

**Supplemental Material**  
**Double CYP11B1/CYP11B2 Immunohistochemistry**  
**and Detection of *KCNJ5* Mutations in Primary Aldosteronism**

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**Disclosures**

The authors have nothing to disclose.

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## Logistic Regression

### Notes

Output Created		28-FEB-2024 17:01:10
Comments		
Input	Data	/Users/utente/Desktop/NGS /FILE 2024/SPSS/BC_NGS_2024_2_26.sav
	Active Dataset	DataSet1
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	Split File	<none>
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Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing
Syntax		LOGISTIC REGRESSION VARIABLES Sanger1POS_2NEG /METHOD=BSTEP (WALD) Tumor_Size Age_calc SBP_AVS DBP_AVS ALL_DRC PAC_ngdl_calc IHC /CRITERIA=PIN(.05) POUT(.10) ITERATE(20) CUT(.5).
Resources	Processor Time	00:00:00,04
	Elapsed Time	00:00:00,00

[DataSet1] /Users/utente/Desktop/NGS /FILE 2024/SPSS/BC\_NGS\_2024\_2\_26.sav

### Case Processing Summary

Unweighted Cases <sup>a</sup>		N	Percent
Selected Cases	Included in Analysis	80	63.0
	Missing Cases	47	37.0
	Total	127	100.0
Unselected Cases		0	.0
Total		127	100.0

a. If weight is in effect, see classification table for the total number of cases.

### Dependent Variable Encoding

Original Value	Internal Value
1.00	0
2.00	1

**Block 0: Beginning Block**

**Classification Table<sup>a,b</sup>**

Observed		Predicted		Percentage Correct
		Sanger1POS_2NEG 1.00	2.00	
Step 0	Sanger1POS_2NEG 1.00	0	40	.0
	2.00	0	40	100.0
Overall Percentage				50.0

- a. Constant is included in the model.
- b. The cut value is .500

**Variables in the Equation**

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	.000	.224	.000	1	1.000	1.000

**Variables not in the Equation**

		Score	df	Sig.	
Step 0	Variables	Tumor_Size	3.501	1	.061
	age calc in yrs at study	13.290	1	<.001	
	SBP baseline	3.313	1	.069	
	DBP baseline	2.435	1	.119	
	DRC_measured_DRCdaPR A	1.764	1	.184	
	Aldo ng_dl Calc da pM	.188	1	.665	
	IHC	1.000	1	.317	
	Overall Statistics	24.739	7	<.001	

**Block 1: Method = Backward Stepwise (Wald)**

### Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	29.408	7	<.001
	Block	29.408	7	<.001
	Model	29.408	7	<.001
Step 2 <sup>a</sup>	Step	-.030	1	.863
	Block	29.378	6	<.001
	Model	29.378	6	<.001
Step 3 <sup>a</sup>	Step	-.366	1	.545
	Block	29.012	5	<.001
	Model	29.012	5	<.001
Step 4 <sup>a</sup>	Step	-.806	1	.369
	Block	28.206	4	<.001
	Model	28.206	4	<.001
Step 5 <sup>a</sup>	Step	-2.438	1	.118
	Block	25.768	3	<.001
	Model	25.768	3	<.001

- a. A negative Chi-squares value indicates that the Chi-squares value has decreased from the previous step.

### Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	81.496 <sup>a</sup>	.308	.410
2	81.525 <sup>a</sup>	.307	.410
3	81.892 <sup>a</sup>	.304	.406
4	82.698 <sup>a</sup>	.297	.396
5	85.136 <sup>b</sup>	.275	.367

- a. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.
- b. Estimation terminated at iteration number 4 because parameter estimates changed by less than .001.

### Classification Table<sup>a</sup>

	Observed	Predicted		Percentage Correct	
		Sanger1POS_2NEG 1.00	2.00		
Step 1	Sanger1POS_2NEG	1.00	26	14	65.0
		2.00	12	28	70.0
	Overall Percentage				67.5
Step 2	Sanger1POS_2NEG	1.00	26	14	65.0
		2.00	12	28	70.0
	Overall Percentage				67.5
Step 3	Sanger1POS_2NEG	1.00	26	14	65.0
		2.00	12	28	70.0
	Overall Percentage				67.5
Step 4	Sanger1POS_2NEG	1.00	24	16	60.0
		2.00	11	29	72.5
	Overall Percentage				66.3
Step 5	Sanger1POS_2NEG	1.00	26	14	65.0
		2.00	11	29	72.5
	Overall Percentage				68.8

a. The cut value is .500

### Variables in the Equation

	B	S.E.	Wald	df	Sig.	
Step 1 <sup>a</sup>	Tumor_Size	.384	.172	4.999	1	.025
	age calc in yrs at study	.138	.037	14.170	1	<.001
	SBP baseline	-.062	.024	6.846	1	.009
	DBP baseline	.047	.032	2.187	1	.139
	DRC_measured_DRCdaPR A	.064	.098	.421	1	.516
	Aldo ng_dl Calc da pM	.002	.011	.029	1	.864
	IHC	.262	.444	.349	1	.555
	Constant	-3.782	2.928	1.669	1	.196
Step 2 <sup>a</sup>	Tumor_Size	.388	.171	5.150	1	.023
	age calc in yrs at study	.139	.037	14.369	1	<.001
	SBP baseline	-.063	.024	7.041	1	.008
	DBP baseline	.047	.031	2.277	1	.131
	DRC_measured_DRCdaPR A	.063	.099	.411	1	.521
	IHC	.267	.442	.363	1	.547
	Constant	-3.766	2.931	1.651	1	.199
Step 3 <sup>a</sup>	Tumor_Size	.383	.169	5.143	1	.023
	age calc in yrs at study	.140	.037	14.543	1	<.001
	SBP baseline	-.063	.024	7.080	1	.008
	DBP baseline	.049	.031	2.497	1	.114
	DRC_measured_DRCdaPR A	.078	.097	.646	1	.422
	Constant	-3.516	2.895	1.475	1	.225

### Variables in the Equation

		Exp(B)
Step 1 <sup>a</sup>	Tumor_Size	1.468
	age calc in yrs at study	1.148
	SBP baseline	.940
	DBP baseline	1.048
	DRC_measured_DRCdaPRA	1.066
	Aldo ng_dl Calc da pM	1.002
	IHC	1.300
	Constant	.023
Step 2 <sup>a</sup>	Tumor_Size	1.474
	age calc in yrs at study	1.149
	SBP baseline	.939
	DBP baseline	1.049
	DRC_measured_DRCdaPRA	1.065
	IHC	1.305
	Constant	.023
	Step 3 <sup>a</sup>	Tumor_Size
age calc in yrs at study		1.150
SBP baseline		.939
DBP baseline		1.051
DRC_measured_DRCdaPRA		1.081
Constant		.030

### Variables in the Equation

		B	S.E.	Wald	df	Sig.
Step 4 <sup>a</sup>	Tumor_Size	.374	.167	4.984	1	.026
	age calc in yrs at study	.143	.037	15.191	1	<.001
	SBP baseline	-.062	.023	6.974	1	.008
	DBP baseline	.045	.030	2.284	1	.131
	Constant	-3.146	2.762	1.297	1	.255
Step 5 <sup>a</sup>	Tumor_Size	.316	.158	4.021	1	.045
	age calc in yrs at study	.123	.033	13.964	1	<.001
	SBP baseline	-.037	.016	5.455	1	.020
	Constant	-1.484	2.539	.341	1	.559

### Variables in the Equation

		Exp(B)
Step 4 <sup>a</sup>	Tumor_Size	1.453
	age calc in yrs at study	1.153
	SBP baseline	.940
	DBP baseline	1.046
	Constant	.043
Step 5 <sup>a</sup>	Tumor_Size	1.372
	age calc in yrs at study	1.131
	SBP baseline	.963
	Constant	.227

a. Variable(s) entered on step 1: Tumor\_Size, age calc in yrs at study, SBP baseline, DBP baseline, DRC\_measured\_DRCdaPRA, Aldo ng\_dl Calc da pM, IHC.

### Variables not in the Equation

		Score	df	Sig.	
Step 2 <sup>a</sup>	Variables	Aldo ng_dl Calc da pM	.029	1	.864
	Overall Statistics		.029	1	.864
Step 3 <sup>b</sup>	Variables	Aldo ng_dl Calc da pM	.044	1	.834
		IHC	.365	1	.546
	Overall Statistics		.391	2	.822
Step 4 <sup>c</sup>	Variables	DRC_measured_DRCdaPRA	.681	1	.409
		Aldo ng_dl Calc da pM	.023	1	.879
		IHC	.682	1	.409
	Overall Statistics		1.099	3	.777
Step 5 <sup>d</sup>	Variables	DBP baseline	2.407	1	.121
		DRC_measured_DRCdaPRA	.460	1	.497
		Aldo ng_dl Calc da pM	.136	1	.712
		IHC	.871	1	.351
	Overall Statistics		3.405	4	.492

a. Variable(s) removed on step 2: Aldo ng\_dl Calc da pM.

b. Variable(s) removed on step 3: IHC.

c. Variable(s) removed on step 4: DRC\_measured\_DRCdaPRA.

d. Variable(s) removed on step 5: DBP baseline.

### Logistic Regression

## Notes

Output Created		28-FEB-2024 17:01:10
Comments		
Input	Data	/Users/utente/Desktop/NGS /FILE 2024/SPSS/BC_NGS_2024_2_26.sav
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	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	127
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing
Syntax		LOGISTIC REGRESSION VARIABLES Sanger1POS_2NEG /METHOD=BSTEP (WALD) Tumor_Size Age_calc SBP_AVS DBP_AVS FR_MUT_KCNJ5 sex1M2F IHC /CONTRAST (sex1M2F)=Indicator /CLASSPLOT /PRINT=CORR ITER(1) SUMMARY CI(95) /CRITERIA=PIN(0.05) POUT(0.10) ITERATE(20) CUT(0.5).
Resources	Processor Time	00:00:00,04
	Elapsed Time	00:00:00,00

## Case Processing Summary

Unweighted Cases <sup>a</sup>		N	Percent
Selected Cases	Included in Analysis	36	28.3
	Missing Cases	91	71.7
	Total	127	100.0
Unselected Cases		0	.0
Total		127	100.0

a. If weight is in effect, see classification table for the total number of cases.

## Dependent Variable Encoding

Original Value	Internal Value
1.00	0
2.00	1



### Categorical Variables Codings

		Frequency	Parameter coding (1)
Sex_1male_2female	1	12	1.000
	2	24	.000

### Block 0: Beginning Block

#### Iteration History<sup>a,b,c</sup>

Iteration		-2 Log likelihood	Coefficients Constant
Step 0	1	26.233	-1.556
	2	25.142	-1.995
	3	25.116	-2.077
	4	25.116	-2.079
	5	25.116	-2.079

- a. Constant is included in the model.
- b. Initial -2 Log Likelihood: 25.116
- c. Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.

#### Classification Table<sup>a,b</sup>

	Observed		Predicted		Percentage Correct
			Sanger1POS_2NEG 1.00	2.00	
Step 0	Sanger1POS_2NEG	1.00	32	0	100.0
		2.00	4	0	.0
Overall Percentage					88.9

- a. Constant is included in the model.
- b. The cut value is .500

#### Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)
Step 0 Constant	-2.079	.530	15.374	1	<.001	.125

### Variables not in the Equation

		Score	df	Sig.
Step 0	Variables			
	Tumor_Size	1.386	1	.239
	age calc in yrs at study	.014	1	.907
	SBP baseline	.355	1	.552
	DBP baseline	.478	1	.489
	FR_MUT_KCNJ5	8.751	1	.003
	Sex_1male_2female(1)	.562	1	.453
	IHC	.129	1	.720
	Overall Statistics	10.316	7	.171

### Block 1: Method = Backward Stepwise (Wald)

#### Iteration History<sup>a,b,c,d,e</sup>

Iteration		-2 Log likelihood	Constant	Coefficients		
				Tumor_Size	age calc in yrs at study	SBP baseline
Step 1	1	20.714	-1.539	.121	.022	-.001
	2	16.290	-2.326	.263	.050	-.002
	3	14.794	-3.155	.453	.086	-.003
	4	14.464	-4.015	.609	.114	-.005
	5	14.438	-4.477	.671	.127	-.006
	6	14.437	-4.550	.679	.128	-.006
	7	14.437	-4.551	.680	.129	-.007
	8	14.437	-4.551	.680	.129	-.007
Step 7	1	21.565	.440			
	2	18.041	1.181			
	3	17.360	1.665			
	4	17.304	1.847			
	5	17.303	1.868			
	6	17.303	1.868			

### Iteration History<sup>a,b,c,d,e</sup>

		Coefficients			
Iteration		DBP baseline	FR_MUT_KCNJ5	Sex_1male_2fe male(1)	IHC
Step 1	1	-.001	-.069	.159	.306
	2	-.014	-.113	.397	.767
	3	-.039	-.142	.756	1.396
	4	-.056	-.158	1.048	1.830
	5	-.061	-.163	1.166	1.961
	6	-.062	-.163	1.183	1.974
	7	-.062	-.163	1.183	1.974
	8	-.062	-.163	1.183	1.974
Step 7	1		-.072		
	2		-.125		
	3		-.160		
	4		-.173		
	5		-.175		
	6		-.175		

a. Method: Backward Stepwise (Wald)

b. Constant is included in the model.

c. Initial -2 Log Likelihood: 25.116

d. Estimation terminated at iteration number 8 because parameter estimates changed by less than .001.

e. Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

### Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	10.678	7	.153
	Block	10.678	7	.153
	Model	10.678	7	.153
Step 7 <sup>a</sup>	Step	-.745	1	.388
	Block	7.813	1	.005
	Model	7.813	1	.005

a. A negative Chi-squares value indicates that the Chi-squares value has decreased from the previous step.

### Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	14.437 <sup>a</sup>	.257	.511
7	17.303 <sup>b</sup>	.195	.388

- a. Estimation terminated at iteration number 8 because parameter estimates changed by less than .001.
- b. Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

### Classification Table<sup>a</sup>

	Observed		Predicted		Percentage Correct
			Sanger1POS_2NEG 1.00	2.00	
Step 1	Sanger1POS_2NEG	1.00	31	1	96.9
		2.00	1	3	75.0
	Overall Percentage				94.4
Step 7	Sanger1POS_2NEG	1.00	32	0	100.0
		2.00	2	2	50.0
	Overall Percentage				94.4

- a. The cut value is .500

### Variables in the Equation

		B	S.E.	Wald	df	Sig.
Step 1 <sup>a</sup>	Tumor_Size	.680	.590	1.325	1	.250
	age calc in yrs at study	.129	.126	1.034	1	.309
	SBP baseline	-.007	.048	.019	1	.892
	DBP baseline	-.062	.076	.662	1	.416
	FR_MUT_KCNJ5	-.163	.085	3.643	1	.056
	Sex_1male_2female(1)	1.183	1.779	.442	1	.506
	IHC	1.974	1.903	1.075	1	.300
	Constant	-4.551	6.824	.445	1	.505
Step 7 <sup>a</sup>	FR_MUT_KCNJ5	-.175	.076	5.302	1	.021
	Constant	1.868	1.514	1.523	1	.217

### Variables in the Equation

	Exp(B)	95% C.I. for EXP(B)	
		Lower	Upper
Step 1 <sup>a</sup> Tumor_Size	1.973	.620	6.276
age calc in yrs at study	1.137	.888	1.457
SBP baseline	.994	.905	1.091
DBP baseline	.940	.810	1.091
FR_MUT_KCNJ5	.850	.719	1.004
Sex_1male_2female(1)	3.263	.100	106.731
IHC	7.197	.173	300.050
Constant	.011		
Step 7 <sup>a</sup> FR_MUT_KCNJ5	.839	.723	.974
Constant	6.477		

a. Variable(s) entered on step 1: Tumor\_Size, age calc in yrs at study, SBP baseline, DBP baseline, FR\_MUT\_KCNJ5, Sex\_1male\_2female, IHC.

### Correlation Matrix

		Constant	Tumor_Size	age calc in yrs at study	SBP baseline
Step 1	Constant	1.000	-.595	-.630	-.054
	Tumor_Size	-.595	1.000	.594	-.192
	age calc in yrs at study	-.630	.594	1.000	-.464
	SBP baseline	-.054	-.192	-.464	1.000
	DBP baseline	.179	-.431	-.148	-.519
	FR_MUT_KCNJ5	-.133	.051	-.130	-.154
	Sex_1male_2female(1)	-.518	.397	.438	-.056
	IHC	-.487	.585	.304	.048
Step 7	Constant	1.000			
	FR_MUT_KCNJ5	-.911			

### Correlation Matrix

		DBP baseline	FR_MUT_KCNJ5	Sex_1male_2fe male(1)	IHC
Step 1	Constant	.179	-.133	-.518	-.487
	Tumor_Size	-.431	.051	.397	.585
	age calc in yrs at study	-.148	-.130	.438	.304
	SBP baseline	-.519	-.154	-.056	.048
	DBP baseline	1.000	.112	-.372	-.631
	FR_MUT_KCNJ5	.112	1.000	.145	.062
	Sex_1male_2female(1)	-.372	.145	1.000	.518
	IHC	-.631	.062	.518	1.000
Step 7	Constant				
	FR_MUT_KCNJ5				

### Correlation Matrix

		FR_MUT_KCNJ5
Step 1	Constant	
	Tumor_Size	
	age calc in yrs at study	
	SBP baseline	
	DBP baseline	
	FR_MUT_KCNJ5	
	Sex_1male_2female(1)	
	IHC	
Step 7	Constant	-.911
	FR_MUT_KCNJ5	1.000

### Variables not in the Equation

		Score	df	Sig.
Step 7 <sup>a</sup>	Variables			
	Tumor_Size	.495	1	.482
	age calc in yrs at study	.767	1	.381
	SBP baseline	.056	1	.813
	DBP baseline	.022	1	.881
	Sex_1male_2female(1)	.029	1	.865
	IHC	.020	1	.888
Overall Statistics		2.218	6	.899

a. Variable(s) removed on step 7: age calc in yrs at study.

Step number: 1

Observed Groups and Predicted Probabilities

	16 +		
		+	
	I		I
	I		I
F	I		I
			I
R	12 +		+
			+
E	I1		I
Q	I1		I
U	I1		I
E	8 +1		+
			+
N	I1		I



