

# Neureglin 3 gene intronic polymorphisms rs10883866, rs6584400, rs1937970 and rs677221 in patients with Schizophrenia

## Supplementary Material

*Supplementary table 1: Components and concentrations of PCR reaction*

<b>Components</b>	<b>Concentrations</b>
DNA	1 $\mu$ l
Reverse Primer (10 pm)	0.5 $\mu$ l
Forward Primer (10 pm)	0.5 $\mu$ l
DreamTaq Master Mix (2X)	8 $\mu$ l
Nuclease free H <sub>2</sub> O	up to 15.0 $\mu$ l

*Supplementary table 2: Components and concentrations of RFLP reaction*

<b>Components</b>	<b>Concentrations</b>
Nuclease free H <sub>2</sub> O	4.7 $\mu$ l
1X Buffer	1 $\mu$ l
PCR product	4 $\mu$ l
Restriction enzyme	0.3 $\mu$ l
Total	10.0 $\mu$ l

*Supplementary table 3: Components and concentrations of ARMS PCR reaction*

<b>Components</b>	<b>Concentrations</b>
DNA	1 $\mu$ l
Outer Reverse Primer (10 pm)	0.5 $\mu$ l
Outer Forward Primer (10 pm)	0.5 $\mu$ l
Inner Forward Primer	0.5 $\mu$ l
Inner Reverse Primer	0.5 $\mu$ l
Green Tag Master Mix	8 $\mu$ l
Nuclease free H <sub>2</sub> O	up to 15.0 $\mu$ l

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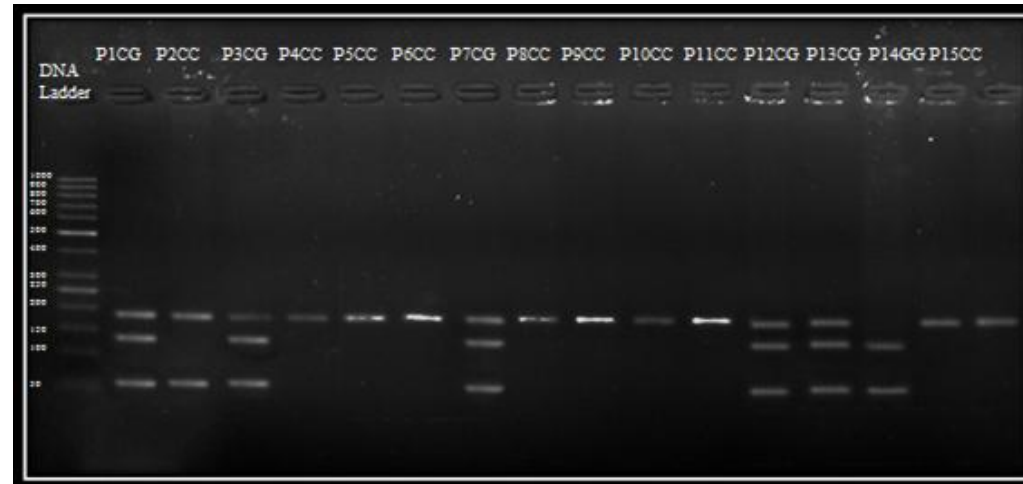
*Supplementary table 4: Primers for SNPs rs10883866 and rs6584400*

No	SNP	Primer	Sequence		Product Size
1. 1	rs10883866	5'- TGTGAATTGCCAGGGAGCTA -3'	5'-↓NNCASTTGN-3' 3'-NGTSACNN↑-5'	Present if G	A=186 bp G=132, 54 bp
2. 2		5'- CACATATGGCAGCTGTTCGTT -3'			
3. 3	rs6584400	5'- TCCAGAGGATGAGAATCTAGG -3'	5'-G↓ANTC-3' 3'-CTNA↑G-5'	Present if G	C=360 bp G=132, 239 bp
4. 4		5'- TGAGACATGCTGAACAGGGTT -3'			

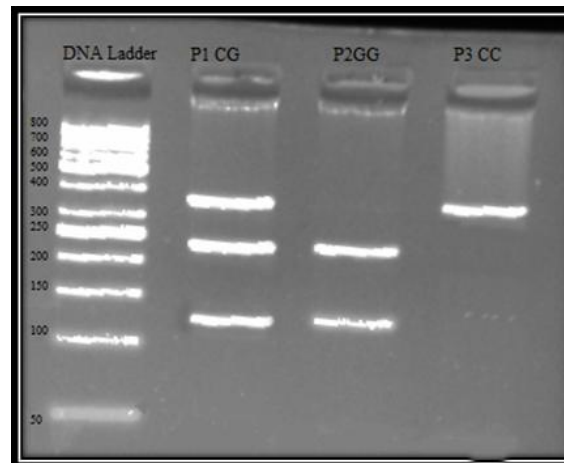
*Supplementary Table 5: Primers for SNPs rs1937970 and rs677221*

5. 1	rs1937970	Inner Forward	5'- GGATAACTGGGCAAAGTCATGCAGCTAACG 3'	N=445bp G allele=229 bp A allele= 273bp
6. 2		Inner Reverse	5'- AGTTGAGGGGTTGGTTCTCTGCCAGGT -3'	
7. 3		Outer Reverse	5'- AGCAGCAAGTGGAAGGGGCGTTGTATTT -3'	
8. 4		Outer Forward	5'- CAGCAACTGCCACTCTCAGTGTCACCTC -3'	
9. 5	rs677221	Inner Forward	5'- GCTGTTATGGTCATGAAATGGAATATTG -3'	N=302bp G allele= 197bp C allele= 159bp
10. 6		Inner Reverse	5'- GCCTGAGCTCCCTTAGGGGTCCTGAG -3'	
11. 7		Outer Reverse	5'- GCACTGCTTTTAGTGGTTACACAAGATCCA-3'	
12. 8		Outer Forward	5'- AACTAGAAAAGGGCAAGGAGCCTGTTCT -3'	

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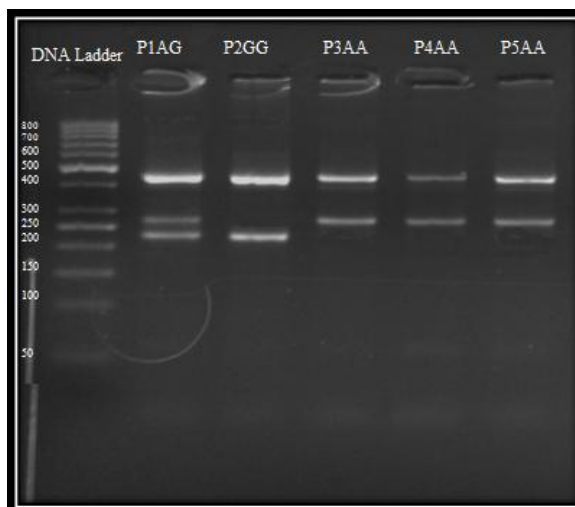


**Supplementary Figure 1:** PCR-RFLP analysis of *NRG3* SNP rs10883866 on 3% agarose gel. The genotypes are shown on the top of the lanes. Lane 1: DNA Ladder, Lane 2-16: Digested PCR products. P=Patient, CC=Homozygous for C allele, CG=Heterozygous, GG=Homozygous for G allele.

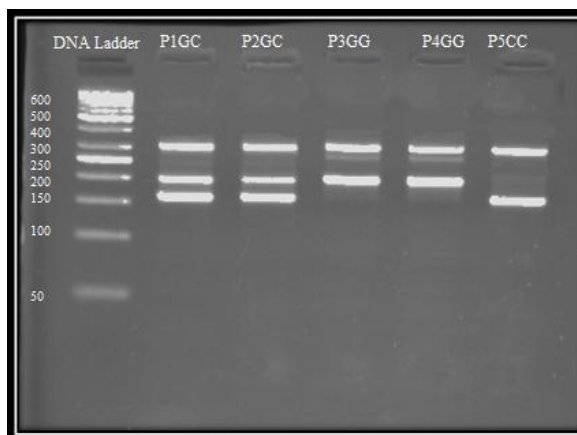


**Supplementary Figure 2:** PCR-RFLP analysis of *NRG3* SNP rs6584400 on 3% agarose gel. The genotypes are shown on the top of the lanes. Lane 1: DNA Ladder, Lane 2, 3, 4: Digested PCR products. P=Patient, CC=Homozygous for C allele, CG=Heterozygous, GG=Homozygous for G allele

## Neureglin 3 gene intronic polymorphisms rs10883866, rs6584400, rs1937970 and rs677221 in patients with Schizophrenia



**Supplementary Figure 3:** *NRG3* rs1937970 analysis by ARMS PCR on 3% agarose gel. The genotypes are shown on the top of the lanes. Lane 1: DNA Ladder, Lane 2-5: PCR products. P=Patient, AA=Homozygous for A allele, AG=Heterozygous, GG=Homozygous for G allele



**Supplementary Figure 4:** *NRG3* rs677221 analysis by ARMS PCR on 3% agarose gel. The genotypes are shown on the top of the lanes. Lane 1: DNA Ladder, Lane 2-5: PCR products. P=Patient, CC=Homozygous for C allele, GC=Heterozygous, GG=Homozygous for G allele

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*Supplementary Table 6: Basic information and minor allele frequency of candidate SNPs in present study*

SNP ID	Gene	Position	Base Change	Role	MAF*			p- Value	OR [95% CI]	p- Value
					Cases	Controls	Database			
rs10883866	NRG3	81883883	(C/G)	Intron	0.211	0.297	0.245	0.002	0.633 [0.4339-0.924]	0.017
rs6584400		81896770	[A/G]	Intron	0.281	0.396	0.292	0.004	0.595 [0.420-0.843]	0.003
rs1937970		82463710	[A/G]	Intron	0.319	0.378	0.312	0.16	0.770 [0.546-1.086]	0.137
rs677221		82554641	[C/G]	Intron	0.277	0.356	0.344	0.05	0.695 [0.489-0.988]	0.042

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Supplementary Table Figure 7: Genotypic frequencies of NRG3 SNPs and associations with the disease

Genotypes/Allele	Controls n (%)	Cases n (%)	p- Value	OR crude [95% CI]	p-Value adjusted*	OR Adjusted* [95% CI]
<b>Genotypes</b>						
<i>rs6584400</i>						
AA	66 (45.5)	80 (55.6)		-----		-----
AG	43 (29.7)	47 (32.6)	0.014	0.902 [0.533-1.527]	<b>0.042</b>	0.77 [0.43-1.39]
GG	36 (24.8)	17 (11.8)		<b>0.390 [0.201-0.756]</b>		<b>0.40 [0.19-0.82]</b>
<i>rs10883866</i>						
CC	59 (40.4)	92 (63.9)		-----		-----
CG	86 (58.9)	43 (29.9)	< <b>0.0001</b>	<b>0.32 [0.20-0.52]</b>	<b>0.042</b>	<b>0.46 [0.24-0.88]</b>
GG	1 (0.68)	09 (6.2)		5.77 [0.71-46.74]		1.70 [0.19-15.30]
<i>rs1937970</i>						
AA	54 (37.5)	62 (43.1)		-----		-----
AG	71 (49.3)	72 (50.0)	0.18	0.883 [0.541-1.442]	0.95	1.10 [0.63-1.92]
GG	19 (13.2)	10 (6.9)		0.458[0.196-1.071]		1.00 [0.33-3.04]
<i>rs677221</i>						
GG	68 (46.6)	77(49.3)		-----		-----
GC	52(35.6)	66(45.8)	<b>0.0013</b>	1.216 [.743-1.989]	0.12	1.18 [0.70-2.00]
CC	26(17.8)	7(4.9)		<b>.258 [.105-.633]</b>		0.43 [0.16-1.15]

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Supplementary Table 8: Haplotype block analysis with Major haplotype frequencies and their odd ratios

Variant position	Block 1				OR Adjusted* [95% CI]	p-value	Variant position	Block 2				OR Adjusted* [95% CI]	p-value
	rs108838866	rs6584400	Haplotype Frquency					rs1937970	rs677221	Haplotype Frquency			
			Cases	Controls						Cases	Controls		
Haplotypes <sup>a,b</sup>	C	G	0.6244	0.4681	1.00	---	Haplotype <sup>c,d</sup>	A	G	0.6072	0.4852	1.00	---
	C	A	0.1638	0.2251	0.85 (0.50 - 1.45)	0.56		G	C	0.2045	0.2322	0.97 (0.59 - 1.58)	0.89
	G	A	0.1174	0.1688	0.47 (0.26 - 0.84)	0.011		G	G	0.115	0.1427	0.96 (0.50 - 1.86)	0.91
	G	G	0.0944	0.1379	1.07 (0.48 - 2.37)	0.87		A	C	0.0733	0.1398	0.65 (0.33 - 1.28)	0.21