

## 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.

```
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(.15) ITERATE(20) CUT(.5).
```

## Logistic Regression

### Notes

<b>Output Created</b>		26-MAY-2024 09:47:16
<b>Comments</b>		
<b>Input</b>	<b>Data</b>	/Users/utente/Downloads/msjcemultimifile/Regression NGS_2024_may_25.sav
	<b>Active Dataset</b>	DataSet1
	<b>Filter</b>	<none>
	<b>Weight</b>	<none>
	<b>Split File</b>	<none>
	<b>N of Rows in Working Data File</b>	127
<b>Missing Value Handling</b>	<b>Definition of Missing</b>	User-defined missing values are treated as missing
<b>Syntax</b>		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(.15) ITERATE(20) CUT(.5).
<b>Resources</b>	<b>Processor Time</b>	00:00:00,04
	<b>Elapsed Time</b>	00:00:00,00

## Case Processing Summary

Unweighted Cases <sup>a</sup>		N	Percent
Selected Cases	Included in Analysis	127	100.0
	Missing Cases	0	0.0
	Total	127	100.0
Unselected Cases		0	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		
			Sanger1POS_2NEG		Percentage Correct
			1.00	2.00	
Step 0	Sanger1POS_2NEG	1.00	0	56	0.0
		2.00	0	71	100.0
	Overall Percentage				

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	0.237	0.179	1.763	1	0.184	1.268

## Variables not in the Equation

	Score	df	Sig.
Step 0 Variables Tumor_Size	4.079	1	0.043

		age calc in yrs at study	13.941	1	<0.001
		SBP_AVS	3.003	1	0.083
		DBP_AVS	1.882	1	0.170
		ALL_DRC	1.908	1	0.167
		PAC_ngdl_calc	0.000	1	0.983
		IHC	2.046	1	0.153
	<b>Overall Statistics</b>		25.723	7	<0.001

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

#### Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	29.773	7	<0.001
	Block	29.773	7	<0.001
	Model	29.773	7	<0.001
Step 2 <sup>a</sup>	Step	-0.010	1	0.922
	Block	29.764	6	<0.001
	Model	29.764	6	<0.001
Step 3 <sup>a</sup>	Step	-0.692	1	0.405
	Block	29.071	5	<0.001
	Model	29.071	5	<0.001
Step 4 <sup>a</sup>	Step	-1.499	1	0.221
	Block	27.572	4	<0.001
	Model	27.572	4	<0.001

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	144.510 <sup>a</sup>	0.209	0.280
2	144.520 <sup>a</sup>	0.209	0.280
3	145.212 <sup>a</sup>	0.205	0.274
4	146.712 <sup>a</sup>	0.195	0.261

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

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

Observed			Predicted		
			Sanger1POS_2NEG		Percentage Correct
			1.00	2.00	
Step 1	Sanger1POS_2NEG	1.00	30	26	53.6
		2.00	20	51	71.8
	Overall Percentage				63.8
Step 2	Sanger1POS_2NEG	1.00	30	26	53.6
		2.00	19	52	73.2
	Overall Percentage				64.6
Step 3	Sanger1POS_2NEG	1.00	30	26	53.6
		2.00	18	53	74.6
	Overall Percentage				65.4
Step 4	Sanger1POS_2NEG	1.00	29	27	51.8
		2.00	18	53	74.6
	Overall Percentage				64.6

a. The cut value is ,500

**Variables in the Equation**

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1 <sup>a</sup>	Tumor_Size	0.259	0.127	4.124	1	0.042	1.296
	age calc in yrs at study	0.092	0.024	14.437	1	<0.001	1.096
	SBP_AVS	-0.041	0.016	6.667	1	0.010	0.960
	DBP_AVS	0.036	0.023	2.446	1	0.118	1.036
	ALL_DRC	0.082	0.074	1.244	1	0.265	1.086
	PAC_ngdl_calc	0.001	0.009	0.010	1	0.922	1.001
	IHC	0.243	0.292	0.694	1	0.405	1.275
	Constant	-3.143	2.397	1.719	1	0.190	0.043
Step 2 <sup>a</sup>	Tumor_Size	0.259	0.127	4.139	1	0.042	1.296
	age calc in yrs at study	0.092	0.024	14.451	1	<0.001	1.096
	SBP_AVS	-0.041	0.016	6.692	1	0.010	0.960
	DBP_AVS	0.036	0.023	2.463	1	0.117	1.037
	ALL_DRC	0.082	0.074	1.237	1	0.266	1.086
	IHC	0.242	0.292	0.689	1	0.407	1.274
	Constant	-3.122	2.387	1.710	1	0.191	0.044
Step 3 <sup>a</sup>	Tumor_Size	0.259	0.127	4.180	1	0.041	1.295
	age calc in yrs at study	0.094	0.024	15.161	1	<0.001	1.098
	SBP_AVS	-0.041	0.016	6.878	1	0.009	0.960
	DBP_AVS	0.037	0.023	2.593	1	0.107	1.037
	ALL_DRC	0.083	0.073	1.294	1	0.255	1.086
	Constant	-2.732	2.325	1.380	1	0.240	0.065
Step 4 <sup>a</sup>	Tumor_Size	0.258	0.126	4.175	1	0.041	1.294
	age calc in yrs at study	0.094	0.024	15.575	1	<0.001	1.099

	<b>SBP_AVS</b>	-0.039	0.016	6.134	1	0.013	0.962
	<b>DBP_AVS</b>	0.032	0.022	2.143	1	0.143	1.033
	<b>Constant</b>	-2.398	2.266	1.120	1	0.290	0.091

a. Variable(s) entered on step 1: Tumor\_Size, age calc in yrs at study, SBP\_AVS, DBP\_AVS, ALL\_DRC, PAC\_ngdl\_calc, IHC.

### Variables not in the Equation

			Score	df	Sig.
<b>Step 2<sup>a</sup></b>	<b>Variables</b>	<b>PAC_ngdl_calc</b>	0.010	1	0.922
	<b>Overall Statistics</b>		0.010	1	0.922
<b>Step 3<sup>b</sup></b>	<b>Variables</b>	<b>PAC_ngdl_calc</b>	0.004	1	0.947
		<b>IHC</b>	0.693	1	0.405
	<b>Overall Statistics</b>		0.702	2	0.704
<b>Step 4<sup>c</sup></b>	<b>Variables</b>	<b>ALL_DRC</b>	1.318	1	0.251
		<b>PAC_ngdl_calc</b>	0.000	1	0.997
		<b>IHC</b>	0.779	1	0.378
	<b>Overall Statistics</b>		1.969	3	0.579

a. Variable(s) removed on step 2: PAC\_ngdl\_calc.

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

c. Variable(s) removed on step 4: ALL\_DRC.

```
LOGISTIC REGRESSION VARIABLES SANGER1POS_2NEG
  /METHOD=BSTEP(WALD) Tumor_Size Age_calc SBP_AVS DBP_AVS ALL_DRC PAC_ngdl_calc
IHC 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).
```

## Logistic Regression

### Notes

<b>Output Created</b>		26-MAY-2024 09:47:16
<b>Comments</b>		
<b>Input</b>	<b>Data</b>	/Users/utente/Downloads/msjcemultimifile/Regression NGS_2024_may_25.sav
	<b>Active Dataset</b>	DataSet1
	<b>Filter</b>	<none>

	<b>Weight</b>	<none>
	<b>Split File</b>	<none>
	<b>N of Rows in Working Data File</b>	127
<b>Missing Value Handling</b>	<b>Definition of Missing</b>	User-defined missing values are treated as missing
<b>Syntax</b>		LOGISTIC REGRESSION VARIABLES SANGER1POS_2NEG  /METHOD=BSTEP(WALD) Tumor_Size Age_calc SBP_AVS DBP_AVS ALL_DRC PAC_ngdl_calc IHC 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).
<b>Resources</b>	<b>Processor Time</b>	00:00:00,06
	<b>Elapsed Time</b>	00:00:00,00

### Case Processing Summary

Unweighted Cases <sup>a</sup>		N	Percent
<b>Selected Cases</b>	<b>Included in Analysis</b>	48	37.8
	<b>Missing Cases</b>	79	62.2
	<b>Total</b>	127	100.0
<b>Unselected Cases</b>		0	0.0
<b>Total</b>		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	20	1.000
	2	28	0.000

### Block 0: Beginning Block

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

Iteration		-2 Log likelihood	Coefficients Constant
Step 0	1	40.679	-1.417
	2	39.888	-1.731
	3	39.880	-1.767
	4	39.880	-1.768

- a. Constant is included in the model.
- b. Initial -2 Log Likelihood: 39,880
- c. Estimation terminated at iteration number 4 because parameter estimates changed by less than ,001.

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

Observed			Predicted		
			Sanger1POS_2NEG		Percentage Correct
		1.00	2.00		
Step 0	Sanger1POS_2NEG	1.00	41	0	100.0
		2.00	7	0	0.0
Overall Percentage					85.4

- 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	-1.768	0.409	18.683	1	<0.001	0.171



### Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	Tumor_Size	1.431	1	0.232
		age calc in yrs at study	1.905	1	0.168
		SBP_AVS	0.219	1	0.640
		DBP_AVS	1.565	1	0.211
		ALL_DRC	0.523	1	0.470
		PAC_ngdl_calc	0.789	1	0.374
		IHC	1.295	1	0.255
		FR_MUT_KCNJ5	14.976	1	<0.001
	Sex_1male_2female	2.987	1	0.084	
Overall Statistics			21.260	9	0.012

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

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

Iteration		-2 Log likelihood	Coefficients				
			Constant	Tumor_Size	age calc in yrs at study	SBP_AVS	DBP_AVS
Step 1	1	27.119	-1.467	0.104	0.018	0.007	-0.010
	2	20.838	-2.266	0.211	0.033	0.008	-0.021
	3	19.031	-2.901	0.327	0.051	0.007	-0.032
	4	18.663	-3.469	0.411	0.069	0.005	-0.038
	5	18.618	-3.633	0.445	0.080	0.002	-0.039
	6	18.615	-3.600	0.452	0.083	0.000	-0.038
	7	18.615	-3.595	0.453	0.084	0.000	-0.038
	8	18.615	-3.595	0.453	0.084	0.000	-0.038
Step 9	1	31.082	0.771				
	2	26.858	1.372				
	3	26.196	1.717				
	4	26.162	1.816				
	5	26.161	1.822				
	6	26.161	1.822				

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

Iteration	Coefficients				
	ALL_DRC	PAC_ngdl_calc	IHC	FR_MUT_KCNJ5	Sex_1male_2female(1)
				5	

<b>Step 1</b>	<b>1</b>	-0.122	0.011	0.215	-0.073	0.606
	<b>2</b>	-0.199	0.023	0.431	-0.109	1.206
	<b>3</b>	-0.274	0.036	0.599	-0.134	1.776
	<b>4</b>	-0.360	0.046	0.671	-0.149	2.122
	<b>5</b>	-0.433	0.050	0.692	-0.155	2.242
	<b>6</b>	-0.460	0.051	0.703	-0.155	2.265
	<b>7</b>	-0.463	0.051	0.704	-0.155	2.267
	<b>8</b>	-0.463	0.051	0.704	-0.155	2.267
<b>Step 9</b>	<b>1</b>				-0.081	
	<b>2</b>				-0.128	
	<b>3</b>				-0.156	
	<b>4</b>				-0.164	
	<b>5</b>				-0.164	
	<b>6</b>				-0.164	

a. Method: Backward Stepwise (Wald)

b. Constant is included in the model.

c. Initial -2 Log Likelihood: 39,880

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.
<b>Step 1</b>	<b>Step</b>	21.264	9	0.012
	<b>Block</b>	21.264	9	0.012
	<b>Model</b>	21.264	9	0.012
<b>Step 9<sup>a</sup></b>	<b>Step</b>	-1.436	1	0.231
	<b>Block</b>	13.718	1	<0.001
	<b>Model</b>	13.718	1	<0.001

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
<b>1</b>	18.615 <sup>a</sup>	0.358	0.634
<b>9</b>	26.161 <sup>b</sup>	0.249	0.441

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		
			Sanger1POS_2NEG		Percentage Correct
			1.00	2.00	
Step 1	Sanger1POS_2NEG	1.00	40	1	97.6
		2.00	3	4	57.1
	Overall Percentage				
Step 9	Sanger1POS_2NEG	1.00	41	0	100.0
		2.00	3	4	57.1
	Overall Percentage				

a. The cut value is ,500

**Variables in the Equation**

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)
								Lower
Step 1 <sup>a</sup>	Tumor_Size	0.453	0.460	0.967	1	0.326	1.573	0.638
	age calc in yrs at study	0.084	0.114	0.537	1	0.464	1.087	0.869
	SBP_AVS	0.000	0.089	0.000	1	1.000	1.000	0.839
	DBP_AVS	-0.038	0.092	0.173	1	0.677	0.962	0.804
	ALL_DRC	-0.463	0.688	0.452	1	0.501	0.630	0.163
	PAC_ngdl_calc	0.051	0.041	1.595	1	0.207	1.053	0.972
	IHC	0.704	1.254	0.316	1	0.574	2.023	0.173
	FR_MUT_KCNJ5	-0.155	0.082	3.555	1	0.059	0.856	0.728
	Sex_1male_2female	2.267	1.481	2.343	1	0.126	9.649	0.530
	Constant	-3.595	6.694	0.288	1	0.591	0.027	
Step 9 <sup>a</sup>	FR_MUT_KCNJ5	-0.164	0.055	8.907	1	0.003	0.849	0.762
	Constant	1.822	1.110	2.694	1	0.101	6.186	

**Variables in the Equation**

		95% C.I. for EXP(B)
		Upper
Step 1 <sup>a</sup>	Tumor_Size	3.877
	age calc in yrs at study	1.360
	SBP_AVS	1.191
	DBP_AVS	1.153
	ALL_DRC	2.425
	PAC_ngdl_calc	1.140
	IHC	23.615
	FR_MUT_KCNJ5	1.006
	Sex_1male_2female	175.761

	<b>Constant</b>	
<b>Step 9<sup>a</sup></b>	<b>FR_MUT_KCNJ5</b>	0.945
	<b>Constant</b>	

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

### Correlation Matrix

		<b>Constant</b>	<b>Tumor_Size</b>	<b>age calc in yrs at study</b>	<b>SBP_AVS</b>	<b>DBP_AVS</b>
<b>Step 1</b>	<b>Constant</b>	1.000	-0.391	-0.162	-0.462	0.164
	<b>Tumor_Size</b>	-0.391	1.000	0.259	0.034	-0.178
	<b>age calc in yrs at study</b>	-0.162	0.259	1.000	-0.574	0.403
	<b>SBP_AVS</b>	-0.462	0.034	-0.574	1.000	-0.811
	<b>DBP_AVS</b>	0.164	-0.178	0.403	-0.811	1.000
	<b>ALL_DRC</b>	-0.208	-0.261	-0.357	0.172	0.088
	<b>PAC_ngdl_calc</b>	-0.104	0.129	0.391	-0.252	0.129
	<b>IHC</b>	0.073	0.216	-0.097	-0.110	-0.222
	<b>FR_MUT_KCNJ5</b>	0.217	0.014	-0.146	-0.201	0.032
	<b>Sex_1male_2female</b>	-0.200	0.243	0.094	0.091	-0.231
<b>Step 9</b>	<b>Constant</b>	1.000				
	<b>FR_MUT_KCNJ5</b>	-0.890				

### Correlation Matrix

		<b>ALL_DRC</b>	<b>PAC_ngdl_calc</b>	<b>IHC</b>	<b>FR_MUT_KCNJ5</b>	<b>Sex_1male_2female(1)</b>
<b>Step 1</b>	<b>Constant</b>	-0.208	-0.104	0.073	0.217	-0.200
	<b>Tumor_Size</b>	-0.261	0.129	0.216	0.014	0.243
	<b>age calc in yrs at study</b>	-0.357	0.391	-0.097	-0.146	0.094
	<b>SBP_AVS</b>	0.172	-0.252	-0.110	-0.201	0.091
	<b>DBP_AVS</b>	0.088	0.129	-0.222	0.032	-0.231
	<b>ALL_DRC</b>	1.000	-0.260	-0.156	-0.009	-0.296
	<b>PAC_ngdl_calc</b>	-0.260	1.000	0.075	-0.458	0.328
	<b>IHC</b>	-0.156	0.075	1.000	0.323	0.128
	<b>FR_MUT_KCNJ5</b>	-0.009	-0.458	0.323	1.000	-0.090
	<b>Sex_1male_2female</b>	-0.296	0.328	0.128	-0.090	1.000
<b>Step 9</b>	<b>Constant</b>					
	<b>FR_MUT_KCNJ5</b>					

### Correlation Matrix

		<b>FR_MUT_KCNJ5</b>
<b>Step 1</b>	<b>Constant</b>	
	<b>Tumor_Size</b>	

	age calc in yrs at study	
	SBP_AVS	
	DBP_AVS	
	ALL_DRC	
	PAC_ngdl_calc	
	IHC	
	FR_MUT_KCNJ5	
	Sex_1male_2female	
<b>Step 9</b>	Constant	-0.890
	FR_MUT_KCNJ5	1.000

### Variables not in the Equation

			Score	df	Sig.
<b>Step 9<sup>a</sup></b>	<b>Variables</b>	<b>Tumor_Size</b>	0.521	1	0.471
		age calc in yrs at study	1.800	1	0.180
		SBP_AVS	0.163	1	0.686
		DBP_AVS	0.012	1	0.914
		ALL_DRC	0.577	1	0.448
		PAC_ngdl_calc	1.408	1	0.235
		IHC	0.032	1	0.859
		Sex_1male_2female	1.299	1	0.254
	<b>Overall Statistics</b>	6.180	8	0.627	

a. Variable(s) removed on step 9: PAC\_ngdl\_calc.

Step number: 1

Observed Groups and Predicted Probabilities

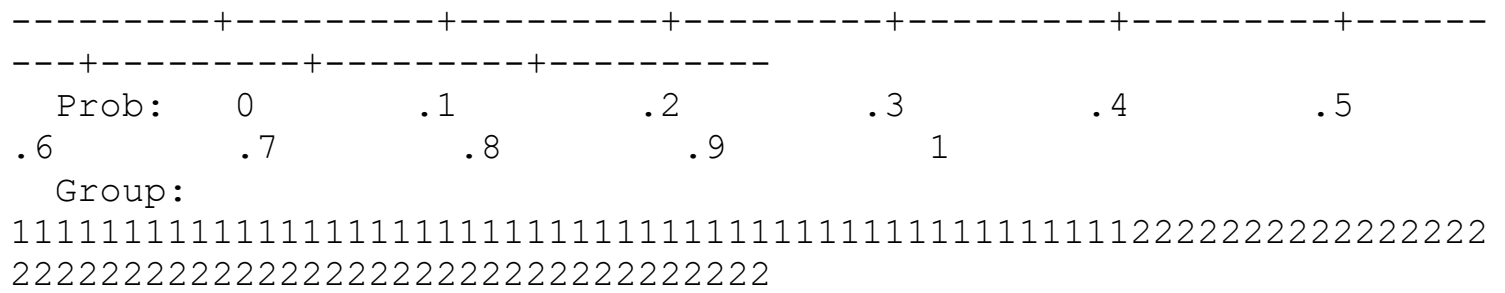
```

+
20 +
+
I
I
I1
I
F
I
R
15 +1
+

```

E I1  
I I1  
Q I1  
I I1  
U I1  
I E 10 +1  
+  
N I1  
I I11  
C I11  
I I11  
Y I11  
I 5 +11  
+  
I I11  
I I1111 2 1  
I I111111 111 1 12 1 1 1 2 1  
2 2 2I

Predicted



Predicted Probability is of Membership for 2.00  
The Cut Value is .50  
Symbols: 1 - 1.00  
2 - 2.00  
Each Symbol Represents 1.25 Cases.

**Step Summary<sup>a,b</sup>**

Step	Improvement			Model			Correct Class %	Variable
	Chi-square	df	Sig.	Chi-square	df	Sig.		
2	0.000	1	1.000	21.264	8	0.006	91.7%	OUT: SBP_AVS
3	-0.350	1	0.554	20.915	7	0.004	91.7%	OUT: IHC
4	-0.260	1	0.610	20.655	6	0.002	91.7%	OUT: DBP_AVS

5	-1.729	1	0.189	18.926	5	0.002	93.8%	OUT: ALL_DRC
6	-0.528	1	0.467	18.398	4	0.001	93.8%	OUT: Tumor_Size
7	-0.796	1	0.372	17.602	3	<0.001	93.8%	OUT: age calc in yrs at study
8	-2.448	1	0.118	15.154	2	<0.001	93.8%	OUT: Sex_1male_ 2female
9	-1.436	1	0.231	13.718	1	<0.001	93.8%	OUT: PAC_ngdl_c alc

- a. No more variables can be deleted from or added to the current model.
- b. End block: 1

Step number: 9

Observed Groups and Predicted Probabilities

```

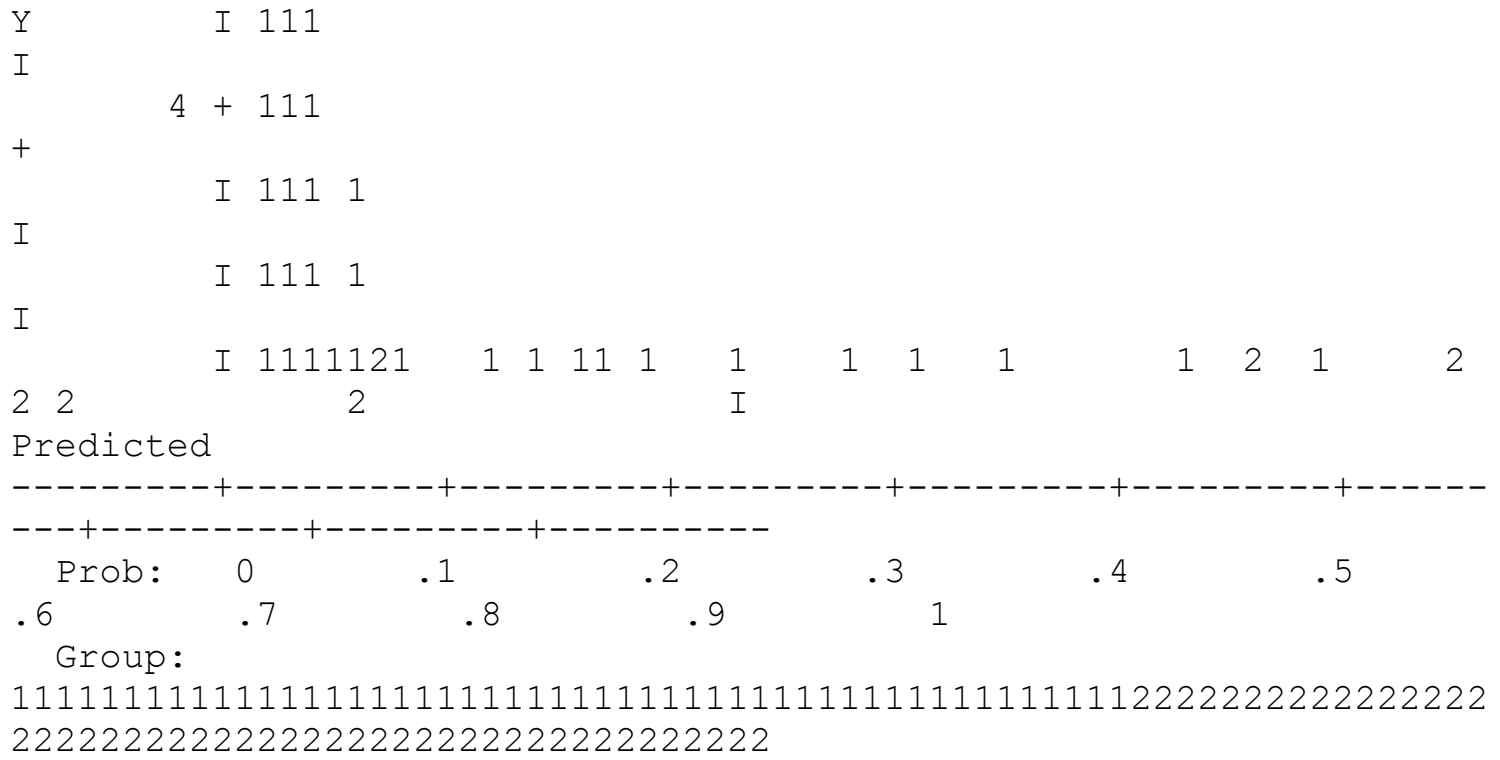
16 +
+
I
I
I
F
I
R
+
E
I
Q
I
U
I
E
+
N
I
C
I

```

```

16 +
I
I
I
I
12 +
I 1
I 12
I 11
8 + 11
I 11
I 11

```



Predicted Probability is of Membership for 2.00  
 The Cut Value is .50  
 Symbols: 1 - 1.00  
 2 - 2.00  
 Each Symbol Represents 1 Case.