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Supplemental Data

A Human Homeotic Transformation Resulting from Mutations in *PLCB4* and *GNAI3* Causes Auriculocondylar Syndrome

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Supplemental Figure 1
Pedigrees of probands used in initial exome sequencing

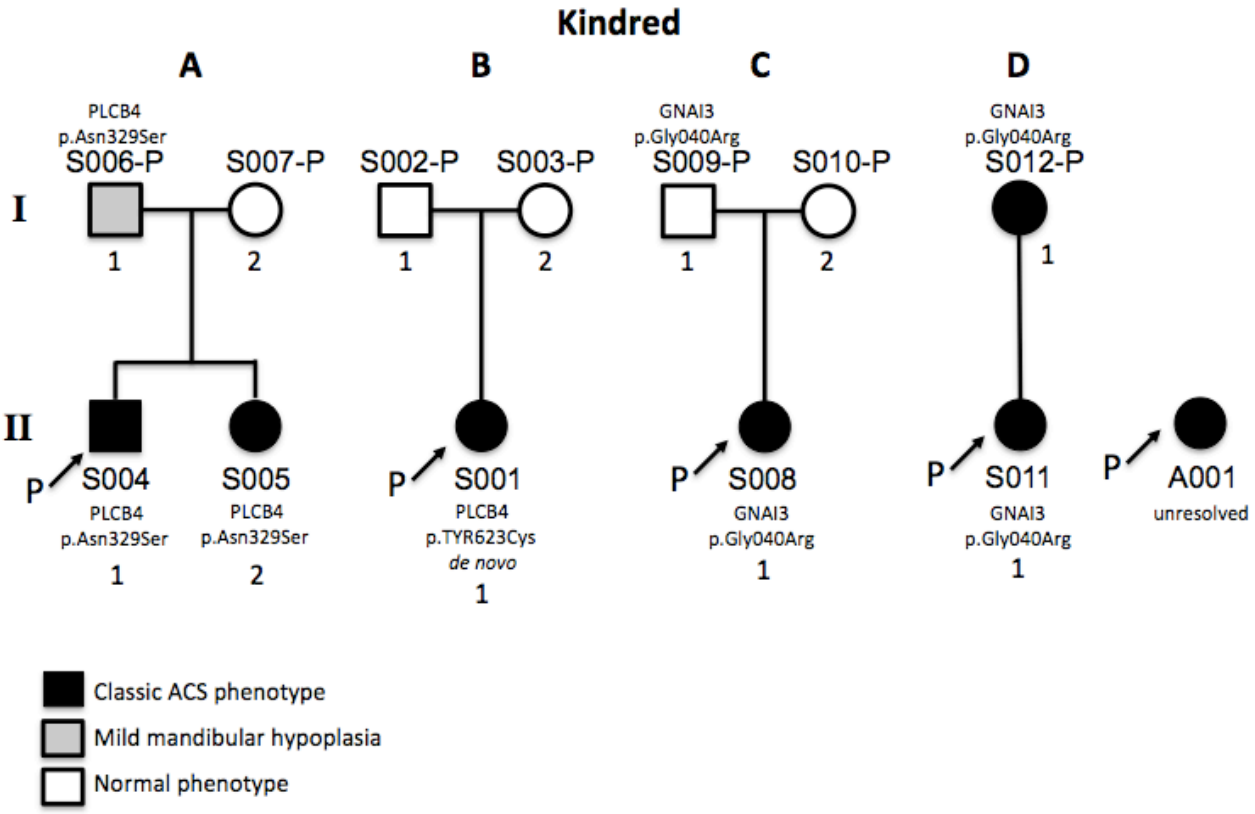
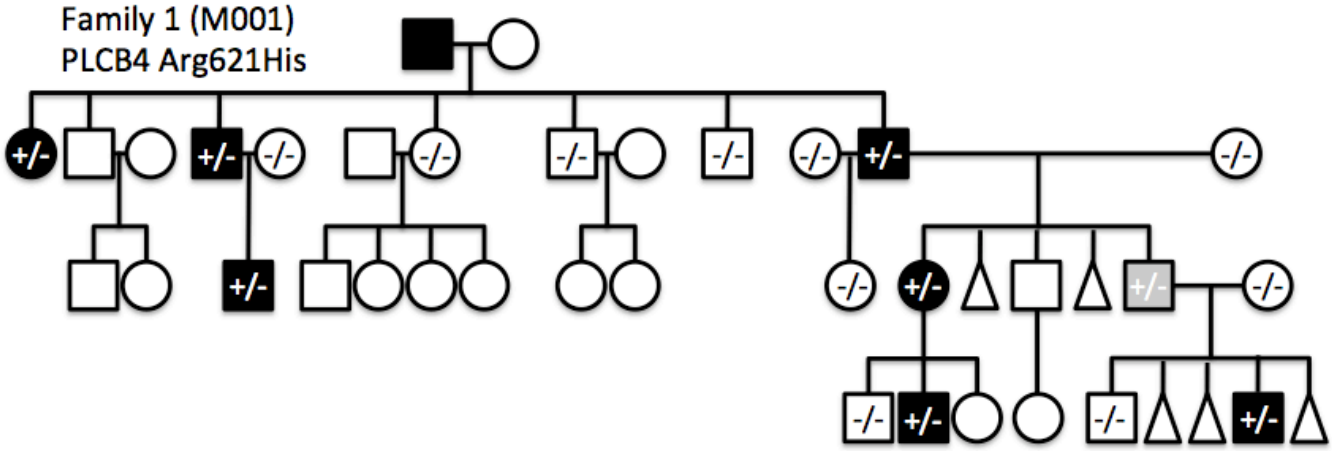
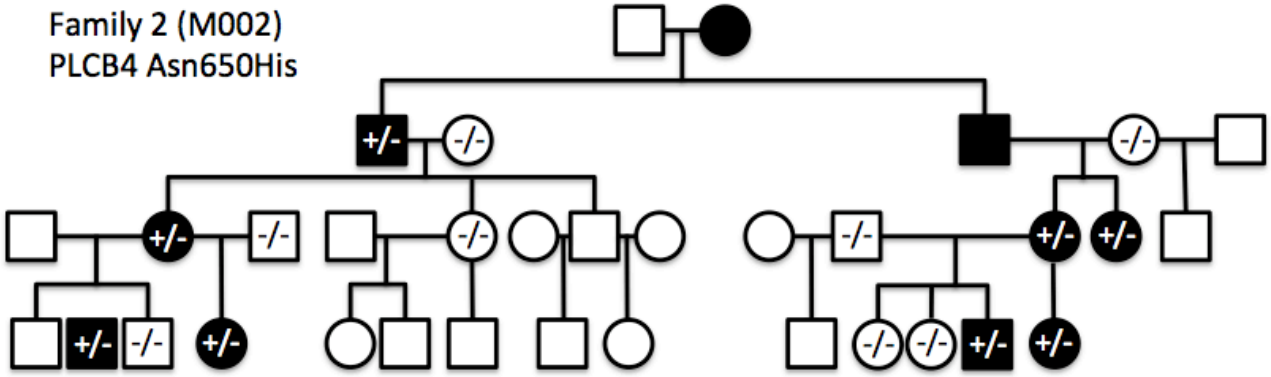


Figure S1. Pedigrees of Proband Kindreds Used in Initial Exome Sequencing

Pedigrees of each of the probands (affected children) and parents used in our initial exome sequencing. Three ACS individuals carried two novel *PLCB4* substitutions and demonstrated variable phenotypic penetrance in one *PLCB4* kindred. The p.Asn329Ser substitution was present in the mildly affected father (S006-P) with two affected two siblings (S004 and S005). A *de novo PLCB4* p.Try623Cys substitution was observed in S001 with a classic ACS phenotype - Figure 1A. Novel mutations in *GNAI3* showed vertical transmission of a classic ACS phenotype in cases S011 and S012-P with the same *GNAI3* p.Gly040RArg substitution seen in the phenotypically normal father (S009-P) and his classically affected daughter S008. A singleton proband (A001) remains unsolved (see Supplemental Table 1). Note: parental sample S012-P was not exome sequenced but the *GNAI3 (*chr1:110091460:G/C; p.Gly040RArg) mutation was confirmed by Sanger sequencing.


Family 1 (M001)
PLCB4 Arg621His
+/-
-/-


Family 2 (M002)
PLCB4 Asn650His
+/-
-/-

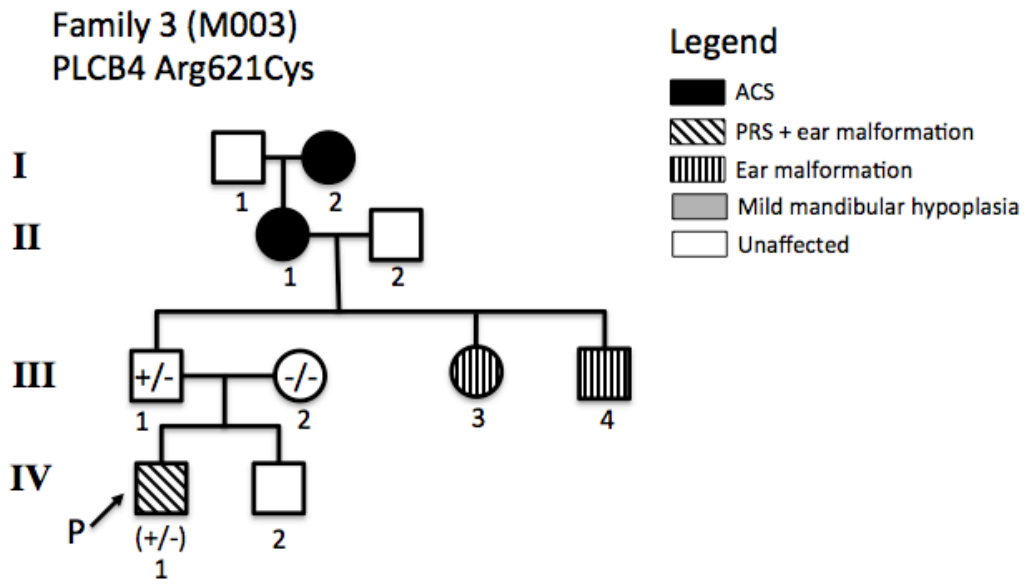


Figure S2. Multigenerational Pedigrees Screened for *PLCB4* Mutations

Identification of two distinct mutations in the regions encoding the catalytic domain of *PLCB4* prompted Sanger sequencing of exons 11-26 in the two large pedigrees with classic features of ACS[1]. The two *PLCB4* mutations identified (chr20:9389727:G/A and chr20:9389813:A/C) were not seen in 10,758 control chromosomes and demonstrated segregation with the ACS phenotype (+/- designation in the pedigree) in family 1 (M001) and 2 (M002). In family 3 (M003) the father carried the p.Arg621Cys and had a very mild ear phenotype, though ACS features were present in pedigree members. These data underscore the variability in clinical phenotype (e.g. one affected case [family 2 – gray box] had mild mandibular hypoplasia and had a classically affected father and son). PRS, Pierre Robin sequence. +/- notation represents a heterozygous genotype for each specific pedigree mutation.

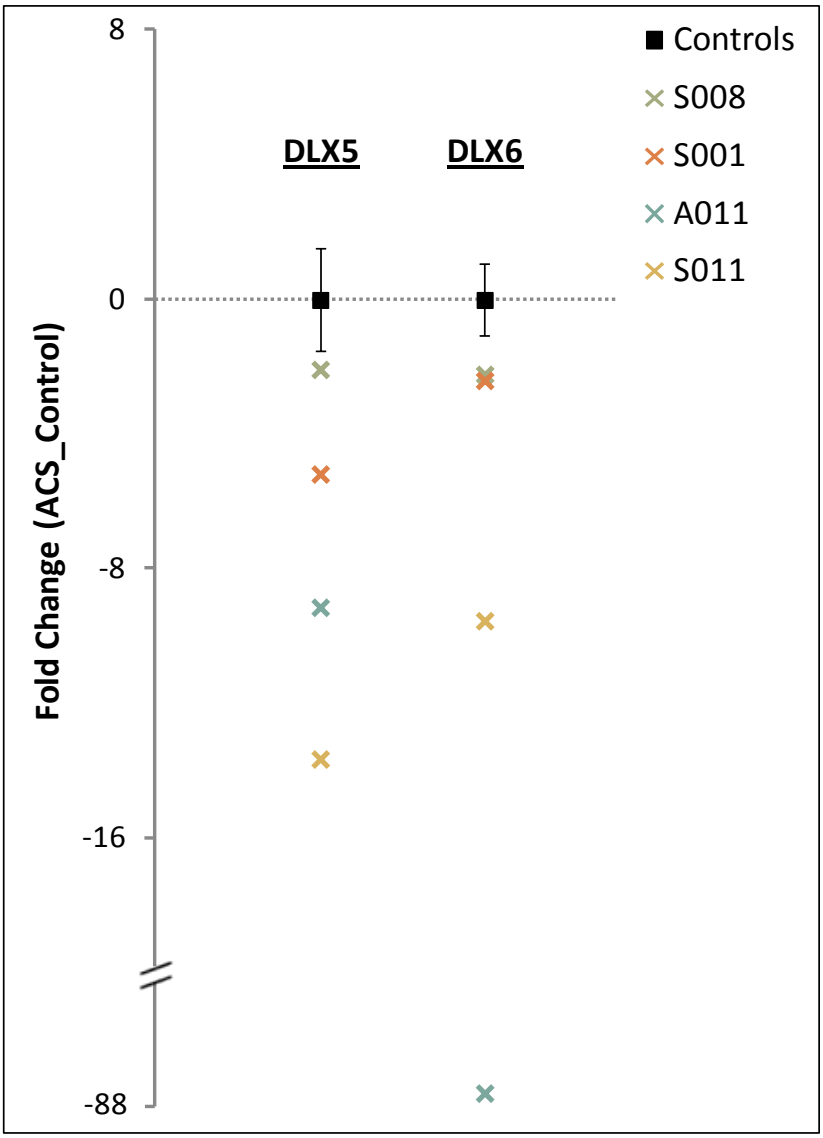


Figure S3. Quantitative Real-Time PCR of DLX5/DLX6 Expression in Mandibular Osteoblasts from ACS Cases

Quantitative real-time PCR of mandibular osteoblasts derived from S001 (PLCB4 p.Tyr623Cys), S008 and S011 (GNAI3 p.Gly040Ar), and A001 (unresolved), demonstrate reduction of DLX5 and DLX6 relative to 17 normalized calvarial osteoblast control cell lines. DLX5 showed a 6-fold reduction and DLX6 a 8-fold reduction relative to controls and support a repression of the endothelin signaling pathway as a unifying cause. The 17 osteoblast control cell lines were randomly selected from 50 previously established lines.

Table S1. Alternative ACS Candidates for Unresolved Case A001 without *PLCB4* or *GNAI3* Mutations

Gene	Protein	Nucleotide	GERP	GS	Control Frequency
PLCH1	Arg033Cys	chr3:155314114:G/A	4.46	21	35/10723
DOCK1	Ser1741Leu	chr10:129242460:C/T	4.28	145	60/10094
DOCK1	Ile111Thr	chr10:128788771:T/C	4.23	89	0/9384
DOCK6	Val405Leu	chr19:11354278:C/G	3.18	32	0/10684
MIOX	Arg149His	chr22:50927506:G/A	4.18	29	2/10756
BMPER	His211Tyr	chr7:34085972:C/T	5.83	81	2/10757[a]

[a] This amino acid position has two substitutions different from A001

Other candidate genes *PLCD1, PLCXD1, PI4KA, HRASLS, RAC1, IGF2BP2, WNT7A* did not contain any functional (missense) variants in this sample.

Supplemental References

1. Storm, A.L., Johnson, J.M., Lammer, E., Green, G.E., and Cunniff, C. (2005). Auriculo-condylar syndrome is associated with highly variable ear and mandibular defects in multiple kindreds. Am. J. Med. Genet. A. 138A, 141–145.