

## CLINICAL STUDY

## A polymorphism in the IGF-I gene influences the age-related decline in circulating total IGF-I levels

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### Abstract

**Objective:** Recent studies have demonstrated an association between a 192 bp polymorphism of the IGF-I gene and total IGF-I serum levels, birth weight, body height and the risk of developing diabetes and cardiovascular diseases later on in life. This IGF-I gene polymorphism in the promoter region of the IGF-I gene may directly influence the expression of IGF-I. In the present study we evaluated the role of this polymorphism in the age-related decline in serum IGF-I levels.

**Subjects and methods:** All subjects were participants of the Rotterdam Study, a population-based cohort study of diseases in the elderly. We studied a total group of 346 subjects, who comprised two subgroups: a randomly selected population-based sample of 196 subjects, and a group of 150 subjects selected on IGF-I genotype. In the total group of 346 individuals the relationship between this 192 bp polymorphism and the age-related decline in circulating total IGF-I levels was studied.

**Results:** Homozygous carriers of the 192 bp allele demonstrated significant decline in serum IGF-I with age ( $r = -0.29$ ,  $P = 0.002$ ). This decline is similar to that seen in the general population. An age-related decline in serum total IGF-I was not observed in heterozygotes ( $r = -0.06$ ,  $P = 0.48$ ) and non-carriers ( $r = -0.12$ ,  $P = 0.32$ ). Interestingly, the relationship between age and serum IGF-binding protein-3 levels showed the same pattern.

**Conclusion:** We observed only in homozygous carriers of the 192 bp alleles of the IGF-I gene an age-related decline in circulating total IGF-I levels, but not in heterozygotes and non-carriers of the 192 bp allele. We hypothesize that this IGF-I gene polymorphism directly or indirectly influences GH-mediated regulation of IGF-I secretion.

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### Introduction

Insulin-like growth factor-I (IGF-I) is a peptide which stimulates skeletal growth, cell differentiation and metabolism and influences body composition. Its secretion is regulated amongst others by growth hormone (GH), nutritional status, liver function and insulin, whereby circulating IGF-I is mainly synthesized by the liver (1). Most of the IGF-I is bound to one of the six IGF-binding proteins (IGFBPs), of which IGFBP-3 is the most abundant. This binding protein forms a ternary complex with IGF-I and an acid-labile subunit, which inhibits the functional properties of IGF-I (1, 2). Most of the effects of GH on linear growth are mediated by IGF-I (1). Deficiency of GH leads to a change in body length and body composition (1, 3, 4). The degree of impaired growth and body size composition depends on age of onset of GH deficiency (GHD) (5). GHD in children is characterized by lowered serum IGF-I levels,

short stature and low body weight (6, 7). In patients who become GHD later in life, circulating IGF-I concentrations are often normal in relation to age- and sex-related normal values (5, 8). This suggests that circulating IGF-I levels later in life become less and less GH-dependent. Furthermore, IGF-I levels are regulated by several variables other than GH (9). Elderly men and women secrete GH less frequently and at lower amplitude than young individuals (10). GH secretion declines to approximately 20% of that in puberty and serum IGF-I and IGFBP-3 levels decline in parallel (11, 12).

Recently, a polymorphism in the promoter region of the IGF-I gene has been identified, which was associated with IGF-I serum levels, birth weight and body height (13, 14). Non-carriers of the most frequent allele (length 192 bp) were demonstrated to have low total serum IGF-I levels and lower height (13) as well as lower birth weight (14). In the present study we investigated the relationship between this polymorphism in

the IGF-I gene and the age-dependent decline of circulating IGF-I levels.

## Subjects and methods

### Study population

All subjects included for the present study were participants from the Rotterdam Study, a population-based cohort study of diseases in the elderly. The Rotterdam Study is a single-center prospective follow-up study in which all residents aged 55 years and over of the Rotterdam suburb Ommoord were invited to take part. The study was approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam and written informed consent was obtained from all participants. The aim of the Rotterdam study is to investigate determinants of chronic and disabling cardiovascular, neurodegenerative, locomotor and ophthalmological diseases. The design of the study has been described previously (15). The baseline examination of the Rotterdam Study was conducted between 1990 and 1993. A total of 7983 participants (response rate 78%) were examined.

A group of 346 subjects aged between 55 and 75 years was studied for the present investigation; they comprised two different sub-samples from the Rotterdam Study: (i) the first study group consisted of 196 subjects who were randomly selected; (ii) the second study group of 150 healthy subjects had been selected based on their IGF-I genotype (50 homozygous carriers of the 192 bp allele, 50 heterozygotes and 50 non-carriers) as described earlier by Vaessen *et al.* (13).

Twenty-two participants who had diabetes, diagnosed on a history of medication, a fasting glucose level of 7.8 mmol/l or above and/or a random glucose of 11.1 mmol/l or above, were excluded since diabetes mellitus and its treatment may affect the GH-IGF-I axis. Also three subjects using estrogen replacement therapy medication were excluded, because this type of medication is known to influence IGF-I concentrations (16). In three individuals, no IGF-I genotype could be determined. After exclusion we studied the remaining group of 318 subjects who comprised a population-based sample of 168 subjects and a sample selected on genotype consisting of 150 subjects.

### Measurements

Blood sampling and storage have been described elsewhere (17). Serum was separated by centrifugation and quickly frozen in liquid nitrogen. Blood measurements were performed on fasting blood samples, unless otherwise specified. Total IGF-I was determined by a commercially available RIA ((Medgenix Diagnostics, Brussels, Belgium) with intra-assay and interassay coefficients of variation of 6.1 and 9.9%). Because of financial restrictions, measurements of IGFBP-1,

IGFBP-3, glucose and insulin were only done in the first study group consisting of 168 subjects. Commercially available IRMAs were used for the measurement of IGFBP-1 and IGFBP-3 ((Diagnostic System Laboratories Inc., Webster, TX, USA) intra-assay and inter-assay coefficients of variation for IGFBP-1 4.0 and 6.0% respectively, and for IGFBP-3 1.8 and 1.9% respectively). Serum glucose levels were determined using a standard glucose hexokinase method. Insulin was measured by a commercially available IRMA (Medgenix Diagnostics; intra-assay and interassay coefficients of variation 3–6% and 5–12%).

Genotypes of the 192 bp IGF-I promoter polymorphism were determined as described earlier (13). This resulted in three possible genotypes: carriers homozygous for the 192 bp allele, carriers heterozygous for the 192 bp allele and non-carriers of the 192 bp allele. At the baseline examination, height and weight were measured wearing indoor clothes and without shoes. Body mass index (BMI) was defined as weight (kg) divided by the square of height (m).

### Statistical analysis

Mean age, serum total IGF-I, BMI and body height were compared between the study groups using an independent samples *t*-test. Distribution of sex between the two study groups was compared using a chi-square test. Body height and serum IGF-I were compared between genotypes using ANOVA. Serum IGF-I values were logarithmically transformed for the analyses to achieve normal distribution and adjusted for the possible confounders age, sex and BMI. Because of ease of interpretation non-transformed IGF-I data are presented. Data on IGF-I levels and height are presented as means  $\pm$  S.E.M.

A linear regression was used to study the correlation between serum IGF-I and age and partial correlation coefficients are given. In all these analyses we adjusted for the same possible confounders, except age. All analyses were performed using the SPSS for Windows software package, version 10.0.5 (SPSS Inc., Chicago, IL, USA).

## Results

We studied the differences in the two study groups with regard to mean age, serum total IGF-I, BMI, body height and the distribution of sex. The subgroup of 150 individuals was significantly younger ( $60.7 \pm 0.3$  vs  $67.0 \pm 0.4$  years,  $P \leq 0.001$ ) and contained less men (36.8 vs 47.6%,  $P = 0.03$ ) than the subgroup of 168 subjects. There were no significant differences in mean serum total IGF-I, BMI and body height.

The total study group comprised 73 non-carriers of the 192 bp allele (23%), 126 heterozygous carriers of the 192 bp allele (39.6%) and 119 homozygous

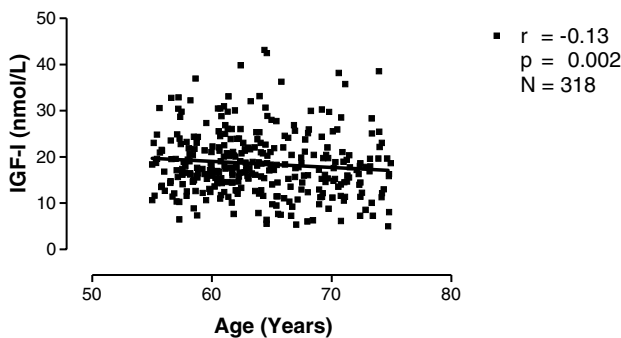
carriers of the 192 bp allele (37.4%). In the group as a whole, non-carriers had lower levels of IGF-I ( $16.5 \pm 0.6$  vs  $18.7 \pm 0.6$  nmol/l,  $P = 0.01$ ) and shorter height compared with homozygous carriers ( $167.0 \pm 0.9$  vs  $169.7 \pm 0.8$  cm,  $P = 0.03$ ). With regard to fasting IGFBP-3, IGFBP-1, insulin and glucose no significant differences were observed between the three IGF-I genotypes (data not shown).

Figure 1 shows the age-related decline of IGF-I in the total study group of 318 subjects. A significant inverse relationship between IGF-I and age was observed ( $n = 318$ ,  $r = -0.14$ ,  $P = 0.002$ ). This relationship remains after adjustment for gender and BMI. Stratification of the relationship between serum IGF-I level and age according to genotype showed that there was only a highly significant relationship between IGF-I and age in homozygous carriers of the 192 bp allele. This was not significant in heterozygous carriers and in non-carriers. Figure 2 shows the relationship between IGF-I levels and age in the three IGF-I genotype strata. Adjustment for gender and BMI did not change these relationships.

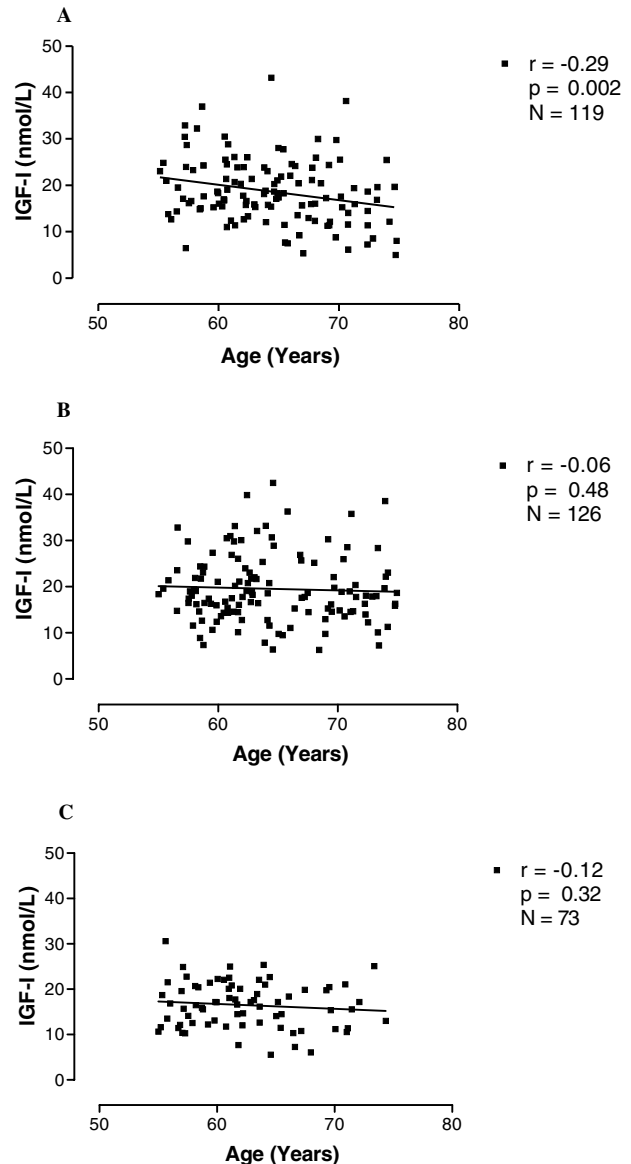
IGFBP-3 levels were only measured in a sample of 168 subjects. In this sample, IGFBP-3 levels decreased with age ( $r = -0.18$ ,  $P = 0.02$ ) and stratification per IGF-I genotype showed again a highly significant correlation in homozygous carriers only ( $n = 72$ ,  $r = -0.35$ ,  $P = 0.002$ ), which was not observed in heterozygous carriers ( $n = 79$ ,  $r = -0.07$ ,  $P = 0.57$ ) and in non-carriers ( $n = 24$ ,  $r = 0.06$ ,  $P = 0.77$ ) (data not shown). These correlation coefficients remained after adjustment for gender and BMI. Fasting insulin, glucose and IGFBP-1 were not related to the presence of the 192 bp allele of the IGF-I gene.

**Discussion**

GH secretion, circulating IGF-I and IGFBP-3 levels in normal individuals demonstrate a gradual continuous decline during aging (12). In the present study we observed that this well-known relationship with



**Figure 1** Relationship between serum total IGF-I and age in the whole study group. The regression line is shown and a partial correlation coefficient calculated from the data after adjustment for gender and BMI.



**Figure 2** Relationship between serum total IGF-I and age according to the IGF-I genotype in (A) homozygous carriers of the 192 bp allele, (B) heterozygous carriers of the 192 bp allele and (C) non-carriers of the 192 bp allele. Regression lines are shown and partial correlation coefficients calculated from the data after adjustment for gender and BMI.

regard to IGF-I and IGFBP-3 was highly influenced by the presence of two 192 bp alleles in the IGF-I gene.

GH is secreted in a pulsatile way, while serum IGF-I and IGFBP-3 levels are constant over the day. The principal factor enhancing the production and secretion of IGF-I and IGFBP-3 is GH (18) and in children and adolescents IGF-I and IGFBP-3 levels correlate well with the 24 h secretion of GH (18). In adults, a significant relationship between 24 h GH levels and IGF-I levels has been reported in normal and GHD subjects (19).

However, in adults there is also an important influence of non-GH factors on IGF-I levels (9). In accordance with this observation, in previous studies of GHD adults, it was demonstrated that determination of serum IGF-I and IGFBP-3 levels become increasingly less discriminative as a diagnostic measurement for GHD as patients get older (5, 8). This suggests that the actual level of circulating IGF-I and IGFBP-3 becomes less and less GH-dependent with age, ultimately leading to a situation in which circulating IGF-I concentrations becomes more and more influenced by nutrition, liver function, sex steroid levels and insulin (1).

In all clinical situations a close correlation between IGF-I and IGFBP-3 levels has been observed (20). GH production and secretion decreases with increasing age (5, 8). In our study a genotype-specific pattern of age-related decrease in circulating IGF-I and IGFBP-3 levels was only observed in homozygous carriers of the 192 bp allele. For heterozygotes and non-carriers no relationship between circulating IGF-I and IGFBP-3 levels with age was observed. This suggests that only in the presence of two 192 bp alleles of the IGF-I gene are the circulating IGF-I levels influenced by GH secretion, but in the presence of only one or none of these alleles this relationship in elderly subjects is absent. In heterozygotes and non-carriers, circulating IGF-I levels seem less GH-dependent and more influenced by other factors such as nutrition, liver function, sex steroids and insulin levels. To test our hypothesis, a study should be performed to investigate whether there is a difference in IGF-I response to recombinant human GH administration between homozygotes compared with heterozygote and non-carriers of the 192 bp allele. In this way, the effect of other non-GH factors on the IGF-I level can be minimized.

A shortcoming of our study is that we did not measure GH secretion and that we did not investigate a population-based sample. In fact, the sample was oversampled with a higher than expected number of non-carriers of the 192 bp allele. This was done in order to maximize the statistical power in the rare subgroup non-carriers. Genotyping a population-based sample would represent homozygous carriers of the 192 bp allele as the most common allele.

In conclusion, in healthy elderly individuals aged between 55 and 75 years, we observed that the age-related decline in circulating IGF-I and IGFBP-3 levels was only present in homozygous carriers of the 192 bp allele of the IGF-I gene. This suggests that this particular polymorphism, which is located 1 kb upstream of the promoter region of the IGF-I gene might, directly or indirectly, be responsible for the GH-driven regulation of IGF-I levels.

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