Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: http://www.e-biomedicine.com

Original article

Appearance of acanthosis nigricans may precede obesity: An involvement of the insulin/IGF receptor signaling pathway

Chung-Hsing Wang [a,b], Wei-De Lin [c,d,h], Da-Tian Bau [e], I-Ching Chou [a,f], Chang-Hai Tsai [a,g], Fuu-Jen Tsai [a,d,*]

[a] Department of Pediatrics, Children's Medical Center, China Medical University Hospital, Taichung, Taiwan
[b] Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan
[c] Department of Medical Research, China Medical University and Hospital, Taichung, Taiwan
[d] School of Post Baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan
[e] Terry Fox Cancer Research Laboratory, China Medical University Hospital, Taichung, Taiwan
[f] Graduate Institute of Integrated Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan
[g] Asia University, Taichung, Taiwan

ARTICLE INFO

Article history:
Received 12 March 2013
Received in revised form
14 March 2013
Accepted 15 March 2013
Available online 11 May 2013

Keywords:
acanthosis nigricans
insulin
obesity
polymorphism

ABSTRACT

Background: Obesity is one of the main causes of preventable death. Complications of childhood obesity include cardiovascular risk, impaired glucose tolerance, type 2 diabetes mellitus, and acanthosis nigricans (AN; associated with obesity as a manifestation of cutaneous insulin resistance). An interaction between AN and obesity as well as a detailed mechanism for the pre- and co-obesity appearance of AN in children are still to be revealed.

Purposes: This research tries to assess involvement of the insulin/insulin-like growth factor (IGF) receptor pathway in childhood pre- and co-obesity AN via a study of the association of polymorphisms of the INSR, IRS1, and IGF1R genes with pre- and co-obesity AN.

Methods: In total, 99 children with pre- and co-obesity AN and 100 healthy controls were genotyped and analyzed by the polymerase chain reaction-restriction fragment length polymorphism method.

Results: Intergroup frequency differed starkly for INSR His1085His and IGF1R IVS7-20, but not in the IRS1 Ala804Ala or IGF1R Thr766Thr genotypes. The T allele of INSR His1085His and C allele of IGF1R IVS7-20 both conferred a starkly(p=0.04 and 2.84E-6 = 2.84×10^{-6}, respectively)higher risk for AN.

Conclusion: The above findings suggest that certain genetic variants in insulin/insulin-like growth factor (IGF) receptor pathway might be correlated with the appearance of AN prior to or concurrent with obesity, and also reveal the insulin/IGF receptor pathway as crucial in pre- and co-obesity AN.

* *Corresponding author*. China Medical University Hospital, 2 Yuh-Der Road, Taichung 40402, Taiwan.
E-mail address: d0704@mail.cmuh.org.tw (F.-J. Tsai).
[h] This author contributed equally to this study.

2211-8020/$ – see front matter
http://dx.doi.org/10.1016/j.biomed.2013.04.001

1. Introduction

Obesity is one of the leading causes of preventable death. With the increasing intake of high-calorie food and a sedentary lifestyle, pediatric obesity looms ever more prevalent and is exerting a major impact on public health in the 21st century. This universal trend of pediatric obesity is reported not only in Occidental countries (up to 25% of children in the USA), but also in Oriental nations like Taiwan [1]. There is no denying that obese children tend to become obese adults, especially those whose obesity lasts to their adolescence. Complications of obesity include cardiovascular risk, hypertension, dyslipidemia, endothelial dysfunction, type 2 diabetes mellitus and impaired glucose tolerance, acanthosis nigricans (AN), hepatic steatosis, precocious puberty, hypogonadism and polycystic ovary syndrome, obstructive sleep disorder, orthopedic complications, cholelithiasis, and pseudotumor cerebri [2–4].

AN is a hyperpigmented, velvety cutaneous thickening easily observed on certain parts of the body, including the axillae, sides of the neck, groin, antecubital and popliteal surfaces, umbilical area, and, in more severe cases, even spread over the whole body and mucosal surface. In the literature, AN is reported to be closely associated with obesity as a manifestation of cutaneous insulin resistance [5]. In addition, endocrinopathies, malignancy (most frequently gastric carcinoma in adults), genetic syndromes, and the use of drugs may also lead to the development of AN [6–10].

From a clinical viewpoint, it is taken for granted that obesity often comes prior to the appearance of AN of cutaneous insulin resistance. Interestingly, from questionnaires given to child patients with AN that we met in the China Medical University Hospital, a large proportion (about 50%) of their AN syndromes appeared prior to or together with obesity, which we will describe as pre- and co-obesity AN (PCOAN).

This clinical observation may challenge the traditional rationale that elevated insulin concentration owing to excessive weight gain and subsequent insulin resistance in obese people results in both direct and indirect activation of insulin-like growth factor (IGF-1) receptors on keratinocytes and fibroblasts, leading to epidermal proliferation and the appearance of AN [11]. Insulin and leptin resistance have been proved to be responsible for a failure of appetite and suppression of excessive energy intake [12]. Also, defective insulin binding and post-insulin receptor function, plus genetic defects within the insulin receptor gene, have been documented in patients with AN [13–16].

From our clinical experience and limited previous reports, we hypothesized that differences in the genetic background of the insulin/IGF receptor and its associated signaling pathway in these children with PCOAN should play a key role in excessive weight gain and AN, no matter which occurred first. Insulin resistance itself may precipitate excessive weight gain via a failure to prevent redundant energy intake, facilitating the concurrent emergence of AN arising from epidermal proliferation. Fig. 1 plots an overall flowchart of our hypothesis.

To understand the genomic role of insulin/IGF receptor pathway-related genes in PCOAN, we chose four single nucleotide polymorphisms (SNPs) from three genes—*INSR* His1085His (rs1799817), *IRS1* Ala804Ala (rs1801123), *IGF1R* IVS7-20 (rs2272037), and *IGF1R* Thr766Thr (rs3743262)—and probed their genotypic distribution in a Taiwanese child population with PCOAN.

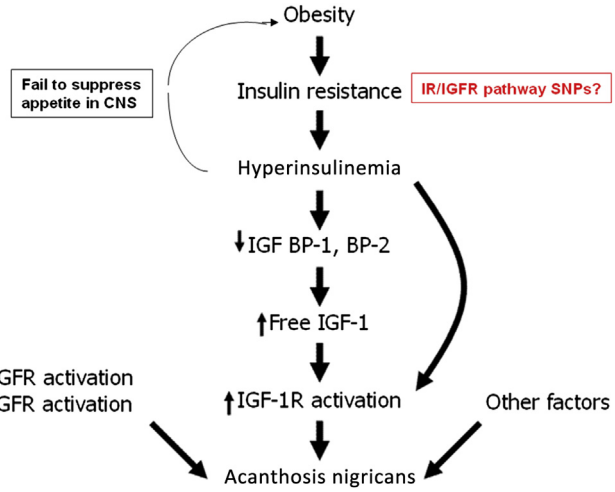

Fig. 1 – Postulated mechanisms for the pathogenesis of pre- and co-obesity acanthosis nigricans. CNS = central nervous system; EGFR = epidermal growth factor receptor; FGFR = fibroblast growth factor receptor; IGF = insulin-like growth factor; IGFR = insulin-like growth factor receptor; IGF-1R = insulin-like growth factor receptor 1; IR = insulin receptor; SNP = single nucleotide polymorphism. *Note.* From Acanthosis nigricans: A practical approach to evaluation and management, by S.P. Higgins, M. Freemark, and N.S. Prose, 2008, *Dermatol Online J* 14(9), p.2. Credit to S.P. Higgins, Copyright 2008, Name of Copyright Holder: The Regents of the University of California, Davis campus, UCDHS Department of Dermatology.

2. Materials and methods

2.1. Study population and sample collection

The children and adolescents (5–18 years old) were recruited to the study group from the Department of Pediatrics at the China Medical University Hospital in central Taiwan. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. All participants with a BMI above the 95th percentile for age and gender were classified as obese on the basis of BMI category by the Department of Health of Taiwan. A morning serum after an 8-hour fast was obtained for glucose and insulin measurement. Insulin resistance was determined using the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index in the fasting state. Detailed medical and family histories were obtained for all participants, and physical examinations were performed. All the participants showed normal thyroid and adrenal function. Controls, enrolled from the Department of Family Medicine, were free of obesity, AN, and other obesity-related diseases. Each person donated samples with informed consent. This study was approved by the Institutional Review Board Committee of China Medical University Hospital.

2.2. Genotyping assays

Genomic DNA was prepared from peripheral blood leukocytes by a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan), and then processed as reported in previous studies [17–25]. Briefly, the following primers were used: *INSR* C3255T rs1799817, 5′-TTGGGTGAGGGCTTGGGTGGAAG-3′ and 5′-CCTGTGTCCTCT GTCGCTCTGTG-3′; *IRS1* C2412T rs1801123, 5′-CTCCTACTACT CATTGCCAAG-3′ and 5′-CAGACAAGTAGCCAGACTGAT-3′; *IGF1R* IVS7-20(C/T) rs2272037, 5′-GACCTCCCATTATAGAAA GTG-3′ and 5′-CCAGTGAGCTTGCGAAGAAG-3′; and *IGF1R* C2298T rs3743262, 5′-TCCACGGTTA AGATTCTTCTG-3′ and 5′-TCCACTAGGTTGTGAGGAAG-3′.

The following cycling conditions were performed: one cycle at 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds; and final extension at 72°C for 10 minute. Polymerase chain reaction (PCR) products, except *IGF1R* IVS7-20(C/T) rs2272037, were studied after digestion with MspA1I, HphI, and DraIII, restriction enzymes for *IRS1* C2412T rs1801123 (cut from 189+257 bp A type into 125+132+189 bp G type), *IGF1R* C2298T rs3743262 (cut from 432 bp T type into 181+251 bp C type), and *INSR* C3255T rs1799817 (cut from 418 bp T type into 92+326 bp C type), respectively. The PCR product of *IGF1R* IVS7-20(C/T) rs2272037 was purified using QIAEX II (Qiagen, Hilden, Germany) and applied to direct sequencing for SNP type detection. Direct sequencing used a BigDye 3.1 Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) with an ABI 3100 Genetic Analyzer (Applied Biosystems).

2.3. Statistical analyses

Our study selected only those matches with all SNP data (case/control = 99/100) for final analysis. Pearson's two-sided Chi-square or Fisher's exact test was used to compare the genotype distribution between cases and controls. Data were recognized as statistically significant at $p < 0.05$.

3. Results

Table 1 outlines the clinical characteristics and analysis of the 99 child patients with PCOAN and the 100 healthy controls, groups similar in gender at enrollment. The control group were of a greater age, but even so had a lower BMI, serum fasting glucose, insulin level and HOMA score ($p < 0.005$), i.e., had higher insulin sensitivity. Defining insulin resistance as a fasting insulin level above 15 μIU/ml, none of the controls but 61 of the 99 patients in the PCOAN group were insulin-resistant. Fourteen patients had impaired fasting glucose, and four fulfilled the criteria for diabetes (Table 1). These differences are detailed in the Discussion section.

Table 2 shows the genotype frequencies of *INSR* His1085His (rs1799817), *IRS1* Ala804Ala (rs1801123), *IGF1R* IVS7-20 (rs2272037), and *IGF1R* Thr766Thr (rs3743262) in the controls and patients with PCOAN. The genotype distributions of various genetic polymorphisms of *INSR* His1085His and *IGF1R* IVS7-20 differed significantly in PCOAN patients versus controls ($p = 0.046$ and 8.77E-6 = 8.77×10^{-6}, respectively), whereas those for *IRS1* Ala804Ala or *IGF1R* Thr766Thr did not ($p > 0.05$) (Table 2). The distributions of *INSR* His1085His *C homozygote/heterozygote/T homozygote in the controls and patients with PCOAN were 36.0%/53.0%/11.0% and 18.2%/61.6%/20.2%, respectively (Table 2). The proportions of IRS1 Ala804Ala *C homozygote/heterozygote/T homozygote in controls and patients with PCOAN were 53.0%/30.0%/9.0% and 39.4%/47.5%/13.1%, respectively (Table 2). The proportions of IGF1R IVS7-20 *A homozygote/heterozygote/G homozygote in controls and patients with PCOAN were 36.0%/51.0%/13.0% and 80.8%/18.2%/1.0% respectively (Table 2). The *IGF1R* Thr766Thr *C homozygote/heterozygote/T homozygote proportions in controls and patients with PCOAN were 43.0%/40.0%/17.0% and 32.6%/46.5%/20.9%, respectively (Table 2). Polymorphisms of *IGF1R* IVS7-20 and *INSR* His1085His thus correlate significantly with PCOAN.

Table 3 shows the frequencies of *IRS1* Ala804Ala (rs1801123), *IGF1R* IVS7-20 (rs2272037), *IGF1R* Thr766Thr (rs3743262), and *INSR* His1085His alleles (rs1799817) for controls and patients with PCOAN. Allele frequency distributions of *INSR* His1085His and *IGF1R* IVS7-20 *C are associated with higher susceptibility to PCOAN. Distributions of the *INSR* His1085His C/T allele in controls and patients with PCOAN

Table 1 – Clinical and biochemical features of the pre-obesity and co-obesity AN patients (PCOAN) and control groups.

	PCOAN group	Control group	p
Age	10.8 (4–18)	24 (19–40)	<0.0001
Sex (male/female)	55/44	50/50	0.435
BMI (kg/m^2)	30.7 (22.4–44.2)	20.1 (18–22)	<0.0001
Fasting glucose (mg/dL)	101.1 (77–327)	81.5 (64–96)	<0.0001
Fasting insulin (μIU/ml)	21.1 (3.9–77.9)	10.3 (6.3–12.4)	<0.0001
HOMA-IR	5.4 (3.7–21.3)	1.8 (0.8–2.6)	<0.0001

Data are presented as mean (range).
HOMA-IR = Homeostasis Model Assessment for Insulin Resistance.

Table 2 – Distributions of *IRS1*, *INSR*, and *IGF1R* genotypes among pre-obesity and co-obesity AN patients and control groups.

Genotype	Controls	Patients	p[a]
INSR His1085His (rs1799817)			0.0462
CC	36 (36.0)	18 (18.2)	
CT	53 (53.0)	61 (61.6)	
TT	11 (11.0)	20 (20.2)	
IRS1 Ala804Ala (rs1801123)			0.2516
AA	53 (53.0)	39 (39.4)	
AG	38 (38.0)	47 (47.5)	
GG	9 (9.0)	13 (13.1)	
IGF1R IVS7-20 (rs2272037)			8.77E-6 = 8.77×10^{-6}
CC	36 (36.0)	80 (80.8)	
CT	51 (51.0)	18 (18.2)	
TT	13 (13.0)	1 (1.0)	
IGF1R Thr766Thr (rs2272037)			0.4253
CC	43 (43.0)	28 (32.6)	
CT	40 (40.0)	40 (46.5)	
TT	17 (17.0)	18 (20.9)	

Data are presented as n (%).

Table 3 – Distributions of *IRS1*, *INSR*, and *IGF1R* alleles among pre-obesity and co-obesity AN patients and control groups.

Allele	Controls	Patients	*p*
INSR His1085His (rs1799817)			0.0255
Allele C	125 (62.5)	97 (49.0)	
Allele T	75 (37.5)	101 (51.0)	
IRS1 Ala804Ala (rs1801123)			0.1025
Allele A	144 (72.0)	125 (63.1)	
Allele G	56 (28.0)	73 (36.9)	
IGF1R IVS7-20 (rs2272037)			2.84E-6 $= 2.84 \times 10^{-6}$
Allele C	123 (61.5)	178 (89.9)	
Allele T	77 (38.5)	20 (10.1)	
IGF1R Thr766Thr (rs2272037)			0.2043
Allele C	126 (63.0)	96 (55.8)	
Allele T	74 (37.0)	76 (44.2)	

Data are presented as *n* (%).

were 62.5%/37.5% and 49.0%/51.0%, respectively (Table 3). Distributions of the *IRS1* Ala804Ala A/G allele in controls and patients with PCOAN were 72.0%/28.0% and 63.1%/36.9%, respectively. The proportions of the *IGF1R* IVS7-20 C/T allele in controls and patients with PCOAN were 61.5%/38.5% and 89.9%/10.1%, respectively. Distributions of the *IGF1R* Thr766Thr C/T allele in controls and patients with PCOAN were 63.0%/37.0% and 55.8%/44.2%, respectively.

4. Discussion

AN reportedly shows a strong ethnic influence and is common in children and adolescents, especially among populations with high rates of adult diabetes [26,27]. However, two recent reports have challenged those who describe AN as a reliable predictor of hyperinsulinemia [28,29]. Hirschler et al reported the BMI of patients with AN as being significantly greater than that of participants without AN, whereas neither fasting immunoreactive insulin nor the HOMA-IR index differed in Hispanic individuals [28]. A Japanese study found a significant difference between AN-positive and AN-negative groups in the duration of obesity, as well as the age and percent overweight [30]. We proposed a clinical observation in a large proportion of PCOAN patients that AN may occur prior to or concurrent with the appearance of obesity, and then investigated its possible mechanism via a pioneering genetic approach.

According to the results shown in Table 1, we can provide some explanations and annotations for the data presented and the stories behind them. Both groups had a similar gender distribution, which prevents gender bias: females were reported to have a higher peripheral insulin sensitivity than males [31]. The PCOAN group had a younger age and higher serum fasting glucose and insulin levels than the control group. Blunted insulin sensitivity in the PCOAN group supports our hypothesis that PCOAN may stem from an indigenous genetic difference in the insulin/IGF receptor pathway, since insulin sensitivity tends to decline with age [32]. In our cohort, 30% of patients with PCOAN showed no insulin resistance, implying that other factors (such as epidermal growth factor receptor, fibroblast growth factor receptor, and leptin) play a key role in such non-insulin-resistant patients [33]. By contrast, the use of fasting serum insulin level or HOMA-IR as an indicator of insulin resistance may be inappropriate if the glucose clamp test is viewed as a gold standard. However, it is nigh on impossible to perform a clamp test in obese children, owing to the parents' wishes and technical aspects [34]. The PCOAN group manifested a higher HOMA-IR index than the controls, i.e., higher insulin resistance. No consensus was reached on a cut-off value for HOMA-IR in obese children, but a value above 3 was generally considered to denote insulin resistance [35,36].

Previous genetic studies of AN have focused largely on the β-adrenergic receptor and discuss the association of AN with obesity, cardiovascular disease, and type 2 diabetes [37–47]. Our paper first evaluated another pathway closely related to obesity—the insulin/IGF receptor—and its genetic association with PCOAN. Of the four SNPs probed in this study, we can report the variant *INSR* His1085His and *IGF1R* IVS7-20 genotypes as being positively correlated with susceptibility to PCOAN (see Tables 2 and 3). This suggests that both insulin and IGF receptor subpathways are involved in PCOAN, in addition to involvement of their downstream genes and crosstalk between them, meriting further investigation.

At the cellular molecular level, insulin interacts with not only the insulin receptor, but also the IGF receptor, activating downstream effectors and even crosstalking with each other [48]. Along with the aforementioned insulin resistance that might result in failure to suppress excessive energy intake, with ensuing obesity, this concept fits our results showing a strong correlation of the insulin/IGF receptor pathway with PCOAN. It is well known that most obesity may originate from a disturbed interaction between genetics and the environment that cannot be explained by a single factor; our study specified one genetic factor, related to the insulin/IGF receptor pathway, as playing a particular role in the complex pathogenesis of obesity, under scrutiny of the patients' phenotype and genotype association.

Future molecular studies are needed to elucidate the complex relationship between polymorphisms and PCOAN. A genetic population study of AN, like an association study, can derive correlations among AN and other diseases, such as obesity and diabetes mellitus. Our preliminary data not only provide evidence that the T allele of *INSR* His1085His and C allele of *IGF1R* IVS7-20 are correlated with the appearance of AN proceeding or concurrent with obesity, but also reveal that the insulin/IGF receptor pathway may play a leading role in PCOAN in Taiwan.

Acknowledgments

This study is funded by grants partly from the China Medical University Hospital (DMR-101-038). We especially appreciate every patient and colleague for their cooperation and contribution.

REFERENCES

[1] Friedman JM. Obesity: cases and control of excess body fat. Nature 2009;459:340–2.

[2] Krebs NF, Himes JH, Jacobson D, Nicklas TA, Guilday P, Styne D. Assessment of child and adolescent overweight and obesity. Pediatrics 2007;120(Suppl. 4):S193–228.
[3] Sturm R. The effects of obesity, smoking, and drinking in medical problems and costs. Health Aff 2002;21: 245–53.
[4] Pomietto M, Docter AD, Van Borkulo N, Alfonsi L, Krieger J, Liu LL. Small steps to health: building, sustainable partnerships in pediatric obesity. Pediatrics 2009;123(Suppl. 5):S308–16.
[5] Guran T, Turan S, Akcay T, Bereket A. Significance of acanthosis nigricans in childhood obesity. J Paediatr Child Health 2008;44:338–41.
[6] Schwartz RA. Acanthosis nigricans. J Am Acad Dermatol 1994;31:1–19.
[7] Schwartz RA. Acanthosis nigricans. In: Demis DJ, editor. Clinical dermatology (unit 12–26). 18th ed. Philadelphia: JB Lippincott; 1999. p. 1–11.
[8] Tasjian D, Jarratt M. Familial acanthosis nigricans. Arch Dermatol 1984;120:1351–4.
[9] Skiljevic DS, Nikolic MM, Jakovljevic A, Dobrosavljevic DD. Generalized acanthosis nigricans in early childhood. Pediatr Dermatol 2001;18:213–6.
[10] Birns J, Badawi RA, Chase AR, Watson T. Acanthosis nigricans associated with acute myeloid leukemia. Eur J Int Med 2004;15:473.
[11] Higgins SP, Freemark M, Prose NS. Acanthosis nigricans: a practical approach to evaluation and management. Dermatol Online J 2008;14:2.
[12] Barsh GS, Schwartz MW. Genetic approaches to studying energy balance: perception and integration. Nat Rev Genet 2002;3:589–600.
[13] Flier JS, Eastman RC, Minaker KL, Matteson D, Rowe JW. Acanthosis nigricans in obese women with hyperandrogenism. Characterization of an insulin resistant state distinct from the type A and B syndromes. Diabetes 1985;34:101–7.
[14] Leme CE, Wajchenberg BL, Lerario AC, Goldman J, Borges JL. Acanthosis nigricans, hirsutism, insulin resistance and insulin receptor defect. Clin Endocrinol (Oxf) 1982;17:43–9.
[15] Rique S, Nogues C, Ibanez L, Marcos MV, Ferragut J, Carrascosa A. Identification of three novel mutations in the insulin receptor gene in type A insulin resistant patients. Clin Genet 2000;57:67–9.
[16] Moller DE, Cohen O, Yamaguchi Y, Assiz R, Grigorescu F, Eberle A, et al. Prevalence of mutations in the insulin receptor gene in subjects with features of the type A syndrome of insulin resistance. Diabetes 1994;43:247–55.
[17] Bau DT, Tseng HC, Wang CH, Chiu CF, Hua CH, Wu CN, et al. Oral cancer and genetic polymorphism of DNA double-strand break gene Ku70 in Taiwan. Oral Oncol 2008;44:1047–51.
[18] Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN, et al. A novel single nucleotide polymorphism in *XRCC4* gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol 2008;44:898–902.
[19] Chiu CF, Tsai MH, Tseng HC, Wang CL, Tsai FJ, Lin CC, et al. A novel single nucleotide polymorphism in *ERCC6* gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol 2008;44:582–6.
[20] Chiu CF, Wang CH, Wang CL, Lin CC, Hsu NY, Weng JR, et al. A novel single nucleotide polymorphism in *XRCC4* gene is associated with gastric cancer susceptibility in Taiwan. Ann Surg Oncol 2008;15:514–8.
[21] Bau DT, Wu HC, Chiu CF, Lin CC, Hsu CM, Wang CL, et al. Association of XPD polymorphisms with prostate cancer in Taiwanese patients. Anticancer Res 2008;27:2893–6.
[22] Chiu CF, Wang HC, Wang CH, Wang CL, Lin CC, Shen CY, et al. A new single nucleotide polymorphism in *XRCC4* gene is associated with breast cancer susceptibility in Taiwanese patients. Anticancer Res 2008;28:267–70.
[23] Yang MD, Hsu YM, Kuo YS, Chen HS, Chang CL, Wu CN, et al. Significant association of Ku80 single nucleotide polymorphisms with colorectal cancer susceptibility in central Taiwan. Anticancer Res 2009;29:2239–42.
[24] Chang CH, Chang CL, Tsai CW, Wu HC, Chiu CF, Wang RF, et al. Significant association of an *XRCC4* single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res 2009;29:1777–82.
[25] Hsu CF, Tseng HC, Chiu CF, Liang SY, Tsai CW, Tsai MH, et al. Association between DNA double strand break gene Ku80 polymorphisms and oral cancer susceptibility in Taiwan. Oral Oncol 2009;45:789–93.
[26] American Diabetes Association. Type 2 diabetes in children and adolescents. Diabetes Care 2000;23:381–9.
[27] Stuart CA, Gilkison CR, Smith MM, Bosma AM, Bruce K, Nagamani M. Acanthosis nigricans as a risk factor for noninsulin dependent diabetes mellitus. Clin Pediatr 1998;37:73–9.
[28] Hirschler V, Aranda C, Oneto A, Gonzalez C, Jadzinsky M. Is acanthosis nigricans a marker of insulin resistance in obese children? Diabetes Care 2002;25:2353.
[29] Nguyen TT, Keil MF, Russell DL, Pathomvanich A, Uwaifo GI, Sebring NG, et al. Relation of acanthosis nigricans to hyperinsulinemia and insulin sensitivity in overweight African American and white children. J Pediatr 2001;138:474–80.
[30] Miura N, Ikezaki A, Iwama S, Matsuoka H, Ito K, Sugihara S. Genetic factors and clinical significance of acanthosis nigricans in obese Japanese children and adolescents. Acta Pediatrica 2006;95:170–5.
[31] Borissova AM, Tankova T, Kirilov G, Koev D. Gender-dependent effect of ageing on peripheral insulin action. Int J Clin Pract 2005;59:422–6.
[32] Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C, Rothman DL, et al. Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science 2003;300:1140–2.
[33] Hermanns L, Hermanns JF, Pierard GE. Juvenile acanthosis nigricans and insulin resistance. Pediatr Dermatol 2000;19:2–14.
[34] DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol Gastrointest Liver Physiol 1979;237:G214–23.
[35] Tresaco B, Bueno G, Pineda I, Moreno LA, Garagorri JM, Bueno M. Homeostatic model assessment (HOMA) index cut-off values to identify the metabolic syndrome in children. J Physiol Biochem 2005;61:381–8.
[36] Madeira IR, Carvalho CN, Gazolla FM, de Matos HJ, Borges MA, Bordallo MA. Cut-off point for Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index established from receiver operating characteristic (ROC) curve in the detection of metabolic syndrome in overweight pre-pubertal children. Arq Bras Endocrinol Metab 2008;52:1466–73.
[37] Walston J, Silver K, Bogardus C, Knowler WC, Celi FS, Austin S, et al. Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the beta 3-adrenergic-receptor gene. N Engl J Med 1995;333:343–7.
[38] Widen E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC. Association of a polymorphism in the b3-adrenergic-receptor gene with features of the insulin resistance syndrome in Finns. N Engl J Med 1995;333:348–51.
[39] Clement K, Vaisse C, Manning BSJ, Basdevant A, Guy-Grand B, Ruiz J, et al. Genetic variation in the b3-adrenergic

receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 1995;333:352–4.
[40] Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, et al. A mutation in the b3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 1995;215:555–60.
[41] Yoshida T, Sakane N, Umekawa T, Sakai M, Takanashi T, Kondo M. Mutation of b3-adrenergic-receptor gene and response to treatment of obesity. Lancet 1995;346:1433–4.
[42] Fujisawa T, Ikegami H, Yamato E, Takekawa K, Nakagawa Y, Hamada Y, et al. Association of Trp64Arg mutation of the b3-adrenergic receptor with NIDDM and body weight gain. Diabetologia 1996;39:349–52.
[43] Arner P. The b3-adrenergic-receptor a cause and cure of obesity. N Engl J Med 1995;333:382–3.
[44] Pulkkinen A, Kareinen A, Saarinen L, Heikkinen S, Lehto S, Laakso M. The codon 64 polymorphism of the beta3-adrenergic-receptor gene is not associated with coronary heart disease or insulin resistance in nondiabetic subjects and noninsulin-dependent diabetic patients. Metabolism 1999;48:853–6.
[45] Nagase T, Aoki A, Yamamoto M, Yasuda H, Kado S, Nishikawa M, et al. Lack of association between the Trp64Arg mutation in the b3-adrenergic receptor gene and obesity in Japanese men: a longitudinal analysis. J Clin Endocrinol Metab 1997;82:1284–7.
[46] Large V, Hellstrom L, Reynisdottir S, Lönnqvist F, Eriksson P, Lannfelt L, et al. Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. J Clin Invest 1997;100:3005–13.
[47] Ishiyama-Shigemoto S, Yamada K, Yuan X, Ichikawa F, Nonaka K. Association of polymorphisms in the b2-adrenergic receptor gene with obesity, hypertriglyceridaemia, and diabetes mellitus. Diabetologia 1999;42:98–101.
[48] Cullen MT, Brice E, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol 2006;7:85–96.