Supplementary Information

A synonymous VHL mutation (c.414A>G, p.Pro138Pro) causes pathogenic familial hemangioblastoma and pheochromocytoma through dysregulated splicing

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Table S1. A concise timeline from the proband’s clinical record and natural history of VHL disease.

| DATE        | Test/Surgery                      | RESULT                                                                 |
|-------------|-----------------------------------|------------------------------------------------------------------------|
| 08.22.2003  | VHL Testing                       | Results + for VHL c.414A>G p. Pro138Pro ; Proband asymptomatic        |
| 10.2.2008   | MRI SPINE MRI BRAIN MRI ABDOMEN/PELVIS | Small enhancing lesions- HGB@ C2, T12, L1 and L2                      |
|             |                                   | Multiple lesions (9) within posterior fossa- Right Cerebellum:         |
|             |                                   | 9mm and 17mm lesions; Left Posterior Cerebellum 4 mm ill-defined       |
|             |                                   | enhancement with mild edema and downward                              |
|             |                                   | displacement of right cerebellar tonsil                               |
|             |                                   | Left Adrenal Gland mass-consistent with PHEO; mild filling             |
|             |                                   | defect right ureter                                                   |
| 10.2008     | SURGERY                           | Left Adrenalectomy                                                    |
| 01.2009     | SURGERY                           | Gamma Knife Radiotherapy to cerebellar lesions                        |
| 06.2009     | MRI SPINE MRI BRAIN               | Stable lesion mid C2 (3.8mm), unchanged,L1,L2                        |
|             |                                   | After gamma knife the two larger right cerebellar lesions (from       |
|             |                                   | 10/2008) now one large lesion 22x25x28; increased vasogenic           |
|             |                                   | edema and increased mass effect on inferior 4th ventricle;           |
|             |                                   | development of hydrocephalus                                          |
| 10.2009     | MRI Abdomen/Pelvis                | S/P left adrenalectomy-no evidence of recurrent tumor                 |
| 11.2009     | SURGERY                           | Craniotomy Posterior Fossa; Resection of large HGB                    |
| 12.2009     | MRI BRAIN                         | Slight increase in size of previously treated right cerebellar        |
|             |                                   | lesion; physicians to monitor Postop MRI area of craniotomy           |
|             |                                   | stable with complete resection of tumor and resolution of edema and   |
|             |                                   | hydrocephalus;                                                       |
| 2010        | MRI SPINE MRI BRAIN MRI ABDOMEN/PELVIS | Stable, leptomeningeal enhancements at C2 and L1, L2                  |
|             |                                   | more prominent than prior MRI                                        |
|             |                                   | Cranial lesions stable                                               |
|             |                                   | S/P adrenalectomy; Hyper intensity noted at lower pole of right       |
|             |                                   | kidney-too small to characterize                                     |
| 2011        | MRI SPINE MRI BRAIN MRI ABDOMEN/PELVIS | No changes in MRI of Spine, Brain or Abdomen/Pelvis from               |
|             |                                   | prior MRI                                                            |
|             |                                   | Nodules in cerebellar hemisphere unchanged; no new nodules            |
| 2012        | MRI SPINE MRI BRAIN MRI ABDOMEN/PELVIS | Slightly thicker plaque are dorsal cervical spine at C2               |
|             |                                   | (5mm, prior study measured 3mm)                                       |
|             |                                   | Right medial cerebellar lesion associated with mild edema but no      |
|             |                                   | mass-effect; no other changes                                         |
|             |                                   | Hyper intense focus at the lower pole of right kidney unchanged       |
| 2013        | MRI SPINE, BRAIN, ABDOMEN/PELVIS  | No new MRI findings from 2012; no changes                             |
| 2014        | MRI SPINE MRI BRAIN MRI ABDOMEN/PELVIS XRAY, MRI KNEE | Dorsal C2 enhancement 4mm unchanged; Stable; no change from 2013   |
|             |                                   | Stable T2 hyper intensity lateral aspect of lower right kidney       |
|             |                                   | Knee pain; small patellar spur noted otherwise normal(X-ray)          |
|             |                                   | MRI revealed moderate medial joint arthrosis; Patello-femoral          |
|             |                                   | chondromalacia                                                       |
| 2015        | MRI SPINE                         | Unchanged cervical spine lesions; mild degenerative changes           |
|             |                                   | cervical spine; small foci of leptomeningeal enhancement dorsal cord   |
|             |                                   | of C2, the conus of L1,L2 and several cauda equine nerve roots        |
| Year | Imaging Studies | Findings |
|------|----------------|----------|
| 2016 | MRI SPINE, BRAIN, ABDOMEN/PELVIS | NO change from prior year |
| 2017 | Xray Knees | Patellofemoral joint degenerative changes |
| 2018 | MRI SPINE, MRI BRAIN, MRI ABDOMEN/PELVIS | Small focus of leptomeningeal enhancement along dorsal cord of C2 unchanged; multiple small foci of leptomeningeal enhancement along the conus and cauda equine nerve roots unchanged; 6 sub centimeter lesions unchanged in posterior fossa; no evidence of disease progression; Unchanged kidney hypertense focus right lower |
Table S2. Summary of clinical cases with synonymous VHL mutation c.414A>G, p.Pro138Pro.

| Family ID | Lenglet, 2018 Family 11 | Lenglet, 2018 Family 12 | Flores, 2019 Family 1 | Flores, 2019 Family 2 | Flores, 2019 Family 3 | Flores, 2019 Family 4 | Flores, 2019 Family 5 | Present study, Family 1 |
|-----------|-------------------------|-------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-------------------------|
| Number of affected individuals in family | 3 | 4 | 7 | 5 | 3 | 3 | 1 | 3 |
| Age at onset of proband (years) | na | na | 32 | 53 | 31 | 20 | 47 | 47 |
| Age range of diagnosis of affected individuals (years) | na | na | 12-73 | 29-64 | 31-50 | 20-27 | 47 | 6-47 |
| Gender of affected individuals (F:M) | 1:2 | 3:1 | 2:5 | 1:4 | 0:3 | 0:3 | 1:0 | 2:1 |
| VHL Related phenotype (# of affected individuals) | PHEO 3 HGB 2 RCC 1 | PHEO 4 | PHEO 7 HGB 1/7 Renal Cyst 1/7 | PHEO 5 PGL 1/5 HGB 1/5 | PHEO 3 | PHEO 3 HGB 1/3 | PHEO + PGL (1) HGB 3/3 PHEO 1/3 |
| Initial Disease Manifestation | ? | PHEO | PHEO | PHEO | PHEO | PHEO | PHEO | HGB PHEO |
| # of individuals with multiple PHEO (% affected in family) | ? | 0.0% | 3 (60%) | 0% | 2 (67%) | 1 (100%) | 0 (0%) |
| Malignancy | Yes renal | na | no | Yes-lung mets | no | no | no | no |
| Other VHL Manifestations | paraganglioma | no | Spinal HGB | Spinal HGB | no | Spinal HGB | no | Spinal HGB Retinal HGB |
| VHL disease subtype | 2A | 2A | 2C | 2A | 2C | 2A |
| Other manifestations (# of individuals) | no | Thyroid Nodule-1 Bladder Carcinoma-1 | no | no | Parathyroid nodule (1) Esophageal carcinoma (1) | no | no |
| Followup time | na | na | 30 years | 18 years | 10 mos | 25 years | 20 years | 10 years |