Available online at www.sciencedirect.com
SCIENCE DIRECT®
Growth Hormone & IGF Research 15 (2005) 363–368
www.elsevier.com/locate/ghir

Genetic variation in the type 2 insulin-like growth factor receptor gene and disparity in childhood height

Clive J. Petry [a], Ken K. Ong [a], Dianne L. Wingate [a], James Brown [b], Carolyn D. Scott [c], The ALSPAC Study Team [d], E. Yvonne Jones [b], Marcus E. Pembrey [d], David B. Dunger [a,*]

[a] Department of Paediatrics, University of Cambridge, Box 116, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK
[b] Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK
[c] Kolling Institute For Medical Research, University of Sydney, Sydney, Australia
[d] Unit of Paediatric and Perinatal Epidemiology, University of Bristol, Bristol, UK

Received 28 February 2005; revised 21 July 2005; accepted 21 July 2005
Available online 19 September 2005

Abstract

Objective: The type 2 insulin-like growth factor receptor (IGF2R) is thought to regulate insulin-like growth factor-II (IGF-II) bioavailability by degrading it in the lysosomes after uptake. We hypothesised that polymorphisms in the IGF2R gene could alter size at birth and childhood growth.
Design and methods: The hypothesis was tested in a normal birth cohort (Avon Longitudinal Study of Parents and Children) by genotyping the IGF2R gene gly1619arg polymorphism, which causes a non-conservative amino acid change in the IGF-II binding region, using PCR and restriction fragment length polymorphism analysis.
Results: The IGF2R gly1619arg genotype was not associated with any measure of size at birth, but A/A homozygotes grew more slowly, as determined by their change in height standard deviation scores (SDS) over the first three years (−0.70 (0.72); $n = 12$), than G/G homozygotes (0.00 (1.09); $n = 561$) ($p = 0.03$). They remained shorter during childhood and by the age of 7 years respective height SDS were: 0.73 (1.02) ($n = 12$) and 0.01 (0.99) ($n = 634$) ($p = 0.01$). These height differences persisted after adjusting for parental heights and gender. There were no detectable differences in weights at 7 years.
Conclusion: Allelic variation in the gly1619arg SNP of the IGF2R gene is associated with disparity in childhood stature which could reflect altered binding of IGF-II to its receptor.

Keywords: IGF-II; Mannose 6-phosphate receptor; Stature; Genetic association study

1. Introduction

Insulin-like growth factor-II (IGF-II) is an important fetal growth factor, acting as a mitogen for many different cell types and in particular modulating growth and differentiation of muscle cells. In a contemporary childhood cohort, the Avon Longitudinal Study of Parents and Children (ALSPAC), we found cord blood IGF-II concentrations to be positively related to size at birth [1]. IGF-II is thought to function through binding to the type 1 IGF receptor. It also binds to the type 2 IGF receptor (IGF2R) which instead of initiating a signalling response causes IGF-II uptake, transportation to lysosomes and ultimately degradation [2]. A soluble circulating form of IGF2R, resulting from the proteolytic cleavage of the intact membrane-bound receptor,

* Corresponding author. Tel.: +44 1223 336886; fax.: +44 1223 336996.
E-mail address: dbd25@cam.ac.uk (D.B. Dunger).

1096-6374/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.ghir.2005.07.003

may function similarly to its membrane-bound partner (namely binding free IGF-II and thus inhibiting its action at the IGF-I receptor) since it has been shown to inhibit IGF-II-mediated DNA synthesis in vitro [3]. In ALSPAC, we found that the ratio of IGF-II to the soluble form of the IGF2R was also related to birth weight in all pregnancies, and that IGF2R concentrations were negatively related to birth weight and length in multiparous pregnancies [1]. This suggests that IGF2R may also have a role in regulating fetal growth.

In utero only the paternal allele is expressed due to imprinting, but postnatally *IGF2*'s role may be different since it is expressed biallelically in humans, at least in the liver [4]. Although its function in postnatal life is not clearly defined, a role in adult body weight regulation is suggested by the findings that in humans low circulating IGF-II concentrations are predictive of future weight gain [5] and in *IGF2*-partial-gene knockout mice there is enhanced adiposity [6]. Possibly related to these findings, there may also be a role for IGF-II in the attainment of stature as a positive relationship between circulating IGF-II concentrations and height has been observed in both children [7,8] and adults [9].

In mice the *IGF2R* gene is imprinted [10] (with only the maternal allele being expressed), whereas in humans there is an uncommon functional polymorphism affecting imprinting in some people [11], but commonly the gene is not imprinted [12]. A number of single nucleotide polymorphisms (SNPs) have been described in *IGF2R* [13]. One of them (G–A), coding for amino acid 1619, causes a non-conservative glycine to arginine substitution in the IGF-II binding region of the receptor. Another SNP (A–G; asparagine to serine), coding for amino acid 2020, falls within an IGF-II affinity-enhancing domain. We hypothesised, therefore, that these polymorphisms would impact upon size at birth and childhood growth by altering IGF-II bioavailability, and tested this in the ALSPAC cohort.

2. Subjects and methods

2.1. Subjects

ALSPAC is a prospective study of 14 541 pregnancies recruited from all pregnancies in three Bristol based District Health Authorities with expected dates of delivery between April 1991 and December 1992. In this study gly1619arg *IGF2R* genotyping was performed in children (and subsequently in their parents) from the ALSPAC "Children in Focus" sub-cohort ($n = 1091$), a random selection from the last 6 months of recruitment, who were measured at birth and every 4 months to 12 months of age, every 6 months to 4 years, and yearly between 5 and 7 years. At age 7 years (mean (SD) age 7.5 (0.1) years; range 6.9–8.2) body weight was measured using electronic scales and standing height was measured by stadiometer (Leicester height measure; Child Growth Foundation, London, UK). Ethical approval was obtained from the ALSPAC and the local ethics committees. Signed or verbal consent to phenotypic and genotypic analyses was obtained from a parent and/or the child. Details of antenatal data collection and measurements of body size from birth to 5 years old have been previously described [14] and further details are available on the ALSPAC website (http://www.ich.bris.ac.uk/alspac).

For assessment of the effect of allele transmission, 621 children were identified with auxological data where microsatellite-validated DNA was available from both parents and the child [15].

2.2. Gly1619arg genotyping

Gly1619arg (rs629849) genotyping was performed using polymerase chain reaction (PCR) amplification and restriction digestion of the resulting PCR products, using the enzyme NciI (New England Biolabs, Hitchin, UK). The sequence of the forward and reverse oligonucleotide primers was 5′-aacaatggttaaagccggattg-gacacttgaagtt-3′ and 5′-ggcccgggtgcagccaggcactg-3′, respectively. 20 ng genomic DNA was incubated with 7.5 pmol of each of these primers, 1× reaction buffer, 1.5 mM magnesium chloride, 0.125U Biotaq DNA polymerase (Bioline, London, UK), 200 μM each dNTP (Promega, Southampton, UK) and glycerol (10% v/v) in a reaction volume of 20 μl. The reaction series started with a 5 min incubation at 94 °C, followed by 20 cycles of 94 °C (45 s), 67 °C (45 s, dropping 0.5 °C per cycle) and 72 °C (45 s). This was followed by 15 cycles of 94 °C (45 s), 57 °C (45 s) and 72 °C (45 s) and a final 10 min incubation at 72 °C. After the restriction digest, separating the products by agarose gel electrophoresis produced a 456 base-pair (bp) band for allele 'A' and 149 and 307 bp bands for allele 'G'.

2.2.1. Asn2020Ser genotyping

Asn2020Ser genotyping was performed using a similar method to that for gly1619arg genotyping, but using the restriction enzyme MspA1I (New England Biolabs) and the following oligonucleotide primers: 5′-ctggggcaa-agctacaaattaacaacatc-3′ and 5′-gagaacccaaaagagcca-accatcgtcaagcagtc-3′. PCR cycling conditions were as those described for gly1619arg but with annealing temperatures 3 °C lower throughout. This produced a 406 bp band with allele 'A' and 373 and 33 bp bands for allele 'G'. Preliminary genotyping of DNA samples from 24 non-ALSPAC Caucasians revealed that for this SNP, allele frequencies were 0.04 for the 'G' allele and 0.96 for the 'A' allele (the genotypes being consistent with Hardy Weinberg equilibrium). Due to the low