receptor-type tyrosine-protein phosphatase S	0.007	0.380
P16070	CD44 antigen	0.012	0.386
P32119	Peroxioredoxin-2	0.017	0.386
Q9NZT1	Calmodulin-like protein 5	0.015	0.388
P00558	Phosphoglycerate kinase 1	0.001	0.399
Q92859	Neogenin	0.000	0.404
B7ZKJ8	ITIH4 protein	0.022	0.412
P02749	Beta-2-glycoprotein 1	0.000	0.414
P28072	Proteasome subunit beta type-6	0.000	0.414
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0.001	0.417
O43505	Beta-1,4-glucuronyltransferase 1	0.019	0.419
A0A2R8Y619;O60814;P06899;P23527;P33778;P57053;P58876;P62807;Q16778;Q5QNW6;Q8N257;Q93079;Q96A08;Q99877;Q99879;Q99880;U3KQK0	Histone H2B	0.002	0.419
Q9P232	Contactin-3	0.003	0.419
P02671	Fibrinogen alpha chain	0.002	0.422
P30530	Tyrosine-protein kinase receptor UFO	0.001	0.432
P08294	Extracellular superoxide dismutase	0.011	0.438
Q14118	Dystroglycan	0.009	0.442
Q6UY14	ADAMTS-like protein 4	0.041	0.443
P26038	Moesin	0.002	0.446
P37802	Transgelin-2	0.021	0.449
P20933	N(4)-(beta-N-acetylglucosaminy)-L-asparaginase	0.002	0.450
O14498	Immunoglobulin superfamily containing leucine-rich repeat protein	0.020	0.451
P00451	Coagulation factor VIII	0.000	0.452
C9Y11;E7EMS7;H7BZ95;M0R0A5	Dermokine	0.002	0.462
A0A0U1RR32;A0A0U1RRH7;A0A3B3IS11;C9J0D1;P04908;P0C0S5;P0C0S8;P16104;P20671;Q16777;Q6F113;Q71UI9;Q7L7L0;Q8IUE6;Q93077;Q96KK5;Q96QV6;Q99878;Q9BTM1	Histone H2A	0.004	0.467
P00734	Prothrombin	0.011	0.471
Q9Y5Y7	Lymphatic vessel endothelial hyaluronic acid receptor 1	0.038	0.474
Q13201	Multimerin-1	0.000	0.477
P11047	Laminin subunit gamma-1	0.041	0.479
P78324;Q5TFQ8	Tyrosine-protein phosphatase non-receptor type substrate 1;Signal-regulatory protein beta-1 isoform 3	0.003	0.484
Q16270	Insulin-like growth factor-binding protein 7	0.006	0.486
P24821	Tenascin	0.018	0.490
P00568;Q5T9B7	Adenylate kinase isoenzyme 1	0.035	0.491
K7ER74	APOC4-APOC2 readthrough (NMD candidate)	0.001	0.496
P15151	Poliiovirus receptor	0.000	0.498
P54108	Cysteine-rich secretory protein 3	0.000	0.504
Q9UNN8	Endothelial protein C receptor	0.014	0.505
P12830	Cadherin-1	0.010	0.507
P05155	Plasma protease C1 inhibitor	0.016	0.519
P08493	Matrix Gla protein	0.001	0.523
Q9UHG3	Prenylcysteine oxidase 1	0.009	0.525
P18206	Vinculin	0.013	0.525
P07357	Complement component C8 alpha chain	0.040	0.526
Q14847	LIM and SH3 domain protein 1	0.019	0.526
P98160	Basement membrane-specific heparan sulfate proteoglycan core protein	0.033	0.528
Q577F0	Neuropilin	0.046	0.538
P07711	Cathepsin L1	0.004	0.540
A0A087X0M8	Neural cell adhesion molecule L1-like protein	0.007	0.545
P12109	Collagen alpha-1(VI) chain	0.033	0.551
P99999	Cytochrome c	0.013	0.554
Q94919	Endonuclease domain-containing 1 protein	0.005	0.555
P02786	Transferrin receptor	0.050	0.570
P13987	CD59 glycoprotein	0.014	0.587
P48637	Glutathione synthetase	0.009	0.591
P11684	Uteroglobin	0.008	0.592
P11142	Heat shock cognate 71 kDa protein	0.001	0.604
P02652	Apolipoprotein A-II	0.028	0.605
Q6UWP8	Suprabasin	0.008	0.605
Q92520	Protein FAM3C	0.003	0.618
P15924	Desmoplakin	0.011	0.622
P12111	Collagen alpha-3(VI) chain	0.028	0.625
Q6UX71	Plexin domain-containing protein 2	0.003	0.632
P27918	Properdin	0.020	0.644
P02774	Vitamin D-binding protein	0.002	0.647
O75144	ICOS ligand	0.012	0.648
A0A0J9YYC8;A6XMV8;A6XMV9;E7EQ64;H0Y8D1;P07477;P07478;Q8NHM4	Trypsin;Protease serine 2 preproprotein	0.001	0.653
P02649	Apolipoprotein E	0.012	0.671
Q96N29	ZNF511-PRAP1 readthrough; Proline-rich acidic protein 1	0.010	0.680
P02753	Retinol-binding protein	0.047	0.684
P05556	Integrin beta-1	0.012	0.686
P33151	Cadherin-5	0.013	0.690
P02533	Keratin, type I cytoskeletal 14	0.044	0.691
Q9UGM5	Fetuin-B	0.010	1.460
Q9NPH3	Interleukin-1 receptor accessory protein	0.009	1.488
P01599	Immunoglobulin kappa variable 1-17	0.037	1.536
Q13740	CD166 antigen	0.030	1.552
Q16853	Membrane primary amine oxidase	0.006	1.583
P01718	Immunoglobulin lambda variable 3-27	0.047	1.629
Q9Y6R7	IgGfC-binding protein	0.019	1.632
P06276	Cholinesterase	0.010	1.661
P01700	Immunoglobulin lambda variable 1-47	0.001	1.706
P06310	Immunoglobulin kappa variable 2-30	0.038	1.711
Q76LX8	A disintegrin and metalloproteinase with thrombospondin motifs 13	0.035	1.733
P23470	Receptor-type tyrosine-protein phosphatase gamma	0.025	1.744
Q6YHK3	CD109 antigen	0.028	1.748
Q9BTY2	Plasma alpha-L-fucosidase	0.030	1.777
P00736	Complement C1r subcomponent	0.021	1.800
P07996	Thrombospondin-1	0.025	1.830
P07942	Laminin subunit beta-1	0.014	1.834
P43652	Afamin	0.025	1.853
A0A0B4J1V0	Immunoglobulin heavy variable 3-15	0.032	1.874
P39060	Collagen alpha-1(XVIII) chain	0.042	1.875
O75882	Attractin	0.011	1.892
P02765	Alpha-2-HS-glycoprotein	0.010	1.895
Q12913	Receptor-type tyrosine-protein phosphatase eta	0.014	1.897
P0DOY2;P0DOY3	Immunoglobulin lambda constant	0.002	1.910
P80748	Immunoglobulin lambda variable 3-9;Immunoglobulin lambda variable 3-21	0.032	1.911
A0A075B6J9	Immunoglobulin lambda variable 2-18	0.038	1.926
Q99784	Noelin	0.001	2.052
A0A075B6H9	Immunoglobulin lambda variable 4-69	0.024	2.056
P07358	Complement component C8 beta chain	0.048	2.071
P27169	Serum paraoxonase/arylesterase 1	0.004	2.074
P17813	Endoglin	0.006	2.146
P02787	Serotransferrin	0.041	2.156
P12821	Angiotensin-converting enzyme	0.012	2.168
P20023	Complement receptor type 2	0.012	2.179
P40189	Interleukin-6 receptor subunit beta	0.002	2.181
Q96IY4	Carboxypeptidase B2	0.015	2.186
A0A2Q2T2Z9	Immunoglobulin kappa variable 1-33	0.000	2.264
P03952	Plasma kallikrein	0.002	2.347
P04003	C4b-binding protein alpha chain	0.002	2.355
A0A0B4J2B5	Immunoglobulin heavy variable 3/OR16-9	0.009	2.371
P35858	Insulin-like growth factor-binding protein complex acid labile subunit	0.010	2.404
P06312	Immunoglobulin kappa variable 4-1	0.020	2.459
A2NJV5	Immunoglobulin kappa variable 2D-29	0.003	2.460
B9A064	Immunoglobulin lambda-like polypeptide 5	0.000	2.469
Q15166	Serum paraoxonase/lactonase 3	0.001	2.493
Q8IWW2	Contactin-4	0.004	2.501
A0A075B6I0	Immunoglobulin lambda variable 8-61	0.016	2.594
P05546	Heparin cofactor 2	0.030	2.625
P01860	Immunoglobulin heavy constant gamma 3	0.000	2.657
P01602	Immunoglobulin kappa variable 1-5	0.003	2.665
P01703	Immunoglobulin lambda variable 1-40	0.001	2.676
Q03591	Complement factor H-related protein 1	0.007	2.702
Q08380	Galectin-3-binding protein	0.007	2.771
P01701	Immunoglobulin lambda variable 1-51	0.002	2.790
A0A075B6I9	Immunoglobulin lambda variable 7-46	0.010	2.817
P02745	Complement C1q subcomponent subunit A	0.000	3.028
P29622	Kallistatin	0.002	3.031
P01857	Immunoglobulin heavy constant gamma 1	0.000	3.067
P22792	Carboxypeptidase N subunit 1	0.001	3.121
Q9Y4L1	Hypoxia up-regulated protein 2	0.048	3.124
P15814	Immunoglobulin lambda-like polypeptide 1	0.003	3.159
P02768	Serum albumin	0.004	3.222
P01859	Immunoglobulin heavy constant gamma 2	0.013	3.228
P01768;P0DP03	Immunoglobulin heavy variable 3-30	0.002	3.289
P05090	Apolipoprotein D	0.006	3.589
P06681	Complement C2	0.001	3.689
P01780	Immunoglobulin heavy variable 3-7	0.000	3.708
P01834	Immunoglobulin kappa constant	0.000	3.734
O75636	Ficolin-3	0.002	4.294
P08603	Complement factor H	0.001	4.380
P01871	Immunoglobulin heavy constant mu	0.010	4.454
P13598	Intercellular adhesion molecule 2	0.004	4.476
A0A0C4DH34	Immunoglobulin heavy variable 4-28	0.000	4.481
P01824;P01825;P06331;P0DP06;P0DP08	Immunoglobulin heavy variable 4-61;Immunoglobulin heavy variable 4-39;Immunoglobulin heavy variable 4-59;Immunoglobulin heavy variable 4-34;Immunoglobulin heavy variable 4-30-4;Immunoglobulin heavy variable 4-38-2	0.018	7.442