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

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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, 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.

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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, IRS1, IGF1R.
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′-TTGGGTGAGGCCTTGTTGGAAG-3′ and 5′-CTCTGCTCCTCTGTGCCTCTG-3′; IRS1 C2412T rs1801123, 5′-CTCCTACTACTCATTGGAAG-3′ and 5′-CATGAGAAGGACAGATGTA-3′; IGF1R IVS7-20(C/T) rs2272037, 5′-GCTCCATTATAGAAAGTGTCGCTCTGTG-3′ and 5′-CCAGTGAGCTTGCGAAGAC for 5′-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).

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 MspAII, HphI, and DraII, restriction enzymes for IGF1R IVS7-20(C/T) rs2272037, to determine the sequence 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 test was applied to compare the genotypes of various genetic polymorphisms of INSIR and IGF1R IVS7-20(C/T) rs2272037. The 36.0%/53.0%/11.0% and 80.8%/61.6%/20.2% differences were statistically significant (p<0.01) among pre-obesity and co-obesity AN patients and controls.

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 was 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 μU/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 INSIR His1085His (rs1799817), IRS1 Ala804Ala (rs1801123), IGF1R IVS7-20(C/T) rs2272037, and IGF1R Thr766Thr (rs3743262) in the controls and patients with PCOAN. The genotype distributions of various genetic polymorphisms of INSIR His1085His and IGF1R IVS7-20(C/T) rs2272037 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 INSIR His1085His C homozygote/heterozygote/T homozygote in the controls and patients with PCOAN were 36.0%/53.0%/11.0% and 82.6%/18.2%/0.0%, 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 48.7%/45.7%/5.6%, respectively (Table 2). The proportions of IGF1R IVS7-20(C/T) rs2272037 A homozygote/heterozygote/G homozygote in controls and patients with PCOAN were 36.0%/51.0%/13.0% and 80.8%/18.2%/0.0%, respectively (Table 2). Polymorphisms of IGF1R IVS7-20(C/T) rs2272037 and INSIR His1085His thus correlate significantly with PCOAN.

Table 3 shows the frequencies of IRS1 Ala804Ala (rs1801123), IGF1R IVS7-20(C/T) rs2272037, IGF1R Thr766Thr (rs3743262), and INSIR His1085His alleles (rs1799817) for controls and patients with PCOAN. Allele frequency distributions of INSIR His1085His and IGF1R IVS7-20(C/T) rs2272037 are associated with higher susceptibility to PCOAN. Distinctions of the INSIR His1085His C/T allele in controls and patients with PCOAN
Distributions of Thr766Thr C/T allele in controls and patients with PCOAN were 63.0%/37.0% and 55.8%/44.2%, respectively. Distributions of the controls and patients with PCOAN were 61.5%/38.5% respectively. The proportions of the patients with PCOAN were 72.0%/28.0% and 63.1%/36.9%, respectively. Distributions of the predictor of hyperinsulinemia[28,29]. Hirschler et al reported reports have challenged those who describe AN as a reliable with high rates of adult diabetes[26,27]. However, two recent in children and adolescents, especially among populations AN reportedly shows a strong ethnic influence and is common in Hispanics[28]. A Japanese study found a significant immunoreactive insulin nor the HOMA-IR index differed in females[31]. The PCOAN group had a younger age and higher reported to have a higher peripheral insulin sensitivity 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 crosstalk 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.

### 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].

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