ONLINE APPENDIX

Spectrum of HNF1A somatic mutations in hepatocellular adenoma differs from that in MODY3 patients and suggests genotoxic damage

Emmanuelle Jeannot[1, 2*], Lucille Mellottee[1], Paulette Bioulac-Sage[3], Charles Balabaud[3], Jean-Yves Scoazec[4], Jeanne Tran Van Nhieu[5], Yannick Bacq[6], Sophie Michalak[7], David Buob[8], Genthep (Inserm network), Pierre Laurent-Puig[9], Ivan Rusyn[2], and Jessica Zucman-Rossi[1]

[1]Inserm, U674, Génomique fonctionnelle des tumeurs solides; Université Paris Descartes, Paris, France
[2]Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC, USA
[3]Inserm, U889, Université Bordeaux 2, IFR66, CHU Bordeaux, Hôpital Pellegrin, Bordeaux, France
[4]Hôpital Edouard Herriot, Lyon, France
[5]AP-HP, pathology department, hôpital Henri Mondor, Créteil, France
[6]Hôpital Trousseau, CHRU de Tours, France
[7]CHU, pathology department, Angers, France
[8]Pôle Pathologie, Centre de Biologie Pathologie, CHR-U de Lille, France
[9]Inserm, U775; Université Paris Descartes, Paris, France
*EJ has worked in Inserm U674 and is now working in the University of North Carolina.

Corresponding author:
Prof Jessica Zucman-Rossi
zucman@cephb.fr

Supplemental Table 1: Conditions for amplification of *MYH* coding exons.

Exons	Primers	Sequence primers 5'-3'	Primer [mM]	Size product PCR	PCR program
1	1F	CTCCACTGAACTGAATCACA	0.3	462	TD 60
	1R	TCTGAACGGAAGTTCGACCC	0.3		
2	2F	CGTGAGCATCTTGAGAGTGC	0.3	345	TD 60
	2R	CTTGATACGTATCACAATCCCT	0.3		
3-4	3F	GCCCTAAGTGGGAGCATAC	0.3	478	TD 64
	4R	TTGGCATGAGGACACTGCT	0.3		
5-6	5F	GCATTGACAGGCAGAAGATGA	0.3	484	TD 60
	6R	GTCAAAGAGATCACCCGTCA	0.3		
7-8	7F	CACCCTAGGGTAGGGGAAAT	0.3	418	TD 56
	8R	ACAGAGGGGCCAAAGAGTTA	0.3		
9-10	9F	TTTGGCCAGGTGATCTCACA	0.3	492	TD 60
	10R	GGCACAGGGTTGAGTGTCAT	0.3		
11	11F	CAGCAGCTCTGGTAGGATGT	0.3	463	TD 56
	11R	TACTCAGGTTAGAGGAAGAAC	0.3		
12	12F	GTTCTTCCTCTAACCTGAGTA	0.3	443	TD 56
	12R	TCACGGACGGGAACTCCCAC	0.3		
13-14	13FLI	AGGGCAGTGGCATGAGTAAC	0.3	507	TD 56
	14RLI	CATGTAGGAAACACAAGGAAGTA	0.3		
15	15F	TGAAGTTAAGGGCAGAACACC	0.3	265	TD 60
	15R	GAAGGTCTCCAGGTCAAGAA	0.3		
16	16F	GCTAAGCCTAGCTAGATCAG	0.6	297	TD 56
	16R	TAAGCACTTTACTAACAACAGGA	0.6		

Abbreviation: TD: touch-down.

Supplemental Table 2: Conditions for amplification of *OGG1* coding exons.

Exons	Primers	Sequence primers 5'-3'	Primer [mM]	Size product PCR	PCR program
1	1F	ACCCCATGCCAGGCAGTGTTG	0.3	486	TD 60
	1R	TCTGGGGGCGGGAGAAGAT	0.3		
2	2F	CGTACATGGAGCTATTGTAGG	0.3	554	TD 56
	2R	ACTTCACTATGGTGATTAGC	0.3		
3	3F	TGGTGTTGCTTTCTCTAACGG	0.3	413	TD 60
	3R	GGAAGCCTTGAGAAGGTAAC	0.3		
4	4F	AGTGTCCACTATCCCATAGA	0.3	383	TD 56
	4R	TGCTCTCCTCTTAGTGTCA	0.3		
5	5F	CAGCCGGCTTTGGGGCTATAA	0.3	340	TD 60
	5R	CTGTGACCCATCAACAGAAGT	0.3		
6	6F	TCAGACCCTACTTCTGTTGATG	0.3	313	TD 56
	6R	GGTGAGACTAGTGACAGTGTT	0.3		
7	7F	AACACTGTCACTAGTCTCACC	0.3	433	TD 56
	7R	TCCTCACTGCATTTTCCATAC	0.3		

Abbreviation: TD: touch-down.

Supplemental Table 3: Alleles frequencies of an *OGG1* single nucleotide polymorphism in the two groups of patients.

Chromosomal loci	Genotypes	Allelic frequencies			Fisher exact test*	Allelic frequencies
			37 patients with a mutated HNF1α adenoma (%)	58 patients with a non-mutated HNF1α benign tumor (%)	*P* value	SNP500 database (%)
3p26.2	OGG1 S326C	Ser	81	78	0.72	78†
		Cys	19	22		22†

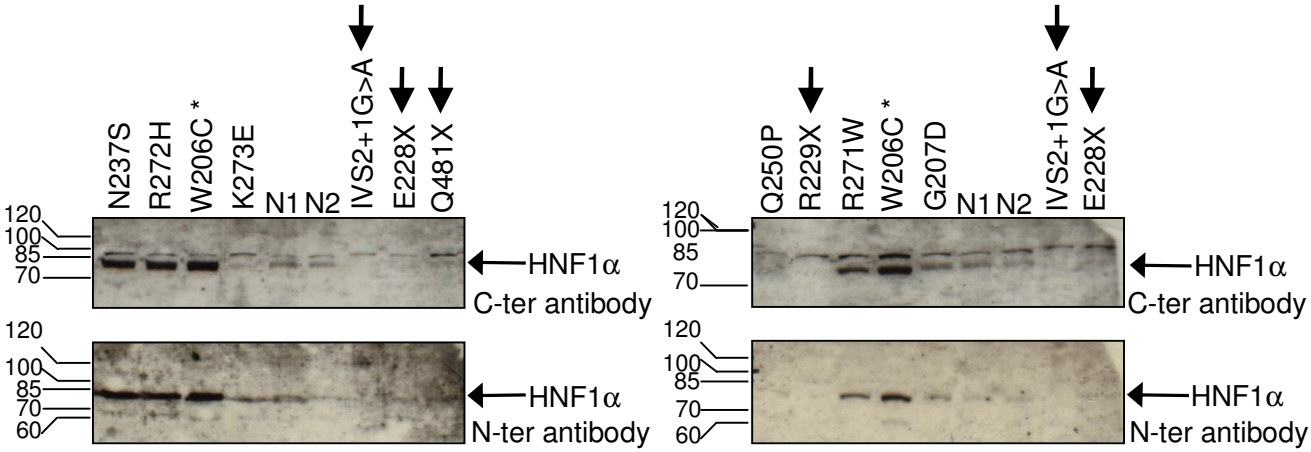
*Comparison between the patients with a mutated HNF1α adenoma and the patients with a non-mutated HNF1α benign tumor.
†European population.

Supplemental Table 4: Percentage of somatic mutations (n=136) identified in HCA and previously described in MODY3 individuals (n=364).

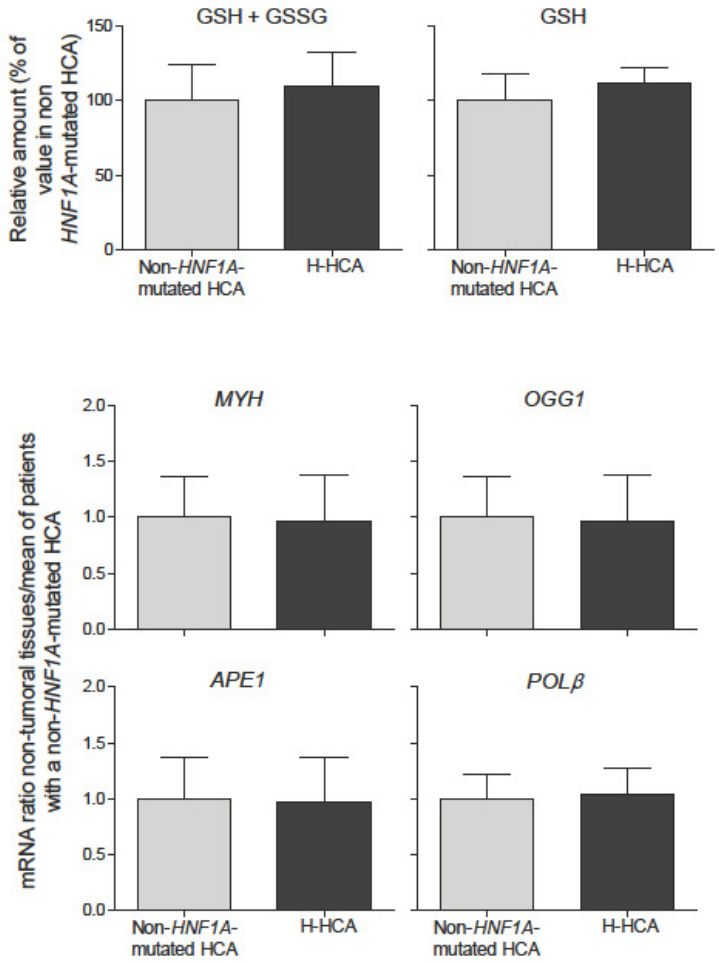
Region	Mutations	Identified in HCA (% of total somatic mutations)	Identified in MODY3 families* (% of total
Missense mutations			
Exon 2	476G>A, R159Q	1 (0.7)	4 (1.1)
Exon 3	607C>T, R203C	1 (0.7)	2 (0.5)
	613A>C, K205Q	1 (0.7)	1 (0.3)
	620G>A, G207D	2 (1.4)	1 (0.3)
	686G>A, R229Q	1 (0.7)	6 (1.6)
Exon 4	779C>T, T260M	2 (1.4)	1 (0.3)
	788G>A, R263H	1 (0.7)	1 (0.3)
	788G>T, R263L	1 (0.7)	1 (0.3)
	815G>A, R272H	1 (0.7)	10 (2.7)
Exon 7	1340C>T, P447L	1 (0.7)	3 (0.8)
Nonsense mutation			
Exon 2	526C>T, Q176X	1 (0.7)	1 (0.3)
Deletions and			
Exon 4	872-873insC,	20 (14.7)	65 (17.9)
	872-873delC,	6 (4.4)	5 (1.4)
Splice site			
Intron 2	IVS2+1G>A	4 (2.9)	1 (0.3)
Intron 5	IVS5-2A>G	1 (0.7)	1 (0.3)

NOTE. *Mutations described in Ellard and Colclough, 2006.

Supplemental figure 1. Expression of the HNF1α protein in H-HCA. Protein levels of HNF1α (75 kDa) were compared between normal liver (N1 and N2) and various types of HNF1α mutants by using an antibody directed against either carboxy (C-ter) or amino (N-ter) terminus. Two mutations, IVS2+1G>A and E228X, were included in each of the experiments to ensure the reproducibility of the results. Asterisk (*) denotes samples from two patients harboring the same mutation. Arrows denote samples with mutations leading to an early stop.



Supplemental figure 2. Oxidative stress markers in liver of patients with a H-HCA (n=6) or a non-*HNF1A*-mutated HCA (n=7). Total glutathione (upper left panel) and reduced glutathione (upper right panel); as well as expression of 4 base excision DNA repair genes, *MYH*, *OGG1*, *APE1*, and *POLβ* were assessed.



Supplemental figure 3. Measurement of 4-HNE protein adducts in liver. (A, C) and (B, D) are representative micrographs (100X) of 4-HNE stained liver sections from patients with a non-HNF1A-mutated adenoma (A, C) and with a H-HCA (B, D), respectively. (E) is an enlargement (400X) of the region indicated.