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Supplemental Data

Germline Mutations in *CDH23*, Encoding Cadherin-Related 23, Are Associated with Both Familial and Sporadic Pituitary Adenomas

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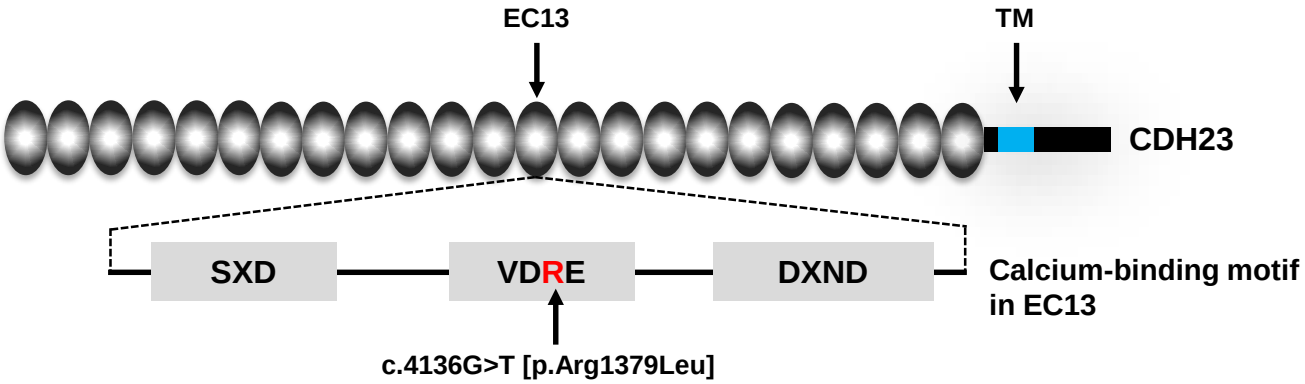
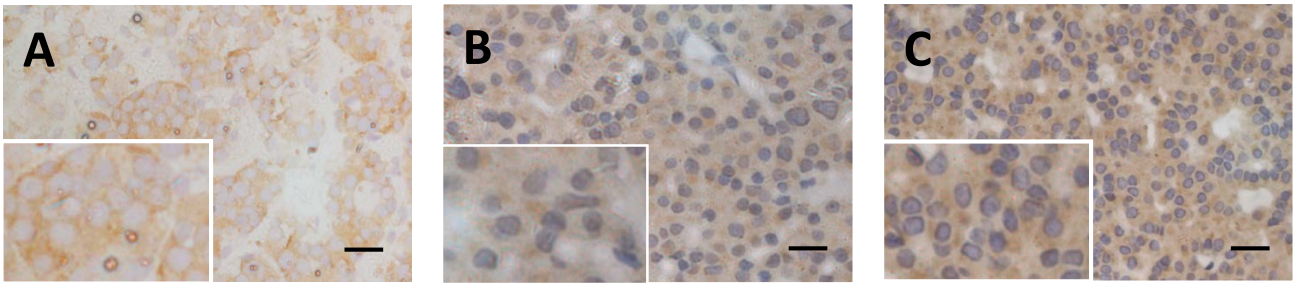
Figure S1.The location of *CDH23* c.4136G>T (p.Arg1379Leu) mutation.



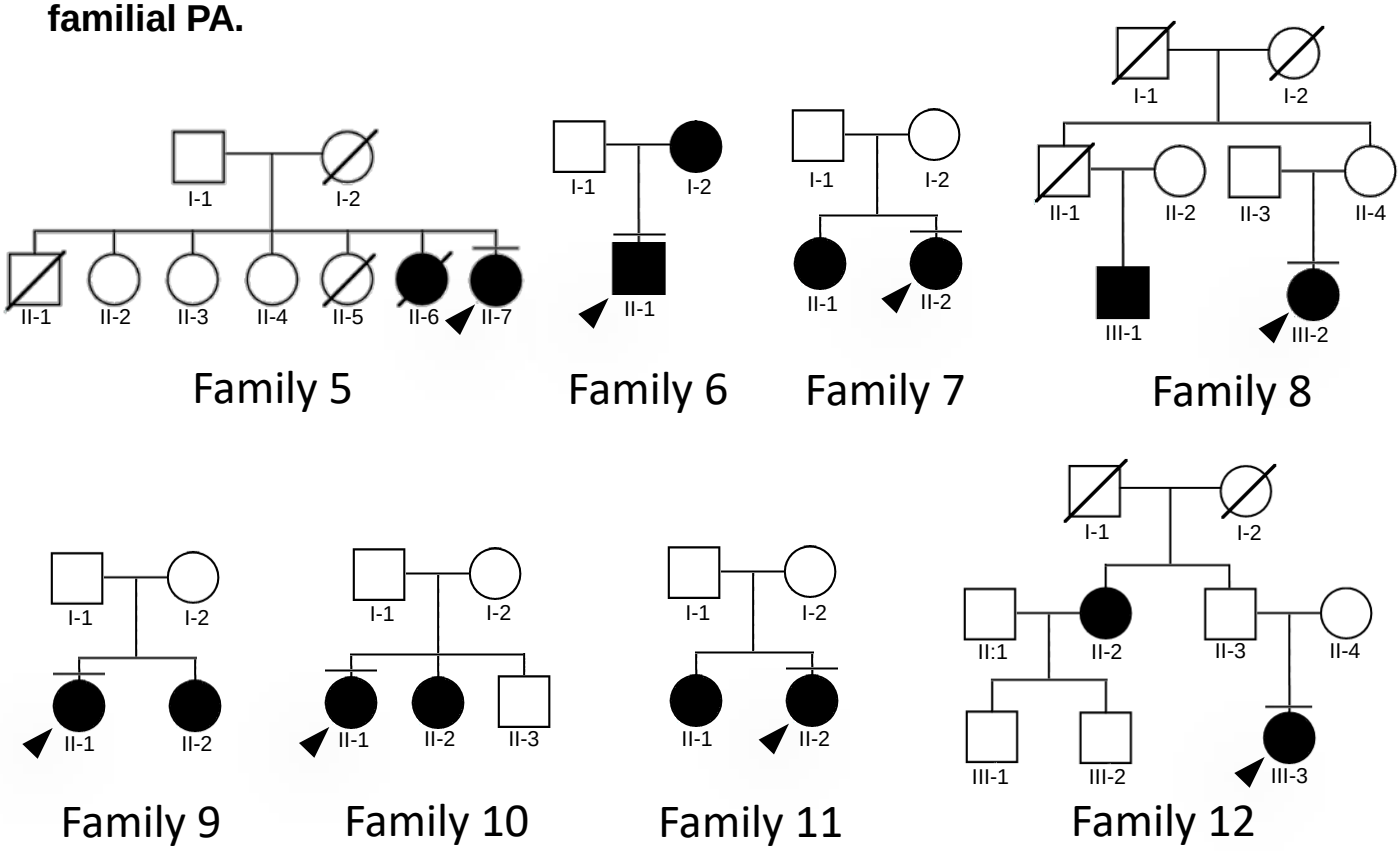
The identified c.4136G>T (p.Arg1379Leu) amino acid substitution located in the second calcium-binding site of extracellular cadherin (EC) 13 domain.

Figure S2. Immunochemistry staining of CDH23 protein.



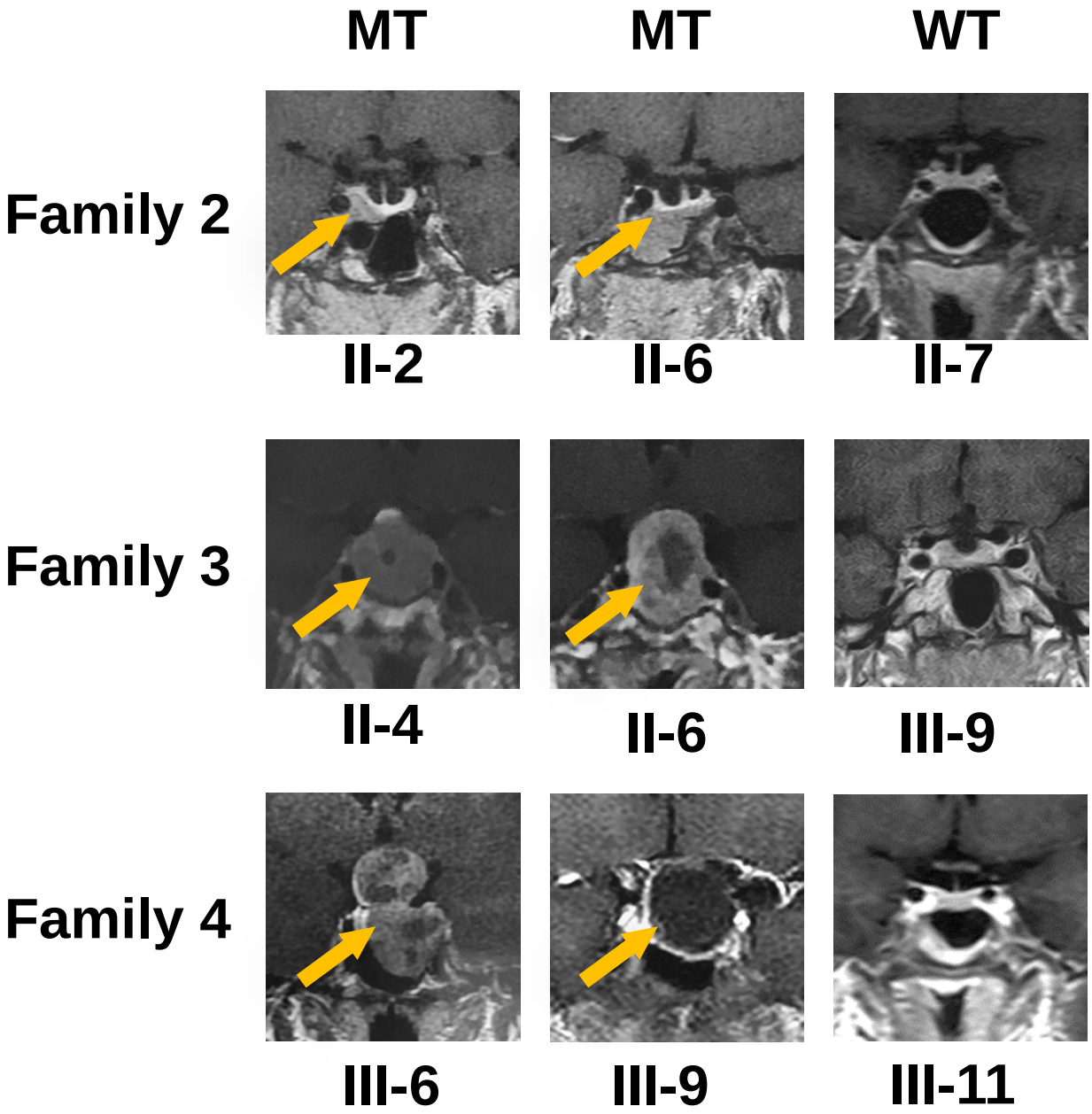
Immunochemistry staining using anti-CDH23 (1:100, Abcam, ab131135) showed that CDH23 protein presented in normal pituitary gland (A), pituitary adenomas with wild-type *CDH23* gene (B) and pituitary adenomas with mutant *CDH23* gene (C). Scale bar, 20 μm.

Figure S3. Family pedigrees of eight *CDH23* wild-type families with familial PA.



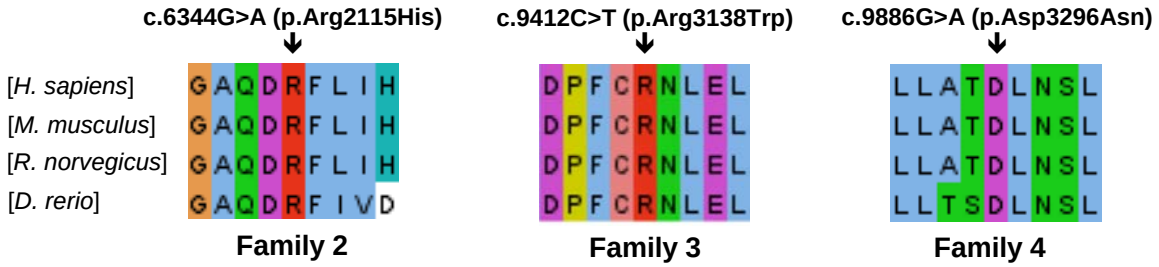
Another eight families with familial PA were included for further exome sequencing. None of these families carried mutant *CDH23*. Black filled shapes represented affected siblings. White shapes were asymptomatic subjects. The family members selected for exome sequencing were noted with a horizontal bar above their respective symbols.

Figure S4. MRI scan of patients and asymptomatic relevants in three families.



The MRI data of individuals with *CDH23* mutations (MT) were shown in the first two columns, while that of asymptomatic *CDH23* wild-type (WT) individuals were shown in the last column. Yellow arrows marked tumors.

Figure S5. Conservation of the alternative residues in CDH23 protein in three families.



Three partial protein alignments of CDH23 showed evolutionary conservation at the site of the identified nucleic acid changes (noted by the black arrow).

Alignments of the CDH23 protein sequences were created using Clustal X, version 2, and checked visually using Jalview, version 2.7.

Table S2: The number of individuals carrying *CDH23* mutations in PA patients(cases) and normal controls

CDH23 **Genotype**	**Pituitary adenoma**		**Normal controls**
	Familial	**Sporadic**	
Mutant	4	15	2
Wild-type	8	110	258
Total	12	125	260

It should be notated that some of the sporadic cases might be masked familial cases because clinical phenotypes of their family members had not been manifested by the time of the diagnosis of these affected persons

Table S3: Comparison of clinical characteristics and *CDH23* genotypes of 145 PA cases

Charateristic		WT (n = 118)	Mutant (n = 27)	*P* value*
Gender				
	Male	68	11	0.113†
	Female	50	16	
Age at diagnosis (Year)				
	Median	40.00	41.00	0.759‡
	Interquartile range	29.75-52.25	31.00-57.00	
Clinical course (Month)				
	Median	24.00	18.00	0.772‡
	Interquartile range	6.00-60.00	6.00-36.00	
Diameter (cm)				
	Median	2.90	1.90	0.005‡
	Interquartile range	1.60-4.00	1.00-2.70	
Invasiveness#				
	Yes	72	5	<0.001†
	No	46	22	
Radical resection				
	Yes	85	18	0.509†
	No	28	4	

* *P* <0.05 was considered to be statistically significant.

† Values were compared by Pearson's $\chi 2$ test.

‡ Values were compared by Mann-Whitney U-test.

Invasive adenomas were defined by fulfilling at least one of the following conditions:

(1) Hardy's modified classification grade III or IV and/or stage C, D, and E;

or (2) Knosp classification grade III and IV.

Intra-operative findings and pathological examinations were also considered as evidence for the identification of invasive PAs

Table S4: Comparison of clinical characteristics and *CDH23* genotypes of 125 sporadic PA cases

Charateristic		WT (n = 110)	Mutant (n = 15)	*P* value*
Gender				
	Male	67	7	0.296†
	Female	43	8	
Age at diagnosis (Year)				
	Median	33.00	40.50	0.187‡
	Interquartile range	26.00-47.00	28.75-53.00	
Clinical course (Month)				
	Median	24.00	24.00	0.625‡
	Interquartile range	12.00-36.00	6.00-60.00	
Diameter (cm)				
	Median	2.90	1.90	0.046‡
	Interquartile range	1.68-4.10	1.00-2.20	
Invasiveness#				
	Yes	67	3	0.003†
	No	43	12	
Radical resection				
	Yes	82	12	0.649†
	No	28	3	

* $P<0.05$ was considered to be statistically significant.

† Values were compared by Pearson's $\chi 2$ test.

‡ Values were compared by Mann-Whitney U-test.

Invasive adenomas were defined by fulfilling at least one of the following conditions:

(1) Hardy's modified classification grade III or IV and/or stage C, D, and E;

or (2) Knosp classification grade III and IV.

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