Published in final edited form as:

Hum Mutat. 2020 January ; 41(1): 103–109. doi:10.1002/humu.23900.

Fumarate hydratase *FH* c.1431_1433dupAAA (p.Lys477dup) variant is not associated with cancer including renal cell carcinoma

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Abstract

Fumarate Hydratase (*FH*) mutations underpin the autosomal recessive syndrome Fumarate Hydratase deficiency and the autosomal dominant syndrome Hereditary Leiomyomatosis and Renal Cell Carcinoma (HLRCC). The *FH* c.1431_1433dupAAA (p.Lys477dup) genomic alteration has been conclusively shown to contribute to fumarate hydratase deficiency when occurring with another *FH* germline alteration. However, a sufficiently large dataset has been lacking to conclusively determine its clinical significance to cancer predisposition in the heterozygous state. We reviewed a series of 7571 cancer patients who received germline results

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Conflict of Interest

Dr. Zhang: Honoraria (Future Technology Research LLC, BGI, Illumina); Honoraria and Travel and accommodation expenses: Roche Diagnostics Asia Pacific. Family member has a leadership position and ownership interest of Shanghai Genome Center. Dr. Cadoo: Institutional support for therapeutic trials (AstraZeneca and Syndax Pharmaceuticals); Travel and accommodation: AstraZeneca. Dr. Carlo: Advisory role (Pfizer). Dr. Robson: Honoraria (Advisory): AstraZeneca; Consulting or Advisory: AstraZeneca, Daiichi-Sanyo (uncompensated), McKesson, Merck (uncompensated); Research Funding: AbbVie (institution); AstraZeneca (Institution); Invitae (Institution, in-kind); Medivation (Institution); Myriad (Institution, in-kind); Pfizer (institution); Tesaro (institution); Travel, Accommodation, Expenses: AstraZeneca; Other transfer of value: Pfizer (editorial services). All other authors declare no conflict of interest

through MSK-IMPACT testing at Memorial Sloan Kettering Cancer Center (MSKCC). The *FH*c.1431_1433dupAAA (p.Lys477dup) variant was detected in 24 individuals, none of whom was affected with renal cancer. 11 of the 372 renal cancer patients were identified to carry pathogenic *FH* variants associated with HLRCC. None of these 372 renal cancer patients carried the *FH*c.1431_1433dupAAA variant. Our data indicate the *FH*c.1431_1433dupAAA is not associated with cancer including renal cell carcinoma.

Keywords

FH; HLRCC; FH deficiency; *FH*c.1431_1433dupAAA; FH p.Lys477dup

Fumarate hydratase (FH) is an enzymatic component of the Krebs cycle catalyzing fumarate to malate. FH deficiency is a severe autosomal recessive disorder resulting from homozygous or compound heterozygous mutations in *FH* (Ewbank, Kerrigan, & Aleck, 1993). Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) is an autosomal dominant disorder caused by *FH* heterozygous loss of function mutations. HLRCC may manifest with multiple cutaneous leiomyomas, uterine leiomyomatosis and/or renal cell cancer (Badeloe et al., 2006; Tomlinson et al., 2002; Toro et al., 2003). Historically, many terms have been cited in the literature for this syndrome including multiple cutaneous leiomyomatosis (MCL), multiple cutaneous and uterine leiomyomatosis (MCUL) and/or leiomyomatosis cutis et uteri. The association of cutaneous and uterine leiomyomas has been referred to as Reed's syndrome (Reed, Walker, & Horowitz, 1973). These are all descriptions of the same disorder, HLRCC, although there is significant intra- and interfamilial variation in terms of disease severity (Wei et al., 2006).

To date, the same *FH* variant has been classified uniformly for both disorders regardless of its status as a homozygous, heterozygous or compound heterozygous variant. Parents of FH deficiency children have variable phenotypes reported, and strong predisposition to MCUL varies (Alam et al., 2003). The location of *FH* mutations associated with FH deficiency differs significantly from those associated with leiomyomatosis (Tomlinson et al., 2002; Toro et al., 2003). In terms of variant types, while missense variants are primarily associated with FH deficiency, truncating variants account for more than half the mutations associated with HLRCC. Classification of in-frame indel variants are less straightforward as functional studies are needed to assess whether these changes sufficiently alter protein activity to predispose to cancer.

The *FH*c.1431_1433dupAAA variant in NM_000143.3 (GRCh37:Chr1: 241661228 – 241661230) results in the duplication of a single Lysine codon at amino acid position 477 (p.Lys477dup). This variant has been described in combination with another *FH* mutation in multiple families with autosomal recessive fumarate hydratase deficiency and is the most common variant found in this disorder (Coughlin et al., 1998). The *FH*c.1431_1433dupAAA variant has been reported as a heterozygous variant in patients with cutaneous leiomyomas (Ezgu, Krejci, & Wilcox, 2013), ovarian mucinous cystadenoma and uterine myomas (Ylisaukko-oja et al., 2006) and leiomyomas (Martinek et al., 2015). A recent study identified this variant in three individuals with multiple primary tumors, so the

presumption of pathogenicity is stronger. However, none of whom had typical hereditary leiomyoma or renal cell carcinoma tumors (Whitworth et al., 2018). Although it is a well-established pathogenic variant for autosomal recessive FH deficiency, its association with renal cell carcinoma is less well defined. The goal of this study was to determine the association of this variant with cancer in heterozygous state.

The *FH*c.1431_1433dupAAA variant is located in the last exon (exon 10) of the *FH* gene and occurs in the C-terminus of the fumarate hydratase protein. In this series, 24/7571 (0.32%) patients were identified carrying the *FH*c.1431_1433dupAAA variant including prostate (n=6), pancreatic (n=2), colorectal (n=3), bladder (n=2), ovarian (n=2), bile duct cholangiocarcinoma (n=2), endometrial (n=1), breast (n=2), lung (n=1), non-seminomatous germ cell tumor (n=1), astrocytoma (n=1) and melanoma (n=1) cancers (Table 1). None of the 24 carriers have a personal or family history of renal cell carcinoma. 20/24 carriers of this variant in our cohort were diagnosed with cancer at age ≥ 50 . 11/24 patients were females with two having prior history of uterine leiomyomata and diagnosed with urothelial cancer and endometrial cancer at ages 50 and 64, respectively. 4/24 carriers also had pathogenic variants in other genes and three matched their phenotypes.

We examined whether the tumors from these individuals showed loss of heterozygosity (LOH) or harbored a somatic second mutation in *FH*. LOH analysis was performed using the FACETS algorithm. LOH was not observed in 21 out of 23 tumors from these patients (one patient's tumor was not available for sequencing). LOH was observed in two tumor specimens, one from a patient who was diagnosed with recurrent melanoma at age 50, and one from a patient with prostate cancer who had an AJ founder mutation, *BRCA2* c.5946delT. None of the 23 tumors had a second hit in *FH* (Table 1).

We then studied the frequency of the *FH*c.1431_1433dupAAA variant in the renal cancer patients tested within the same period. 372 renal cancer patients (Supplementary Table 1 for subtypes) were included in this study and none of them carried the *FH*c.1431_1433dupAAA variant, while 11/372 patients carried other pathogenic *FH* variants that are associated with HLRCC (Table 2).

Evaluating the gnomAD database across populations for frequency of the *FH*c.1431_1433dupAAA showed that this variant is present in 0.0015 (188/127,286 alleles with 1 homozygote) in European (Non-Finnish) population (<http://gnomad.broadinstitute.org/variant/1-241661227-A-ATTT>). The allele frequency in our cohort is 0.0015 (24/(7571*2)), which is very close to that in the European (Non-Finnish) population. Our data indicate that there was no association of this variant with cancer in our cohort of patients (n=7571) ($p = 0.74$, OR=1.07) (Supplementary Table 2).

Since 6 out of 24 patients (predominantly AJ) were affected with prostate cancer, we investigated whether this variant is associated with increased risk of prostate cancer. We generated genotypes for a hospital based ascertainment of AJ prostate cancer (Sullivan et al., 2015; Vijai et al., 2011) patients (5/886) vs. non-cancer diagnosed AJ controls (Carmi et al., 2014) (3/557) and found no evidence of association for this variant with prostate cancer in AJ population ($p = 0.87$, OR=1.12) (Supplementary Table 3).

To determine the FH activity in such carriers, we measured the fumarase activity from skin fibroblasts harvested from one carrier, who had a history of uterine leiomyomas. The enzymatic activity in the patient (22.3 nmol/min/mg protein) is significantly reduced (24%) relative to controls (91.5+/-22.8 nmol/min/mg protein, n=43) (Supplementary Fig. 1). However, it is significantly higher than the residual activity in *FH* deficiency cases which lies between <1% to 10% (Coughlin et al., 1998; Deschauer et al.; Rustin et al., 1997; Tregoning et al., 2013).

This study highlights the need to extensively sequence ethnically diverse populations in order to accurately interpret genetic variations and their clinical significance. *FH* c.1431_1433dupAAA is a relatively common variant in the general population with Minor Allele Frequencies (MAFs) of 0.5% and 0.15% in AJ and European (non-Finnish) populations in gnomAD cohort, respectively, including two homozygous individuals. All but 1 of 24 carriers in our cohort self-identified as White or AJ. The allele frequency of the *FH* c.1431_1433dupAAA variant in our cohort is consistent with that of European (Non-Finnish) population in the gnomAD database, suggesting this variant was detected in our cohort simply because it is relatively common in the general population and that it may not be related to HLRCC.

None of the 372 RCC patients in our cohort were carriers of this variant while 11 had other *FH* pathogenic variants. In addition, none of the 24 *FH* c.1431_1433dupAAA carriers have a personal or family history of renal cell cancer. Two patients reported a history of uterine fibroids but not cutaneous leiomyomas. Uterine fibroids are the most common benign tumors in women worldwide (Merrill, 2008; Zimmermann, Bernuit, Gerlinger, Schaefer, & Geppert, 2012), and in isolation are not indicative of HLRCC by themselves (Menko et al., 2014; Pithukpakorn Manoj). None of the 24 *FH* c.1431_1433dupAAA carriers in our cohort meet either the major or minor criteria for HLRCC diagnosis, supporting our observations for a possible non-pathogenic role for this variant in the heterozygous state. 3/24 carriers also had pathogenic variants in other genes that matched their phenotypes. As noted, two of the 24 heterozygous germline carriers in our cohort showed loss of heterozygosity of the wild type allele in the tumor and the remaining tumors do not have another somatic mutation in the tumor. Since these two patients were affected with cutaneous melanoma and prostate cancer which are not associated with HLRCC, we do not speculate that LOH of *FH* contributes to the tumorigenesis in these two cases. Of note, the *FH* c.1431_1433dupAAA was detected in one case of HLRCC, but the patient also had a second germline variant in *FH*, c.683T>A (p.I228N), which is predicted to affect the protein function using *in silico* prediction tools, hence the role of the c.1431_1433dupAAA in this case remains unclear (Chen et al., 2014).

While *FH* c.1431_1433dupAAA has been reported as one of the most common mutations associated with autosomal recessive *FH* deficiency, none of the published *FH* deficient cases carry homozygous c.1431_1433dupAAA (Coughlin et al., 1998; Deschauer et al.; Rustin et al., 1997; Tregoning et al., 2013). The presence of two homozygous individuals in the gnomAD database suggests this variant is only pathogenic in conjunction with a different loss of function variant.

This report emphasizes the value of a large and diverse dataset in clinical interpretation. One caveat is that most of the patient's family history is self-reported and there is a selection bias in our cohort as AJ are over represented and our cancer patients are predominantly white. Nevertheless, the large cohort of patients in this study indicate the *FHc.1431_1433dupAAA* is not associated with cancer including renal cell cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This research was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA008748.

References

- Alam NA, Rowan AJ, Wortham NC, Pollard PJ, Mitchell M, Tyrer JP, . . . Tomlinson IP (2003). Genetic and functional analyses of FH mutations in multiple cutaneous and uterine leiomyomatosis, hereditary leiomyomatosis and renal cancer, and fumarate hydratase deficiency. *Hum Mol Genet*, 12(11), 1241–1252. [PubMed: 12761039]
- Badeloe S, van Geel M, van Steensel MA, Bastida J, Ferrando J, Steijlen PM, . . . Poblete-Gutierrez P (2006). Diffuse and segmental variants of cutaneous leiomyomatosis: novel mutations in the fumarate hydratase gene and review of the literature. *Exp Dermatol*, 15(9), 735–741. doi:10.1111/j.1600-0625.2006.00470.x [PubMed: 16881969]
- Carmi S, Hui KY, Kochav E, Liu X, Xue J, Grady F, . . . Pe'er I (2014). Sequencing an Ashkenazi reference panel supports population-targeted personal genomics and illuminates Jewish and European origins. *Nat Commun*, 5, 4835. doi:10.1038/ncomms5835 [PubMed: 25203624]
- Chen YB, Brannon AR, Toubaji A, Dudas ME, Won HH, Al-Ahmadie HA, . . . Reuter VE (2014). Hereditary leiomyomatosis and renal cell carcinoma syndrome-associated renal cancer: recognition of the syndrome by pathologic features and the utility of detecting aberrant succination by immunohistochemistry. *Am J Surg Pathol*, 38(5), 627–637. doi:10.1097/PAS.0000000000000163 [PubMed: 24441663]
- Coughlin EM, Christensen E, Kunz PL, Krishnamoorthy KS, Walker V, Dennis NR, . . . Shih VE (1998). Molecular analysis and prenatal diagnosis of human fumarase deficiency. *Mol Genet Metab*, 63(4), 254–262. doi:10.1006/mgme.1998.2684 [PubMed: 9635293]
- Deschauer M, Gizatullina Z, Schulze A, Pritsch M, Knöppel C, Knape M, . . . Gellerich FN. Molecular and biochemical investigations in fumarase deficiency. *Molecular Genetics and Metabolism*, 88(2), 146–152. doi:10.1016/j.ymgme.2006.01.007
- Ewbank C, Kerrigan JF, & Aleck K (1993). Fumarate Hydratase Deficiency In Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH, & Stephens K (Eds.), *GeneReviews(R)*. Seattle (WA).
- Ezgu F, Krejci P, & Wilcox WR (2013). Mild clinical presentation and prolonged survival of a patient with fumarase deficiency due to the combination of a known and a novel mutation in FH gene. *Gene*, 524(2), 403–406. doi:10.1016/j.gene.2013.03.026 [PubMed: 23612258]
- Martinek P, Grossmann P, Hes O, Bouda J, Eret V, Frizzell N, . . . Ondic O (2015). Genetic testing of leiomyoma tissue in women younger than 30 years old might provide an effective screening approach for the hereditary leiomyomatosis and renal cell cancer syndrome (HLRCC). *Virchows Arch*, 467(2), 185–191. doi:10.1007/s00428-015-1783-y [PubMed: 25985877]
- Menko FH, Maher ER, Schmidt LS, Middleton LA, Aittomäki K, Tomlinson I, . . . Linehan WM (2014). Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance

- and treatment. *Familial Cancer*, 13(4), 637–644. doi:10.1007/s10689-014-9735-2 [PubMed: 25012257]
- Merrill RM (2008). Hysterectomy surveillance in the United States, 1997 through 2005. *Med Sci Monit*, 14(1), Cr24–31. [PubMed: 18160941]
- Pithukpakorn Manoj, T. J. R. J. U. A. I. A. M., Ardinger HH, Pagon RA, et al., editors. Seattle (WA): University of Washington, Seattle; 1993-2018 Hereditary Leiomyomatosis and Renal Cell Cancer. GeneReviews® [Internet].
- Reed WB, Walker R, & Horowitz R (1973). Cutaneous leiomyomata with uterine leiomyomata. *Acta Derm Venereol*, 53(5), 409–416. doi:10.2340/000155553409416 [PubMed: 4127477]
- Rustin P, Bourgeron T, Parfait B, Chretien D, Munnich A, & Rötig A (1997). Inborn errors of the Krebs cycle: a group of unusual mitochondrial diseases in human. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1361(2), 185–197. doi:10.1016/S0925-4439(97)00035-5 [PubMed: 9300800]
- Sullivan J, Kopp R, Stratton K, Manschreck C, Corines M, Rau-Murthy R, . . . Klein RJ (2015). An analysis of the association between prostate cancer risk loci, PSA levels, disease aggressiveness and disease-specific mortality. *Br J Cancer*, 113(1), 166–172. doi:10.1038/bjc.2015.199 [PubMed: 26068399]
- Tomlinson IP, Alam NA, Rowan AJ, Barclay E, Jaeger EE, Kelsell D, . . . Multiple Leiomyoma, C. (2002). Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet*, 30(4), 406–410. doi:10.1038/ng849 [PubMed: 11865300]
- Toro JR, Nickerson ML, Wei MH, Warren MB, Glenn GM, Turner ML, . . . Zbar B (2003). Mutations in the fumarate hydratase gene cause hereditary leiomyomatosis and renal cell cancer in families in North America. *Am J Hum Genet*, 73(1), 95–106. doi:10.1086/376435 [PubMed: 12772087]
- Tregoning S, Salter W, Thorburn DR, Durkie M, Panayi M, Wu JY, . . . Coman DJ (2013). Fumarase Deficiency in Dichorionic Diamniotic Twins. *Twin Research and Human Genetics*, 16(6), 1117–1120. doi:10.1017/thg.2013.72 [PubMed: 24182348]
- Vijai J, Kirchhoff T, Gallagher D, Hamel N, Guha S, Darvasi A, . . . Klein RJ (2011). Genetic architecture of prostate cancer in the Ashkenazi Jewish population. *Br J Cancer*, 105(6), 864–869. doi:10.1038/bjc.2011.307 [PubMed: 21829199]
- Wei MH, Toure O, Glenn GM, Pithukpakorn M, Neckers L, Stolle C, . . . Toro JR (2006). Novel mutations in FH and expansion of the spectrum of phenotypes expressed in families with hereditary leiomyomatosis and renal cell cancer. *J Med Genet*, 43(1), 18–27. doi:10.1136/jmg.2005.033506 [PubMed: 15937070]
- Whitworth J, Smith PS, Martin JE, West H, Luchetti A, Rodger F, . . . Maher ER (2018). Comprehensive Cancer-Predisposition Gene Testing in an Adult Multiple Primary Tumor Series Shows a Broad Range of Deleterious Variants and Atypical Tumor Phenotypes. *Am J Hum Genet*, 103(1), 3–18. doi:10.1016/j.ajhg.2018.04.013 [PubMed: 29909963]
- Ylisaukko-oja SK, Cybulski C, Lehtonen R, Kiuru M, Matyjasik J, Szymanska A, . . . Aaltonen LA (2006). Germline fumarate hydratase mutations in patients with ovarian mucinous cystadenoma. *Eur J Hum Genet*, 14(7), 880–883. doi:10.1038/sj.ejhg.5201630 [PubMed: 16639410]
- Zimmermann A, Bernuit D, Gerlinger C, Schaefer M, & Geppert K (2012). Prevalence, symptoms and management of uterine fibroids: an international internet-based survey of 21,746 women. *BMC Women's Health*, 12(1), 6. doi:10.1186/1472-6874-12-6 [PubMed: 22448610]

Table 1.

Characteristics of 24 cancer patients identified in our cohort that carry the *FH* c.1431_1433dupAAA variant.

Case #	Primary Site and Tumor Type	Gender	Age at Dx	Race	Ancestry	LOH	Other FH mutations in the tumor	Other pathogenic variant identified
1	Bile Duct Cholangiocarcinoma	Male	64	White	German/Welsh/Scottish/Irish	NO LOH	Not detected	
2	Bladder Urothelial Carcinoma	Female	50	White	Ashkenazi Jewish *	NO LOH	Not detected	
3	Bladder Urothelial Carcinoma	Female	76	White	German/Irish	NO LOH	Not detected	
4	Colon Adenocarcinoma	Male	48	White	Unknown	NO LOH	Not detected	
5	Colon Adenocarcinoma	Female	60	White	Ashkenazi Jewish	NO LOH	Not detected	
6	Endometrial Carcinoma	Female	64	White	French/Canadian/Irish	NO LOH	Not detected	
7	Lung Adenocarcinoma	Male	61	White	Ashkenazi Jewish	NO LOH	Not detected	
8	Mediastinum, Non-Seminomatous Germ Cell Tumor	Male	25	White	German/Irish	NO LOH	Not detected	
9	Breast Carcinoma	Female	60	African American	African American	Tumor not sequenced	Tumor not sequenced	
10	High-Grade Serous Ovarian Cancer	Female	68	White	Irish	NO LOH	Not detected	
11	High-Grade Serous Ovarian Cancer	Female	48	White	Ashkenazi Jewish *	NO LOH	Not detected	
12	Pancreatic Adenocarcinoma	Female	55	White	Ashkenazi Jewish	NO LOH	Not detected	
13	Bile Duct Cholangiocarcinoma	Male	60	White	Unknown	NO LOH	Not detected	
14	Prostate Adenocarcinoma	Male	58	White	Ashkenazi Jewish/Jamaican	NO LOH	Not detected	
15	Prostate Adenocarcinoma	Male	71	White	Ashkenazi Jewish *	NO LOH	Not detected	
16	Cutaneous Melanoma	Female	50	White	Ashkenazi Jewish	LOH	Not detected	
17	Prostate Adenocarcinoma	Male	65	White	Unknown	NO LOH	Not detected	MSH2 (NM_000251.2):c.942+3A>T (p.?)
18	Colon Adenocarcinoma	Male	64	White	Ashkenazi Jewish	NO LOH	Not detected	MSH6 (NM_000179.2):c.3743_3744insT (p.Tyr1249LeufsTer26); APC (NM_000038.2):c.3920T>A (p.Ile1307Lys)
19	Pancreatic Adenocarcinoma	Female	58	White	Unknown	NO LOH	Not detected	BRCA2 (NM_000059.3):c.1796_1800delCTAT (p.Ser599Ter)

Case #	Primary Site and Tumor Type	Gender	Age at Dx	Race	Ancestry	LOH	Other FH mutations in the tumor	Other pathogenic variant identified
20	Prostate Adenocarcinoma	Male	64	White	Ashkenazi Jewish	LOH	Not detected	BRCA2 (NM_000059.3) c.5946delT (p.Ser1982ArgfsTer22);
21	Astrocytoma	Male	40	White	Irish	NO LOH	Not detected	
22	Breast Invasive Ductal Carcinoma	Female	66	White	Unknown	NO LOH	Not detected	
23	Prostate Adenocarcinoma	Male	61	White	Unknown	NO LOH	Not detected	
24	Prostate Adenocarcinoma	Male	70	White	Italian/Western European	NO LOH	Not detected	

* -AJ ancestry presumed based on reported religion

Table 2.

11 patients who carried *FH* pathogenic variants that cause their renal cancers.

Cases	Gender	Tumor Type	Sample Type	FH mutations
1	Male	FH-Deficient Renal Cell Carcinoma	Metastasis (Lymph node)	FH (NM_000143.3)c.-62_132+1del (del exon 1)
2	Male	FH-Deficient Renal Cell Carcinoma	Primary	FH(NM_000143.3)c.584T>C(p.Met195Thr)
3	Male	Unclassified Renal Cell Carcinoma	Primary	FH(NM_000143.3)c.1394A>G(p.Tyr465Cys)
4	Female	Unclassified Renal Cell Carcinoma	Metastasis (Adrenal Gland)	FH (NM_000143.3)c.-62_132+1del (del exon 1)
5	Female	FH-Deficient Renal Cell Carcinoma	Primary	FH (NM_000143.3)c.556-2A>T
6	Female	Papillary Renal Cell Carcinoma	Metastasis (Pleura)	FH(NM_000143.3) c.1083_1086delTGAA(p.Glu362Glnfs*10)
7	Female	FH-Deficient Renal Cell Carcinoma	Primary	FH (NM_000143.3) c.824_835delGAGGCAC(TGCTG(p.Gly275_Ala278del)
8	Male	FH-Deficient Renal Cell Carcinoma	Primary	FH (NM_000143.3)c.905_1533del (del exons 7–10)
9	Male	FH-Deficient Renal Cell Carcinoma	Primary	FH(NM_000143.3)c.988A>C(p.Thr330Pro)
10	Male	FH-Deficient Renal Cell Carcinoma	Primary	FH (NM_000143.3)c.267+1G>C
11	Male	Renal Cell Carcinoma	Primary	FH (NM_000143.3)c.557G>A(p.Ser186Asn)