

Examination of a Genomic Region on Chromosome 3q for Susceptibility Genes for Diabetic Nephropathy (DN) in type 1 diabetes (T1D): Haplo-block Approach

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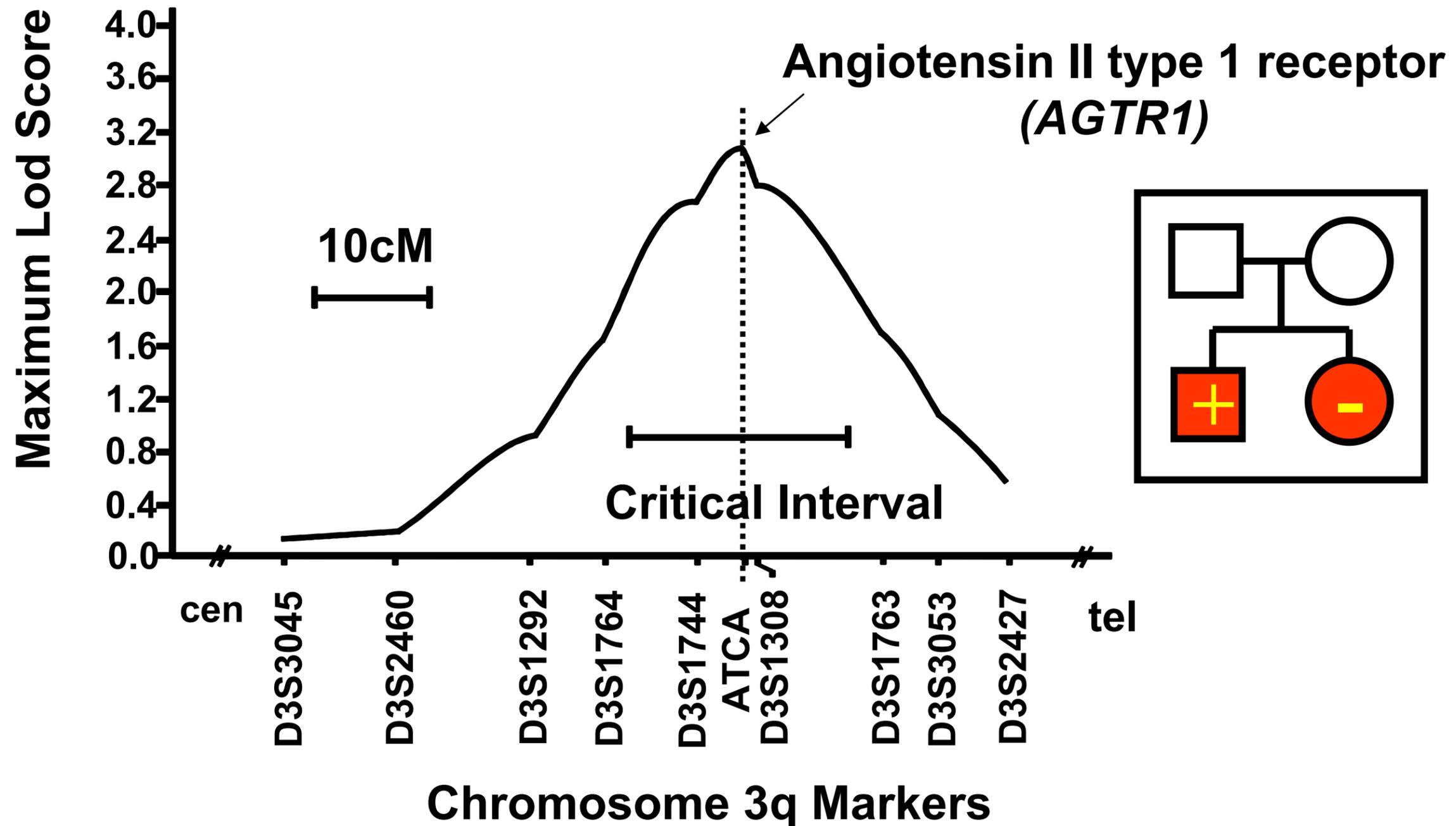
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Introduction (1)

- Diabetic nephropathy (DN) is one of the most serious complications resulting from diabetes and is the main cause of end stage renal disease (ESRD) in the U.S.
- Besides hyperglycemia, risk of developing diabetic nephropathy has a strong genetic component.
- Chromosomal regions contains loci that encode for proteins of the renin-angiotensin system have been considered candidate genes for DN.

Linkage results pointing to the presence of a major susceptibility gene for DN on human chromosome 3q has been previously reported.

Moczulski DK et al., Diabetes 47



Introduction (3)

- The most obvious candidate gene encoding for angiotensin II type 1 receptor (*AGTR1*) does not seem to be associated with DN in T1DM patients.

Ng DPK et al., ADA 2001

- However, the studied interval contains several plausible candidate genes for DN, including:
 - *CBP1*
 - *CPA3*
 - *GYG*
 - *SMARCA3*
 - *HPS3*
 - *CP*
 - *TM4SF18*
 - *TM4SF1*

Aim of the Study

- **To search for susceptibility gene(s) in the critical interval around the linkage peak**
 - We focused on the genomic interval encompassing AGTR1 and the telomeric 640 kb (149964086 – 150603793)
 - We decided to use haploblock-tagging SNPs to search indirectly for association within a genomic region as the most cost-efficient approach.

Study Groups and Methods (1)

- Our study group comprises 331 cases with advanced DN and 276 controls with normoalbuminuria and at least 15 years duration of T1DM. All of them were Caucasians.
- Diabetes was classified as type 1 if it was diagnosed before age 30 years and continuous treatment with insulin began within one year of diagnosis.

Study Groups and Methods (2)

Patients were classified as Controls if they had:

- diabetes duration ≥ 15 years
- the albumin creatinine ratio (in mg/g) was < 17 (men) or < 25 (women) in at least 2 out of the last 3 urine specimens (i.e. normoalbuminuric).

Patients were considered Cases if they had one of the following:

- persistent proteinuria (PROT)
- ESRD due to diabetic nephropathy.

Patients with microalbuminuria or intermittent proteinuria were excluded from the study.

Selected clinical characteristics of individuals in the study group with type 1 diabetes according to nephropathy status at time of examination.

	Control	PROT + ESRD
Men (%)	52	45
Age at diabetes diagnosis (years)	12.0 ± 7.0	11.8 ± 6.5
Duration of diabetes (years)	22.6 ± 7.9	25.2 ± 8.0
HbA1c (%)	8.0 ± 1.3	9.1 ± 1.8
Systolic blood pressure (mmHg)	118 ± 14	136 ± 19
Diastolic blood pressure (mmHg)	72 ± 8	80 ± 10
Treated for hypertension (%)	15.3	86.0
ACE inhibition (%)	5.9	44.15

Data are means ± SD.

Study Groups and Methods (4)

- Haplotype blocks were identified using the D' 90% confidence bounds method of *Gabriel et al.* using *Haploview*
- Haplotype tagging SNPs (htSNPs) were selected using the *tagSNP* software (*Stram et al.*).
- The minimum set of htSNPs within each block was selected to ensure an R_h^2 of at least 0.80 for all haplotypes observed at a frequency of at least 5%. With this set of htSNPs, the minimum R_h^2 was always greater than or equal to 0.80.
- Public databases were used to select SNPs
- Finally, 122 SNPs were selected as tags for the 25 haplo-blocks that virtually cover the genomic region including 5 inter-blocks.
- All individuals were genotyped using GoldenGate® Assay on a Bead Array™ platform by Illumina Genotyping Services, Illumina Inc (San Diego, CA).

Analysis

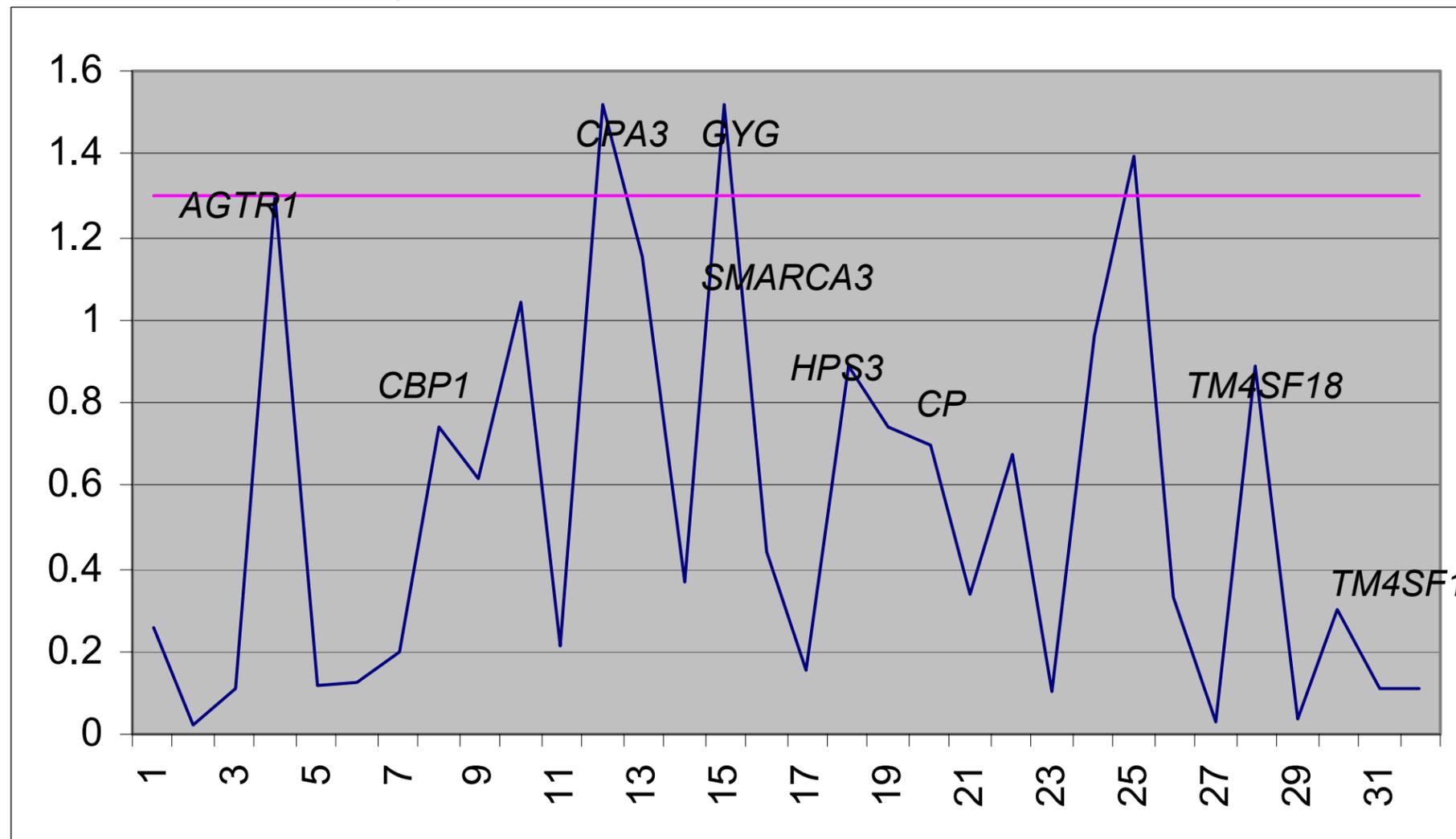
- The data from the study were analyzed using SAS (version 8.02) and R version 1.9.
- All markers were tested for HWE (*Wigginton et al.*)
- Block-centric haplotype tests of association were performed using the R haplo.stats package.
- Although selected as a haplotype tagging SNPs, we analyzed single markers under assumptions of **additive, recessive, dominant and co-dominant models**.
- Similarly, we used logistic regression to compare haplotype frequencies in the two groups, for each of the four models.
- Experiment-wide **significance threshold** required to keep Type I error rate at 5% equals **0.00043** (*Nyholt DR*)

Results (1)

- The distributions of individual SNP alleles did not differ significantly between DN cases and controls (the lowest nominal p-value was 0.005).
- Block-centric haplotype tests did not reveal any difference between study groups (see plots).
- Finally, **the distributions of the haplotypes in the logistic regression did not differ significantly between DN cases and controls under the assumption of the four models** (the lowest nominal p-value was 0.004).

Results (2)

Global p – value according to Haplo.Stat: (haplo skip option set to 0.05) controls versus proteinurics



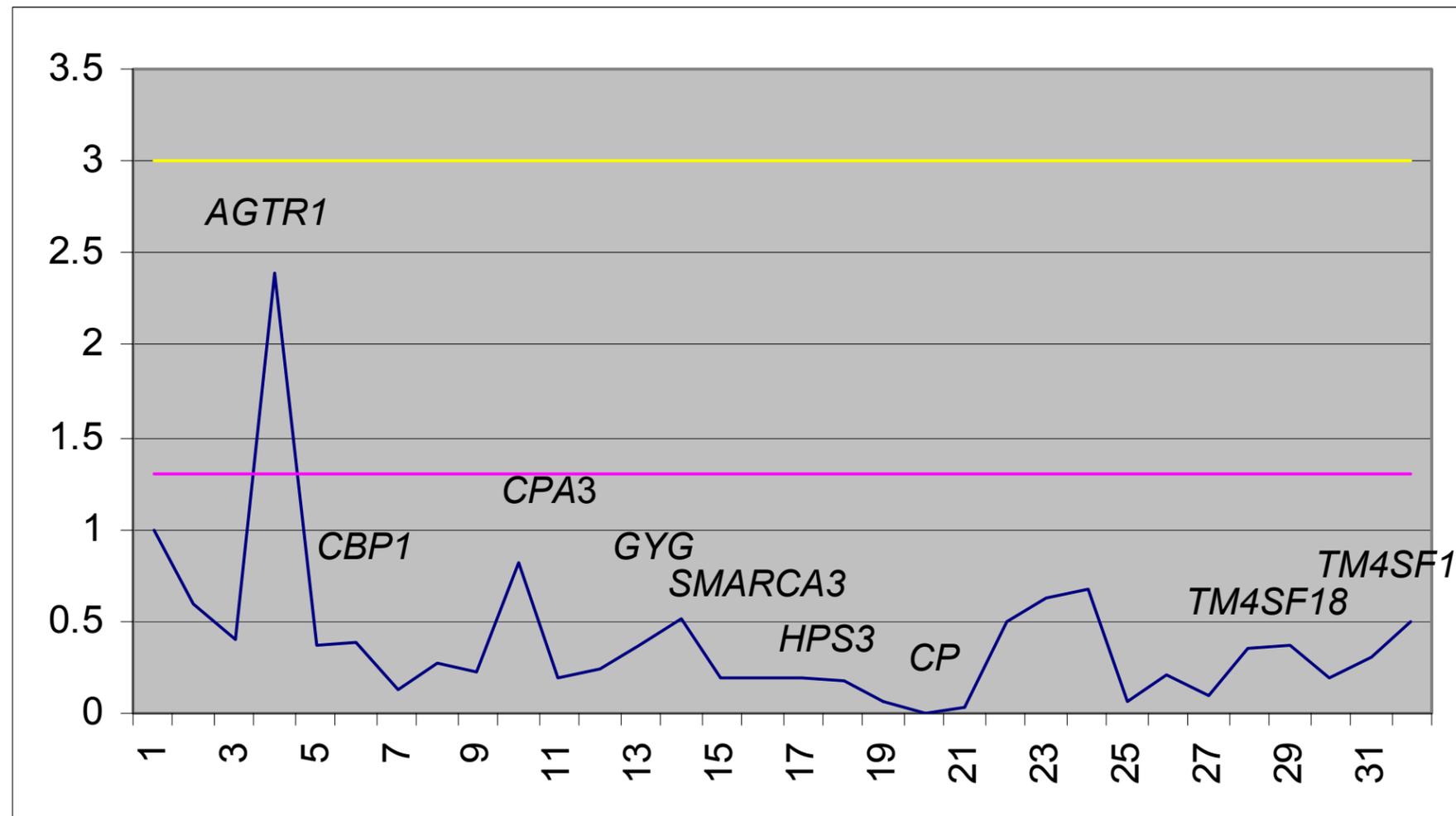
y: - log of p value

X: haploblocks and interblocks

Horizontal line: nominal p = 0.05

Results (3)

Global p – value according to Haplo.Stat: (haplo skip option set to 0.05) controls versus ESRDs



y: - log of p value

X: haploblocks and interblocks

Lower horizontal line: nominal p = 0.05

Upper horizontal line: nominal p = 0.001

Conclusions

- In conclusion, our study does not support the existence of a susceptibility gene for DN within the examined 720 kb genomic interval encompassing *AGTR1*.