A large-scale SNP evaluation of associations with sporadic neuroendocrine tumor (NET) risk

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Introduction

Characteristics of NET

- **Incidence** ~ 5.25 /100,000
  - Diagnosis is increasing
- **Prevalence estimated at >100,000**
- **Often pursue indolent course**
- **Hormone-secreting**
- Common subtypes:
  - **Carcinoid** – small bowel is most common site
  - **Pancreatic Endocrine tumor**

Known Risk Factors

- **Rare Mendelian diseases** TSC, VHL, NF1, MEN1 and MEN2
  - However 95% of NET are sporadic
  - genetic risk factors for sporadic NET are not defined

Potential Risk Factors

- **Female gender**
- **Family history** of any cancer
- **Smoking, Diabetes**

Methods

Study Populations

Two separate case/control sets used for discovery and replication:

- Case recruitment at the Dana-Farber Cancer Institute (DFCI) from 2003-2009
- 261 Discovery cases diagnosed from 1967 to 2007
- 319 Discovery controls from Harvard Lung Cancer Susceptibility study at MGH
- 235 Replication cases diagnosed 1969-2009
- 113 Replication spousal/ friend controls from DFCI

- Cases & controls **restricted to Caucasians** and known familial cases excluded

SNP selection

1334 SNPs genotyped on the Illumina GoldenGate platform for **discovery**

Functional and tagging SNPs in 354 cancer genes from these pathways:

Pathway	Number of Genes	Number of SNPs
MTOR	28	216
Inflammation	38	228
Apoptosis	36	233
Other	18	33
Transporter	8	16
DNA Repair	50	136
Cell Cycle	46	165
Cell Growth	33	62
Metastasis	15	18
Metabolism	71	202
Angiogenesis	11	126
Hormone	8	45
Epigenetic	3	15
Immunity	8	14
Total*	354	1334

* Total is not a sum due to overlap in gene and SNP count

25 SNPs from discovery chosen for **replication** genotyped on the Sequenom platform

Descriptive characteristics of the study populations

Characteristics	Discovery set			Replication set		
	Cases (n= 261)	Controls (n=319)	Multivariate p-value[b]	Cases (n= 235)	Controls (n=113)	Multivariate p-value[b]
Age[a]	52 (15-86)	56 (30-83)	**0.0006**	55 (18-83)	53 (26-85)	0.47
Gender						
Females	140 (53.6%)	138 (43.3%)		112 (47.7%)	72 (63.7%)	
Males	121 (46.4%)	181 (56.7%)	**0.04**	123 (53.3%)	41 (36.3%)	**0.005**
Smoking status						
Non-Smoker	118 (45.9%)	112 (35.1%)		117 (51.5%)	56 (50%)	
Ex-Smoker	124 (48.3%)	143 (44.8%)	0.62	96 (42.3%)	44 (39.3%)	0.93
Current Smoker	15 (5.8%)	64 (20.1%)	**<0.001**	14 (7.0%)	12 (10.7%)	0.19
Missing	4	0		8	1	
Site of origin						
Pancreatic Islet Cell	54 (20.7%)			47 (20%)		
Small Bowel	91 (34.9%)			92 (39.2%)		
Lung	24 (9.1%)			25 (10.6%)		
Appendix	19 (7.3%)			11 (4.7%)		
Stomach	8 (3.1%)			5 (2.1%)		
Other[c]	23 (8.8%)			23 (9.8%)		
Unknown primary	42 (16.1%)			32 (13.6%)		
Stage[d]						
M0	118 (45.2%)			106 (45.1%)		
M1	143 (54.7%)			129 (54.9%)		

a. median (range)
b. adjusted for age, sex, and smoking status for the discovery set and age and sex for the replication set
c. Other sites include colon, rectum, anus, thorax, larynx, heart, and thyroid
d. M0 = no metastasis after resection at initial diagnosis, M1 = metastasis at initial diagnosis

Results

Polymorphism associations[a] with NET in the discovery set and in the replication set

Gene	Variable	Discovery set			Replication set			
		Dominant Adjusted OddsRatio (95%CI)	Dominant p-value	Additive p-value	Dominant Adjusted OddsRatio (95%CI)	Dominant p-value	Additive Adjusted OddsRatio (95%CI)	Additive p-value
TSC2	rs13337626	2.816 (1.894, 4.189)	3.18E-07	2.69E-06	Failed genotyping			
IL1RN	rs380092	1.87 (1.321, 2.649)	0.0004	0.0002	0.611 (0.381, 0.98)	0.04	0.687 (0.485, 0.973)	0.03
CYP1B1	rs162562	1.749 (1.232, 2.483)	0.002	0.008	1.208 (0.747, 1.952)	0.44	1.195 (0.785, 1.819)	0.41
BIRC5	rs1508147	1.755 (1.219, 2.528)	0.002	0.014	0.925 (0.57, 1.501)	0.75	0.915 (0.647, 1.293)	0.61
AKAP9	rs6964587	0.58 (0.405, 0.831)	0.003	0.006	1.455 (0.894, 2.37)	0.13	1.266 (0.906, 1.768)	0.17
IL12A	**rs2243123**	**1.676 (1.181, 2.377)**	**0.004**	**0.011**	1.533 (0.965, 2.434)	0.07	**1.473 (1.027, 2.115)**	**0.04**
BCL2	rs7234941	0.582 (0.403, 0.841)	0.004	0.013	1.205 (0.72, 2.017)	0.48	1.183 (0.743, 1.882)	0.48
APAF1	rs1007573	0.53 (0.343, 0.821)	0.004	0.012	0.887 (0.481, 1.637)	0.70	0.948 (0.55, 1.635)	0.85
BCL2	rs1982673	0.568 (0.382, 0.845)	0.005	0.003	Failed genotyping			
DAD1	**rs8005354**	**1.645 (1.154, 2.346)**	**0.006**	**0.028**	1.52 (0.96, 2.405)	0.07	**1.434 (1.016, 2.023)**	**0.04**
APAF1	rs2288713	0.552 (0.357, 0.853)	0.007	0.018	0.903 (0.486, 1.677)	0.75	0.977 (0.546, 1.75)	0.94
CYP1B1	rs10916	1.616 (1.134, 2.303)	0.008	0.019	Failed genotyping			
MS4A6A	rs1019670	0.672 (0.474, 0.954)	0.026	0.002	1.002 (0.621, 1.617)	0.99	0.944 (0.679, 1.314)	0.73
FRAP1	rs12124983	1.515 (1.071, 2.144)	0.019	0.002	0.834 (0.526, 1.322)	0.44	0.896 (0.633, 1.266)	0.53
CASP7	rs4342983	1.8 (1.135, 2.856)	0.013	0.007	0.945 (0.48, 1.859)	0.87	0.92 (0.49, 1.726)	0.79
FRAP1	rs1064261	1.421 (1.006, 2.006)	0.046	0.007	0.84 (0.527, 1.34)	0.46	0.987 (0.707, 1.378)	0.94
TERT	rs2075786	1.383 (0.972, 1.969)	0.072	0.007	1.471 (0.928, 2.331)	0.10	1.229 (0.866, 1.742)	0.25
ADH1C	rs698	1.458 (1.023, 2.079)	0.037	0.011	1.107 (0.681, 1.799)	0.68	1.138 (0.815, 1.588)	0.45
IFNGR2	rs1059293	1.673 (1.122, 2.494)	0.012	0.019	0.766 (0.464, 1.264)	0.30	0.975 (0.71, 1.338)	0.88
IL17RB	rs1043261	1.667 (1.04, 2.673)	0.034	0.02	0.953 (0.466, 1.949)	0.90	0.89 (0.452, 1.756)	0.74
TSC2	**rs8050755**	**1.513 (1.047, 2.187)**	**0.027**	**0.137**	**2.05 (1.131, 3.731)**	**0.02**	**1.95 (1.105, 3.353)**	**0.02**

*Adjusted for age, sex and smoking (discovery), adjusted for age and sex (replication)

Small Bowel and Pancreatic NET subgroups

Discovery set – All 1334 SNPs

Small Bowel (91 cases)
- 23 SNPs p ≤0.01
- Top 10 genes: *TSC2, CYP1B1 (3), CFLAR, IL1RN, ALOX5, DAD1, PIK3CA, TNFRSF6, IGFBP1, MS4A6A*

Pancreatic (54 cases)
- 19 SNPs p ≤0.01
- Top 10 genes: *LIG3, CDKN2A(4), TSC2, BCL2, ADPRT, IFNGR2, TNFA, VEGFR1, SLC10A2, TSC1*

Replication set – 25 SNPs

Small Bowel (92 cases)
- *IL1RN* rs380092, p=0.018
- *IL12A* rs2243123, p=0.04
- *DAD1* rs8005354, p=0.02

Pancreatic (47 cases)
- *IL12A* rs2243123, p=0.004
- *AKAP9* rs6964587, p=0.035
- *DAD1* rs8005354, p=0.054
- *TSC2* rs8050755, p=0.08

Combined sets – 16 SNPs

Combined small bowel
- *CYP1B1* rs16256, p=0.003
- *IL12A* rs2243123, p=0.02
- *DAD1* rs8005354, p=0.04

Combined pancreatic
- *IL12A* rs2243123, p=0.005
- *TSC2* rs8050755, p=0.01

For SNPs evaluated in both discovery and replication sets we combined the subgroups after testing for a non-significant interaction between the sets

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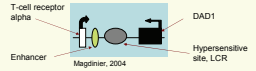
Discussion

IL12A **(interleukin 12A) rs2243123**
- **IL-12 promotes anti-tumor cell-mediated immunity and anti-angiogenesis by activating the T helper 1 (Th1) response**
- **rs2243123 is in LD with *IL12A* rs568408 (3'UTR G>A) found to be associated w/ increased risk of cervical cancer in Chinese**
- **Other *IL12A* SNPs have shown mixed reults with gastric cancer**

DAD1 **(defender against apoptotic cell death) rs8005354**
- **Previously DAD1 shown to be highly expressed in small bowel carinoid compared to normal tissue from DFCI cases**
- **A Locus Control region of DAD1 and T-cell receptor (TRAC) was also shown to be amplified in a SNP array**



- **rs8005354 is located in a region of high LD with the Locus control region**

TSC2 **(tuberous sclerosis 2) rs8050755**
- **Mutations in *TSC2* are associated with the rare familial form of NET**

Conclusions

First large scale evaluation of genetic variation and risk in sporadic neuroendocrine tumor

Genetic variation in *IL12A, DAD1 and TSC2* strongly associated with sporadic NET risk

Further investigation into linked variation in these gene regions is warranted

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