*Romanian Biotechnological Letters* *Vol. 18, No.4, 2013*

**ORIGINAL PAPER**

# ASSOCIATION STUDY OF *NEURONAL APOPTOSIS INHIBITORY PROTEIN* GENE IN OVERWEIGHT AND OBESITY

Received for publication, March 14, 2013
Accepted, August 19, 2013

**APOSTOL POMPILIA[1], CIMPONERIU DANUT[1], SPANDOLE SONIA[1], TOMA MIHAI[1], STAVARACHI MONICA[1], RADU IRINA[1], SERAFINCEANU CRISTIAN[2], CRACIUN ANNE MARIE[2], BERCA LAVINIA-MARIANA[3], ION DANIELA ADRIANA[4]**

[1] *Department of Genetics, University of Bucharest, Bucharest, Romania*
[2] *"Nicolae C. Paulescu" National Institute for Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania*
[3] *Molecular Biology Laboratory, National Institute of Research&Development for Food Bioresources – IBA Bucharest, Bucharest, Romania.*
[4] *"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania*
** Corresponding author: PhD. Cimponeriu Danut*
*Postal address: Department of Genetics, University of Bucharest, Intrarea Portocalelor, No. 1-3, 6th District, Bucharest, 060101, Romania*
*Telephone: 0040213181576, Fax: 0040213181565, E-mail: dancimponeriu@yahoo.com*

## Abstract

*The Neuronal Apoptosis Inhibitory Protein (NAIP) has anti-apoptotic effects in different cells and is up-regulated during pre-adipocytes differentiation. Our aim was to test the potential association between NAIP exon 5 homozygous deletion and overweight or obesity.*

*DNA samples from overweight (n=75), obese (n=75), overweight type 2 diabetes (T2DM) with proteinuria (n=75) and healthy subjects with normal weight (n=225) were used. NAIP mutation was detected using PCR based methods. Fisher, Pearson and* Mann-Whitney U *tests were used for comparison between lots. A P-value <0.05 was considered statistically significant.*

*The frequency of NAIP exon 5 homozygous deletion in the cases was in the range between 0% and 1.33%, whereas in the control lots the frequency of the deletion was 1.33%. The frequency of this homozygous deletion in control group (1.33%) was in the range of data reported for other populations.*

*The present study showed that the NAIP exon 5 homozygous deletion is not an important contributor to predisposition for the common form of obesity or overweight (with or without T2DM) status in our patients.*

**Key words:** NAIP, mutation, type 2 diabetes

## Introduction

Obesity is a highly heritable disease [1] determined by regulatory networks which involve genes, environmental factors and behavior. Obesity also represents a risk factor for other pathologies like metabolic syndrome, type 2 diabetes (T2DM) or its chronic complications.

Neuronal Apoptosis Inhibitory Protein (NAIP or BIRC1) is encoded within a region (5q) undergoing rapid evolution driven by positive selection forces [2]. It carries 3 BIR (Baculovirus Inhibitor of apoptosis protein Repeat) domains, followed by a NACHT domain (essential for the oligomerization of the molecules involved in signal transduction) and a C-terminal LRR (Leucine-Rich Repeat) domain (for sensing microbial motif) [3]. NAIP might be involved in susceptibility to infectious [4, 5], inflammatory [6], neurodegenerative [7, 8]

or malignant [9-11] diseases. Several lines of evidences support also a potential role of NAIP in obesity, such is described below.

1. NAIP has anti-apoptotic properties (by BIR domains which bind and inhibit caspase-3, -7, and -9 activities [12-17]) and its up-regulated levels were associated with preadipocytes survival or differentiation [18, 19]. Disequilibrium of the balance between preadipocytes proliferation and apoptosis may be reflected in obesity.

2. NAIP is expressed at high levels in human placenta [8, 20, 21] and its normal development and function is required for the normal fetal growth. Alteration of fetal and postnatal growth rate increases the risk for obesity [22, 23].

3. NAIP may be an inhibitor of apoptosis in transmigrated monocytes, which are differentiated into macrophages when migrate to the site of infection and activate the innate immune response in response to infection [24, 25]. NAIP mediates the assembly of cytoplasmic "inflammasomes" which can activate inflammatory caspases [10, 26]. Caspase 1 contributes to some cytokine maturation (e.g. pro-IL1 b, pro-IL18) involved in the innate immunity [27-29] and in obesity-induced insulin resistance development [30-32].

4. "Infectobesity" hypothesis [33-36] is sustained by phagocytic and microbicidal activity of preadipocytes [37]. NAIP seems to be a pathogen response protein [38].

The primary infection with TTV may occur early in life or even *in utero* [39, 40] and is ubiquitous distributed in the body [41]. TTV DNA drives immune cells to secrete proinflammatory cytokines [42] associated with obesity and its related phenotypes. Despite of these evidences the disease-inducing potential of TTV remains poorly understood [43].

In this context, the purpose of our study was to test if there is any relationship between homozygous deletion of *NAIP* exon 5 and overweight or obesity.

## Materials and methods

***Subjects***. Blood samples were collected from unrelated overweight subjects (n=75, BMI-Body Mass Index: 25-29.9 kg/m$^2$), obese patients (n=75, BMI: 30.0-38.9 kg/m$^2$ independent of gender) and overweight T2DM patients with proteinuria (n=75, BMI: 26.3-29.5). We also selected clinically healthy subjects (n=225) with normal weight (BMI: 20.01-24.96 kg/m$^2$), blood pressure (<120mmHg) and fasting glycemia (<110 mg/dl) to be matched for sex, age and ethnicity with the cases.

***Exclusion criteria.*** Subjects suspected of monogenic form of obesity or with heredo-collateral antecedents for monogenic obesity, cancers, neurological diseases or muscular atrophies were excluded from our lots.

***Laboratory assays.*** Genomic DNA was isolated from peripheral blood samples with a commercial kit and was quantified with Quant-iT™ PicoGreen® (Invitrogen *Corp., Carlsbad CA*) in a Rotor -Gene 6000 (Corbett Research). The *NAIP* gene exon 5 was detected using $^{5'}$CTC TCA GCC TGC TCT TCA GAT$^{3'}$ and $^{5'}$AAA GCC TCT GAC GAG AGG AT$^{3'}$ primers [8]. The PCR program consisted in an initial melting step of 1 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 58°C, and 1 min at 72°C, and a final elongation step of 2 min at 72°C. The presence of *NAIP* exon 5 was detected after PCR products electrophoresis in agarose gels (2%, TBE 1x, 5V/cm) (Figure 1a). The presence or homozygous deletion of *NAIP* exon 5 was confirmed by melting analysis and high resolution melting (HRM) analysis using the same primers and SYBR® Green I and SensiMix® with EvaGreen® (Bioline, USA) respectively (Figure 2). TTV infection was detected in samples from patients and controls by heminested-PCR using primer with sequences shown in Table 1. The second round amplicons

were analyzed by polyacrylamide gel (8%, TBE 1x, 5V/cm) electrophoresis and visualized after silver staining (Figure 1b.).

**Table 1.** Primer sequences used for TTV amplification [39].

| Primer | Polarity | Sequence | Amplicon size |
|---|---|---|---|
| First round of PCR | | | |
| NG779 | Forward | ACWKMCGAATGGCTGAGTTT | ~130 bp |
| NG780 | Forward | RGTGRCGAATGGYWGAGTTT | |
| NG781 | Reverse | CCCKWGCCCGARTTGCCCCT | |
| NG782 | Reverse | AYCTWGCCCGAATTGCCCCT | |
| Second round | | | |
| NG770 | Forward | RGTGRCGAATGGYWGAGTTT | 112-117 bp |
| NG780 | Forward | ACWKMCGAATGGCTGAGTTT | |
| NG785 | Reverse | CCCCTTGACTBCGGTGTGTAA | |

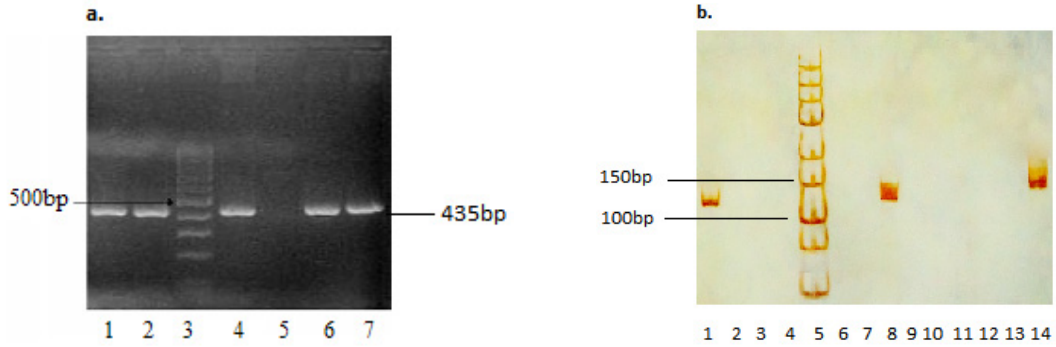
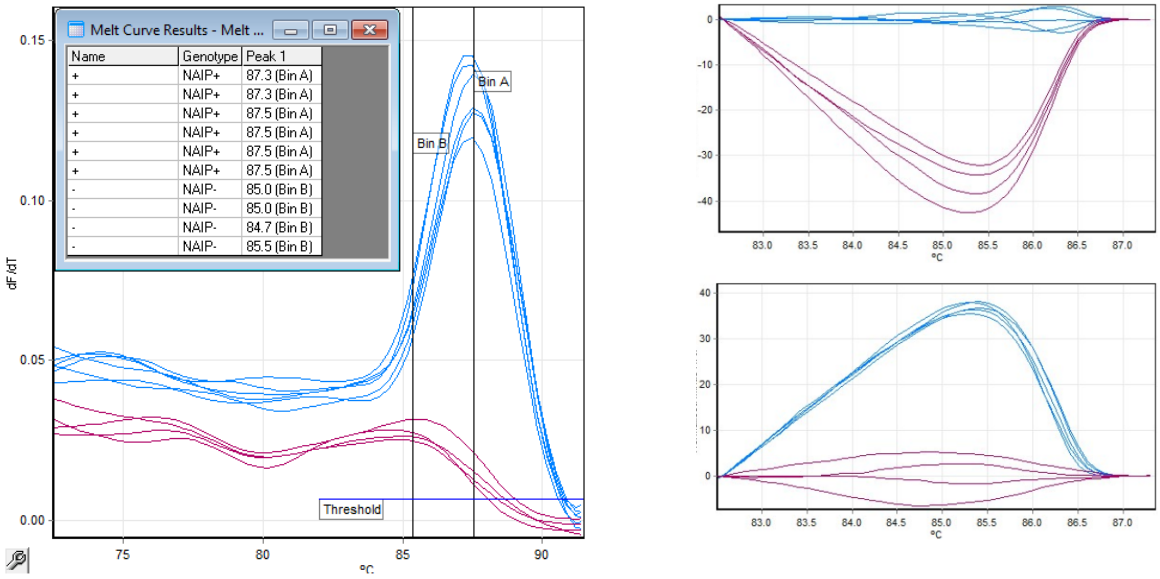



**Figure 1. a.** Gel electrophoresis for NAIP exon 5: Line 3- GeneRuler 100bp DNA Ladder, Fermentas; Lines 1,2,4,6,7- presence of NAIP exon 5, Line 5- homozygous deletion of NAIP exon 5; **b.** Gel electrophoresis for TTV detection: Line 5 - GeneRuler$^{TM}$ Low RangeDNA ladder, Fermentas; Lines 1,8,14- presence of TTV amplicons with a length of ~ 112 bp - 117bp depending on the genetic variant of the virus; Lines 2-4,6,7,9-13 – absence of TTV.




A) B) C)

**Figure 2.** Genotyping of NAIP exon 5 homozygous deletion by melting analysis (A) and HRM analysis [Difference Graphs normalized according to presence (B) or homozygous deletion (C) of NAIP exon 5].

***Statistical analysis.*** The Pearson tests were used to compare categorical data. Fisher's exact test was used when the expected number of individuals in any of the table cells was less than five. Kolmogorow-Smirnow test was used to decide if quantitative data comes from a specific distribution. Mann–Whitney U-test was used to compare quantitative data. A p value <0.05 was considered statistically significant.

## Results and Discussions

The main clinical characteristics of the subjects are shown in Table 2. Overweight patients with proteinuria were diagnosed with T2DM at 49.76±2.72 years (women: 49.66±2.58, range 44-54 years, men 49.86±2.88 range 45-56 years) and have had diabetes for 9.39±2.17 years (women: 9.18±2.36 years, range 5-12 years; men: 9.59±1.98 years, range: 6-13 years) when selected for this study.

Significant differences can be noticed between cases and their matched controls for weight, BMI and total cholesterol (Mann–Whitney U- test $p<0.001$). Triglyceride levels were also significantly higher in overweight and obese patients compared to healthy control lots HC1 and HC2 (Mann–Whitney U-test $p<0.001$).

The frequencies of *NAIP* exon *5* homozygous deletion in overweight (with or without T2DM) or obese patients and control lots were similar. In the control lots 1.33% of subjects had homozygous deletion of *NAIP* exon 5 (Table 2).

The TTV was detected more frequently in overweight subjects (69.33% vs. 60%, p=0.2, O.R.=1.5, 95% CI: 0.8 >1.5> 3.0), obese patients (68% vs. 64%, p=0.6, O.R.= 1.2, 95% CI: 0.6>1.2> 2.4) or overweight T2DM patients with proteinuria (76% vs. 62.67, p=0.08, O.R.=1.9, 95% CI: 0.9 >1.9> 3.82) than in control lots; however, the presence of infection was not associated with these diseases. When gender was included in the analysis, the frequency of TTV was found to be significantly higher in overweight (p=0.007, O.R.=4.57, 95% CI: 1.45 >4.57> 14.39) or obese (p=0.01, O.R.=3.57, 95% CI: 1.27 >3.57> 10.01) women compared with controls.

The presence of TTV infection was detected in 62.2% of healthy controls and was more frequent in men than in women (73.77% vs. 48.54%, p=0.0001). When healthy controls were grouped according to age, the differences between men and women were statistically significant for those in the range of 36-40 (93.8% vs. 47.6%, p = 0.003) and 61-65 (90.9% vs. 0%, p = 0.01) years (Table 3).

Our case-control study was carried out on 450 subjects of Romanian ethnicity. The frequency of *NAIP* exon 5 homozygous deletion in control group (1.33%) was in the range of data reported for other populations (0-2%) [44] (Table 4). To our knowledge, there are no data regarding *NAIP* gene exon 5 homozygous deletion in Romanian population or a synopsis for this mutation frequency in different countries. The frequency of *NAIP* homozygous deletion was ranged between 0% (in the T2DM group) and 1.33% (in obese, overweight and control subjects). Consistent with these data, our results shown that *NAIP* exon 5 homozygous deletion is not associated with development of overweight, obesity or overweight T2DM with proteinuria.

NAIP protein belongs to the IAP family (*Inhibitor of Apoptosis Protein*) initially identified in baculoviruses [45]. The IAP proteins contain an N-terminal domain with one to three repeats of a ~70 amino acids domain termed the BIR [46]. In NAIP protein, this domain is present in three copies (BIR1-3); the BIR2 inhibits caspases -3 and -7, while BIR3 mediates inhibition of caspase -9 in the presence of ATP [16]. The homozygous deletions of *NAIP* exon 5 and 6 leads to a truncated protein synthesis (without BIR domains) with failed

caspase inhibition capacity and a diminished anti-apoptotic effect. Also, the deletion of some highly conserved amino acids in BIR2 affects the interaction of this domain with caspase -3 [14]. The extensively deletion of *NAIP* exons 10 and 11, which may lead to deletion of BIR3 (partially) and C- terminal region (entirely), was also associated in some tumoral cell lines with the reduction of anti-apoptotic activity of NAIP [47].

**Table 2.** Mean and standard deviation of anthropometric and biochemical characteristics of the study groups.

| Lots Characteristics | Overweight | Normal weight (HC1) | Obesity | Normal weight (HC2) | Overweight proteinuric T2DM | Normal weight (HC3) |
|---|---|---|---|---|---|---|
| Gender (W/M) | 30/45 | 30/45 | 35/40 | 35/40 | 38/37 | 38/37 |
| Smokers | 24 | 18 | 31 | 19 [a] | 28 | 21 |
| Women | 10 | 7 | 13 | 8 | 11 | 8 |
| Men | 14 | 11 | 18 | 11 | 17 | 13 |
| Drinkers | 11 | 10 | 18 | 12 | 11 | 13 |
| Women | 0 | 0 | 0 | 0 | 0 | 0 |
| Men | 11 | 10 | 18 | 12 | 11 | 13 |
| TTV infections | 52 | 45 | 51 | 48 | 57 | 47 |
| Women | 24 | 14 [b] | 27 | 17 [c] | 26 | 19 |
| Men | 28 | 31 | 24 | 31 | 31 | 28 |
| NAIP del/del | 1 | 0 | 1 | 2 | 0 | 1 |
| Women | 1 | 0 | 1 | 1 | 0 | 1 |
| Men | 0 | 0 | 0 | 1 | 0 | 0 |
| Age | 33.69±7.76 (19-49) | 34.01±7.07 (21-49) | 48.03±5.42 (34-57) | 46.09±5.97 (35-57) | 59.15±2.67 (52-64) | 58.85±2.95 (53-65) |
| Women | 33.43±7.97 (21-49) | 33.53±7.47 (23-49) | 46.63±5.79 (34-57) | 46.23±6.19 (37-57) | 58.84±2.8 (52-64) | 58.47±3.16 (53-64) |
| Men | 33.87±7.69 (19-48) | 34.33±6.86 (21-47) | 49.25±4.81 (39-57) | 45.98±5.84 (35-56) | 59.46-±2.53 (56-64) | 59.24±2.7 (55-65) |
| Weight (Kg) | 81.43±4.64 (70-93) | 68.28±4.16 (61-81) [d] | 96.4±5.66 (85-110) | 67.53±5.11 (57-84) [d] | 84.61±4.12 (75-95) | 70.99±3.93 (60-79) [d] |
| Women | 78.63±4.39 (70-88) | 70.53±3.23 (65-81) [d] | 93.34±4.42 (85-102) | 68.34±5.74 (57-84) [d] | 84.03±3.87 (75-91) | 69.13±4.17 (60-79) [d] |
| Men | 83.29±3.82 (75-93) | 66.76±4.03 (61-77) [d] | 99.08±5.3 (89-110) | 66.83±4.44 (59-77) [d] | 85.22±4.33 (79-95) | 72.89±2.55 (67-79) [d] |
| Height (m) | 1.71±0.03 (1.66-1.78) | 1.71±0.03 (1.67-1.82) | 1.72±0.03 (1.65-1.81) | 1.71±0.03 (1.66-1.84) | 1.73±0.04 (1.67-1.84) | 1.72±0.03 (1.66-1.8) [e] |
| Women | 1.7± 0.02 (1.66-1.76) | 1.71±3.23 (1.67-1.82) | 1.71±0.03 (1.65-1.76) | 1.72±0.04 (1.67-1.84) | 1.73±0.04 (1.67-1.8) | 1.71±0.03 (1.66-1.8) |
| Men | 1.72±0.03 (1.66-1.78) | 1.71±0.03 (1.67-1.76) | 1.73±0.04 (1.68-1.81) | 1.71±0.03 (1.66-1.76) | 1.73±0.04 (1.69-1.84) | 1.72±0.03 (1.68-1.79) |
| BMI ($Kg/m^2$) | 27.76±1.12 (25.16-29.76) | 23.27±1.2 (20.48-24.96)[d] | 32.62±1.90 (30.11-38.97) | 22.97±1.36 (20.05-24.86)[d] | 28.25±0.83 (26.37-29.41) | 24.05±0.96 (21.01-24.96)[d] |
| Women | 27.16±1.02 (25.16-29.07) | 24.07±0.79 (21.47-24.96)[d] | 32.05±1.36 (30.11-35.71) | 23.2±1.44 (20.18-24.81)[d] | 28.12±0.91 (26.37-29.41) | 23.57±1.06 (21.01-24.86)[d] |
| Men | 28.16±1.01 (26.09-29.76) | 22.73±1.14 (20.48-24.96)[d] | 33.11±2.17 (30.32-38.97) | 22.77±1.28 (20.05-24.86)[d] | 28.38±0.72 (27.02-29.41) | 24.54±0.52 (22.13-24.96)[d] |
| Triglycerides (mg/dl) | 113.36±12.79 (90-142) | 98.76±12.2 (75-126)[d] | 137.56±22.43 (102-189) | 110.21±15.54 (75-139)[d] | 183.89±4.48 (105-258) | 173.09±10.87 (150-189) |
| Women | 118.1±13.66 (96-142) | 100.6±11.98 (79-121)[d] | 122.71±12.4 (102-147) | 112.86±14.14 (80-138)[d] | 181.68±40.43 (115-256) | 173.08±10.63 (150-189) |
| Men | 110.2 (90-135) | 97.53±12.33 (75-126)[d] | 150.55±21.2 (105-189) | 107.9±16.49 (75-139)[d] | 186.16±48.75 (105-258) | 173.11±11.26 (151-189) |
| Total Cholesterol (mg/dl) | 151.71±29.01 (103-221) | 121.95±8.79 (105-139)[d] | 182.91±35.32 (103-239) | 128.73±12.64[d] (101-154) | 241.71±17.5 (197-274) | 161.11±13.84 (83-157)[d] |
| Women | 154.97±32.19 (103-221) | 118.17±7.9 (105-135)[d] | 156.06±31.77 (103-221) | 127±12.07 (105-154)[d] | 242.08±17.24 (200-274) | 131.34±14.37 (100-157)[d] |
| Men | 149.53 (110-215) | 124.47±8.51 (107-139)[d] | 206.38±16.61 (177-239) | 130.25±13.08 (101-152)[d] | 241.32±18 (197-271) | 130.86±13.47 (94-153)[d] |

Chi2 test: [a] p=0.04; [b] p= 0.007, O.R.=4.57, 95% CI: 1.45 >4.57> 14.39; [c] p=0.01, O.R.=3.57, 95% CI: 1.27 >3.57> 10.01; Mann-Whitney U test two tailed test: [d] p<0.001; e p<0.05.

**Table 3.** Rates of TTV infection in different age interval.

| | Men | | Women | |
|---|---|---|---|---|
| Age interval | Number of subjects | Number (percent) of infected subjects | Number of subjects | Number (percent) of infected subjects |
| 21-25 | 5 | 5 (100%) | 5 | 5 (100%) |
| 26-30 | 12 | 6 (50%) | 9 | 2 (22.2%) [a] |
| 31-35 | 12 | 8 (66.7%) | 3 | 2 (66.7%) |
| 36-40 | 16 | 15 (93.8%) | 21 | 10 (47.6%) [b] |
| 41-45 | 16 | 13 (81.3%) | 5 | 2 (40%) |
| 46-50 | 15 | 9 (60%) | 12 | 7 (58.3%) |
| 51-55 | 10 | 6 (60%) | 21 | 8 (38.1% |
| 56-60 | 25 | 18 (72%) | 24 | 14 (58.3%) |
| 61-65 | 11 | 10 (90.9%) | 3 | 0 (0%) [c] |
| TOTAL | 122 | 90 (73.77%) | 103 | 50 (48.54%) [d] |

Differences from the preceding age interval [a] p (two sided Fisher exact test) = 0.01. Differences between men and women [b] p (two sided Fisher exact test) = 0.003, [c] p (two sided Fisher exact test) = 0.01, [d] p (Pearson test) =0.0001.

**Table 4.** The frequency of homozygous deletion of NAIP exon 5 in different countries.

| Continent | Country | Normal / unaffected /controls | Parents and healthy relatives of SMA patients | References |
|---|---|---|---|---|
| South America | Brazil | 104 | 0 of 138 | [48] |
| North Africa | Morocco | 30 | | [49] |
| Europe | Europeans | 532* | 7 of 373 | [50] |
| | Slovakia | 25 | 0 of 81 | [51] |
| | Macedonia | 30 | 2 of 30 | [52] |
| | Serbia | 32 | 1 of 100 | [53] |
| | Bulgaria | 32 | 8 of 100 | [54] |
| Eurasia | Turkey | 34 | | [55] |
| | | 150 | | [56] |
| Asia | Saudi Arabia | 90 | 0 of 110 | [57] |
| | Kuwait | 44 | 0 of 41 | [58] |
| | India | 50 | | [59] |
| | | 100 | | [60] |
| | Vietnam | 52 | | [61] |
| | China | 40 | | [62] |
| | | 30 | 0 of 30 | [63] |

*Heterogeneous normal controls; SMA – Spinal Muscular Atrophy, a neurodegenerative disease whose severity is modified by NAIP gene copy numbers

The data about involvement of NAIP in obesity are very scarce. The evidences for apoptosis occurring in mature adipocytes have been obtained through *in vivo* studies in rodents and humans as well as by *in vitro* studies on cell culture models (3T3-L1 and 3T3-F442A), where a high level of *NAIP* expression was observed [19]. The preadipocytes (fibroblast-like adipocyte precursor cells) can also undergo apoptotic cell death, yet during

the differentiation these cells acquire a relative resistance to apoptosis induced by growth factor deprivation. During adipogenesis, an increased level of the cell survival proteins Bcl-2 and NAIP has been observed. Thus, NAIP expression is differentiation-dependent and its upregulation accompanies adipocytes differentiation.

It is known that during the adult life, adipose tissue undergoes a dynamic remodeling process which is represented by the cellular turnover of adipose cells, consisting in the adipocytes formation from preadipocytes, possible de-differentiation back to adipocytes and apoptosis of preadipocytes and adipocytes. The increase of adipocytes number can occur at any stage of life and can be the result of one or more cycles of preadipocytes replication followed by their differentiation, while the volume can increase or decrease by lipogenesis or lipolysis [64]. Also, the change of adipocytes number or volume can lead to pathological conditions of adipose tissue, like obesity and lipoatrophy.

TTV infection is similar distributed in the patients and corresponding control lots ($p \leq 0.2$). When the gender was included into analysis, TTV was found to be more frequent in overweight ($p=0.007$, O.R.=4.57, 95% CI: 1.45 >4.57> 14.39) or obese women ($p=0.01$, O.R.=3.57, 95% CI: 1.27 >3.57> 10.01) than in their matched controls. The two overweight and obese women with homozygous deletion of NAIP exon 5 were also TTV positive. In the control lots, from three carriers of these mutations, two also had TTV infections.

We considered that truncated NAIP isoform lack the potential roles related to the fetal growth and development, the differentiation of preadipocytes and response to some pathogens and thus it may change the risk for obesity. Our results showed that deletion of *NAIP* exon 5 was encountered in one overweight and one obese woman and in three individuals from the control group. Thus, this *NAIP* gene mutation may not represent a factor significantly associated with overweight or obesity.

The prevalence of TTV DNA in biological samples from healthy persons was found to vary widely in different countries and the results are influenced by the characteristics of subjects and method used for detections. We also found that the gender and age of subjects may influence the frequency of TTV infections in healthy individuals. From healthy controls, 62.2% were infected with TTV. This results is in concordance with previously reports in which the prevalence of TTV vary from 46% (in Brazil) [65] to 94% (in Russia) [66].The highest prevalence of TTV infections in the cases lots was calculated for overweight T2DM patients (76%). This result is also similar with those reported for previous studies performed in other populations [67, 68].

## Conclusions

The present study showed that the *NAIP* exon 5 homozygous deletion is not an important contributor to predisposition for the common form of obesity or overweight status (with or without T2DM) in our patients. The frequency of this homozygous deletion in control group (1.33%) was in the range of data reported for other populations.

## Acknowledgments

This paper is supported by the Sectoral Operational Programme Human Resources Development, financed from the European Social Fund and by the Romanian Government under the contract number POSDRU/89/1.5/S/64109 and by the Romanian Ministry of Education and Research under the research project PN II 42 –161/2008.

## References

1. W. YANG, T. KELLY, J. HE, Genetic epidemiology of obesity. *Epidemiol Rev.*, **29**, 49, 61 (2007).
2. F. KONG, Z. SU, C. ZHOU, C. SUN, Y. LIU, D. ZHENG, H. YUAN, J. YIN, J. FANG, S. WANG, H. XU, Role of positive selection in functional divergence of mammalian neuronal apoptosis inhibitor proteins during evolution. *J Biomed Biotechnol.*, **2011**(809765) (2011).
3. L. DUBREZ-DALOZ, A. DUPOUX,J. CARTIER, IAPs: more than just inhibitors of apoptosis proteins. *Cell Cycle*, **7**(8), 1036, 1046 (2008).
4. E. DIEZ, S.H. LEE, S. GAUTHIER, Z. YARAGHI, M. TREMBLAY, S. VIDAL, P. GROS, Birc1e is the gene within the Lgn1 locus associated with resistance to Legionella pneumophila. *Nat Genet.*, **33**(1), 55, 60 (2003).
5. E.K. WRIGHT, S.A. GOODART, J.D. GROWNEY, V. HADINOTO, M.G. ENDRIZZI, E.M. LONG, K. SADIGH, A.L. ABNEY, I. BERNSTEIN-HANLEY, W.F. DIETRICH, Naip5 affects host susceptibility to the intracellular pathogen Legionella pneumophila. *Curr Biol.*, **13**(1), 27, 36 (2003).
6. M. CHAMAILLARD, S.E. GIRARDIN, J. VIALA, D.J. PHILPOTT, Nods, Nalps and Naip: intracellular regulators of bacterial-induced inflammation. *Cell Microbiol.*, **5**(9), 581, 592 (2003).
7. L.A. CHRISTIE, J.H. SU, C.H. TU, M.C. DICK, J. ZHOU,C.W. COTMAN, Differential regulation of inhibitors of apoptosis proteins in Alzheimer's disease brains. *Neurobiol Dis.*, **26**(1), 165, 173 (2007).
8. N. ROY, M.S. MAHADEVAN, M. MCLEAN, G. SHUTLER, Z. YARAGHI, R. FARAHANI, S. BAIRD, A. BESNER-JOHNSTON, C. LEFEBVRE, X. KANG, The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell*, **80**(1), 167, 178 (1995).
9. O. GRZYBOWSKA-IZYDORCZYK, B. CEBULA, T. ROBAK,P. SMOLEWSKI, Expression and prognostic significance of the inhibitor of apoptosis protein (IAP) family and its antagonists in chronic lymphocytic leukaemia. *Eur J Cancer*, **46**(4), 800, 810 (2010).
10. S. MAZROUEI, A. ZIAEI, A.P. TANHAEE, K. KEYHANIAN, M. ESMAEILI, A. BARADARAN, M. SALEHI, Apoptosis inhibition or inflammation: the role of NAIP protein expression in Hodgkin and non-Hodgkin lymphomas compared to non-neoplastic lymph node. *J Inflamm. (Lond)*, **9**(1), 4 (2012).
11. K. YAMAMOTO, S. ABE, Y. NAKAGAWA, K. SUZUKI, M. HASEGAWA, M. INOUE, M. KURATA, K. HIROKAWA, M. KITAGAWA, Expression of IAP family proteins in myelodysplastic syndromes transforming to overt leukemia. *Leuk Res.*, **28**(11), 1203, 1211 (2004).
12. Q.L. DEVERAUX, E. LEO, H.R. STENNICKE, K. WELSH, G.S. SALVESEN, J.C. REED, Cleavage of human inhibitor of apoptosis protein XIAP results in fragments with distinct specificities for caspases. *EMBO J*, **18**(19), 5242, 5251 (1999).
13. B. NACHMIAS, Y. ASHHAB,D. BEN-YEHUDA, The inhibitor of apoptosis protein family (IAPs): an emerging therapeutic target in cancer. *Semin Cancer Biol.*, **14**(4), 231, 243 (2004).
14. J.K. MAIER, Z. LAHOUA, N.H. GENDRON, R. FETNI, A. JOHNSTON, J. DAVOODI, D. RASPER, S. ROY, R.S. SLACK, D.W. NICHOLSON, A.E. MACKENZIE, The neuronal apoptosis inhibitory protein is a direct inhibitor of caspases 3 and 7. *J Neurosci.*, **22**(6), 2035, 2043 (2002).
15. G.M. KASOF, B.C. GOMES, Livin, a novel inhibitor of apoptosis protein family member. *J Biol Chem.*, **276**(5), 3238, 3246 (2001).
16. J. DAVOODI, L. LIN, J. KELLY, P. LISTON,A.E. MACKENZIE, Neuronal apoptosis-inhibitory protein does not interact with Smac and requires ATP to bind caspase-9. *J Biol Chem.*, **279**(39), 40622, 40628 (2004).
17. J. DAVOODI, M.H. GHAHREMANI, A. ES-HAGHI, A. MOHAMMAD-GHOLI, A. MACKENZIE, Neuronal apoptosis inhibitory protein, NAIP, is an inhibitor of procaspase-9. *Int J Biochem Cell Biol.*, **42**(6), 958, 964 (2010).
18. R. MAGUN, D.L. BOONE, B.K. TSANG, A. SORISKY, The effect of adipocyte differentiation on the capacity of 3T3-L1 cells to undergo apoptosis in response to growth factor deprivation. *Int J Obes Relat Metab Disord.*, **22**(6), 567, 571 (1998).
19. R. MAGUN, A. GAGNON, Z. YARAGHI, A. SORISKY, Expression and regulation of neuronal apoptosis inhibitory protein during adipocyte differentiation. *Diabetes*, **47**(12), 1948, 1952 (1998).
20. J.K. MAIER, S. BALABANIAN, C.R. COFFILL, A. STEWART, L. PELLETIER, D.J. FRANKS, N.H. GENDRON, A.E MACKENZIE, Distribution of neuronal apoptosis inhibitory protein in human tissues. *J Histochem Cytochem.*, **55**(9), 911, 923 (2007).

21. K. YAMAMOTO, H. SAKAI, S. HADANO, Y. GONDO, J.E. IKEDA, Identification of two distinct transcripts for the neuronal apoptosis inhibitory protein gene. *Biochem Biophys Res Commun.*, **264**(3), 998, 1006 (1999).
22. D.J. BARKER, J.G. ERIKSSON, T. FORSEN, C. OSMOND, Fetal origins of adult disease: strength of effects and biological basis. *Int J Epidemiol.*, **31**(6), 1235, 1239 (2002).
23. M. BARKER, S. ROBINSON, C. OSMOND, D.J. BARKER, Birth weight and body fat distribution in adolescent girls. *Arch Dis Child.*, **77**(5), 381, 383 (1997).
24. M.R. WILLIAMS, Y. SAKURAI, S.M. ZUGHAIER, S.G. ESKIN, L.V. MCINTIRE, Transmigration across activated endothelium induces transcriptional changes, inhibits apoptosis, and decreases antimicrobial protein expression in human monocytes. *J Leukoc Biol.*, **86**(6), 1331, 1343 (2009).
25. E. DIEZ, Z. YARAGHI, A. MACKENZIE, P. GROS, The neuronal apoptosis inhibitory protein (Naip) is expressed in macrophages and is modulated after phagocytosis and during intracellular infection with Legionella pneumophila. *J Immunol.*, **164**(3), 1470, 1477 (2000).
26. F. MARTINON, O. GAIDE, V. PETRILLI, A. MAYOR,J. TSCHOPP, NALP inflammasomes: a central role in innate immunity. *Semin Immunopathol.*, **29**(3), 213, 229 (2007).
27. L. FRANCHI, T. EIGENBROD, R. MUNOZ-PLANILLO,G. NUNEZ, The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. *Nat Immunol*, **10**(3), 241, 247 (2009).
28. F. MARTINON,J. TSCHOPP, Inflammatory caspases and inflammasomes: master switches of inflammation. *Cell Death Differ.*, **14**(1), 10, 22 (2007).
29. J.P. TING, B.K. DAVIS, CATERPILLER: a novel gene family important in immunity, cell death, and diseases. *Annu Rev Immunol.*, **23**, 387, 414 (2005).
30. J. JAGER, T. GREMEAUX, M. CORMONT, Y. LE MARCHAND-BRUSTEL, J.F. TANTI, Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology*, **148**(1), 241, 251 (2007).
31. M.G. NETEA, L.A. JOOSTEN, E. LEWIS, D.R. JENSEN, P.J. VOSHOL, B.J. KULLBERG, C.J. TACK, H. VAN KRIEKEN, S. H. KIM, A.F. STALENHOEF, F.A. VAN DE LOO, I. VERSCHUEREN, L. PULAWA, S. AKIRA, R.H. ECKEL, C.A. DINARELLO, W. VAN DEN BERG, J.W. VAN DER MEER, Deficiency of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. *Nat Med.*, **12**(6), 650, 656 (2006).
32. E.P. ZORRILLA, M. SANCHEZ-ALAVEZ, S. SUGAMA, M. BRENNAN, R. FERNANDEZ, T. BARTFAI, B. CONTI, Interleukin-18 controls energy homeostasis by suppressing appetite and feed efficiency. *Proc Natl Acad Sci U S A*, **104**(26), 11097, 11102 (2007).
33. N.V. DHURANDHAR, Infectobesity: obesity of infectious origin. *J Nutr.*, **131**(10), 2794S, 2797S (2001).
34. V. VAN GINNEKEN, L. SITNYAKOWSKY, J.E. JEFFERY, "Infectobesity: viral infections (especially with human adenovirus-36: Ad-36) may be a cause of obesity. *Med Hypotheses*, **72**(4), 383, 388 (2009).
35. M. PASARICA, N.V. DHURANDHAR, Infectobesity: obesity of infectious origin. *Adv. Food. Nutr. Res.*, **52**(61), 102 (2007).
36. H.N. NA, J.H. NAM, Adenovirus 36 as an obesity agent maintains the obesity state by increasing MCP-1 and inducing inflammation. *J Infect Dis.*, **205**(6), 914, 922 (2012).
37. B. COUSIN, O. MUNOZ, M. ANDRE, A.M. FONTANILLES, C. DANI, J.L. COUSIN, P. LAHARRAGUE, L. CASTEILLA, L. PENICAUD, A role for preadipocytes as macrophage-like cells. *FASEB J.*, **13**(2), 305, 312 (1999).
38. E. MEYLAN, J. TSCHOPP, M. KARIN, Intracellular pattern recognition receptors in the host response. *Nature*, **442**(7098), 39, 44 (2006).
39. M. NINOMIYA, M. TAKAHASHI, T. NISHIZAWA, T. SHIMOSEGAWA,H. OKAMOTO, Development of PCR assays with nested primers specific for differential detection of three human anelloviruses and early acquisition of dual or triple infection during infancy. *J Clin Microbiol.*, **46**(2), 507, 514 (2008).
40. T. POLLICINO, G. RAFFA, G. SQUADRITO, L. COSTANTINO, I. CACCIOLA, S. BRANCATELLI, C. ALAFACI, MG. FLORIO, G. RAIMONDO, TT virus has a ubiquitous diffusion in human body tissues: analyses of paired serum and tissue samples. *J Viral Hepat.*, **10**(2), 95, 102 (2003).
41. Y.H. PENG, T. NISHIZAWA, M. TAKAHASHI, T. ISHIKAWA, A. YOSHIKAWA, H. OKAMOTO, Analysis of the entire genomes of thirteen TT virus variants classifiable into the fourth and fifth genetic groups, isolated from viremic infants. *Arch Virol.*, **147**(1), 21, 41 (2002).

42. J. ROCCHI, V. RICCI, M. ALBANI, L. LANINI, E. ANDREOLI, L. MACERA, *M*. PISTELLO, L. CECCHERINI-NELLI, M. BENDINELLI, F. MAGGI, Torquetenovirus DNA drives proinflammatory cytokines production and secretion by immune cells via toll-like receptor 9. *Virology*, **394**(2), 235, 242 (2009).
43. H. OKAMOTO, History of discoveries and pathogenicity of TT viruses. *Curr Top Microbiol Immunol.*, **331**(1), 20 (2009).
44. S. LEFEBVRE, L. BURGLEN, S. REBOULLET, O. CLERMONT, P. BURLET, L. VIOLLET, B. BENICHOU, C. CRUAUD, P. MILLASSEAU, M. ZEVIANI, Identification and characterization of a spinal muscular atrophy-determining gene. *Cell*, **80**(1), 155, 165 (1995).
45. R.J. CLEM,C.S. DUCKETT, The iap genes: unique arbitrators of cell death. *Trends Cell Biol.*, **7**(9), 337, 339 (1997).
46. E.A. MERCER, L. KORHONEN, Y. SKOGLOSA, P.A. OLSSON, J.P. KUKKONEN, D. LINDHOLM, NAIP interacts with hippocalcin and protects neurons against calcium-induced cell death through caspase-3-dependent and -independent pathways. *EMBO J.*, **19**(14), 3597, 3607 (2000).
47. M. NOTARBARTOLO, M. CERVELLO, L. DUSONCHET, N. D'ALESSANDRO, NAIP-deltaEx10-11: a novel splice variant of the apoptosis inhibitor NAIP differently expressed in drug-sensitive and multidrug-resistant HL60 leukemia cells. *Leuk Res.*, **26**(9), 857, 862 (2002).
48. C.A. KIM, M.R. PASSOS-BUENO, S.K. MARIE, A. CERQUEIRA, U. CONTI, M.J. MARQUES-DIAS, C.H. GONZALEZ, M. ZATZ, Clinical and molecular analysis of spinal muscular atrophy in brazilian patients. *Genet Mol Biol*. **22**(4), 487, 492 (1999).
49. A. BOUHOUCHE, A. BENOMAR, N. BIROUK, N. BOUSLAM, R. OUAZZANI, M. YAHYAOUI, T. CHKILI, High incidence of SMN1 gene deletion in Moroccan adult-onset spinal muscular atrophy patients. *J Neurol.*, **250**(10), 1209, 1213 (2003).
50. N.R. RODRIGUES, N. OWEN, K. TALBOT, S. PATEL, F. MUNTONI, J. IGNATIUS, V. DUBOWITZ, K.E. DAVIES, Gene deletions in spinal muscular atrophy. *J Med Genet*, **33**(2), 93, 96 (1996).
51. A. ZAT'KOVA, E. HAHNEN, B. WIRTH, L. KADASI, Analysis of the SMN and NAIP genes in slovak spinal muscular atrophy patients. *Hum Hered.*, **50**(3), 171, 174 (2000).
52. S.A. KOCHEVA, S. VLASKI-JEKIC, M. KUTUREC, G.D. EFREMOV, Molecular analysis of survival motoneuron and neuronal apoptosis inhibitory protein genes in Macedonian spinal muscular atrophy pathients. *BJMG* **10**(2), 55, 60 (2007).
53. M. MISKOVIC, T. LALIC, D. RADIVOJEVIC, S. CIRKOVIC, G. VLAHOVIC, D. ZAMUROVIC, M. GUC-SCEKIC, Lower incidence of deletions in the survival of motor neuron gene and the neuronal apoptosis inhibitory protein gene in children with spinal muscular atrophy from Serbia. *Tohoku J Exp Med.*, **225**(3), 153, 159 (2011).
54. A. JORDANOVA, V. STOYANOVA, M. UZUNOVA, I. LITVINENKO,I. KREMENSKY, Deletion analysis of Bulgarian SMA families. *Hum Mutat.*, **12**(1), 33, 38 (1998).
55. H. ERDEM, S. PEHLIVAN, H. TOPALOGLU, M. OZGUC, Deletion analysis in Turkish patients with spinal muscular atrophy. *Brain Dev.*, **21**(2), 86, 89 (1999).
56. S. SAVAS, N. GOKGOZ, H. KAYSERILI, F. OZKINAY, M. YUKSEL-APAK, B. KIRDAR, Screening of deletions in SMN, NAIP and BTF2p44 genes in Turkish spinal muscular atrophy patients. *Hum Hered.*, **50**(3), 162, 165 (2000).
57. M. AL-JUMAH, R. MAJUMDAR, S. AL-RAJEH, A. AWADA, E. CHAVES-CARBELLO, M. SALIH, S. AL-SHAHWAN, K. AL-SUBIEY, S. AL-UTHAIM, Molecular analysis of the spinal muscular atrophy and neuronal apoptosis inhibitory protein genes in Saudi patients with spinal muscular atrophy. *Saudi Med J.*, **24**(10), 1052, 1054 (2003).
58. E. SAMILCHUK, B. D'SOUZA, L. BASTAKI,S. AL-AWADI, Deletion analysis of the SMN and NAIP genes in Kuwaiti patients with spinal muscular atrophy. *Hum Genet.*, **98**(5), 524, 527 (1996).
59. A. KESARI, U.K. MISRA, J. KALITA, V.N. MISHRA, S. PRADHAN, S.J. PATIL S.R. PHADKE, B. MITTAL, Study of survival of motor neuron (SMN) and neuronal apoptosis inhibitory protein (NAIP) gene deletions in SMA patients. *J Neurol*., **252**(6), 667, 671 (2005).
60. A. KESARI, M.M. IDRIS, G.R. CHANDAK, B. MITTAL, Genotype-phenotype correlation of SMN locus genes in spinal muscular atrophy patients from India. *Exp Mol Med.*, **37**(3), 147, 154 (2005).
61. V.K. TRAN, T.H. SASONGKO, D.D. HONG, N.T. HOAN, V.C. DUNG, M.J. LEE, GUNADI, Y. TAKESHIMA, M. MATSUO, H. NISHIO, SMN2 and NAIP gene dosages in Vietnamese patients with spinal muscular atrophy. *Pediatr Int.*, **50**(3), 346, 351 (2008).

62. S. MA, L. YUAN, T. LIU, T. YANG, W. ZHOU, H. WU, Survival motor neuron gene and neuronal apoptosis inhibitory protein gene deletion in patients with spinal muscular atrophy. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao*, **22**(6), 551, 554 (2000).
63. L. ZHANG, X. YANG,B. XIAO, [A study of survival motor neuron and neuronal apoptosis inhibitory protein gene in spinal muscular atrophy]. *Zhonghua Nei Ke Za Zhi*, **40**(6), 401, 404 (2001).
64. H. HAUNER, G. ENTENMANN, M. WABITSCH, D. GAILLARD, G. AILHAUD, R. NEGREL, E.F. PFEIFFER, Promoting effect of glucocorticoids on the differentiation of human adipocyte precursor cells cultured in a chemically defined medium. *J Clin Invest*., **84**(5), 1663, 1670 (1989).
65. S. DEVALLE, C. NIEL, Distribution of TT virus genomic groups 1-5 in Brazilian blood donors, HBV carriers, and HIV-1-infected patients. *J Med Virol.*, **72**(1), 166, 173 (2004).
66. E.V. VASILYEV, D.Y. TROFIMOV, A.G. TONEVITSKY, V.V. ILINSKY, D.O. KOROSTIN, D.V. REBRIKOV, Torque Teno Virus (TTV) distribution in healthy Russian population. *Virol J.*, **6**, 134 (2009).
67. P. GALLIAN, Y. BERLAND, M. OLMER, D. RACCAH, P. DE MICCO, P. BIAGINI, S. SIMON, D. BOUCHOUAREB, C. MOUREY, C. ROUBICEK, M. TOUINSSI, J.F. CANTALOUBE, B. DUSSOL, X. DE LAMBALLERIE, TT virus infection in French hemodialysis patients: study of prevalence and risk factors. *J Clin Microbiol*, **37**(8), 2538, 2542 (1999)
68. C. GUNEY, A. KADAYIFCI, M.C. SAVAS, A. UYGUN, A. BALKAN, A. KUBAR, Frequency of hepatitis G virus and transfusion-transmitted virus infection in type II diabetes mellitus. *Int J Clin Pract.*, **59**(2), 206, 209 (2005).