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Supplemental Data

A Point Mutation in *PDGFRB* Causes Autosomal-Dominant Penttinen Syndrome

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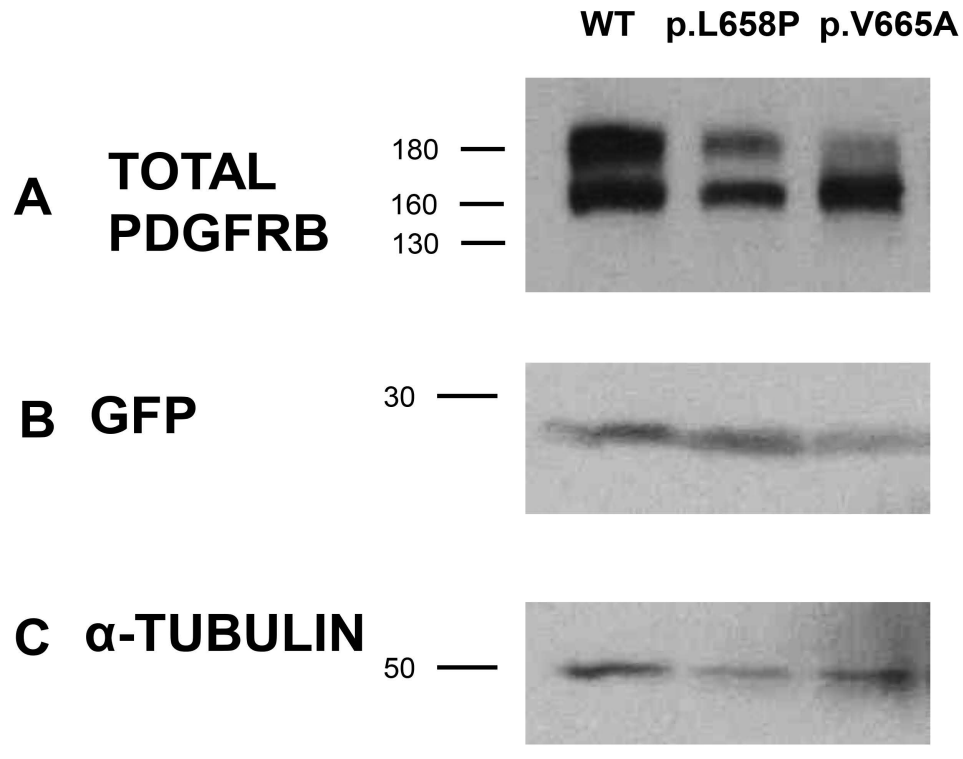
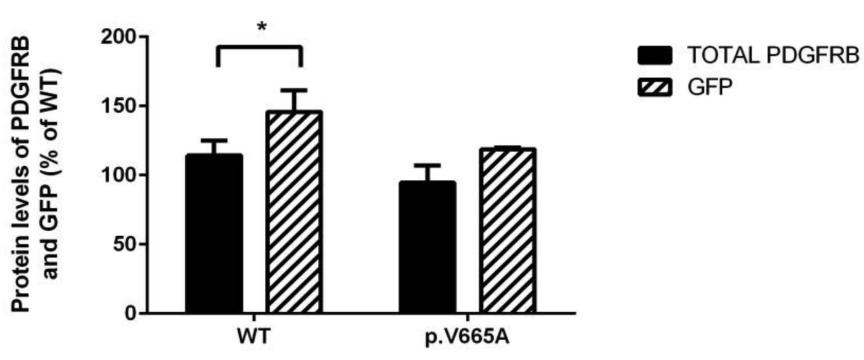
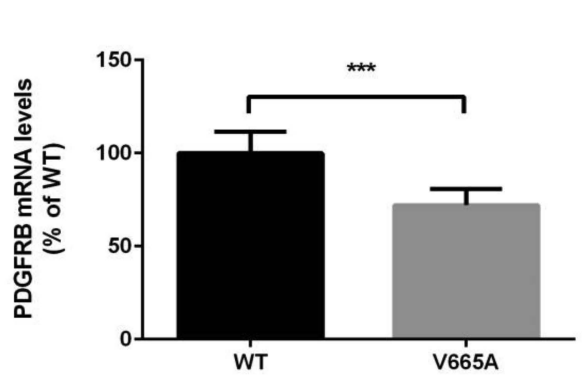
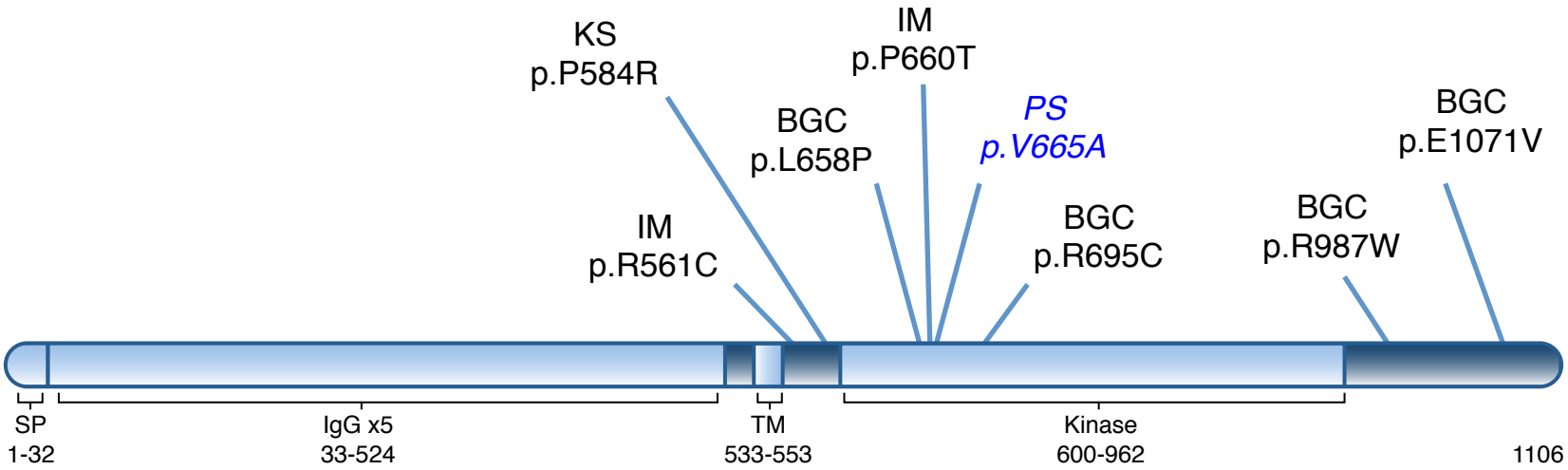

WT p.L658P p.V665A
A
TOTAL PDGFRB
180
160
130
B GFP
30
C α-TUBULIN
50


D
Protein levels of PDGFRB and GFP (% of WT)
200
150
100
50
0
*
WT
p.V665A
TOTAL PDGFRB
GFP


E
PDGFRB mRNA levels (% of WT)
150
100
50
0
***
WT
V665A



Variant	Disease	ExAC alleles*	Cellular Effect
p.Arg561Cys	Infantile myofibromatosis	0	Unknown
p.Pro584Arg	Kosaki syndrome	0	Unknown
p.Leu658Pro	Basal ganglia calcification	0	Reduced protein levels, no phosphorylation with PDGFR-BB stimulation
p.Pro660Thr	Infantile myofibromatosis	1	Unknown
p.Val665Ala	Penttinen syndrome	0	Reduced protein levels, phosphorylation in absence of ligand, predominant species is immature 160kD
p.Arg695Cys	Basal ganglia calcification	10	Reduced protein levels, reduced phosphorylation with PDGFR-BB stimulation
p.Arg987Trp	Basal ganglia calcification	2	Reduced protein levels
p.Glu1071Val	Basal ganglia calcification	0	Unknown

*Approximately 120,000 alleles were screened for each variants with the exception of p.Glu1071Val, for this variant approximately 85,000 alleles were screened.

Fig S1. Co-transfection Efficiency and mRNA Expression studies of mutant vectors. Representative western blots showing total levels of PDGFRB (A), GFP (B) and α−tubulin (C). Quantitative analysis of total PDGFRB and GFP observed by western blot (n=2) (D). Difference in protein level between wild-type and pVal665Ala construct was not found to be significant. Expression level of *PDGFRB* in cells transfected with wild-type or p.Val665Ala construct (E). p.Val665Ala construct was determined to express 28% less *PDGFRB* mRNA (P=0.0007, n=6).

Fig. S2. Location of Mutations Identified in PDGFRB. Eight mutations have been identified in *PDGFRB* in individuals with four distinct phenotypes. Protein alterations and corresponding phenotypes are shown above the protein model (IM, Infantile myofibromatosis; KS, Kosaki syndrome; BGC, Basal ganglia calcification; PS, Penttinen syndrome). Protein domains are indicated below the protein model and include the signal peptide (SP), extracellular immunoglobulin-like domains (IgG), trans membrane domain (TM) and split tyrosine-kinase domain. Allele counts for each variant as reported in ExAC and cellular effect if known are summarized in the table.

Table S1. Quantitative analysis of the three maturation forms of total PDGFRB observed by Western Blot. The percentage of the each PDGFRB band was calculated from the total PDGFRB in WT.

PDGFRB form	WT (n=3)	p.L658P (n=3)	p.V665A (n=3)
Total PDGFRB	100.0%	71.8% ±1.9 (**<0.0001**)	29.4% ±5 (**<0.0001**)
180 kDa	52.1% ±2	14.0% ±0.6	5.5% ±0.9
160 kDa	45.9% ±3.2	55.1% ±1.6	21.8% ±3.9
Ratio of 180 kDa/160 kDa forms	1.1	0.3 (**<0.0001**)	0.3 (**<0.0001**)

(Significant P values for difference between WT and mutant protein.)

Table S2. Quantification of the time-dependent change in the PDGF-BB induced phosphorylation of PDGFRB and downstream pathways. Data is expressed as the fold change (average ± S.E.M.) in p-Tyr751 PDGFRB, p-Tyr1021 PDGFRB, pAKT, pSTAT3, pPLCγ1, pSRC and pERK immunoreactivity compared to total PDGFRB, AKT, STAT3, PLCγ1, SRC and ERK respectively.

	PDGF-BB Time Course				
	0 min (n=3)	**5 min** (n=3)	**15 min** (n=3)	**30 min** (n=3)	**60 min** (n=3)
PDGFRB p-Tyr751/180					
WT	0.2 ± 0.1	2 ± 0.4	4.2 ± 1.1	5.5 ± 0.7	3.9 ± 0.5
V665A	1 ± 0.2	3.1 ± 0.6	1.7 ± 0.1 **(<0.05)**	1.4 ± 0.2 **(<0.0001)**	1.3 ± 0.2 **(<0.01)**
PDGFRB p-Tyr751/160					
WT	0.4 ± 0.1	0.7 ± 0.2	0.9 ± 0.3	1.1 ± 0.2	0.7 ± 0.1
V665A	22.6 ± 1.2 **(<0.0001)**	27.3 ± 5.8 **(<0.0001)**	47 ± 2.3 **(<0.0001)**	44.4 ± 3.4 **(<0.0001)**	35.7 ± 2.8 **(<0.0001)**
PDGFRB p-Tyr1021/180					
WT	0.1 ± 0.1	1.3 ± 0.3	6.5 ± 1.1	10.5 ± 0.1	8.7 ± 0.8
V665A	1.7 ± 0.2	3.2 ± 1.1	1.8 ± 0.3 **(<0.0001)**	1.7 ± 0.2 **(<0.0001)**	1.4 ± 0.2 **(<0.0001)**
PDGFRB p-Tyr1021/160					
WT	0.5 ± 0.2	0.9 ± 0.1	0.7 ± 0.3	1.5 ± 0.4	1 ± 0.2
V665A	72.6 ± 6.2 **(<0.001)**	78.2 ± 21 **(<0.001)**	121 ± 3.8 **(<0.0001)**	116.2 ± 16.7 **(<0.0001)**	100.1 ± 8.6 **(<0.0001)**
p-AKT					
WT	17.2 ± 5	232 ± 20.7	304.4 ± 43.6	239.6 ± 56.1	201.6 ± 27
V665A	73.7 ± 40	237.6 ± 41	241.5 ± 39.7	134.4 ± 25.8	93.8 ± 3
p-STAT3					
WT	34.4 ± 19.3	69 ± 7.7	276.2 ± 44.8	204 ± 51.8	137 ± 39.7
V665A	155.6 ± 36.3	253.1 ± 60.8 **(<0.05)**	198.3 ± 42.2	157.8 ± 26.3	116.6 ± 12.2
p-PLCγ1					
WT	10.2 ± 6	57.2 ± 14.3	90.6 ± 14.6	61.5 ± 8.4	41.7 ± 7.6
V665A	36.3 ± 1.8	106 ±9.5 **(<0.01)**	83.1 ± 1.1	65.9 ± 6.6	58.3 ± 13
p-SRC					
WT	57.1 ± 17.8	125.9 ± 36.8	122.7 ± 32.4	114.2 ± 33.7	101.7 ± 32.7
V665A	72.9 ± 20.2	123.6 ± 36.1	95.7 ± 26.2	105.7 ± 36.4	90.6 ± 25.2
pERK					
WT	56.2 ± 16.3	1437 ± 261.3	604.9 ± 119.5	269.9 ± 127.7	90.2 ± 20.6
V665A	38.4 ± 10.7	1136 ± 119.7	801.2 ± 123.5	129 ± 20.5	87.2 ± 8