Table 1 (*continued*)

cDNA nucleotide (or splice site) change	Mutation	Amino acid change	CGD type	Accession number	Ref.	Kindred (patients)[a]	
c.771C>A	Nonsense	p.Cys257X	het[d]		[1]	1(1)	
c.779C>G	Missense	p.Pro260Arg	X91°		Unpubl.	1(1)	*
c.781_782delCA	Deletion	p.Gln261ValfsX22	X91°	A0001	[1,11]	1(1)	
c.781C>T	Nonsense	p.Gln261X	X91°	A0020	[1,11]	5(5)	
				A0286	unpubl.		
				A0424			
				A0425			
c.785_804+1dup21del9ins2[c]	Splice site	del. exon7 p.Arg226LeufsX5	X91[?]		Unpubl.	1(1)	*
c.788delC	Deletion	p.Ala263ValfsX6	X91°	A0582	[27]	1(1)	*
c.797delC	Deletion	p.Pro266LeufsX3	X91°	A0316	[1,23]	2(2)	
c.799_800insAA	Insertion	p.Pro267GlnfsX3	X91[?]		Unpubl.	1(1)	*
c.804+1G>A[c]	Splice site	del exon 7 p.Arg226LeufsX5	X91°	A0522	[1,77,80]	2(2)	
c.804+1G>T[c]	Splice site	del exon 7 p.Arg226LeufsX5	X91°	A0523	[1] unpubl.	3(3)	
c.804+2T>A[c]	Splice site	del. exon 7 p.Arg226LeufsX5	X91°	A0005	[1,32]	1(1)	
c.804+2T>C[c]	Splice site	del. exon 7 p.Arg226LeufsX5	X91°	A0236	[1,11]	3(4)	
				A0237	unpubl.		
c.805−2A>C[c]	Splice site	del exon 8? p.Thr269_Lys299del?	X91°	A0238	[1,19]	1(1)	
c.805−2A>T[c]	Splice site	del. exon 8? p.Thr269_Lys299del?	X91°		Unpubl.	1(1)	*
c.805−2A>G[c]	Splice site	del exon 8 p.Thr269_Lys299del	X91°	A0520	[1,17,44]	1(1)	
c.805−1G>A[c]	Splice site	del exon 8? p.Thr269_Lys299del?	X91°	A0521	[1,12] unpubl.	2(2)	
c.805−1G>C[c]	Splice site	del. exon 8? p.Thr269_Lys299del?	X91[?]		Unpubl.	1(1)	*
c.805−7[c]	Splice site	del exon 8 p.Thr269_Lys299del	X91°		[1,25]	1(1)	
c.810G>A	Nonsense	p.Trp270X	X91°	A0624	[53] unpubl.	2(2)	*
c.811A>T	Nonsense	p.Lys271X	X91°		Unpubl.	1(1)	*
c.815G>A	Nonsense	p.Trp272X	X91°	A0099	[1,12,19]	3(3)	
				A0287			
				A0482			
c.816G>A	Nonsense	p.Trp272X	X91°	A0047	[1,11]	1(1)	
c.831_853del23	Deletion	p.Met277IlefsX63	X91[?]		Unpubl.	1(1)	*
c.840T>A	Nonsense	p.Tyr280X	X91[?]		Unpubl.	1(1)	*
c.844_874del31	Deletion	p.Cys282AsnfsX21	X91°	A0536	Unpubl.	1(1)	*
c.845dupG	Insertion	p.Cys282TrpfsX2	X91°		Unpubl.	1(1)	*
c.867G>A	Nonsense	p.Trp289X	het[d]		[81]	1(1)	*
c.868C>T	Nonsense	p.Arg290X	X91°	A0045	[1,11,12,20,23,29,33,	38(42)	
			2 het[d]	A0046	39,42,43,49,53,61]		
				A0145	unpubl.		
				A0159			
				A0194			
				A0198			
				A0288			
				A0289			
				A0443			
				A0444			
				A0445			
				A0446			
				A0447			
				A0448			
				A0449			
				A0450			
				A0451			
				A0452			
				A0453			
				A0639			
c.871_880del10	Deletion	p.Ser291ArgfsX19	X91°	A0321	[1,17]	1(1)	
c.883_887dupGTGGT	Insertion	p.Ile297TrpfsX18	X91[?]		Unpubl.	1(1)	*
c.890_904del15	Deletion	p.Ile297_Val301del	X91+	A0310	[1,25]	1(1)	
c.894delC	Deletion	p.Lys299ArgfsX14	het[d]		[82]	1(2)	*
c.897G>C	Missense Splice site?	p.Lys299Asn	X91−		Unpubl.	1(1)	*
c.897G>T	Missense Splice site?	p.Lys299Asn	X91[?]	A0402	[1]	1(1)	
c.897G>A (3′ end of exon 8)[c]	Splice site	del exon 8 p.Thr269_Lys299del	X91[?]		[23] unpubl.	2(2)	*

(*continued on next page*)

Table 1 (*continued*)

cDNA nucleotide (or splice site) change	Mutation	Amino acid change	CGD type	Accession number	Ref.	Kindred (patients)[a]	
c.897+1G>A[c]	Splice site	del exon 8 p.Thr269_Lys299del	X91°	A0524	[1,29]	2(2)	
c.897+1G>T[c]	Splice site	del. exon 8 p.Thr269_Lys299del	X91°	A0239 A0240 A0303	[1,11,23]	3(4)	
c.898−1G>A[c]	Splice site	del exon 9? p.Val300AspfsX47	X91?		Unpubl.	2(2)	*
c.903dupC	Insertion	p.Thr302HisfsX46	X91°		Unpubl.	1(1)	*
c.904A>C	Missense	p.Thr302Pro	X91?		[23]	1(1)	*
c.906_909delTCAC	Deletion	p.His303LeufsX9	X91?		Unpubl.	1(1)	*
c.907C>T	Missense	p.His303Tyr	X91+		Unpubl.	1(1)	*
c.[907C>A;911C>G]	Missense	p.[His303Asn; Pro304Arg]	X91+	A0538	[1,83,84]	1(2)	
c.909C>A	Missense	p.His303Gln	X91°		Unpubl.	1(7)	*
c.911C>T	Missense	p.Pro304Leu	X91?		Unpubl.	1(2)	*
c.915delC	Deletion	p.Phe305LeufsX8	X91?	A0558	[24]	1(1)	*
c.916A>T	Nonsense	p.Lys306X	X91°	A0290	[1,11]	1(1)	
c.919A>C	Missense	p.Thr307Pro	X91°	A0469 A0590	[27,39]	2(2)	*
c.919delA	Deletion	p.Thr307ProfsX6	X91°	A0221	[1,11]	1(1)	
c.[922_923insCCTTTCA; 935_937delTGA][f]	Deletion/insertion	p.Ile308ProfsX39	X91?		[1,12]	1(1)	
c.922_923insGTTTC	Insertion	p.Ile308SerfsX7	X91°		[39]	1(1)	*
c.925G>A	Missense	p.Glu309Lys	X91−	A0101 A102 A0368 A0369 A0370 A0371 A0372	[1,17,20,43] unpubl.	9(15)	
c.925G>T	Nonsense	p.Glu309X	X91°		Unpubl.	1(2)	*
c.929T>C	Missense	p.Leu310Pro	X91°		Unpubl.	1(1)	*
c.931delC	Deletion	p.Gln311ArgfsX2	X91?		Unpubl.	1(1)	*
c.935T>G	Missense	p.Met312Arg	X91?	A0253	[1,11] unpubl.	2(2)	
c.935T>A	Missense	p.Met312Lys	X91?	A0629	[33]	1(1)	*
c.943_945delAAG	Deletion	p.Lys315del	X91−	A0163 A0208 A0209	[1,11,12,43,48,79] unpubl.	3(4)	
c.948delG	Deletion	p.Phe317SerfsX26	X91°	A0222	[1,19]	1(1)	
c.958delG	Deletion	p.Glu320LysfsX23	X91?		Unpubl.	1(1)	*
c.[958delG;962T>G]	Deletion/missense	p.Glu320LysfsX23	X91°		Unpubl.	1(1)	*
c.958G>T	Nonsense	p.Glu320X	X91°	A0569	[35]	1(1)	*
c.960delA	Deletion	p.Val321TrpfsX22	X91°	A0306	[1]	1(1)	
c.965delG	Deletion	p.Gly322AspfsX21	X91°	A0118	[1,12,52]	1(1)	
c.965G>A	Missense	p.Gly322Glu	X91−	A0377	[1,12] unpubl.	2(2)	
c.967C>T	Nonsense	p.Gln323X	X91°	A0067	[1,17,19]	1(1)	
c.972C>A	Nonsense	p.Tyr324X	X91°		Unpubl.	1(1)	*
c.973A>T	Missense	p.Ile325Phe	X91−	A0401	[1,12]	1(1)	
c.979delG	Deletion	p.Val327SerfsX16	X91°	A0584	[27]	1(1)	*
c.981_985delCAAGT	Deletion	p.Lys328ProfsX18	X91°		Unpubl.	1(1)	*
c.985T>C	Missense	p.Cys329Arg	X91°		[42]	1(1)	*
c.992_997 delAGGTGTinsGGGGG	Deletion/insertion	p.Lys331ArgfsX12	X91°		Unpubl.	1(1)	*
c.994delG	Deletion	p.Val332CysfsX11	X91°	A0571	[35]	1(1)	*
c.997T>C	Missense	p.Ser333Pro	X91?	A0200	[1,12,52] unpubl.	2(2)	
c.1006G>T	Nonsense	p.Glu336X	X91°	A0087 A088 A0291	[1,20,52]	4(5)	
c.1010G>A	Nonsense	p.Trp337X	X91°	A0292 A0535	[1,11] unpubl.	3(3)	
c.1011G>A	Nonsense	p.Trp337X	X91? 1 het[d]		[53] unpubl.	3(3)	*
c.1012C>T	Missense	p.His338Tyr	X91−	A0014 A0084 A0254 A0397 A0398	[1,11,26,49,80,85] unpubl.	7(7)	
c.1012C>A	Missense	p.His338Asn	X91?	A0396	[1]	1(1)	
c.1013A>G	Missense	p.His338Arg	X91+		Unpubl.	2(4)	*
c.1014C>A	Missense	p.His338Gln	X91?		Unpubl.	3(3)	*
c.1016C>A	Missense	p.Pro339His	X91+/−	A0070 A0096 A0416 A0417	[1,11,12,17,19, 22,26,29,44,86] unpubl.	11(13)	
c.1016C>T	Missense	p.Pro339Leu	X91?		Unpubl.	1(1)	*

Table 1 (*continued*)

cDNA nucleotide (or splice site) change	Mutation	Amino acid change	CGD type	Accession number	Ref.	Kindred (patients)[a]	
c.1016delC	Deletion	p.Pro339LeufsX4	X91[?]	A0317	[1]	1(1)	
c.1016dupC	Insertion	p.Thr341TyrfsX7	X91[+]	A0347	[1,22,87]	1(2)	
c.1022C>A	Missense	p.Thr341Lys	X91[+]	A0255	[1,19,88]	1(1)	
c.1022C>T	Missense	p.Thr341Ile	X91[−]	A0470	[1]	1(1)	
c.1025T>A	Missense	p.Leu342Gln	X91[+]	A0064 A0256 A0404	[1,11,33,55] unpubl.	4(4)	
c.1027A>C	Missense	p.Thr343Pro	X91[+]	A0591	[27] unpubl.	2(2)	*
c.1030T>C	Missense	p.Ser344Pro	X91[+]		Unpubl.	2(2)	*
c.1030_1031insCT	Insertion	p.Ala345ProfsX42	X91[+]		Unpubl.	1(2)	*
c.1031C>T	Missense	p.Ser344Phe	X91[+]	A0467 A0468	[1,17,44] unpubl.	3(4)	
c.1032delC	Deletion	p.Ala345ProfsX41	X91[+]	A0324 A0325	[1]	2(2)	
c.1038delT	Deletion	p.Glu347ArgfsX39	X91[+]	A0129 A130 A0161 A0318	[1,12,19,54] unpubl.	5(6)	
c.1046delA	Deletion	p.Asp349AlafsX37	X91[?]		Unpubl.	1(1)	*
c.1061A>C	Missense	p.His354Pro	X91[+]		Unpubl.	2(2)	*
c.1061A>G	Missense	p.His354Arg	X91[−]	A0399	Unpubl.	1(2)	*
c.1062_1071del10	Deletion	p.His354GlnfsX29	X91[+]	A0207	[1,11]	1(1)	
c.1063delA	Deletion	p.Ile355SerfsX31	X91[+]		[23]	1(1)	*
c.1063_1070delATCCGCAT	Deletion	p.Ile355ArgfsX15	X91[?]		Unpubl.	1(1)	*
c.1067G>C	Missense	p.Arg356Pro	X91[?]	A0454	[12]	1(1)	*
c.1075G>A	Missense	p.Gly359Arg	X91[+]	A0056 A0378	[1,11,23] unpubl.	3(4)	
c.1076G>T	Missense	p.Gly359Val	X91[?]	A0379	[1]	1(1)	
c.1076G>C	Missense	p.Gly359Ala	X91[−]	A0611	[20]	1(1)	*
c.1081T>C	Missense	p.Trp361Arg	X91[+]		[22] unpubl.	3(3)	*
c.1082G>A	Nonsense	p.Trp361X	X91[?]		Unpubl.	1(1)	*
c.1083G>A	Nonsense	p.Trp361X	X91[+]	A0484	[1,23,28]	3(3)	
c.1085C>T	Missense	p.Thr362Ile	X91[+]		Unpubl.	2(3)	*
c.1085C>G	Missense	p.Thr362Arg	X91[?]		Unpubl.	1(1)	*
c.1094T>C	Missense	p.Leu365Pro	X91[+]	A0405	[1] unpubl.	2(2)	
c.1094dupT	Insertion	p. Phe366ValfsX7	X91[+]		Unpubl.	1(1)	*
c.1095delG	Deletion	p.Phe366SerfsX20	X91[?]		Unpubl.	1(1)	*
c.1105T>C	Missense	p.Cys369Arg	X91[+] het[d]	A0257	[1,11,88]	1(1)	
c.1120C>T	Nonsense	p.Gln374X	X91[+]		Unpubl.	2(2)	*
c.1123G>T	Nonsense	p.Glu375X	X91[+]	A0610	[20] unpubl.	3(3)	*
c.1129C>T	Nonsense	p.Gln377X	X91[+]	A0293	[1,11]	1(1)	
c.1136dupC	Insertion	p.Trp380ValfsX5	X91[?]		Unpubl.	1(1)	*
c.1139G>A	Nonsense	p.Trp380X	X91[+]	A0086 A0174	[1,12,52] unpubl.	3(3)	
c.1140G>A	Nonsense	p.Trp380X	X91[+]		Unpubl.	3(3)	*
c.1144_1145insAGGT	Insertion	p.Leu382GlnfsX4	het[d]		Unpubl.	1(1)	*
c.1147_1150delCCTA	Deletion	p.Pro383ArgfsX2	X91[?]	A0315	[1]	1(1)	
c.1150_1151+2delAAGT[c]	Splice site	del exon 9? p.Val300AspfsX4?	X91[+]	A0156	Unpubl.	9(9)	
c.1151+4A>T[c]	Splice site	del exon 9? p.Val300AspfsX4?	X91[?]		Unpubl.	1(1)	*
c.1151+5G>A[c]	Splice site	del exon 9? p.Val300AspfsX4	X91[+]	A0525	[1] unpubl.	2(2)	
c.1152−11T>G[c]	Splice site	del exon 10/ins10 into exon 10> pAla488PhefsX12	X91[−]		[1,26]	1(2)	
c.1152−2A>G[c]	Splice site	del exon 10? p.Ile385SerfsX63?	X91[?]	A0580	[38]	1(1)	*
c.1152−2A>T[c]	Splice site	del exon 10? p.Ile385SerfsX63?	X91[+]		Unpubl.	2(2)	*
c.1152−1G>A[c]	Splice site	alt splice site>1 nt del p.Ile385X	X91[+]		Unpubl.	2(2)	*
c.1154T>G	Missense	p.Ile385Arg	X91[+]		Unpubl.	1(1)	*
c.1163delA	Deletion	p.Asp388ValfsX17	X91[?]		Unpubl.	1(1)	*
c.1165G>A	Missense	p.Gly389Arg	X91[?]		Unpubl.	1(1)	*
c.1166G>A	Missense	p.Gly389Glu	X91[+]	A0380	[1,17] unpubl.	2(2)	
c.1166G>C	Missense	p.Gly389Ala	X91[−]	A0004	[1,11,49,51]	1(1)	
c.1166G>T	Missense	p.Gly389Val	X91[?]		Unpubl.	1(1)	*
c.1166_1170del insTGTTCAGC	Deletion/insertion	p.Gly389_Pro390del insValPheSer	X91[?]		Unpubl.	1(1)	*

(continued on next page)

Table 1 (*continued*)

cDNA nucleotide (or splice site) change	Mutation	Amino acid change	CGD type	Accession number	Ref.	Kindred (patients)[a]	
c.1167delG	Deletion	p.Phe391LeufsX14	X91[?]		Unpubl.	1(1)	*
c.1169C>T	Missense	p.Pro390Leu	X91[+]	A0258 A0259	[1,11] unpubl.	2(3)	
c.1177delA	Deletion	p.Thr393LeufsX12	X91[?]		Unpubl.	1(1)	*
c.1180_1182delGCC/ ins ATGTGATGAACACAT	Deletion/insertion	p.Ala394MetX2	X91[+]	A0072 A0073	[1,89]	1(2)	
c.1186_1195del10	Deletion	p.Glu396SerfsX6	X91?		Unpubl.	1(1)	*
c.1190_1191delAT	Deletion	p.Asp397GlyfsX5	X91[+]	A0213	[1,11]	1(1)	
c.1214T>G	Missense	p.Met405Arg	X91[+]	A0142	[1,12,19]	1(1)	
c.1222G>A	Missense	p.Gly408Arg	X91[?]	A0157 A0158	[1,12,19]	2(3)	
c.1222G>C	Missense	p.Gly408Arg	X91[+]		[90]	1(1)	*
c.1223G>A	Missense	p.Gly408Glu	X91[+] 1 het[d]	A0013 A0016 A0190 A0191	[1,11,12,19,88]	3(4)	
c.1234G>A	Missense	p.Gly412Arg	X91[?]		Unpubl.	1(1)	*
c.1234G>C	Missense	p.Gly412Arg	X91[+]		Unpubl.	1(2)	*
c.1235G>A	Missense	p.Gly412Glu	X91[?]		Unpubl.	1(1)	*
c.1234_1257dup24	Insertion	p.Gly412Ile419dup	X91[?]		[1]	1(1)	
c.1237dupG	Insertion	p.Val413GlyfsX18	X91[+] 1 het[d]	A0144	[1,12,19] unpubl.	2(2)	
c.1244C>A	Missense	p.Pro415His	X91[+]	A0048 A049 A0076 A077 A0107	[1,11,12,91]	3(5)	
c.1244C>G	Missense	p.Pro415Arg	X91[?]	A0419	[1]	1(1)	
c.1244C>T	Missense	p.Pro415Leu	X91[+]	A0112	[1,3,12,19] unpubl.	3(3)	
c.1253C>A	Missense	p.Ser418Tyr	X91[+]		Unpubl.	1(1)	*
c.1255dupA	Insertion	p.Ile419AsnfsX12	X91[?]	A0574	[38]	1(1)	*
c.1259T>C	Missense	p.Leu420Pro	X91[+]	A0406	[1,17,64]	1(1)	
c.1264T>C	Missense	p.Ser422Pro	X91[?]	A0202	[1,12,19]	1(1)	
c.1265C>A[f]	Nonsense	p.Ser422X	X91[?]		[23]	1(1)	*
c.1265_1273delTCAGTCTGG	Deletion	p.Ser422_Trp424del	X91[−]		Unpubl.	1(1)	*
c.1271G>A	Nonsense	p.Trp424X	het[d]		Unpubl.	1(1)	*
c.1272delG	Deletion	p.Trp424CysfsX11	X91[+]	A0330	[1,12]	1(1)	
c.1272G>A	Nonsense	p.Trp424X	X91[+]	A0622 A0623	[53] unpubl.	4(4)	*
c.1275C>G	Nonsense	p.Tyr425X	X91[?]	A0496	[1]	1(1)	
c.1281T>G	Nonsense	p.Tyr427X	X91[?]		Unpubl.	1(1)	*
c.1284C>A	Nonsense	p.Cys428X	X91[+]	A0598	Unpubl.	1(1)	*
c.[1287delT;1290delC]	Deletion	p.Asn429LysfsX23	X91[+]	A0617	[20]	1(1)	*
c.1294delA	Deletion	p.Thr432ProfsX3	X91[+]		Unpubl.	1(1)	*
c.1309A>T	Nonsense	p.Lys437X	X91[+]	A0403	[1,17] unpubl.	2(2)	
c.1313delA[c]	Deletion Splice site?	p.Lys438ArgfsX64	X91[+]	A0053 A0054	[1,11] unpubl.	2(3)	
c.1313_1314delAGinsT[c]	Deletion/insertion Splice site?	p.Lys438IlefsX64	X91[+]	A0091	[1,12,19]	1(1)	
c.1314delG[c]	Deletion Splice site?	p.Ile439SerfsX63	X91[?]		Unpubl.	2(2)	*
c.1314+1G>A[c]	Splice site	del exon 10? p.Ile385SerfsX63?	X91[?] 1 het[d]	A0526 A0579	[1,12,38]	2(2)	
c.1314+1G>T[c]	Splice site	del exon 10 p.Ile385SerfsX63	X91[?]	A0529	[1]	1(1)	
c.1314+1G>C[c]	Splice site	del exon 10 p.Ile385SerfsX63	X91[+]		Unpubl.	1(1)	*
c.1314+2T>A[c]	Splice site	del exon 10 p.Ile385SerfsX63	X91[+]	A0527	[1]	1(1)	
c.1314+2T>G[c]	Splice site	del exon 10 p.Ile385SerfsX63	het[d]		Unpubl.	1(1)	*
c.1314+4_+5AG>GC[c]	Splice site	del exon 10 p.Ile385SerfsX63	X91[−]		Unpubl.	1(1)	*
c.1315−2A>C[c]	Splice site	del exon 11? p.Ile439_Gln487del?	X91[?]		Unpubl.	2(2)	*
c.1315−1G>C[c]	Splice site	del exon 11 p.Ile439_Gln487del	X91[+]	A0528	[1,12]	1(1)	
c.1315−1G>T[c]	Splice site	del. exon 11? p.Ile439_Gln487del?	X91[?]		Unpubl.	1(1)	*
c.1315delA[c]	Deletion Splice site?	p.Ile439SerfsX63	X91[+]	A0081	[1,19,25]	1(1)	
c.1320C>A	Nonsense	p.Tyr440X	X91[?]	A0151	[1,12,52]	1(1)	
c.1320C>G	Nonsense	p.Tyr440X	X91[?]		Unpubl.	1(1)	*
c.1326C>G	Nonsense	p.Tyr442X	X91[+]	A0589	[27]	1(1)	*

Table 1 (*continued*)

cDNA nucleotide (or splice site) change	Mutation	Amino acid change	CGD type	Accession number	Ref.	Kindred (patients)[a]	
c.1327delT	Deletion	p.Trp443GlyfsX59	X91$^{?}$	A0630	[33]	1(1)	*
c.1329G>A	Nonsense	p.Trp443X	X91*	A0168	[1,12,52]	2(2)	
				A0485			
c.1333T>C	Missense	p.Cys445Arg	X91$^{?}$	A0605	Unpubl.	1(1)	*
c.1335C>A	Nonsense	p.Cys445X	X91*	A0361	[1]	2(3)	
					unpubl.		
c.1340delA	Deletion	p.Asp447AlafsX55	X91*		[23]	1(1)	*
c.1348_1352delGCCTT	Deletion	p.Ala450X	X91$^{?}$	A0308	[1]	1(1)	
c.1350delC	Deletion	p.Phe451LeufsX51	X91$^{?}$	A0192	[1,12,19]	1(1)	
c.1354G>T	Nonsense	p.Glu452X	X91*	A0373	[1,17]	1(1)	
c.1357T>C	Missense	p.Trp453Arg	X91*	A0486	[1,12,23]	3(3)	
				A0487	unpubl.		
				Kuhns unp			
c.1357T>A	Missense	p.Trp453Arg	X91*	A0613	[20]	1(2)	*
c.1357_1358delTG	Deletion	p.Trp453ValfsX32	X91*		Unpubl.	1(1)	*
c.1358G>A	Nonsense	p.Trp453X	X91*	A0488	[1,12,19]	1(1)	
c.1359G>A	Nonsense	p.Trp453X	X91*	A0489	[1]	2(3)	
					unpubl.		
c.1363_1375del13	Deletion	p.Ala455AsnfsX43	X91*		Unpubl.	1(1)	*
c.1375C>T	Nonsense	p.Gln459X	X91*		Nunoi unp	1(1)	*
c.1382delT	Deletion	p.Leu461ArgfsX41	X91$^{?}$		Unpubl.	1(1)	*
c.1384G>T	Nonsense	p.Glu462X[b]	X91*	A0374	[39,72]	2(2)	*
c.1396C>T	Nonsense	p.Gln466X	X91*	A0426	[1,17]	2(2)	
					unpubl.		
c.1399G>T	Nonsense	p.Glu467X	X91^{-}		Unpubl.	1(2)	*
c.1407−1414del8/ins TGTTGTA[f]	Deletion/insertion	p.Asn470ValfsX14	X91*		[1]	1(2)	
					unpubl.		
c.1415delG	Deletion	p.Gly472AlafsX30	X91*	A0092	[1,12,19]	1(2)	
				A0493			
c.1421T>G	Missense	p.Leu474Arg	X91^{-}		Unpubl.	1(1)	*
c.1428C>A	Nonsense	p.Tyr476X	X91*	A0071	[1,11,17,44,86]	1(1)	
c.1437C>A	Nonsense	p.Tyr479X	X91$^{?}$	A0631	[33]	3(3)	*
					unpubl.		
c.1441A>C	Missense	p.Thr481Pro	X91^{+}		Unpubl.	1(1)	*
c.1447_1459del13	Deletion	p.Trp483ArgfsX15	X91*	A0331	[1,17]	1(1)	
c.1448G>A	Nonsense	p.Trp483X	X91*		Unpubl.	3(3)	*
c.1449G>A	Nonsense	p.Trp483X	X91*	A0294	[1,19]	1(1)	
c.1455delG	Deletion	p.Glu485AspfsX17	X91$^{?}$	A0165	[1,12,19]	1(1)	
c.1456dupT	Insertion	p.Ser486PhefsX11	X91*	A0184	[1,12,19]	2(3)	
				A0185	unpubl.		
c.1461+1G>A[c]	Splice site	del exon 11?	X91$^{?}$	A0241	[1,19]	2(2)	
		p.Ile439_Gln487del?			unpubl.		
c.1461+1G>T[c]	Splice site	del exon 11	X91*	A0530	[1,24]	3(4)	
		p.Ile439_Gln487del		A0560	unpubl.		
c.1461+2delT[c]	Splice site	del exon 11?	X91$^{?}$		[29]	1(1)	*
		p.Ile439_Gln487del?					
c.1461+668_1462−807del1558	Deletion	intronic deletion; no phenotype	het[d,j]		[68]	1(1)	*
c.1462−809_1586+819 del1753ins12	Deletion/insertion	del exon 12	X91*		[1,68]	1(1)	
		p.Ala488TyrfsX11					
c.1462−2A>G[c]	Splice site	partial del exon 12	X91^{+}	A0078	[1,92]	3(4)	
		p.Ala488_Glu497del		A0104	unpubl.		
				A0531			
c.1462−2A>C[c]	Splice site	(partial) del exon 12?	X91^{+}		Unpubl.	1(1)	*
		p.Ala488_Glu497del?					
c.1462−1G>A[c]	Splice site	altern. splicing exon 12	X91*	A0532	[1]	1(1)	
		G del exon 12>p.Ala488ProfsX14					
c.1462−?[c]	Splice site	del exon 12	X91*		[1,17]	1(1)	
		p.Ala488TyrfsX11					
c.1464delC	Deletion	p.Asn489IlefsX13	X91*	A0114	[1,12,19]	1(1)	
c.1484A>C	Missense	p.His495Pro	X91^{-}		Unpubl.	1(2)	*
c.1488_1490delTGA	Deletion	p.Asp496del	X91^{+}		Unpubl.	1(1)	*
c.1497delA	Deletion	p.Asp500MetfsX2	X91*	A0312	[1,17]	1(1)	
c.1498G>A	Missense	p.Asp500Asn	X91^{-}	A0366	Unpubl.	1(3)	*
c.1498G>C	Missense	p.Asp500His	X91$^{?}$	A0632	[23,33]	2(2)	*
c.1498G>T	Missense	p.Asp500Tyr	X91^{+}	A0367	[23,56]	2(3)	*
c.1499A>G	Missense	p.Asp500Gly	X91^{+}	A0019	[1,93]	1(1)	
c.1500T>G	Missense	p.Asp500Glu	X91^{+}		[23]	1(1)	*
c.1509delA	Deletion	p.Gly504AlafsX2	X91*		[23] unpubl.	2(2)	*
c.1514T>G	Missense	p.Leu505Arg	X91^{+}	A0408	[35,39]	3(3)	*
				A0573	unpubl.		
c.1514T>C	Missense	p.Leu505Pro	X91^{-}	A0407	[1]	1(2)	
c.1515_1525del11	Deletion	p.Lys506PhefsX9	X91$^{?}$		Unpubl.	1(1)	*
c.1519C>T	Nonsense	p.Gln507X	X91*	A0116	[1,12,52]	2(5)	
c.1521_1525delAAAGA/ ins CATCTGGG	Deletion/insertion	p.Gln507_Thr509del/ insHisIleTrpAla	X91^{+}	A0068	[1,94]	1(1)	

(*continued on next page*)

Table 1 (*continued*)

cDNA nucleotide (or splice site) change	Mutation	Amino acid change	CGD type	Accession number	Ref.	Kindred (patients)[a]	
c.1522_1523delAA	Deletion	p.Lys508AspfsX10	X91$^{?}$	A0313	[1]	1(1)	
c.1523delA	Deletion	p.Lys508ArgfsX25	X91^{o}	A0314	[1,23]	2(3)	
c.1524_1527delGACT	Deletion	p.Lys508AsnfsX24	X91$^{?}$		Unpubl.	1(1)	*
c.1528_1529delTT	Deletion	p.Leu510ValfsX8	X91^{o}	A0143	[1,19] unpubl.	3(3)	
c.1532_1538delATGGACGinsTTCA	Deletion/insertion	p.Tyr511_Arg513del/insPheGln	X91$^{?}$	A0645	[95]	1(1)	*
c.1533T>A	Nonsense	p.Tyr511X	X91^{o}		[23]	1(1)	*
c.1546T>A	Missense	p.Trp516Arg	X91$^{?}$		[23]	1(1)	*
c.1546T>C	Missense	p.Trp516Arg	X91^{o}	A0494	[1,64]	1(1)	
c.1547G>A	Nonsense	p.Trp516X	X91^{o}	A0570	[35] unpubl.	3(3)	*
c.1548G>T	Missense	p.Trp516Cys	X91$^{?}$	A0094	[1,12,19]	1(1)	
c.1548G>A	Nonsense	p.Trp516X	X91^{o}	A0295 A0296	[1,19] unpubl.	3(3)	
c.1549delG	Deletion	p.Asp517IlefsX16	X91^{o}		Unpubl.	1(1)	*
c.1555G>T	Nonsense	p.Glu519X	X91^{o}	A0059	[1,33,55]	1(1)	
c.1561A>T	Nonsense	p.Lys521X	X91^{o}	A0025	[1,11]	1(1)	
c.1565delC	Deletion	p.Thr522LysfsX11	X91^{o}	A0326	[1,12,34]	1(1)	
c.1570_1586+?del	Deletion	p.Ala524X	X91^{o}		Unpubl.	1(1)	*
c.1571C>T	Missense	p.Ala524Val	X91^{o}		[96] unpubl.	2(2)	*
c.1578delA	Deletion	p.Gln526HisfsX7	X91^{o}	A0319	[56]	1(1)	*
c.1579dupC	Insertion	p.His527ProfsX3	X91^{o}		[23]	1(1)	*
c.1585_1586+9del11[c]	Splice site	del 17 from 3′ exon 12 p.Ala524TyrfsX11	X91^{-}	A0572	[35]	1(1)	*
c.1586+1G>C[c]	Splice site	del exon 12? p.Ala488TyrfsX11?	X91$^{?}$		Unpubl.	1(1)	*
c.1586+3A>T[c]	Splice site	del exon 12? p.Ala488TyrfsX11?	X91$^{?}$		Unpubl.	1(1)	*
c.1587−2A>G[c]	Splice site	altern. splicing G insert exon 13>p.Asn529LysfsX12	X91^{o}	A0533	[1,17,61] unpubl.	3(4)	
c.1598_1600delGAG	Deletion	p.Gly533del	X91^{-}		[28] unpubl.	2(2)	*
c.1600_1614del15	Deletion	p.Val534_Gly538del	X91^{-}	A0329	[1]	1(1)	
c.1601T>A	Missense	p.Val534Asp	X91$^{?}$	A0147	[1,12,19]	1(1)	
c.1603_1609delTTCCTCT	Deletion	p.Phe535ValfsX10	X91^{o}		Unpubl.	1(1)	*
c.1607dupT	Insertion	p.Cys537LeufsX4	X91$^{?}$	A0345	[1]	1(1)	
c.1609T>C	Missense	p.Cys537Arg	X91^{+}	A0199 A0545	[1,12,19,41] unpubl.	2(2)	
c.1611_1612delTG	Deletion	p.Cys537TrpfsX3	X91^{o}		[23]	1(1)	*
c.1618delG	Deletion	p.Glu540LysfsX7	X91^{o}	A0537	[97] unpubl.	2(3)	*
c.1622_1625dupCCTT	Insertion	p.Leu542PhefsX4	X91^{o}	A0183	[1,12,19]	1(1)	
c.1625T>C	Missense	p.Leu542Ser	X91^{o}	A0082	[1,19,26]	1(1)	
c.1637T>C	Missense	p.Leu546Pro	X91^{+}	A0409	[1,26]	1(1)	
c.1637T>G	Missense	p.Leu546Arg	X91^{-}		Unpubl.	1(1)	*
c.1642A>T	Nonsense	p.Lys548X	X91$^{?}$		Unpubl.	1(1)	*
c.1645C>T	Nonsense	p.Gln549X	X91$^{?}$	A0297	[1,19]	1(1)	
c.1658delA	Deletion	p.Ser554LeufsX24	X91$^{?}$		Unpubl.	1(1)	*
c.1661_1662delCT	Deletion	p.Ser554X	X91$^{?}$		[29]	1(1)	*
c.1662dupT	Insertion	p.Glu555X	X91^{o}	A0302 A0348 A0556	[1,19,24] unpubl.	3(5)	
c.1662_1663insGT	Insertion	p.Glu555ValfsX23	X91$^{?}$		Unpubl.	1(1)	*
c.1663_1693dup31	Insertion	p.Phe565X	X91$^{?}$	A0300	[1,39,79]	2(2)	
c.1678G>T	Nonsense	p.Gly560X	X91$^{?}$		Unpubl.	1(1)	*
c.1679delG	Deletion	p.Gly560GlufsX17	X91^{o}	A0307	[77,80,98]	3(3)	*
c.1682−1712del31	Deletion	p.Val561AspfsX6	X91^{o}	A0155	[1,12,19] unpubl.	2(2)	
c.1694dupT	Insertion	p.Asn566GlnfsX28	X91^{-}		Unpubl.	1(1)	*
c.1702G>A	Missense	p.Glu568Lys	X91^{+}	A0259	[1,19,88]	1(1)	

Acc. #, accession number in the X-CGD database (see text); *mutation added since last tabulation; unpubl., not previously published; ND, not determined; NA, not applicable; del, deletion; ins, insertion; dup, duplication; bp, base pairs; AA, amino acids; w.t., wild type.

[a] Number of unrelated kindreds and (number of patients).

[b] These promoter mutations lead to loss of gp91-*phox* expression on neutrophils and monocytes, but normal expression on eosinophils [14–16,18].

[c] Position of introns in *CYBB*: intron 1 c.45_46; intron 2 c.141_142; intron 3 c.252_253; intron 4 c.337_338; intron 5 c.483_484; intron 6 c.674_675; intron 7 c.804_805; intron 8 c.897_898; intron 9 c.1151_1152; intron 10 c.1314_1315; intron 11 c.1461_1462; and intron 12 c.1586_1587.

[d] Female heterozygote patient or female heterozygote relative of a deceased patient.

[e] This patient has a *TMF1* retrogene insertion in *CYBB* intron 1, resulting in an extra exon between exons 1 and 2 in the *CYBB* mRNA. This extra exon contains TAG as the second codon (De Boer et al., unpublished).

[f] Corrected after consultation of the author.

[g] Due to insertion of a LINE-1 element [60,70].

[h] Two patients with c.388C>T (Kuhns et al., unpubl.), one patient with c.667G>T and one patient with c.1384G>T [72] have somatic mosaicisms of cells with the mutated *CYBB* sequence and a small proportion of reverse mutated cells with the wild-type *CYBB* sequence.

[i] Due to unequal crossing over between a GT repetition at the 3′ region of intron 5 and a GT repetition at the 5′ region of intron 8 (Van Leeuwen, Stasia, et al., unpublished).

[j] This woman is a triple mosaic carrier of two different mutations and the wild-type of *CYBB* [68].

Table 2
Large (≥1 exon) deletions in the *CYBB* region known to cause X-linked CGD.

Approximate size of deletion (associated disease)	Affected exon(s)	CGD type	Acc. #	Ref	Kindred (patients)	
~6000 kb (+DMD, McLeod)	NA	X91°		Unpubl.	1(1)	*
~5650 kb (+DMD, RP, McLeod)	NA	X91°		[99]	1(1)	*
~5000 kb (+DMD, RP, McLeod)	NA	X91°	A0030	[1,100]	1(1)	
~4000 kb (+DMD, McLeod)	NA	X91°	A0031	[1,101]	1(1)	
~3900 kb (+OTC, RP, McLeod)	NA	X91?		[102]	1(1)	*
~3500 kb (+OTC, McLeod)	NA	X91°		Unpubl.	1(1)	*
913 kb	del promoter_exon 1	X91°		[1,12]	1(1)	
~800 kb (+McLeod)	NA	X91°	A0032	[1,103]	1(1)	
~800 kb (+McLeod)	NA	X91°		Unpubl.	1(1)	*
~550 kb (+McLeod)	NA	X91°		[20]	1(1)	*
~500 kb (+RP, McLeod)	NA	X91°		[1,104]	1(1)	
~500 kb (+RP, McLeod)	NA	X91°	A0066	[1,17]	1(1)	
~500 kb	NA	X91°		[20]	1(1)	*
ND (+RP, McLeod)	NA	X91°	A0033	[1,105]	1(1)	
~450 kb (+McLeod)	NA	X91°		Unpubl.	3(4)[a]	*
ND (+McLeod)	NA	X91°		Unpubl.	1(1)	*
ND (+McLeod)	NA	X91°		[29]	1(1)	*
ND (+McLeod)	NA	X91°		[29]	1(1)	*
ND (+McLeod)	del exons 1_13	X91?		[106]	1(1)	*
ND (+McLeod)	del exons 1_13	X91?		[106]	1(1)	*
ND (+McLeod)	del exons 1_13	X91?		[106]	1(1)	*
ND (+McLeod)	del exons 1_13	X91?		[106]	1(1)	*
~320 kb (+DMD, McLeod)	NA	X91°		[1,12]	1(1)	
~320 kb (+McLeod)	NA	X91°		Unpubl.	1(1)	*
>300 kb (+McLeod)	del exons 1_13	X91°		Unpubl.	1(1)	*
>300 kb (+McLeod)	del exons 1_13	X91°		Unpubl.	1(1)	*
>150 kb (+McLeod)	del exons 1_13	X91°		Unpubl.	1(1)	*
>150 kb (+McLeod)	del exons 1_13	X91°		Unpubl.	1(1)	*
>100 kb (+McLeod)	del exons 1_3	X91°		Unpubl.	1(1)	*
>100 kb (+McLeod)	del exons 1_13	X91°		[107]	1(3)	*
~100 kb	del exons 1_13	X91°		Unpubl.	1(1)	*
~80 kb	del exons 1_13	X91°		Unpubl.	1(2)	*
>60 kb (+McLeod)	del exons 1_3	X91?		[20]	1(2)	*
≫30 kb (+DMD+McLeod)	NA	X91°	A0119	[1,12]	1(1)	
>27 kb (+McLeod)	del. exons 1_13	X91o	A0035	[1,11,19]	1(1)	
>27 kb	del exons 1_13[a]	X91°	A0034	[1,11,12,20,23,29,54]	30[a](32)	
			A0036	unpubl.		
			A0037			
			A0038[b]			
			A0119			
			A0153			
			A0167			
			A0169			
			A0201			
25 kb	del promoter_exon 7	X91°		[1,17,108,109]	1(1)	
>20 kb	del exons 1_10	X91°	A0039[b]	[1,11]	1(1)	
>20 kb	del exons 1_10	X91°		Unpubl.	1(1)	*
ND	del promoter_exon 4	X91°		Unpubl.	1(1)	*
~19 kb	del exons 6_13	X91°		[1]	1(3)	
>15 kb	del exons 4_13	X91°	A0040	[1,19]	1(1)	
~14 kb	del exons 4_9	X91°	A0026	[1,34]	1(1)	
>13 kb	del exons 6_13	X91°	A0041	[1,110]	1(2)	
>13 kb	del exons 6_13	X91°		Unpubl.	1(1)	*
ND	del exons 7_13	X91?		Unpubl.	1(1)	*
>10 kb	del exons 8_13	X91°	A0042	[1,11]	1(1)	
>10 kb	del exons 8_13	X91°		[1]	1(1)	
~10 kb	del exons 7_12	X91° (het[b])		Unpubl.	1(1)	*
~10 kb	del exons 7_11	X91°		[1]	1(1)	
>9 kb	del exons 9_13	X91°		[1]	1(1)	
ND	del exons 6_8	X91°		[29]	1(1)	*
ND	del. exons 4_6	X91?		Unpubl.	1(1)	*
7 kb	del exons 3_4	X91°		[1,17]	1(1)	
>6.5 kb	del exons 11_13	X91°	A0043	[1,11]	1(1)	
~6 kb	del exons 12_13	X91°	A0044	[1,67]	1(1)	
>5.3 kb	del exons 1_3	X91°	A0204	[1,19]	1(1)	
ND	del exons 1_3	X91°		[56]	1(2)	*
~4.3 kb	del exons 11_13	X91°	A0172	[1,52]	1(2)	
			A0173			
ND	del. exons 11–13	X91?		Unpubl.	1(1)	*
ND	del exons 7_8	X91°		Unpubl.	1(1)	*
~3.5 kb	del exons 6_7	X91°	A0028[c]	[1,34,111]	1(1)	
~3.2 kb	del exon 6	X91°		[1]	1(1)	
~3.2 kb	del exon 7	X91?	A0205	[1,11]	1(1)	
~3 kb	del exon 5	X91°	A0027[c]	[1,34,111]	1(1)	

(continued on next page)

Table 2 (*continued*)

Approximate size of deletion (associated disease)	Affected exon(s)	CGD type	Acc. #	Ref	Kindred (patients)	
~3 kb	del exon 7	X91$^{?}$		Unpubl.	1(2)	*
~2.2 kb	del exon 5	X91*	A0206	[1,19]	1(1)	
~2 kb	del exon 3	X91*		[1]	1(1)	
~2 kb	del exon 7	X91*		[23]	1(1)	*
ND	del exon 3	X91$^{?}$		Unpubl.	1(1)	*
~2 kb	del promoter_exon 1	X91*		[1]	1(1)	
ND	del promoter_exon 1	X91$^{?}$		[23]	1(1)	*
~2 kb	del exon 8	X91*		[1] unpubl.	2(2)[a]	
2 kb	del exons 12_13	X91*		[1,17]	1(1)	
~2 kb	del. exon 7	X91*		[23]	1(1)	*
ND	del. exon 7	X91$^{?}$		Unpubl.	1(1)	*
~1.1 kb	del exon 6	X91$^{?}$		[23]	1(2)	*
~1 kb	del intron 12_ 3′UTR	X91*	A0203	[1,101] unpubl.	1(1)	
ND	del exon 9	X91$^{?}$		Unpubl.	1(1)	*
ND	del. exon 9	X91$^{?}$		Unpubl.	1(1)	*
0.35 kb	del exon 3	X91*		[1]	1(2)	
0.22 kb	del promoter	X91*		[1]	1(1)	

DMD, Duchenne muscular dystrophy; RP, X-linked retinitis pigmentosa; OTC, ornithine transcarbamylase deficiency; McLeod, McLeod hemolytic anemia; 3′UTR, 3′ untranslated region.

[a] These mutations are not necessarily identical.

[b] Patients A0038 and A0039 are brothers with different deletions.

[c] Patients A0027 and A0028 are brothers with different deletions; their mother has both mutations and the wild-type *CYBB* sequence (triple mosaic) [34,111].

Table 3
Known polymorphisms in the *CYBB* gene.

Nucleotide change	Effect	Approximate frequency
c.−270C/A	N.A.	Unknown [112]
c.141+48C/G	N.A.	Unknown (Maddalena, unpubl.)
c.142−12C/T	N.A.	Unknown (internet, unpubl.)
c.484−60delT	N.A.	Unknown (Jianxin He, unpubl.)
c.484−4G/A	Splice	Unknown [1]
c.654C/A	Silent (p.Gly218)	2% A in sub-Sahara Africans (internet, unpubl.)
c.804+118A/G	N.A.	Unknown (Maddalena, unpubl.)
c.1002G/A	Silent (p.Lys334)	4% A in sub-Sahara Africans (internet, unpubl.) [1]
c.1090G/C	p.364Gly/Arg	Unknown [1,113]
c.1414G/A	p.472Gly/Ser	2% A in Asians (internet, unpubl.)
c.1551T/A	p.517Asp/Glu	Unknown (Hill, unpubl.) [1]
c.1581C/T	Silent (p.His527)	Unknown (Di Matteo, unpubl.)

Table 4
Total number of kindreds with X-CGD patients, total number of X-CGD patients, total number of different mutations and total number of mutations unique for one kindred.

	Kindreds	Mutations
Deletions	281 (22.2%)	242 (35.6%)
Insertions	89 (7.0%)	54 (7.9%)
Deletion/insertions	19 (1.5%)	19 (2.8%)
Splice site mutations	247 (19.5%)	120 (17.6%) (2 undefined)
Missense mutations	246 (19.4%)	145 (21.3%)
Nonsense mutations	377 (29.8%)	96 (14.1%)
Promoter mutations	8 (0.6%)	5 (0.7%)
	Total 1267 unrelated kindreds with 1415 patients	Total 681 different mutations in the patients (all large deletions considered different). Of these 681 mutations, 498 (73.1%) are unique for one kindred.

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A Phase I Study of Aromatic L-Amino Acid Decarboxylase Gene Therapy for Parkinson's Disease

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Gene transfer of dopamine-synthesizing enzymes into the striatal neurons has led to behavioral recovery in animal models of Parkinson's disease (PD). We evaluated the safety, tolerability, and potential efficacy of adeno-associated virus (AAV) vector–mediated gene delivery of aromatic L-amino acid decarboxylase (AADC) into the putamen of PD patients. Six PD patients were evaluated at baseline and at 6 months, using multiple measures, including the Unified Parkinson's Disease Rating Scale (UPDRS), motor state diaries, and positron emission tomography (PET) with 6-[^{18}F]fluoro-L-*m*-tyrosine (FMT), a tracer for AADC. The short-duration response to levodopa was measured in three patients. The procedure was well tolerated. Six months after surgery, motor functions in the OFF-medication state improved an average of 46% based on the UPDRS scores, without apparent changes in the short-duration response to levodopa. PET revealed a 56% increase in FMT activity, which persisted up to 96 weeks. Our findings provide class IV evidence regarding the safety and efficacy of AADC gene therapy and warrant further evaluation in a randomized, controlled, phase 2 setting.

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INTRODUCTION

Dopamine replacement has been the standard pharmacotherapy for motor impairment in Parkinson's disease (PD). Although virtually all patients benefit from levodopa at an early stage of the disease, severe loss of nigrostriatal nerve terminals in advanced PD leads to profoundly decreased activities of dopamine-synthesizing enzymes, including aromatic L-amino acid decarboxylase (AADC), an essential enzyme that converts levodopa to dopamine. Failure to respond to levodopa therapy may result from a reduction in AADC activity, decreased dopamine storage capacity in synaptic vesicles, postsynaptic changes in striatal output neurons, and abnormalities of nondopaminergic neurotransmitter systems.[1,2] Systemic administration of high-dose levodopa enhances oscillations in motor performance and complications, including hallucinations, due to dopaminergic stimulation of the mesolimbic system.

One potential treatment for advanced PD is gene therapy to restore striatum-selective dopamine production. In addition to AADC, tyrosine hydroxylase, which converts L-tyrosine to levodopa, and guanosine triphosphate cyclohydrolase I, which catalyzes biosynthesis of the essential tyrosine hydroxylase cofactor, tetrahydrobiopterine, are necessary for efficient synthesis of dopamine.[2] Viral vector–mediated gene transfer of these dopamine-synthesizing enzymes has been shown to achieve behavioral recovery in animal PD models, with efficient transduction of striatal neurons that escape degeneration.[3–6] When tyrosine hydroxylase and guanosine triphosphate cyclohydrolase I are expressed in the striatum, levodopa can be synthesized continuously. This strategy would be useful for reducing motor fluctuations associated with intermittent levodopa intake. Gene transfer of AADC alone in combination with oral levodopa administration would be a safer strategy for initial clinical trials. In the latter approach, the patients still need to take levodopa to control motor symptoms, but excess production of dopamine could be avoided by reducing the dose of levodopa. We assessed the safety, tolerability, and the potential efficacy of intraputaminal infusion of recombinant adeno-associated virus (AAV) serotype 2 vector encoding human AADC (AAV-hAADC-2) in patients with mid- to late-stage PD. We also examined whether the short-duration response to levodopa, the antiparkinsonian response that parallels the plasma levodopa levels, would change after gene therapy.[7]

RESULTS

Patient disposition and baseline characteristics

Six patients (4 men, 2 women), mean age 60 (range, 51–68) years, were enrolled (**Table 1**). The mean disease duration was 10 (range, 5–18) years, and time on levodopa was 9.3 (range, 5–15) years. The average baseline daily levodopa and levodopa equivalent doses were 642 and 808 mg, respectively.

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Table 1 Patients' baseline characteristics

Subject	Age (years)	Sex	Disease duration (years)	Time on levodopa (years)	Levodopa dose (mg)	Levodopa equivalents (mg)
A-1	51	M	11	9	600	900
A-2	63	M	9	9	450	650
A-3	66	F	7	7	500	700
A-4	58	M	11	11	700	700
A-5	68	F	18	15	1,000	1,100
A-6	56	M	5	5	600	800
Mean (SD)	60 (6.5)	67% M	10 (4.5)	9.3 (3.4)	642 (196)	808 (169)

Abbreviations: F, female; M, male.
Patients are listed in the order in which they received treatment. Levodopa equivalents were estimated as follows: 100 mg of levodopa with a dopa-decarboxylase inhibitor is equivalent to 0.8 mg talipexole, 1 mg pergolide, 1 mg pramipexole, and 1.5 mg cabergoline.

Primary end point

The procedure was well tolerated. All patients completed all protocol-defined visits. One patient (patient A-2) had a venous hemorrhage in the right frontal lobe just below a burr hole that was found on CT scan 3 days after infusion. The patient used his left arm less frequently than his right arm for 3 weeks; this was assumed to reflect mild frontal lobe dysfunction and resolved completely. Mild, transient headache around the burr holes was present for 2 days after surgery in all patients. There were no significant laboratory test abnormalities. All patients had mildly increased titers of anti-AAV2-neutralizing antibodies 6 months after treatment, which tended toward baseline concentrations thereafter (**Table 2**).

Table 2 Changes in neutralizing AAV2 antibody titers in sera following gene therapy

Subject	Pre	2 weeks	6 months	1 year
A-1	1:2	1:4	1:4	1:4
A-2	<1	1:32	1:4	1:2
A-3	1:32	1:64	1:64	1:32
A-4	1:32	1:32	1:256	1:64
A-5	1:4	1:32	1:32	1:32
A-6	<1	1:16	1:32	1:32

Abbreviations: AAV, adeno-associated virus.
Titers are determined by *in vitro* assay and represented as "1:" dilutions.

Clinical evaluations

The clinical results are summarized in **Table 3**. Intraputaminal AAV-hAADC-2 infusion significantly improved both total and motor scores of the unified Parkinson's disease rating scale (UPDRS) in the OFF state. Five of six patients showed substantial improvement in UPDRS motor ratings in the OFF state (**Figure 1**). Changes in the UPDRS ON state and the percent of ON state hours in a day were not significant. One patient with relatively mild motor symptoms at baseline did not improve on UPDRS (A-3 in **Figure 1**). However, this patient showed a remarkable increase in mobile time as measured by the diaries (28% at baseline to 58% at 6 months after gene transfer; **Figure 2**). The daily dose of levodopa was unchanged in two patients (A-2 and A-5) and reduced in three patients (A-1, A-3, and A-5) at 6 months. Patient A-6, who had daytime sleepiness, preferred to reduce pramipexole instead of levodopa after gene therapy.

The last three patients underwent the levodopa test after our institutional review board confirmed the safety of AADC gene transfer in the first three patients. The short-duration response to levodopa did not change significantly after gene therapy in these three patients, though UPDRS motor scores at 6 months showed slight improvement at 30 minutes in patient 5 and at 120 minutes in patient 4 after levodopa intake (**Figure 3**). Significantly higher peak plasma levodopa concentrations were observed in these two patients after gene therapy.

The mini-mental state examination (MMSE) and geriatric depression scale (GDS) scores did not change significantly.

Table 3 Clinical outcomes of six patients

	Baseline	6 months	*P* value
UPDRS Total OFF	53 (12.4)	38 (10.1)	0.049*
UPDRS Total ON	15 (7.2)	10.7 (2.9)	0.262
UPDRS Part III (Motor) OFF	25.3 (9.4)	13.7 (6.0)	0.024*
UPDRS Part III (Motor) ON	5.2 (4.6)	1.8 (1.5)	0.120
Percent day spent in mobile state	48.8 (12.9)	55.4 (14.8)	0.348
Daily levodopa equivalents dose, mg	808 (169)	707 (233)	0.097

Abbreviations: OFF, off-medication state; ON, on-medication state; UPDRS, Unified Parkinson's Disease Rating Scale.
Data are presented as means (SD). The UPDRS scores in each patient did not change during the 2 months of the screening period.
*$P < 0.05$.

PET analysis

PET imaging revealed increased 6-[^{18}F]fluoro-L-*m*-tyrosine (FMT), a tracer for AADC, activity 4 weeks postoperatively, which persisted at 6-month evaluation (**Figure 4**). The mean increase in FMT uptake from baseline in the combined (right and left) putamen at 24 weeks was 56%. Two patients (A-1 and A-2) who had PET scans 96 weeks after surgery showed persistently increased FMT uptake. In these two patients, motor performance in the OFF state also maintained its improvement at 96 weeks.

DISCUSSION

Extensive preclinical studies on both rodent and nonhuman primate models of PD have shown that AAV vectors can express exogenous genes for a long time in the brain target areas without

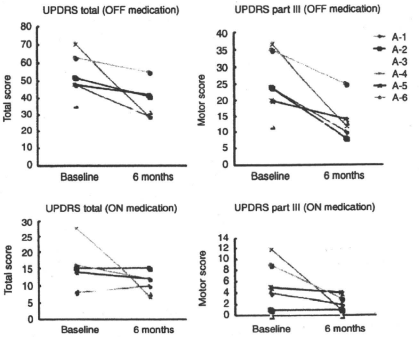

Figure 1 Changes in UPDRS scores. Absolute changes in scores from baseline to 6 months for individual patients. OFF, off-medication state; ON, on-medication state; UPDRS, Unified Parkinson's Disease Rating Scale.

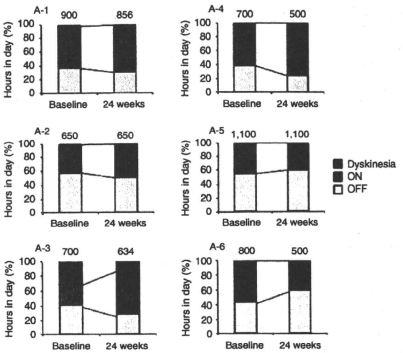

Figure 2 Evaluation of patients' diaries and daily doses of levodopa equivalents. For each 30-minute interval throughout the day, the patients recorded whether they were mobile (ON), immobile (OFF), or asleep. They also recorded the time with troublesome dyskinesias (Dyskinesia). The graph shows the percentage of hours in a day spent in each condition at baseline and at 6 months. The numbers on the bars indicate the mean daily doses of levodopa equivalents (mg). OFF, off-medication state; ON, on-medication state.

significant toxicity.[3,4,6,8,9] Recently, three phase I clinical trials of gene therapy for advanced PD demonstrated that AAV vector–mediated gene delivery into the subthalamic nucleus or putamen was safe and tolerable.[10–13] In this study, the safety of the AAV vectors for clinical use in the human brain was confirmed. Although one patient developed a venous hemorrhage in the subcortical white matter along the trajectory, it is well known that cerebral bleeding occasionally occurs in association with surgical procedures for deep brain stimulation in which electrodes are inserted into the basal ganglia through the frontal lobe white matter.[14,15] PET imaging in this patient showed that putaminal AADC expression was not affected by the subcortical venous hemorrhage and persisted up to 96 weeks. Thus, the venous hemorrhage was probably due to the surgical procedure and not gene transduction.

Although the present trial was a small, open-label study, and the nonblinded, uncontrolled analysis limits the interpretation, the initial efficacy outcomes are encouraging. Our patients showed improved motor performance in the OFF state. Levodopa has a relatively short plasma half-life (60–90 minutes), and antiparkinsonian effects observed after levodopa administration have generally been recognized as short- and long-duration responses. The short-duration response roughly parallels the plasma levodopa concentrations and is thought to be closely linked to dyskinesia, whereas the long-duration response builds up over weeks and improves trough (worst) motor performance in the OFF state.[7] Because the pattern of the short-duration response to levodopa did not change after gene therapy in our patients, the beneficial effect on the OFF state appears to be attributed to augmentation of the long-term response to levodopa.[16] In the preclinical studies with animal models of PD, AAV vectors mainly transduced medium spiny neurons that have dopamine receptors, and extracellular dopamine was increased in the striatum after administration of levodopa.[5,17] The mechanism underlying the long-duration response is not sufficiently understood, and future study is necessary to determine how nonphysiologic production of dopamine in the striatal neurons could enhance the response. It has been reported that the sustained long-duration response to levodopa is greater in patients treated with higher single doses of levodopa.[18] Thus, it is likely that increased dopamine in the putamen after gene transfer may enhance the stable long-duration response. Motor fluctuations in PD are associated with increased response to levodopa with a deeper trough in motor performance, rather than shortening of the response. Improving trough or OFF state motor function by augmenting the long-term response would likely reduce motor fluctuation.[16] Two of three patients in whom the short-duration response to levodopa was studied showed increased peak plasma levodopa concentrations after gene therapy. This finding may simply reflect variable absorbance of levodopa, and it remains to be elucidated whether changes in gastrointestinal absorption could be related to better motor performance in the OFF state.[19]

Activities and levels of AADC mRNA and protein are profoundly reduced in advanced PD,[2] but there are still several types of AADC-containing cells in the striatum, such as serotonin neurons, intrinsic dopamine neurons, AADC-containing "D" neurons, and glial cells.[20] These cells may act as a local source of dopamine. However, dopamine produced in nondopamine cells may not be taken up into dopamine cells and stored in synaptic vesicles, as dopamine transporter and vesicular monoamine transporter 2 are also reduced in advanced PD. The functional efficacy of dopamine produced from exogenous levodopa in these cells may be limited, at least in primates.[2,3] Striatal output neurons, main targets in AADC gene therapy, play a principal role in dopamine modulation of motor function in the basal ganglia. Dopamine synthesized in the striatal neurons themselves may more easily stimulate both synaptic and extrasynaptic receptors.

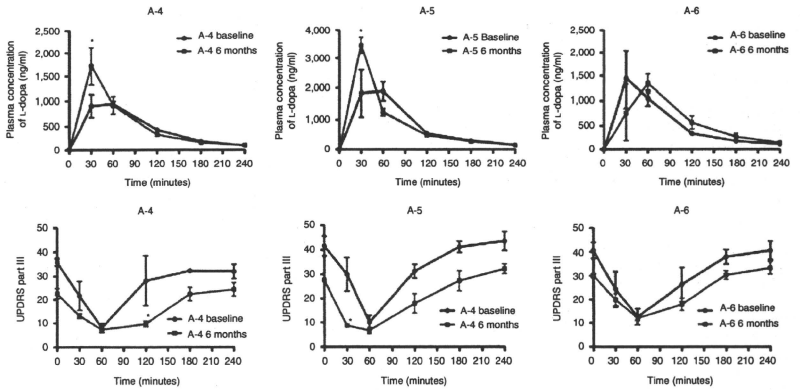

Figure 3 Short-duration response to levodopa. Comparison of short-duration response to levodopa before (blue) and after gene therapy (brown) in three patients (A-4, A-5, and A-6). Patients took 100 mg of levodopa with 25 mg benserazide orally after 20 hours without dopaminergic medication. Values represent means and SE of three trials. Upper panels: plasma levodopa levels; lower panels: Unified Parkinson's Disease Rating Scale motor scores. $^{*}P < 0.05$.

Results of a similar phase I protocol were reported recently for the 10 patients treated with AAV-hAADC-2 (ref. 10). That study used the same vector preparations as this study. The subjects were divided into two groups that received the same or one-third dose of the vector used in this study, respectively. Although the present patients had slightly milder initial symptoms, the patients treated with the same dose of vector in the two studies showed similar improvement in the OFF state and putaminal FMT uptake on PET. These findings provide independent confirmation of the safety, tolerability, and potential efficacy of AADC gene therapy. Future studies focusing on optimal vector dosing and defining the relationship between vector dose and clinical effects are necessary.[21]

In conclusion, these data indicate that AAV vector–mediated gene transfer of AADC is safe and may benefit advanced PD patients.

MATERIALS AND METHODS

Study design. The protocol and consent forms were approved by the institutional review board. The protocol was also reviewed by the committee of the Ministry of Health, Labour and Welfare of Japan. A data safety monitoring board reviewed the ongoing study. All subjects reviewed the consent form and provided their written, informed consent.

This 24-week, phase I, open-label study was primarily designed to evaluate the safety and tolerability of intraputaminal AAV-hAADC-2 infusion in idiopathic PD. Patients were evaluated preoperatively and monthly postoperatively for 6 months, using multiple measures, including the UPDRS, motor state diaries, the MMSE, the short form of the GDS, and laboratory tests. The UPDRS was done in the practically defined OFF state 12 hours after withdrawal of all antiparkinsonian medications, and in the ON state 1 hour after administration of the usual morning dose of medication. Motor scores for the UPDRS can range from 0 to 56, with higher scores indicating poorer function. Using diaries that separated the day into half-hour segments, the patients recorded their mobility during the 4 days before admission and for another 4 days at 6 months

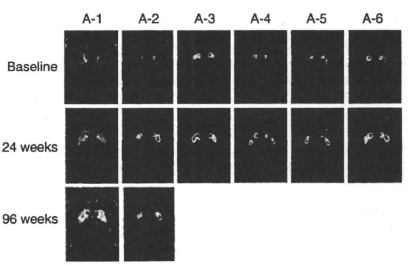

Figure 4 FMT-PET images. Axial images at the level of the putamen are shown before and 24 weeks after gene therapy for all six patients. Increased FMT uptake persisted until 96 weeks in two patients. The 4-week images are not shown because they are similar to the 24-week images. FMT, 6-[^{18}F]fluoro-L-*m*-tyrosine; PET, positron emission tomography.

after admission. They were trained to rate their condition as sleeping, immobile, mobile without troublesome dyskinesias, or mobile with troublesome dyskinesias. The total number of hours spent in each of these categories was calculated, and the differences between the baseline and the 6-month scores were compared between the groups.

The short-duration response to levodopa was evaluated in three patients (patients 4–6) at baseline and 6 months after gene transfer; they took 100 mg of levodopa orally with 25 mg benserazide after 20 hours without dopaminergic medication. Motor symptoms based on UPDRS motor (part III) and plasma levodopa concentrations were assessed at baseline and 30 minutes, 1, 2, 3, and 4 hours after levodopa intake.

Patients. The main entry criteria were: age 45–75 years; diagnosis of moderate to advanced PD, defined as Hoehn and Yahr Stage IV and UPDRS in the practically defined OFF condition of at least 20; at least

5 years of levodopa therapy; a minimum 8-point improvement in the UPDRS motor score after levodopa intake; and motor complications not satisfactorily controlled with medical therapy. The main exclusion criteria were atypical parkinsonism, dementia (MMSE score <20), and previous neurosurgical treatment for PD.

Vector and stereotaxic infusion. The vector used in this trial was a recombinant AAV2 with an expression cassette consisting of a human cytomegalovirus immediate-early promoter, followed by the human growth hormone first intron, complementary DNA of human AADC, and simian virus 40 polyadenylation signal sequence.[3–6] Clinical grade AAV-hAADC-2 was manufactured by Avigen (Alameda, CA) and provided by Genzyme (Boston, MA). The patients received AAV-hAADC-2 *via* bilateral intraputaminal infusions. Two target points were determined in the putamen that were sufficiently separated from each other in dorsolateral directions and identified on a magnetic resonance image. One burr hole was trepanned in each side of the cranial bone, through which the vector was injected into the two target points *via* the two-track insertion route. The vector-containing solution was prepared to a concentration of 1.5×10^{12} vector genome/ml, and 50 µl per point of the solution were injected at 1 µl/min; each patient received 3×10^{11} vector genome of AAV-hAADC-2.

Neutralizing antibody titers against AAV2 were determined by measuring β-galactosidase activities in HEK293 cells transduced with 5×10^3 vector genome/cell of AAV2 vectors expressing β-galactosidase in various dilutions of sera.[22]

PET. The AADC expression level in the putamen was assessed on PET imaging with FMT 6 days before surgery and 1 and 6 months after gene transfer. All patients stopped dopaminergic medications 18 hours before PET and took 2.5 mg/kg of carbidopa orally 1 hour before FMT injection. Subsequently, 0.12 mCi/kg of FMT in saline were infused into an antecubital vein, and a 90-minute dynamic acquisition sequence was obtained. The PET and magnetic resonance imaging data were co-registered with a fusion processing program (Syntegra; Philips, Amsterdam, The Netherlands) to produce the fusion images. Radioactivities within volumes of interest drawn in the putamen and occipital lobe were calculated between 80 and 90 minutes after tracer injection. A change in putaminal FMT uptake from baseline to 24 weeks was assessed using the putaminal–occipital ratio of radioactivities.

Statistical analysis. Values at baseline and 6 months after gene transfer were compared using Student's *t*-test (paired analyses). A two-sided *P* value <0.05 was taken to indicate significant differences. Two-way analysis of variance with Bonferroni correction of *P* values was used for the short-duration response to levodopa.

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Gain-of-function *c-CBL* mutations associated with uniparental disomy of 11q in myeloid neoplasms

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***c-CBL (CBL)* encodes a multifunctional protein engaged in the regulation of intracellular signaling pathways.[1,2] It was first identified as a cellular counterpart of the viral oncogene, *v-CBL*, that causes murine lymphoma.[3,4] Although no genetic evidence existed suggesting its role in human carcinogenesis, the recent discovery of c-*CBL* mutations in myeloid cancers has unveiled a unique oncogenic mechanism mediated by gain-of-function of a mutated tumor suppressor, closely associated with allelic conversion of 11q arms.[5-9] In this review, we summarize our current knowledge about *c-CBL* mutations and discuss the molecular mechanisms of their gain-of-function.**

Key words: c-CBL, 11qUPD, myeloproliferative neoplasms, gain-of-function, MDS/MPN, tyrosine kinases

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Myeloproliferative Neoplasms and Related Disorders

Myeloproliferative neoplasms (MPNs) are a heterogeneous group of blood cancers, characterized by clonal hematopoiesis that causes excessive production of one or more components of mature blood cells with hypercellular bone marrow and extramedullary hematopoiesis.[10] Some patients also show abnormalities in cell morphology and differentiation with dysplastic bone marrow, and are classified into myelodysplastic/myeloproliferative neoplasms (MDS/MPN) in the World Health Organization (WHO) classification.[11] A genetic hallmark of MPN and MDS/MPN is frequent mutations of genes on signal transduction pathways, which have been causally linked to hypersensitivity of neoplastic progenitors to growth factors and cytokines.[10] A notable example is *JAK2 V617F* mutations found in most cases of polycythemia vera (PV), a form of MPNs that is characterized by overproduction of mature erythrocytes together with other blood components.[12-14]

JAK2 Mutations in MPNs

These mutants encode constitutive active kinases that transmit signals from erythropoietin receptor, and induce a hypersensitive proliferative response to erythropoietin.[12] Of particular interest about *JAK2* mutations in PV is the presence of one or more subclones showing acquired uniparental disomy (aUPD) involving the 9p arm that leads to homozygous *JAK2* mutations (*JAK2*$^{mut/mut}$) by allelic conversion (**Fig. 1**).[15] One of the initial discoveries of *JAK2* mutations relied on the detailed mapping of loss of heterozygosity (LOH) caused by aUPD in 9p.[13] The consequence of 9p-aUPD is loss of wild type *JAK2* and duplication of mutated *JAK2*, but the latter seems to be more important for the clonal selection of UPD clones, because mutated *JAK2* is duplicated without loss of wild-type allele in 9p trisomy in some cases.[16] Similarly gain-of-function mutations of *cMPL* are

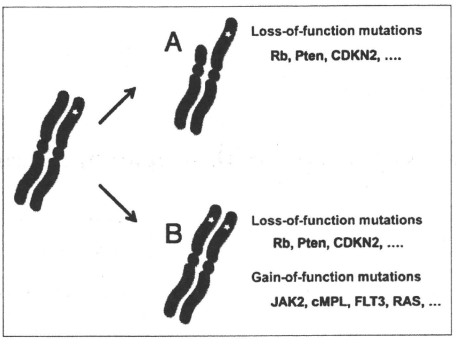

Figure 1. In cancer cells, LOH is frequently associated with a mutated tumor suppressor locus, in which a normal copy of the tumor suppressor is lost by simple allelic deletion (A), or replaced by the mutated copy through allelic conversion that leads to copy number neutral LOH or aUPD (B). In either case, the common consequence is biallelic loss-of-function of the tumor suppressor. In addition, LOH caused by aUPD is also implicated in the common mechanism of homozygous mutations of proto-oncogenes. A number of gain-of-function oncogenic mutations found in aUPD regions have been shown to exist in a homozygous state, including mutations of *JAK2* (9pUPD), *MPL* (1pUPD), *NRAS* (1pUPD), *KRAS* (12pUPD), *BRAF* and *FLT3* (13qUPD). The clonal outgrowth of aUPD-positive clones indicates that two copies of mutations confer a growth advantage to aUPD positive cells through their gain-of-function.

frequently found in primary myelofibrosis in close association with 1p-aUPD.[17] Thus, aUPD, or copy number neutral LOH, is associated not only with biallelic loss-of-function of classical tumor suppressor genes in the Knudson's paradigm,[18] but also with gain-of-function of proto-oncogenes. Moreover, genome-wide analysis of genetic imbalances in a variety of myeloid neoplasms revealed that aUPD is another genetic feature of MPNs, where 42% of chronic myelomonocytic leukemia (CMML) cases had one or more regions of aUPD and were grouped into several discrete clusters, which may or may not harbor mutations of known cancer related genes.[9] Among these one of the most prominent is the cluster that is defined by 11q-aUPD, from which mutated *c-CBL* proto-oncogene was identified.[9]

c-CBL Mutations in MDS/MPNs

Although *c-CBL* mutations have been reported in a variety of myeloid neoplasms including acute myeloid leukemia, myelodysplastic syndromes, as well as classical myeloproliferative disorders, the majority of *c-CBL*-mutated cases are MDS/MPN, including CMML (~15%), juvenile myelomonocytic leukemia (JMML) (~17%), and atypical chronic myeloid leukemia (~5%).[5-9,19,20] In most cases, *c-CBL* mutations are associated with 11q-aUPD involving *c-CBL* locus, which converts these mutations into a homozygous state. Loss of wild-type *c-CBL* is rarely caused by chromosomal deletion.[6,7,9] *c-CBL* mutations exclusively occur independent of *RAS* and *PTPN11* in CMML and JMML.[8,9] Notably, *c-CBL* mutations have a germline origin in some JMML cases.[8] Approximately half of the *c-C-BL* mutations in JMML cases involve Y371, while mutations are widely distributed within linker/RING finger domain in other neoplasms. *c-CBL* mutants strongly transform fibroblasts and enhance proliferation of hematopoietic progenitors in methylcellulose culture.[9] These genetic and functional observations indicate that mutant *c-CBL* may have some gain-of-function, which promotes clonal evolution, especially of aUPD-positive clones carrying two copies of the mutations.

c-CBL as a Tumor Suppressor Gene

c-CBL proto-oncogene is a cellular homologue of a viral oncogene, *v-CBL*, isolated from the Casitas-NS-lymphoma virus that induces murine lymphoma.[3,4] Together with other two homologues CBL-b and CBL-c, it comprises the CBL family of proteins. All c-CBL proteins have an N-terminal domain for binding to phosphorylated tyrosine kinases (TKB domain) connected through a linker sequence to the RING finger, but CBL-c lacks most of the C-terminal domains shared by c-CBL and CBL-b (Fig. 2A). While c-CBL has multivalent molecular functions in signal transduction and cytoskeletal regulation, the most intensively studied-function is its role in negative regulation of receptor tyrosine kinase (RTK) signalings, which depends on the E3 ubquitin ligase activity of this molecule.[1,21] After RTKs are phosphorylated on cytokine stimulation, c-CBL binds to the phosphorylated RTKs through the TKB domain, and monoubiquitinates these RTKs at multiple sites in concert with the E2 conjugating enzyme, which is followed by internalization and degradation/recycling of the phosphorylated RTKs.[21] Thus, c-CBL prevents excessive RTK signaling after cytokine/growth factor stimulation and potentially acts as a tumor suppressor. *c-CBL*$^{-/-}$ mice have an enlarged thymus, splenomegaly with extramedullary hematopoiesis.[22,23] In these mice, hematopoietic progenitor pools are expanded,[9,24] and their hematopoietic progenitors exhibit hypersensitive proliferative responses to cytokine stimulations. When introduced into *BCR/ABL* transgenic mice, a *c-CBL*$^{-/-}$ allele accelerates blastic crisis.[9] Moreover, *c-CBL*$^{-/-}$ mice developed invasive cancer spontaneously (in preparation), further supporting that *c-CBL* has tumor suppressor functions.

Gain of Function of CBL Mutants

How can we reconcile with the tumor suppressor functions of c-CBL on the one hand, and the oncogenic properties of c-CBL mutants on the other? A simple explanation would be an inhibition of

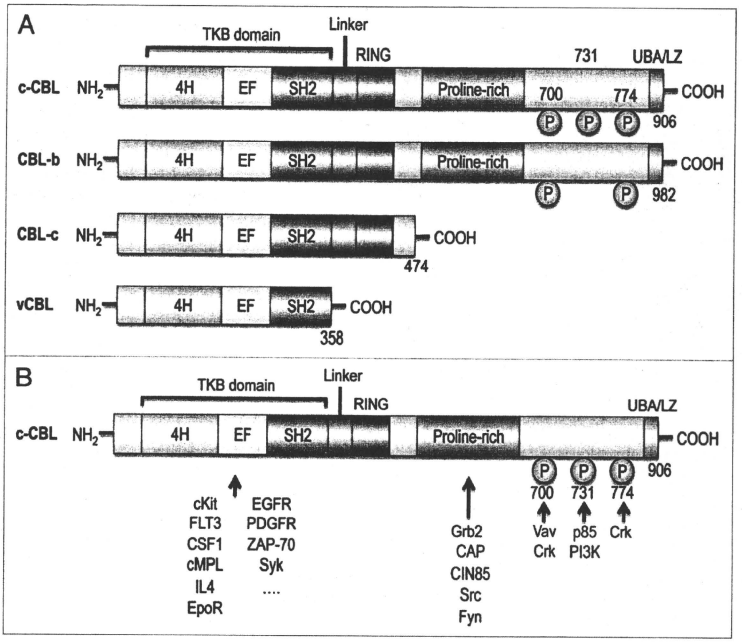

Figure 2. (A) Structure of CBL family proteins. CBL family proteins in mammals have highly conserved domains, where an N-terminal TKB domain, consisting of a four-helix bundle (4H), a Ca^{2+}-binding EF (EF), and a src-homology (SH2) domains, is connected to a RING finger domain via a linker. c-CBL and CBL-b, but not CBL-c, have a proline-rich and other C-terminal components that end with a ubiquitin-associated and leucine zipper (UBA/LZ) domain. Their viral form, v-CBL, is truncated just after its SH2 domain. (B) CBL family proteins interact with a number of signal transducing molecules. Through their TKB domain, CBL family proteins target phosphrylated tyrosine kinases, including growth factor receptors and cytokine receptors, as well as, non-receptor tyrosine kinases. Ubiquitin conjugating enzymes have contact with CBL proteins via the linker/RING finger domain, which is central to the E3 ubiquitin ligase activity. The proline-rich domain provides a binding site for SH3 domains of Grab2, CAP and Src-family kinases. The C-terminal portion contains three tyrosine residues, Y700, Y731 and Y774, which are the major phosphorylated tyrosines, and which bind to the p85 subunit of PI3 kinase (Y731), Vav (Y700) and Crk proteins (Y700 and Y774).

tumor suppressor function of wild type c-CBL by mutant c-CBL. Most *c-CBL* mutations in MPNs occur within the linker/RING finger domains, through which c-CBL binds E2 conjugating enzymes, and thus are expected to compromise the E3 ligase activity of the molecule. In fact, when expressed in fibroblasts, tumor-derived linker and RING finger mutants show severely compromised E3 ubiquitin ligase activity.[9,20] Moreover, two linker mutants (Q367P and Y371S) have been shown to inhibit the activity of wild-type c-CBL protein, although they do not make direct contact with E2 enzymes but with the TKB domain.[25] As expected from the inhibitory action of these mutants with regard to E3 ubiquitin ligase activity, transduction of the latter mutants into NIH3T3 or hematopoietic cells lead to prolonged activation of tyrosine kinases after stimulation with a variety of cytokines and growth factors, including epidermal growth factor, stem cell factor (SCF), Interleukin 3 (IL3), thrombopoietin, and FLT3 ligand.[9,20] Given the diverse spectrum of kinase targets of CBL, the enhanced sensitivity of these cells to a variety of cytokines is well expected.

Although these experimental data support a dominant negative mechanism of mutant *c-CBL*, a simple dominant negative model is defied by an experiment, in which mutant *c-CBL* was transduced into *c-CBL*$^{-/-}$ hematopoietic progenitors. Lin$^-$ Sca1$^+$cKit$^+$ (LSK) hematopoietic progenitors from *c-CBL*$^{-/-}$ mice showed enhanced survival or proliferative responses after stimulation with a variety of cytokines, including SCF, IL3, or thrombopoietin, as compared to those from *c-CBL*$^{+/+}$ mice. However, transduction of mutant *c-CBL* into *c-CBL*$^{-/-}$ progenitors dramatically augmented the responses to these cytokines and also to FLT3 ligand, while the effect of mutant *c-CBL*-transduction into *c-CBL*$^{+/+}$ progenitors was unremarkable even as compared to mock-transduced

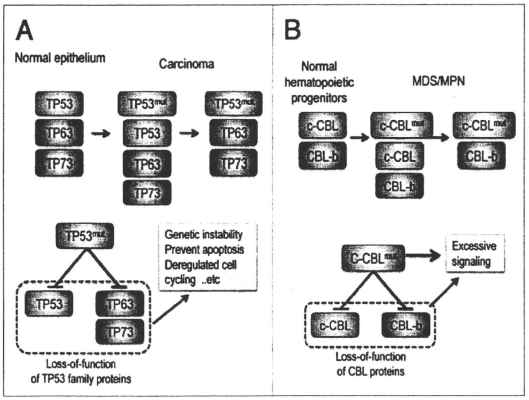

Figure 3. Possible mechanisms of gain-of-function of mutated TP53 and c-CBL. The gain-of-function of TP53 mutants is associated with their potential to induce carcinoma in mice as well as in human, which is considered to be mediated by inhibition of TP63 and TP73. TP53-deficient mice frequently develop sarcomas and lymphomas but only rarely carcinomas, which are thought to be suppressed by TP53 homologues, TP63 and TP73, in epithelial tissues, in the face of loss of TP53. Mutant TP53 inhibits tumor suppressor functions of TP63 and TP73, and compromises TP53-like activity. Similarly, the gain-of-function of CBL mutants found in MDS/MPN may be explained by the inhibition of CBL-b (red arrow), which would result in more profound defects in negative regulation of tyrosine kinase signaling compared to simple loss of c-CBL. On the other hand, c-CBL is thought to have positive regulatory functions that are not directly related to the E3 ubiquitin ligase activity and could be the source of the gain-of-function of c-CBL mutants (blue arrow).

CBL$^{-/-}$ progenitors.[9] The augmented sensitivity to these cytokines in *c-CBL*$^{-/-}$ cells was nothing to do with the inhibition of c-CBL functions, and thus is considered to represent a true gain-of-function of the mutant c-CBL. The gain-of-function nature of *c-CBL* mutations is also predicted from the fact that in myeloid neoplasms, 11qLOH is caused by aUPD in most cases and rarely accompanies 11q deletion, although in this case the target gene has tumor suppressor functions. Interestingly, the effect of the gain-of-function effect largely disappears by introducing wild type *c-CBL* or in the presence of the wild-type *c-CBL* allele,[9] which might explain the observation that the wild type *c-CBL* allele was lost in most MDS/MPN cases with *c-CBL* mutations as a result of allelic conversion or aUPD.

Origin of the Gain-of-Function of Mutant CBL

The exact mechanism through which mutant c-CBL acquires oncogenic functions even in *c-CBL*$^{-/-}$ cells is still elusive. Because the gain-of-function of mutant c-CBL is largely neutralized by the presence of wild type c-CBL, one possibility is that it could be mediated by the inhibition of some 'CBL-like' activity still present in *c-CBL*$^{-/-}$ cells, most likely CBL-b. Both c-CBL and CBL-b are expressed in immature hematopoietic progenitors, and c-CBL mutant inhibits E3 ubiquitin ligase activity of both c-CBL proteins.[9,26] Although *c-CBL/CBL-b*-double knockout mice are embryonic lethal, conditional double knockout in T cells shows hypersensitive to anti-CD3 stimulations and prolonged TCR-signaling, as compared to *c-CBL* or *CBL-b* single null T cells.[27] This reminds us of the gain-of-function of mutated TP53, which explains the difference in the phenotypes between *TP53*$^{-/-}$ and *TP53*$^{mut/-}$ mice. *TP53*$^{-/-}$ mice develop tumors at a high frequency, but they are mostly sarcomas or lymphomas and development of carcinoma is very rare, whereas *TP53*$^{mut/-}$ mice also develop carcinoma in various organs. Thus, TP53 mutant has more than null functions, which are thought to be mediated by the inhibition of its homologues, TP63 and TP73, expressed in epithelial tissues (**Fig. 3**).[28,29] Like c-CBL, TP53 tumor suppressor gene was first identified as an oncogene through its mutated, oncogenic forms in cancer cells. On the other hand, the model of gain-of-function mediated through CBL-b inhibition fails to explain why *CBL-b* mutations are extremely rare in CMML. According to this model, essentially no difference would be expected between the mutations of *c-CBL* and *CBL-b*, as long as in either case, compromised E3 ubiquitin ligase activity would result. The linker-RING finger mutants of c-CBL would be expected also to be able to inhibit E3 ubiquitin ligase activity of the wild-type c-CBL.

Another, but not necessarily exclusive, explanation of the gain-of-function of mutant c-CBL would be related to positive roles of c-CBL as a signal transducer rather than an attenuator (**Figs. 3A and 4**). c-CBL not only binds to a number of phosphorylated tyrosine kinases through its TKB domain, which is indispensable for the negative regulation of these kinases, but also interacts with more than 150 different proteins through a number of C-terminal domains and residues, and acts as a multi-domain adaptor protein, involved in signal transduction (**Fig. 2B**).[2] When recruited to phosphorylated tyrosine kinases, c-CBL is also phosphorylated at multiple tyrosine residues, and provides docking sites for the SH2 domains of Vav (pY700),[30] CrkL (pY700 and pY774)[31-34] and the p85 subunit of PI3 kinase (Y731).[35-37] c-CBL also binds to Grab2,[38-40] CAP,[41] and Src family tyrosine kinases[36] through the proline-rich domain. Several lines of evidence suggest that c-CBL positively transmits signals through these interactions. For example, c-CBL promotes cell survival and proliferation, depending on the PI3 kinase pathway,[42,43] and also enhances activation of MAP kinases after stimulation of Met tyrosine kinase.[44] c-CBL is also a key substrate/effector of Src kinase, which plays a central role in bone resorption and osteoclast migration.[45,46] It also is involved in cytoskeletal rearrangements through activation of Rac1, Cdc42, and R-Ras.[47,48] Normally, mediated by its E3