Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. Supplement methods

Study population

The rhGH-treated population included all patients who had received at least one dose of rhGH before 18 years of age between January 1, 1985, and December 31, 2010. The patient data were collected from the Swedish National GH Register for Children and from clinical trials with rhGH between 1985-2010 with the following clinical trial numbers: TRN89-071, TRN88-080, TRN88-177, TRN89-070-01, TRN89-071-01, TRN98-0198-003, TRA6280-003, TRN151:142/01, and TRN2009/529-31. Informed consent was obtained for all participants in the National Growth Hormone Register for Children as well as for the clinical trials and ethical permission for the register and the studies have been approved by the appropriate Swedish ethics committees.[1] The coverage of the National GH Register is considered high, at an estimated approximately 85% based on an earlier evaluation.[2] Visits are registered 2-4 times a year through a specific protocol.

The total cohort consisted of 6,804 patients, and after the exclusions described below, the final cohort remaining in the analysis included 3,408 patients. The treatment indications of isolated growth hormone deficiency (GHD), small for gestational age (SGA) without catch-up growth, and idiopathic short stature (ISS) were selected to exclude more severe underlying diagnoses associated with rhGH treatment. These treatment indications correspond to the low-risk group defined in earlier publications including parts of our cohort.[3]

Exclusion criteria:

1. Treatment indication other than GHD, ISS, or SGA, thus belonging to risk groups 2 and 3 of previously published long-term follow-up studies within the SAGhE (Safety and Appropriateness of Growth Hormone Treatments in Europe) collaboration (n=2,888).[3]
2. Previous treatment with pituitary-derived GH (n=78).
3. Other hormonal treatment during the rhGH treatment period, indicating multiple pituitary hormonal deficiency (n=79).
4. Start of rhGH treatment after 18 years of age (n=4).
5. Other severe diagnoses in the Swedish National Patient Register (see eTable 1) or Swedish National GH Register indicating underlying conditions associated with increased risk of premature mortality or morbidity (n=346).
6. Other reasons (obviously incorrect starting date of rhGH in the register, n=1).

For each patient, 15 controls were randomly selected by Statistics Sweden (SCB) from the Swedish Total Population Register.[4] The selected controls were matched for sex, birth year and county, and alive at study start (date of rhGH treatment start). Thus, for the total cohort of 6,804 patients, a total of 102,060 controls were selected. The controls to the patients excluded above were also excluded (n=50,081), as well as controls with severe diagnoses in the Swedish National Patient Register (n=1,161), prescription of rhGH (n=20), or re-used personal identity number (n=22). A total of 50,036 controls remained in the analysis.

The classification of treatment indications followed the same criteria as those described by Albertsson-Wikland et al in a previous study of the same cohort of treated patients.[1] In summary, isolated GHD (n=1,837) was defined as having a GH peak level (GH_{max}) <10 µg/L on either provocation test (mainly Arginine-Insulin Tolerance Test) or during spontaneous 12h or 24h GH secretion profiles. Subgroup classification of GH_{max} from 0-4 µg/L (n=485) or 5-9 µg/L (n=1352) within the GHD group was also performed. The SGA group (n=672) consisted of treated patients born SGA (birth length and/or birth weight <-2 SDS compared to the Swedish healthy newborn population reference)[5] and a having a GH_{max} ≥10 µg/L. Hence, patients born SGA but with a GH_{max} <10 µg/L were only included in the GHD group. The ISS treatment indication (n=899) was defined as having a GH_{max} ≥10 µg/L and *not* being SGA at birth.[5] If information on GH_{max} (n=691) or birth characteristics (n=285) were missing, the patient was categorized according to the diagnosis of the referring physician.

All treatment data (start of treatment, mean daily dose, duration of treatment, and cumulative dose) were gathered from the National GH registry and the clinical trials stated above. Information about rhGH treatment in adulthood was collected from the nationwide Prescribed Drug Register, which is updated automatically on a monthly basis and includes all prescribed drugs (ATC codes) in Sweden.[6]

The rhGH-treated patients had a mean age of 9.3 years at the start of rhGH treatment (1st percentile: 2.51 years, 99th percentile: 15.98 years), which equaled the age at study start, and a mean age of 16.35 years at the end of rhGH treatment (1st percentile: 6.59 years, 99th percentile: 20.03 years). The HRs of patients treated with rhGH after 18 years of age, based on information from the Prescribed Drug Registry (2005-2015), are presented in eTable 3. The mean follow-up time was 14.93 years (1st percentile: 2.24 years, 99th percentile: 25.00 years).

Linkage of individual information, for both patients and controls, from various healthcare and population-based registers was carried out through the individuals' unique personal identity numbers.[7]

Study outcome data

Information on morbidity was gathered from the Swedish National Patient Register, which contains information on all in-patient care since 1963 (nationwide since 1987) and since 2001, also includes information on all outpatient visits to specialized caregivers (both public and private). The coverage of this register is very high, with an estimated drop-out rate of less than 1% in 2007.[8] Information on cause of death was gathered from the Swedish Cause of Death Register, which includes information on all deaths in Sweden since 1963. The coverage rate is over 99% of all deaths in Sweden and the register is updated annually. The dose-response analyses (duration of treatment, cumulative and mean dose) was performed with a lag-period of two years since the end of treatment to avoid the risk of reverse causality (protopathic bias) due to the potential association of treatment termination and the outcome or early manifestations of the outcome.

Independent variables

The Swedish Medical Birth Register was used to collect background data regarding gestational age, birth weight, and birth length for both patients and controls and also to collect information about the mothers' height. The standard deviation scores (SDS) for birth length and birth weight were calculated from the current Swedish healthy population reference.[5] As described in Table 1, the patients were to a larger extent than the controls born prematurely and smaller at birth both regarding birth length and birth weight. All three birth characteristics, and the differences therein between the groups, were adjusted for in the Cox regression model that contained all the included covariates, used for our main analyses of overall CVD and severe CVD (Tables 3 and 4).

The Swedish Military Conscription Register includes measured height information of all young Swedish men and volunteering women who enroll for military service at approximately 19 years of age. The mandatory military service was gradually dismantled from mid-1990 and completely abolished in 2010. Information was gathered from the National Archives for data before 1997 and from the Swedish Defense Recruitment Agency for more recent data. Height data were also collected from the Swedish National Passport Registry, which is administered by the Swedish National Police Agency and includes height data for all individuals that issued a Swedish passport from 1991 and onwards. Height data are either stated or measured when applying for a passport.

To investigate the validity of height data from the Passport Register, we compared measured heights from the Conscription Register with height data in the Passport Register for individuals who had applied for a passport in the same year as their conscription (n=4,534). The results showed a very high correlation (r=0.98), with a mean difference of only 0.6 cm (5th - 95th percentile: -1 to +3 cm) between the height values.

Statistics Sweden (SCB) has several population-based registries (Income and Taxation Register, The Swedish Register of Education, Multi-Generation Register and The Swedish Total Population Register) that were used to provide information on vital status, migration, and socioeconomic information such as total income and educational level.

To account for inflation and change in absolute income over the study period, total disposable income within the household at study inclusion was categorized in five income classes (quintiles) for each year, with income class = 1 the lowest quintile and income class = 5 the highest. The highest achievable education level for each parent to the patients or controls was coded in the register from 1 to 7, where 1 = primary school <9 years and 7 = postgraduate/doctoral studies. For each family, the highest educational level of either of the parents was included in the dataset.

The proportions of missing values within each baseline characteristic are presented in eTable 4. The possible interactions between patient status and all different independent variables were also checked without any significant interactions detected, including testing for interaction between patient status and sex (p=0.325, full model).

Estimation of the height at study entry for children in the control group

Inclusion in the study for children in the patient group corresponded to the time when they started treatment. The control group included children who were of the same age as that of patients at the initiation of treatment. Therefore, the measure for height at study inclusion was available for children in the patient group but not for those in the control group. Because height was an important determinant of starting treatment, its value at the age of study inclusion of the controls was estimated. The estimation was based on all available height values measured on the controls at other time points.

The estimated value was the best linear unbiased predictor from a mixed-effects model. The analysis was conducted on the control children only and separately by sex. The mixed-effects model included age as the only predictor. Because the relationship between mean height and age was nonlinear, age was introduced in the model by means of natural cubic splines. The regression coefficients associated with the splines variables were considered fixed, not random, parameters. The splines knots were placed at -21, -18, -13, -6, -2, and 2 years. Some knots were at negative ages because age was centered at 20 years. The number and placement of the knots were determined to maximize the goodness of fit as measured by the likelihood. The fit was also evaluated visually through graphical representations.

An initial mixed-effects model was estimated separately for each sex. Along with the fixed coefficients for the age splines variables, the model included two random coefficients, one for the intercept and one for the linear slope. The two-by-two covariance matrix of the two random coefficients was left unstructured. The values of height with standardized residuals greater than 5 were considered outliers and were excluded from the subsequent analyses. After this exclusion, a second model was estimated, which included the same fixed and random effects as the first.

The fixed-effect coefficients for the age splines were all statistically significant. The variances of the random coefficients for the intercept and linear slope were significantly greater than zero.[9] The variance of the random effects for the remaining spline coefficients was not significant and these random coefficients were excluded from the models.

The value of height at study inclusion was calculated as the best linear unbiased predictor from the mixed models described above.[10]

Sensitivity analysis

A sensitivity analysis was performed on a subset of the total cohort, in which only controls similar in height at study start were kept in the analysis. Of the 15 controls per patient, only those up to 5 cm taller than their corresponding patient at study start remained. Since the groups, as expected, differed a great deal on this variable, being one of the main reasons for considering growth hormone treatment, many controls were dropped from the analysis and subsequently only patients that had at least one control left where kept. In addition, patients and controls <-5 SDS in birth length and birth weight were excluded. Thus, the groups were in this analysis matched on height at study start and more similar in size at birth, in order to analyze if the point estimates for the hazard ratios (HRs) between the groups would differ or not compared with our main analyses.

The basal characteristics for this subset of patients and controls are summarized in eTable 5. The number of events, person-years, incidence rate ratios, crude and adjusted HRs for overall cardiovascular disease (CVD) and severe CVD are presented in eTable 6.

eTable 1. *ICD* Codes Corresponding to Severe Diagnoses for Exclusion of Patients and Controls			
	ICD-10	**ICD-9**[*]	**ICD8**
Malignant neoplasms	C00-99	140-209	140-209,99
Cancer in situ	D00-D099	230-234	140-209,99
Benign pituitary tumour	D35.2	227D	226,20
Neoplasms of uncertain or unknown behaviour	D37-48	235-239	230-239
Haemolytic anaemias	D55-59	282	282
Anaemia due to enzyme disorders	D55	.	.
Thalassemia[†]	D56	282E	282.4
Sickle-cell anaemia[‡]	D57	282G	.
Other hereditary haemolytic anaemias	D58	282	282
Acquired haemolytic anaemias	D59	282	282
Aplastic and other anaemias	D60-64	284	284
Acquired pure red cell aplasia	D60	284W	284
Other aplastic anaemias	D61.0	284A	284
Other specified diseases with lymphoreticular and reticulohistiocytic tissue (e.g. HLH)	D76.1	.	.
SCID	D81.0-2	279L	.
Diabetes Mellitus	E10-14	250	250
Hyperfunction of pituitary gland	E22,0	253A, 253B	253,00-01
Cushing syndrome	E24,0	255A	258,00
Disorders of aromatic amino acids	E70	270	270
Disorders of branched-chain amino-acids	E71	270	270
Other disorders of amino-acid metabolism	E72	270	270
Other disorders of carbohydrate metabolism[§]	E74	271	271
Disorders of sphingolipid metabolism and other lipid storage disorders	E75	272, 330A,330B	272
Disorders of glycosaminoglycan metabolism	E76	277F	.
Disorders of glycoprotein metabolism	E77	272H	.

	ICD-10	ICD-9*	ICD8
Disorders of lipoprotein metabolism and other lipidemias, purine and pyrimidine metabolism and porphyrin and bilirubin metabolism	E78-83	272, 277	279
Cystic fibrosis‖	E84	277A	273
Amyloidosis	E85	277D	276.99
Crohn's disease	K50	555	563
Ulcerative colitis	K51	556	563
Liver diseases	K70-74	571	571
Juvenile rheumatic arthritis	M08	714	712
Chronic kidney disease & Unspecified kidney failure	N18	585	582,00
Congenital malformations of the nervous system	Q00-07	740-742	740-743
Cardiovascular malformations¶	Q20-28	745-747	746-747
Thanatophoric short stature‖	Q77.1	756E	.
Achondroplasia‖	Q77.4	756E	"756,40"
OI, McCune-Albright, Osteopetrosis, etc.‖	Q78.0-3	756F	756,50, 756,60
NF, Tuberous sclerosis, etc.‖	Q85.0-9	237H	743,40,
Other malformations of face (Rubinstein, Marfan, etc.) ‖	Q87	758A-X, 759F/G	759,30-99
Chromosomal anomalies‖◊	Q90-99	758A-X, 759F/G	759,30-99
Status post-transplantation (bone marrow, stem cells, etc.)	Z94.8	.	.

* The Swedish version of ICD-9 is alphanumeric with the fourth and fifth digit replaced by a letter (A=0, B=1, C=2, D=3, E=4, F=5, G=6, H=7, W=8, X=9). This was done in the adoption of ICD-9 in order to more easily distinguish between earlier similar coding in ICD-8.
† Exception: Thalassemia trait (D56.3, 282E).
‡ Exception: Sickle-cell trait (D57.3, 282F).
§ Exception: Lactase deficiency, renal glukosuria, unspecified cause (E74.8-9, 270X,271D/E/W, 270/271.88/99).
‖ Exclusion diagnosis independent of received before or after study start.
¶ Exception: Patent ductus arteriosus (Q25.0, 747A, 747,09).
◊ Exception: Healthy individuals with balanced translocations or inversions (Q95.0-1).

eTable 2. List of *ICD* Codes for Cardiovascular Diseases and Number of Total Events for Each Category

Cardiovascular diagnose	ICD-8	ICD-9	ICD-10	Patient cohort (n=3,408)	Control cohort (n=50,036)	*p* value*
				n (%)	n (%)	
Hypertensive disease	400,00-405,99	400-405X	I10.0-I15.9	14 (0.41)	199 (0.40)	0.89
Ischemic heart disease†	410,00-414,99	410-414X	I20.0-I25.9	5 (0.15)	18 (0.04)	0,01
Pulmonary heart disease	426,01-02, 426,08-09	415-417X	I26.0-I28.9	2 (0.06)	48 (0.10)	0.77
Aneurysm of pulmonary artery†	.	417B	I28.1	0	0	.
Valvular heart disease	424,00-424,99	424A-424X	I34.0-I37.9	7 (0.21)	52 (0.10)	0.10
Cardiomyopathy†	425,00, 425,08-09	425A-E, 425W-X	I42.0-2, 4-5, I42.7-9	5 (0.15)	19 (0.04)	0,02
Arrhythmia	427,20, 427,27-29, 427,90-92, 427,98-99	426A-X	I44.0-49.9	32 (0.94)	445 (0.89)	0.71
Heart failure†	427,00-10, 428,99	428A-X	I50.0-9	4 (0.12)	21 (0.04)	0.07
Subarachnoid bleeding†	430,00-99	430	I60.0-9	1 (0.03)	17 (0.03)	1.00
Intracerebral bleeding†	431,00, 431,90	431	I61.0-9	0	15 (0.03)	0.62
Other or unspecified (non-traumatic) intracranial bleeding†	431,01, 431,08-09, 431,91, 431,98-99	432, 432A, 432B, 432X	I62.0-9	1 (0.03)	3 (0.01)	0.23
Cerebral infarction†	432-434	433-434	I63.0-I63.9	3 (0.09)	28 (0.06)	0.45
Unspecified stroke†	436-437	436-437	I64.0-9	1 (0.03)	4 (0.01)	0.28
Transient cerebral ischemia†	435-436	435-436	G45.0-9	2 (0.06)	11 (0.02)	0.20
Other cerebrovascular disease†	438,00-99	437-437X	I65.0-I67.9	1 (0.03)	17 (0.03)	1.00
Late effects of cerebrovascular disease†	.	438A-X	I69.0-I69.9	3 (0.09)	27 (0.05)	0.44
Atherosclerosis	440	440	I70.0-I70.9	0	11 (0.02)	1.00
Aneurysm†	441-442	441-442	I71.0-I72.9	3 (0.09)	12 (0.02)	0.07
Claudicatio intermittens	443.9	443X	I73.9	0	8 (0.02)	1.00
Emboli and thrombosis in arteries	444	444	I74.0-I74.9	0	11 (0.02)	1.00
Other diseases of circulatory system	443,00-80, 443,99	443A-W, 445-449	I73.0-I73.9, I77.0-I99.9	78 (2.30)	977 (1.96)	0.18

* Comparison between patients and controls with Fischer exact test.
† These diagnoses are included in the subgroup of "Severe CVD" diagnoses.

eTable 3. Incidence Rate Ratios (IRRs) and Crude and Adjusted Hazard Ratios (HRs) for Overall and Severe CVD if rhGH Treatment Prescribed After 18 Years of Age in the Prescribed Drug Register (2005-2015)

	Events (n)	Pyrs	IRR [95% CI]	Crude HR [95% CI]	*p*-value	Adjusted HR [95% CI], restricted model†	*p*-value	Adjusted HR [95% CI], full model‡	*p*-value
Overall CVD									
Controls* (n=40,229)	1,590	678,329	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Patients (n=174)	16	2567	2.66 [1.52-4.33]	2.88 [1.76-4.72]	<0.001	4.60 [2.71-7.84]	<0.001	5.14 [2.94-8.98]	<0.001
Severe CVD									
Controls* (n=40,229)	148	688,985	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Patients (n=174)	4	2644	7.04 [1.89-18.42]	7.80 [2.87-21.18]	<0.001	8.04 [2.44-26.46]	0.001	12.49 [3.64-42.92]	<0.001

* Only controls with same age range (17-45 years) kept as comparison group to exclude difference of age range between the patients and controls.
† Restricted model adjusted only for age at start, height at start and sex.
‡ Full model adjusted for gestational age, birth length, birth weight, age at start, height at start, parental educational level, family income and sex.

eTable 4. Percentage Missing Values of Baseline Characteristics		
	Patients	**Controls**
	(n=3,408)	(n=50,036)
Sex		
Missing, n (%)	0 (0)	0 (0)
Gestational age		
Missing, n (%)	276 (8.1)	4,622 (9.2)
Birth length		
Missing, n (%)	294 (8.6)	5,072 (10.1)
Birth weight		
Missing, n (%)	198 (5.8)	4,702 (9.4)
Age at start		
Missing, n (%)	0 (0)	0 (0)
Height at start		
Missing, n (%)	180 (5.3)	2,257 (4.5)
Family income level		
Missing, n (%)	12 (0.4)	251 (0.5)
Parental educational level		
Missing, n (%)	2 (0.1)	154 (0.3)

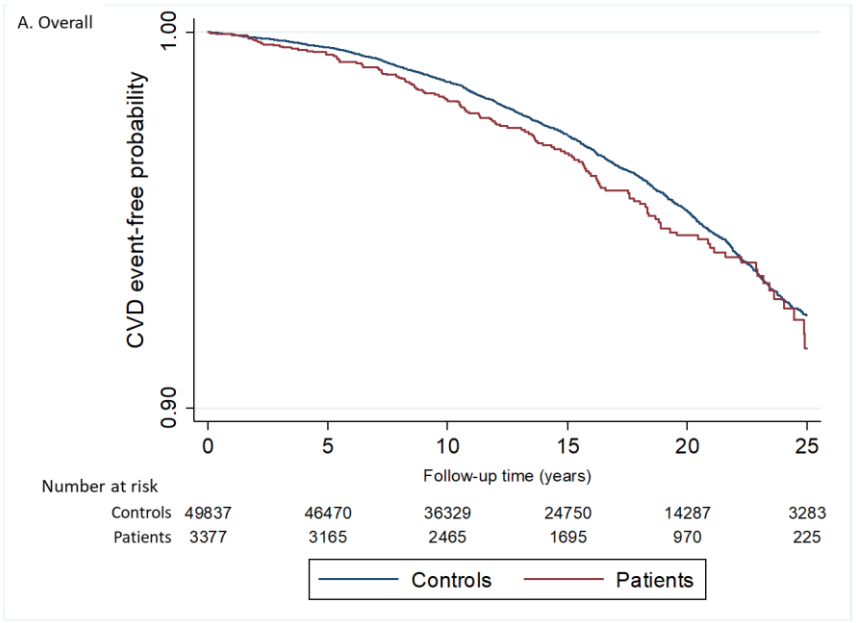
eTable 5. Basal Characteristics for the Patients and Controls in the Sensitivity Analysis		
	Patients	**Controls**
	(n=837)	(n=1,695)
Sex		
Males, n (%)	583 (69.7)	1,131 (66.7)
Females, n (%)	254 (30.3)	564 (33.3)
Gestational age (week)		
Mean (SD)	38.5 (3.0)	39.1 (2.2)
Birth length (SDS)		
Mean (SD)	-1.30 (1.4)	-0.90 (1.3)
Birth weight (SDS)		
Mean (SD)	-0.81 (1.3)	-0.52 (1.1)
Age at start (years)		
Mean (SD)	7.94 (2.99)	7.60 (3.26)
Height at start (cm)		
Mean (SD)	115.6 (17.5)	118.3 (19.7)
Family income level		
Mean (SD)	3.2 (1.4)	2.8 (1.4)
Parental educational level		
Mean (SD)	4.7 (1.4)	4.4 (1.4)

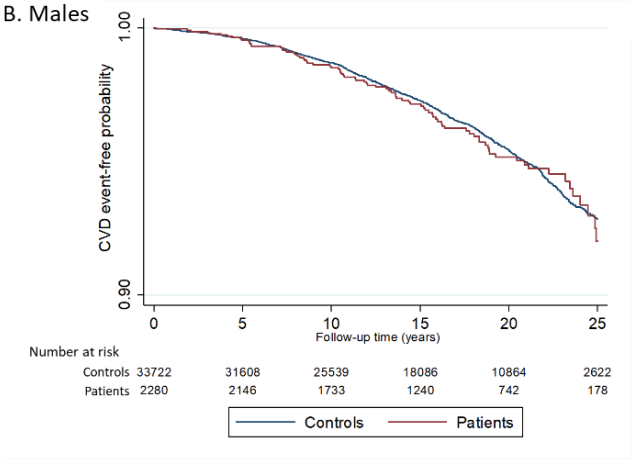
eTable 6. Number of events, person-years (pyrs), incidence rate ratios (IRRs), crude and adjusted hazard ratios (HRs) for overall CVD and severe CVD in the sensitivity analysis on a subset of patients and controls

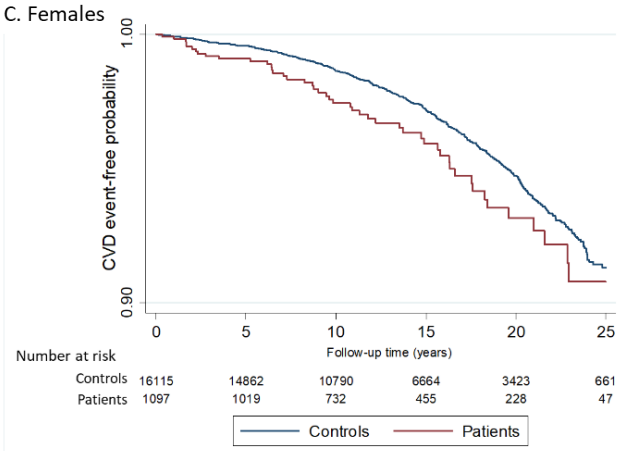
	Events (n)	Pyrs	IRR [95% CI]	Crude HR [95% CI]	*p*-value	Adjusted HR [95% CI], restricted model*	*p*-value	Adjusted HR [95% CI], full model†	*p*-value
Overall CVD									
Controls (n=1,695)	44	24,498	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Patients (n=837)	36	12,042	1.66 [1.04-2.65]	1.66 [1.07-2.58]	0.024	1.42 [0.88-2.31]	0.154	1.56 [0.89-2.71]	0.119
Severe CVD									
Controls (n=1,695)	5	24,771	1.00 (Ref)	1.00 (Ref)		1.00 (Ref)		1.00 (Ref)	
Patients (n=837)	8	12,291	3.22 [0.93-12.53]	3.21 [1.04-9.95]	0.043	2.13 [0.67-6.81]	0.201	4.33 [0.81-23.25]	0.087

* Restricted model adjusted only for age at start, height at start and sex.
† Full model adjusted for gestational age, birth length, birth weight, age at start, height at start, parental educational level, family income and sex.

eFigure. Kaplan-Meier Curve of CVD Event-Free Probability in Patients vs Controls, Overall and Separated by Sex







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