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Effect of nicotinic acetylcholine receptors polymorphism in Egyptian males with chronic obstructive pulmonary disease

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ABSTRACT

The major reinforcing component of tobacco smoke is Nicotine which acts through neuronal nicotinic acetylcholine receptors (nAChRs). It is a strong predisposing factor for the development of chronic obstructive pulmonary disease (COPD). The aim of this study was to evaluate the associations between single nucleotide polymorphism (SNP) of nAChRs rs1051730 and demographic (including smoking index and cigarette consumption per day), physiologic and lab characteristics of Egyptian males with COPD. This study was conducted on 68 COPD smoker patients and 32 non-COPD smokers who were selected from Chest Department, Faculty of Medicine, Menoufia University during the period from October 2015 to January 2016. A blood sample was taken and a spirometry was performed. Genotyping was performed for nAChRs rs1051730 by SNP assay real time PCR methods. The distribution of nAChRs rs1051730 AA genotypes is more frequent in COPD patients with increase susceptibility to COPD by 5.19 fold. AA genotypes were also associated with higher smoking index and more cigarette smoking per day. Increase frequency of AA genotypes in patient's heavy smokers as an indicator of nicotine dependence. The Nicotinic acetylcholine receptors (nAChRs) SNP rs1051730 AA genotype and A allele may influence the development of COPD but not its severity. AA genotype of rs1051730 may also have more influence in nicotine dependence.

Key words: Smokers, Nicotine, COPD, Polymorphism

INTRODUCTION

COPD is the fourth leading cause of death in the United States [1]. The development of airflow limitation is the main character of COPD. It is not fully reversible and may be progressive [2]. Based on the spirometric results, the severity of COPD divided into mild, moderate, severe and very severe according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy [3]. Over 90% of causation of COPD is due to cigarette smoking in the western world [4]. Prevalence of smoking is 54–77% in mild COPD patients and 38–51% in severe COPD patients [5].

Nicotine acts through neuronal nicotinic acetylcholine receptors (nAChRs) as a major reinforcing component of tobacco smoking [6]. Smoking less than 10 to 15 pack years of cigarettes is unlikely to result in COPD without the

presence of a genetic/environmental/occupational predisposition [7]. Nicotine leads to activation of the reward pathway by stimulation of dopamine release in the brain, which leads to the development of substance dependence [8].

Neuronal nAChRs are ligand-gated ion channels composed of five transmembrane subunit proteins arranged around a central pore. Neuronal nAChR consists of α (α2–α10) and β (β2–β4) subunits, each of which is encoded for by a single gene [9].

A kind of subunit combination responsible for different biological receptor function [10]. Variation in genes coding for nAChRs could change nAChR function and increased predisposition to nicotine dependence [11].

The aim of this study was to evaluate the associations between single nucleotide polymorphism (SNP) of nAChRs rs1051730 and demographic (including smoking index and cigarette consumption per day), physiologic and lab characteristics of Egyptian males with COPD.

MATERIALS AND METHODS

This study was performed in Hospital of Menoufia University during the period from October 2015 to January 2016, Including 100 male subjects classified into two groups: Group I: included 68 cigarette smokers with COPD diagnosed by spirometric evidence of airflow obstruction (forced expiratory volume in one second /forced vital capacity ((FEV1/FVC) < 0.70. and sub-classified according to severity of airflow obstruction by FEV1 into mild, moderate, severe and very severe grade < 80% , 50–79% , 30–49% and <30% pred respectively (3). Group II (control): included 32 apparently healthy cigarette smokers with no COPD. Patients with history of lung or cardiovascular diseases were excluded.

All participants were current smokers defined as Individuals, who smoked cigarettes either daily or occasionally, at the time of the study [12]. Nicotine consumption was estimated for all participants with cigarettes smoked per day and the pack-year smoking index which was calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoked. subjects were divided into heavy smokers with 20 or more cigarettes per day and light smokers with less than 20 cigarettes per day to assess nicotine dependence, [13].

The exclusion criteria were: acute exacerbation of COPD, chronic pulmonary disease (other than COPD), coronary artery disease, hypertension, heart failure, a history of stroke, diabetes mellitus, , and chronic renal disease.

Menoufia Hospital's Review Board have given ethics approval and all participants have written an informed consent prior to subject characterization and sample collections.

All persons included in the study were subjected to the following: Complete history taking, include previous diseases, cigarette smoking per day and calculation of the pack-year smoking index. Complete general and local examination, plain X-ray and measurements of pulmonary functions in Pulmonary Function Test Unit in Menoufia University Hospital. Pulmonary functions were measured with a turbine spirometer "Quark PFT3, COSMED, Italy".

Blood sampling:
Six milliliters (ml) of blood samples were taken from each subject and divided into: three, one for complete blood count (CBC) and the other for DNA extraction in EDETA tubes, while last portion was put in a plain tube, left to clot for 30 minutes at room temperature, then subjected to centrifugation for 10 minutes at 4000 rotations per minute (RPM) and the serum obtained was put in aliquot, stored at -80◦C until the time of assay of lipid profile.

Assay methods
Complete blood picture was measured with Pentra-80 automated blood counter (ABX– France –Rue du Caducee-Paris Euromedecine-BP-7290.34184 Montpellier-Cedex 4.)

Quantitative determination of total cholesterol, HDLc and triacylglycerides (TG) using colorimeteric enzymatic method [14]. LDLc was calculated by the formula of Friedewald et al. [15].

SNP assay.
After DNA extraction by Pure link genomic DNA extraction kits (USA) The DNA extract was used for SNP assay in a total reaction volume 20 ul with 10 ul of Taqman Genotyping Master Mix, 1.25 ul of 20X TaqMan genotyping assay kits containing both primers and probes and nuclease free water. By using the 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA), the Reaction conditions were as following: 50ºC for 1 min Pre-PCR read, then 95 ºC for 10 min and 45 cycles of 95 ºC for 15 s, 60 ºC for 1 min (cycling), and 60 ºC for 1 min (Post-PCR).

The primer sequence of Nicotinic acetylcholine receptors (nAChRs) rs1051730 SNP was as follows: Forward AGGGAGAGGAGGACAGAAA reverse AAGGACTATTGGGAGAGCG and probe sequence labeled with VIC and FAM fluorescent dyes was as follows:

AGCAGTTGTACTTGATGTCGTGTTT[A/G]TAGCCTGGGGCTTTGATGATGGCCC. Figure 1 shows the allelic discrimination type of three genotypes of SNP (AA, AG and GG) while, figure 2 shows the amplification plot of the two alleles A and G.

Statistical analysis
All data were expressed as mean ± standard deviation, number and percent . A p-value of < 0.05 was considered statistically significant using SPSS version 16 (SPSS Inc. Chicago, Illinois, USA).

RESULTS

The results of this study showed a statistically significant decrease in lung function tests (FEV1%, FVC% and FEV1/FVC), Hemoglobin level, red blood cell count and significant increase in cigarette smoking per day, smoking index and white blood cells in patients compared to the controls. While, there was a statistically non-significant difference between the studied groups regarding age, platelet count and lipid profile (Table 1).

Table (1): Comparison between the studied groups regarding demographic, spirometric and laboratory data

	Group 1 (n=65) Mean± SD	Group 2 (n=35) Mean ±SD	t- test	P value
Age (years)	62.86 ± 6.97	60.57 ± 5.41	1.68	0.07
Cigarette per Day (CPD)				
> 20 No %	29 44.6	09 25.7		
< 20 No %	36 55.4	26 74.3	χ^2 = 5.00	0.02
Smoking index	25.93 ± 13.89	13.52 ± 11.15	U=2.03	0.03
FEV1%	45.36 ± 10.33	97.22 ± 6.27	31.16	0.000
FVC%	65.81 ± 5.41	84.77 ± 4.02	18.16	0.000
FEV1/FVC	59.97 ± 5.56	83.03 ± 13.12	12.29	0.000
Platelets ($\times 10^3$/L)	322.66 ± 84.22	352.31 ± 68.23	1.78	0.07
WBCs ($\times 10^3$/L)	7.05 ± 2.34	5.73 ± 1.24	3.67	0.000
RBCs ($\times 10^6$/L)	4.71 ± 0.33	4.88 ± 0.33	2.44	0.01
Hb (gm/dl)	11.86 ± 1.33	13.20 ± 1.17	5.00	0.000
Cholesterol (mg/dl)	172.00 ± 34.49	175.13 ± 38.52	0.41	0.67
Triglyceride (mg/dl)	133.20 ± 17.27	175.13 ± 18.40	0.09	0.92
HDL (mg/dl)	38.53 ± 4.94	38.97 ± 5.28	0.40	0.68
LDL (mg/dl)	107.01 ± 35.83	109.55 ± 40.47	U=0.09	0.92

Table (2): Comparison between the studied groups regarding genotype and allele frequency

	Group 1 (n=65) No. %	Group 2 (n=35) No. %	χ^2	P value	OR (CI 95%)
Genotype:					
A/A:	14 21.5	3 8.6			OR1 5.91(1.43;24.43)
A/G:	36 55.4	13 37.1			OR2 3.51(1.39;8.87)
G/G:	15 23.1	19 54.3	10.31	0.006	Reference group
Allele frequency:					
A allele:	64 49.2	19 27.1			
G allele:	66 50.8	51 72.9	8.26	0.004	2.60 (1.39;4.88)

Table (3): Comparison between COPD genotypes regarding demographic, spirometric and laboratory data

	AA (n=14) Mean ±SD	AG (n=36) Mean ±SD	GG (n=15) Mean ±SD	ANOVA	P value
No. of cig/d: <20: No % >20: No %	 2 14.3 12 85.7	 22 61.1 14 38.9	 12 80.0 3 20.0	χ^2 = 13.7	0.001*
Smoking index	38.42 ± 19.23	23.12 ± 11.04	21.03 ± 5.59	K=6.65	0.03*
FEV1%	46.07 ± 9.65	44.36 ± 11.33	47.13 ± 8.59	0.41	0.66
FVC%	65.64 ± 6.12	66.13 ± 5.39	65.20 ± 5.08	0.16	0.84
FEV1/FVC	59.82 ± 6.79	60.40 ± 5.23	59.09 ± 5.38	0.29	0.74
Platelets (×10³/L)	292.92 ± 85.71	334.58 ± 82.29	321.80 ± 86.18	1.23	0.29
WBcs (×10³/L)	7.70 ± 2.38	7.13 ± 2.44	6.26 ± 1.94	1.43	0.24
RBCs (×10^6/L)	4.82 ± 0.37	4.71 ± 0.33	4.61 ± 0.26	1.53	0.22
Hb (gm/dl)	11.92 ±0.86	11.90 ± 1.40	11.76 ± 1.57	0.12	0.88
Cholesterol (mg/dl)	163.02 ±25.39	175.36 ± 39.33	172.30 ± 29.37	0.63	0.53
Triglyceride(mg%	131.78 ±15.15	136.25 ± 18.72	127.20 ± 14.52	1.53	0.22
HDL (mg/dl)	39.14 ± 3.54	38.30 ± 5.59	38.53 ± 4.62	0.14	0.86
LDL (mg/dl)	97.56 ± 26.92	109.74 ± 40.77	109.28 ± 30.42	0.61	0.54

**AA genotype has significantly higher smoking index than both AG and GG (p<0.05)*

Table (4): Comparison between heavy and light smokers of COPD patients regarding genotype and allele frequency

	Heavy smokers (n=29) No. %	Light smokers (n=36) No. %	χ^2	P value	OR (CI 95%)
Genotype: A/A: A/G: G/G:	 12 41.4 14 48.3 3 10.3	 2 5.6 22 61.1 12 33.3	13.72	0.001	 OR1 24.00(3.38;170.38) OR2 4.53 (0.61;10.65) Reference group
Allele frequency: A allele: G allele:	 38 65.5 20 34.5	 26 36.1 46 63.9	9.97	0.001	 3.36 (1.63;6.94)

Table (5): Comparison between COPD severity subgroups regarding demographic, spirometric and laboratory data

	Moderate (n=22) Mean ± SD	Severe (n=33) Mean ± SD	very severe (n=10) Mean ± SD	ANOVA	P value
Cigarette per Day (COPD) > 20 < 20	 8 36.4 14 63.6	 19 57.6 14 42.4	 2 20.0 8 80.0	5.30	0.07
Smoking index	26.15 ± 15.55	27.20 ± 13.80	21.25 ± 9.97	K=1.56	0.45
FEV1%	56.09 ± 4.56	43.48 ± 4.78	28.00 ± 0.47	K=53.21	0.000
FVC%	70.59 ± 4.06	64.39 ± 4.32	60.00 ± 1.63	K=33.13	0.000
FEV1/FVC	64.58 ± 3.65	58.40 ± 5.19	55.00 ± 2.20	K=27.76	0.000
Platelets (×10³/L)	323.90 ± 97.53	321.96 ± 70.77	322.20 ± 102.13	0.10	0.94
WBcs (×10³/L)	6.54 ± 2.01	7.59 ± 2.48	6.40 ± 2.32	3.88	0.14
RBCs (×10^6/L)	4.73 ± 0.36	4.67 ± 0.31	4.82 ± 0.31	1.68	0.43
Hb (gm/dl)	11.69 ± 1.07	11.81 ± 1.39	12.39 ± 1.63	1.29	0.52
Cholesterol (mg/dl)	177.41 ± 31.23	167.58 ± 32.18	174.66 ± 48.47	1.61	0.44
Triglyceride(mg/dl)	130.40 ± 18.50	132.93 ± 15.57	140.20 ± 19.71	1.72	0.42
HDL (mg/dl)	38.81 ± 4.57	39.33 ± 4.39	35.30 ± 6.49	4.71	0.09
LDL (mg/dl)	323.90 ± 97.53	321.96 ± 70.77	322.20 ± 102.13	0.10	0.94

Table (6): Comparison between the different grades of severity group regarding genotype distribution among the patients group

	Moderate (n=22) No. %	Severe (n=33) No. %	very severe (n=10) No. %	χ^2	P value
Genotype: A/A: A/G: G/G:	 6 27.3 12 54.5 4 18.2	 7 21.2 16 48.5 10 30.3	 1 10.0 8 80.0 1 10.0	4.05	0.39
Allele frequency: A allele: G allele:	 24 54.5 20 45.5	 30 45.5 36 54.5	 10 50.0 10 50.0	0.88	0.64

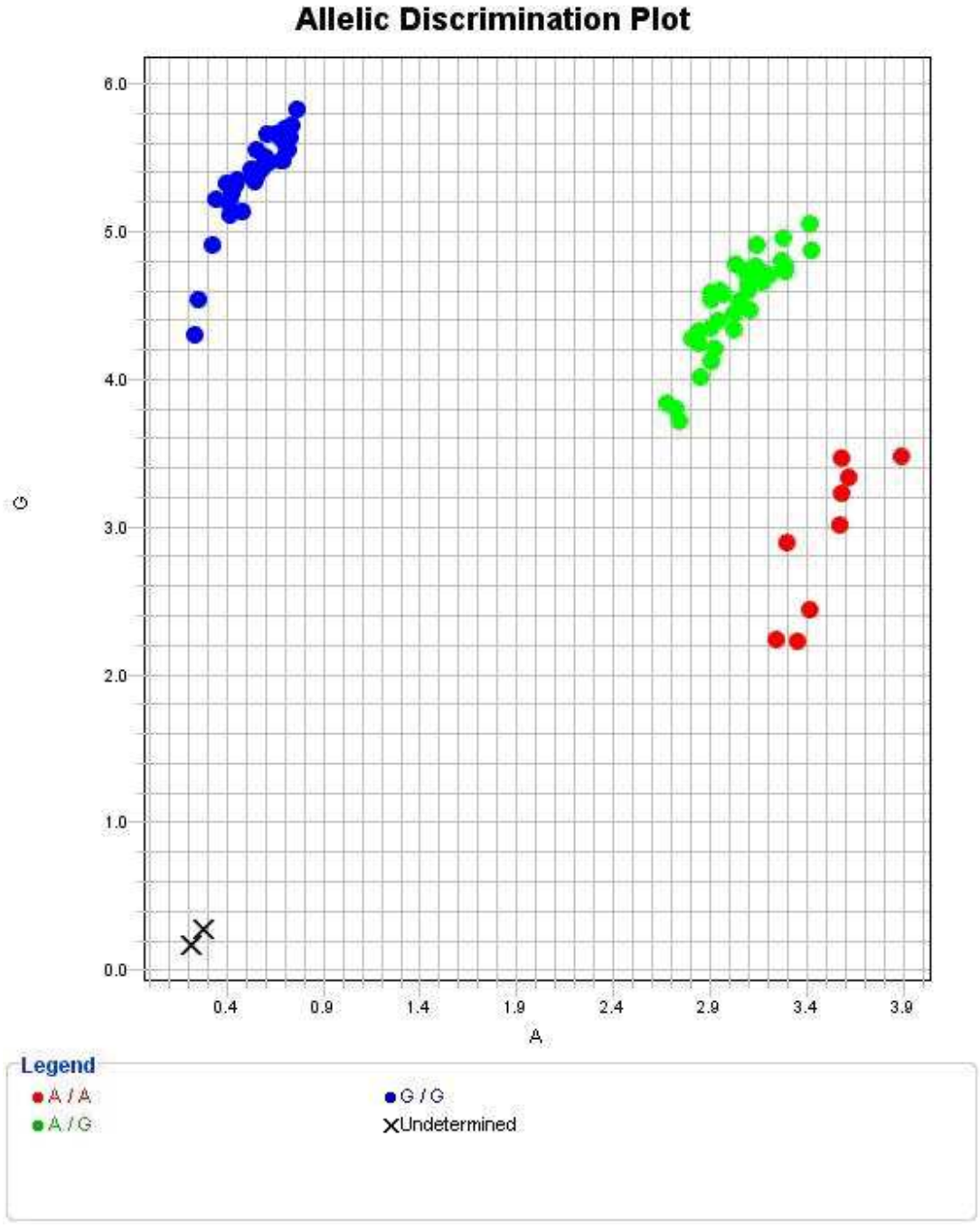

Figure (1) Allelic Discrimination plot

There was a significantly increased frequency of the AA genotype and A allele of the nicotinic acetylcholine receptors (nAChRs) SNP rs1051730 in patients group comparing to controls. AA genotypes increase the risk of COPD by 5.9 fold and A allele increases the risk by 2.6 fold (Table 2).

There was significant differences among AA, AG and GG types of rs1051730 of nicotinic acetylcholine receptors as regard number of cigarette smoking per day and smoking index with AA genotype has significantly higher smoking index and more cigarette smoking per day than both AG and GG ($p<0.05$). While there was a non significant difference as regard lung function tests, complete blood picture and lipid profile (Table 3).

There was a significantly increased frequency of the AA genotype and A allele of the nicotinic acetylcholine receptors (nAChRs) SNP rs1051730 in heavy smokers compared light smokers. AA genotypes increase the risk of heavy smoking as indicator of nicotine dependence by 24.0 fold and A allele increases the risk by 3.36 fold (Table 4).

On subclassification of patients group according to severity of airway obstruction, 22 patients had moderate disease, 33 patients had severe disease and 10 patients had very severe disease with a highly significant difference between the three groups regarding pulmonary function test parameters (FEV1%, FVC% and FEV1/FVC). Moreoever, there was a non significant difference as regard cigarette smoking per day, smoking index, complete blood picture and lipid profile (Table 5). Also, There was a non significant change in frequency of the genotypes and alleles of the nicotinic acetylcholine receptors (nAChRs) SNP rs1051730 in COPD subgroups (Table 6).

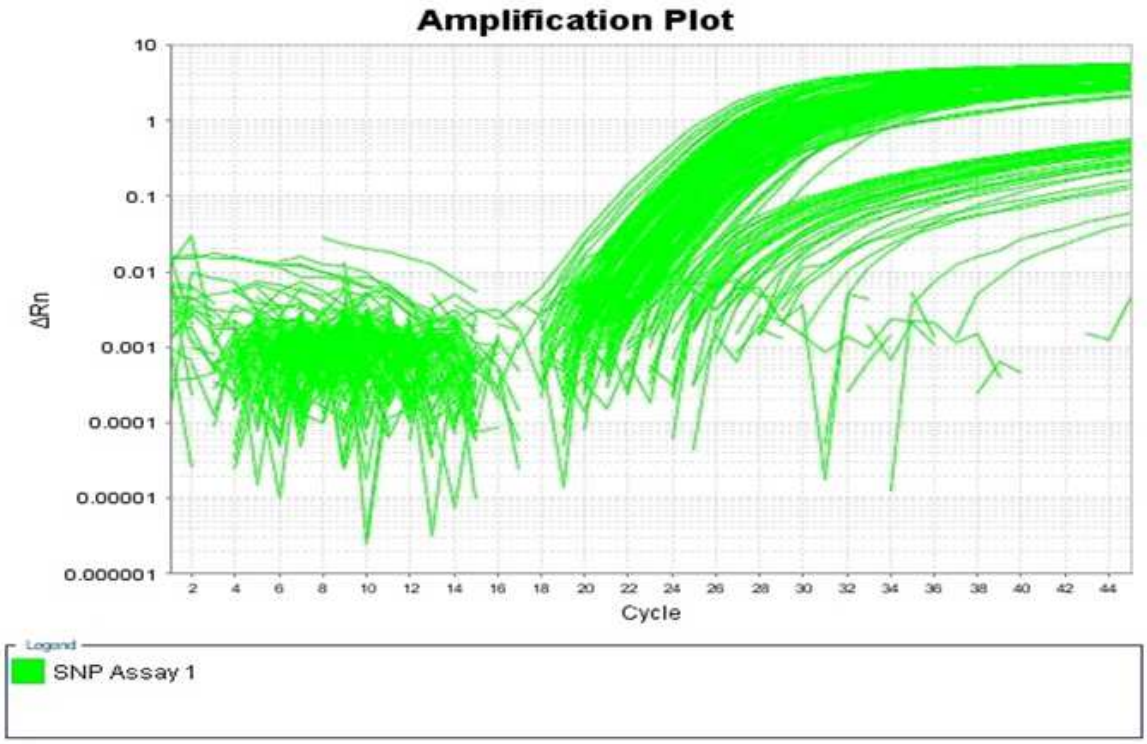

Figure (2) Amplification Plot

DISCUSSION

The most environmental risk factor of COPD is cigarette smoking, In spite of this, only a minority of smokers develops clinically symptomatic COPD. So, the genetic component may contribute to the development of COPD, a major health burden [16]. Nicotine dependence and smoking behavior can be influenced by genetic variants in the a-nAChR 3/5 subunit (CHRNA3/5) locus [17]. The main aim of the present study was to evaluate the associations between single nucleotide polymorphism (SNP) of nAChRs rs1051730 and demographic (including smoking index and cigarette consumption per day), physiologic and lab characteristics of Egyptian males with COPD.

In the present study AA genotypes of CHRNA3 SNP rs1051730 increase the risk of COPD by 5.9 fold and A allele increases the risk by 2.6 fold. While, there was a non significant change in frequency of the genotypes and alleles as regard severity of COPD.

This matched with the study of Budulac et al., who found a relationship of rs1051730 SNP with the risk of COPD with no direct effects on lung function [18] they suggest that CHRNA3 SNP may be increase smoking consumption lead to the development of COPD without effect on lung function decline. Also, the study of Lambrechts et al stated

that rs1051730 is associated with COPD signs. [19]. On the contrary the study of Kaur-Knudsen et al found that a reduction of lung function and severity of COPD associated with CHRNA3 rs1051730 genotype this may be as a result of differing on sample size and ethic groups [20].

In this study a significantly increased frequency of the AA genotype of rs1051730 found in heavy smokers compared to light smokers. This may be due to change in the binding sites numbers on the nAChRs and this requires the consumption of a large amount of nicotine [21].

This result is matched with the study on Polish population, which demonstrated a correlation between cigarette smoking per day >10 and allele A of rs1051730 [22]. and the study of Diljit et al who confirmed that smokers carry homozygous mutant genotype for nicotinic acetylcholine receptor inhale more often than others genotypes [23]. Also, Kaur-Knudsen et al found that a variant allele of CHRNA3 is more nicotine dependent [20].

This may explained by that the A allele of rs1051730 may be associated with increased tobacco consumption as a result of decrease sensitivity to nicotine plasma levels [24].

On the contrast the study of Lambrechts et al found that A allele of rs1051730 not correlated with the number of pack years smoked [19].

It could be concluded that a correlation may be present between A allele rs1051730 of Nicotinic acetylcholine receptors (nAChRs) SNP and development of COPD but not its severity. Heavy smoking and nicotine dependence rates may also affected by this allele. However this result needs to be approved by a study that includes males and female patients, larger scale of smoker, non smoker and previous smoker populations, more data about age of onset of smoking and COPD, forms of nicotine smoking other than cigarette.

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Ethical Approval: Research involving Human Participants. The study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent, and the Ethics Committee of Faculty of Medicine, Menoufia University approved the study protocol.

REFERENCES

[1] Heron, M. et al, Deaths: final data for 2006. *Natl Vital Stat Rep* **2009**, 57,1-134.
[2] Rabe KF, Hurd S, Anzueto A, et al. *Am J Respir Crit Care Med*, **2007**, 176,532–55.
[3] Global Initiative for Chronic Obstructive Pulmonary Disease (GOLD). COPD: Global Strategy for Diagnosis, Management, and Prevention of COPD.www.goldcopd.org/guidelines-global-strategy-for-diagnosis and management. html Date last accessed: December 17, **2015**. Date last updated: **2015**.
[4] Weiss S, DeMeo D, Postma DS. *Eur Respir J*, **2003**, 21, suppl.41, 4-12.
[5] Watson L, Vonk JM, Löfdahl CG, et al. *Respir Med*, **2006**, 100, 746–53.
[6] Picciotto MR, Kenny PJ. *Cold Spring Harb Perspect Med*, **2013**, 3(1), a012112.
[7] Qaseem A, Wilt TJ, Weinberger SE, et al. *Ann Intern Med*, **2011**, 155-179.
[8] Salokangas RK, Vilkman H, Ilonen T, et al: *Am J Psychiatry*, **2000**, 157,632–34.
[9] Gotti C, Zoli M, Clementi F, *Trends in Pharmacological Sciences* **2006**, 27 (9), 482-91.
[10] Bierut LJ. *Trends in Pharmacological Sciences* **2010**, 31(1), 46–51.
[11] Kuryatov A, Berrettini W, Lindstrom J, *Molecular Pharmacology* **2011**, 79(1),119-25.
[12] World Health Organization, Guidelines for Controlling and Monitoring the Tobacco Epidemic, World Health Organization, Geneva, Switzerland, **1998**.
[13] Broms U, Wedenoja J, Largeau MR, et al., *Nicotine and Tobacco Research* **2012**,14 (6), 720-33.
[14] N. Rifai, R. Warnick A.B. Carl, R.A. Edward, E.B. David (Eds.), Lipids, lipoproteins, apolipoproteins and other cardiovascular risk factors, Tietz textbook of Clinical Chemistry and Molecular Diagnosis (4th ed.), Saunders **2006**, 918–922.
[15] Wallach J. Metabolic and hereditary disorders. In: Interpretation of diagnostic tests. Lippincott. Williams and wilkns, 6th edition. **1996** Little brown company.

[16] Lokke Ae, Lange P, Scharling H, et al. *Thorax*. **2006**, 61, 935–9.
[17] Chen X, Chen J, Williamson VS, et al, *Am J Med Genet B Neuropsychiatr Genet* **2009**, 150B, 926-33.
[18] Budulac SE, Vonk JM, Postma DS, Siedlinski M, Timens W, Boezen MH. *PLOS ONE*, **2012**, 7(3), 1-7.
[19] Lambrechts D, Buysschaert I, Zanen P, et al. *Am J Respir Crit Care Med* **2010**, 181,486-93.
[20] Kaur-Knudsen D, Nordestgaard BG, Bojesen SE. *Eur Respir J*, **2012**,40(6),1538-44.
[21] Benowitz NL. *The New England Journal of Medicine* **2010**,362,(24), 2295–303.
[22] Kita-Milczarska K, Sieminska A , Jassem E. Association Between *CHRNA3* and *CHRNA5* Nicotine Receptor Subunit Gene Variants and Nicotine Dependence in an Isolated Population of Kashubians in Poland Med Sci Monit, **2016**, 22, 1442-50.
[23] Kaur-Knudsen D, Bojesen SE, Tybjærg-Hansen A Nordestgaard BG. *J Clin Onc*ol. **2011**,29(21),2875-82.
[24] Bierut LJ, Stitzel JA, Wang JC, et al. *Am J Psychiatry*, **2008**, 165, 1163-71.