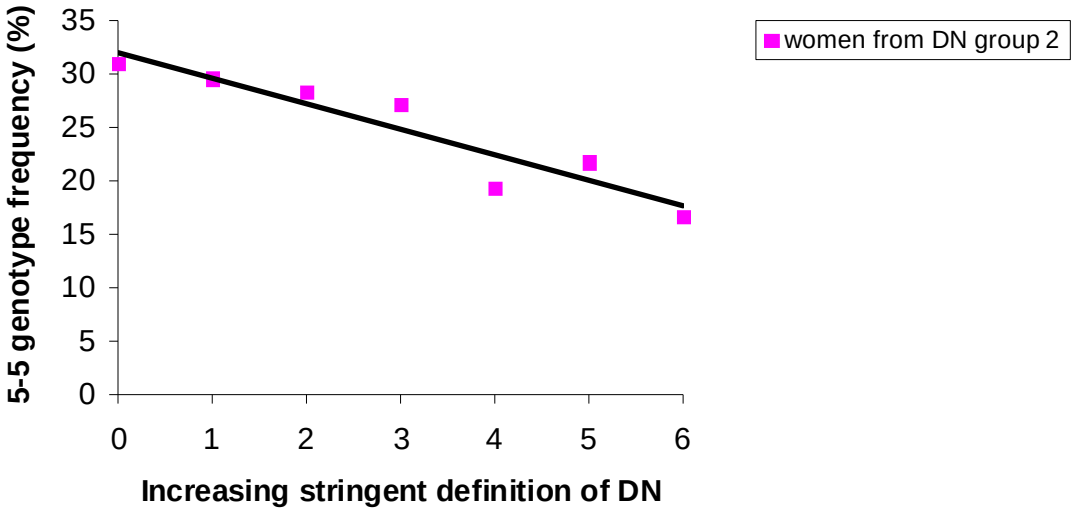
ONLINE APPENDIX

Hardy-Weinberg equilibrium

	Population (n=472)		DM population (n=562)		DN group 1 (n=114)		DN group 2 (n=90)		DN group 3 (n=66)		Diabetic controls (n=93)	
CNDP1 genotype	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
5-5	197	201.0	214	205.7	32	35.4	32	31.2	27	25.2	40	35.8
5-6	194	186.0	230	243.8	58	52.3	39	38.9	23	27.4	34	52.8
5-7	28	28.1	22	24.8	4	2.8	3	4.7	4	3.1	1	2.8
6-6	40	43.0	79	72.2	17	19.3	11	12.1	10	7.4	15	19.3
6-7	11	13.0	15	14.7	1	2.1	5	2.9	1	1.7	1	2.1
7-7	2	1.0	2	0.7	0	0.1	0	0.2	0	0.1	0	0.1
P	0.57		0.24		0.50		0.50		0.50		0.43	

Sensitivity analysis



Increasing stringent definition of DN:		women (n)	5-5 homozygous frequency (%)
0	MDRD < 60	220	31
1	MDRD < 60 and age < 70	88	30
2	DN as in manuscript	60	28
3	MDRD < 60 + microalbuminuria	59	27
4	MDRD < 45	52	19
5	MDRD < 45 + microalbuminuria	23	22
6	MDRD < 30	6	17

With increasing stringent definition of the diagnosis diabetic nephropathy the frequency of the 5-5 homozygous genotype decreases in women, suggesting that the protective effect will only be stronger with a more stringent definition of diabetic nephropathy.

Permutation studies

We first analyzed Hardy-Weinberg Equilibrium (HWE) for the total dataset and various subgroups (table 1) to see if there would be indications for population stratification. Stratification by sex and disease status does not reveal any deviation from HWE. Hence, HWE analysis does not give an indication on population strata.

Table 1. Tests for deviation from HWE in several subgroups of the data set. Subgroups are characterized by *Sex* (F=female, M=male) and disease status. P-values are given for the Chi-Square goodness of fit test. *N* denotes the sample size in the subgroups.

Sex	Disease status	P-value	N
F	no DN	0.53	44
M	no DN	0.90	47
Both	no DN	0.43	91
F	DN	0.56	139
M	DN	0.74	128
Both	DN	0.95	267
F	All	0.92	183
M	All	0.48	175
Both	All	0.85	358

As individual ethnicity is not known for all patients in this sample and the sample is in almost perfect HWE, it is difficult to construct a permutation scheme that incorporates population strata. We therefore first performed a permutation test without incorporating population strata by randomly permuting phenotype status across the whole data set. Such a procedure can primarily account for small sample size. The permuted P-values are lower than P-values based on the asymptotic Chi-Square distribution (table 2), indicating that small sample size cannot explain the P-values in our study. The asymptotic P-values behave conservative in this situation.

Table 2. P-values for genetic association of the 5-5 genotype in a recessive model. Column *Total* lists P-values for the combined sample (all cases are treated as a single group).

	Total	DN group 1	DN group 2	DN group3
Asymptotic P-value	0.0000358	0.000542	0.00102	0.00698
Permuted P-value	0.0000073	0.000234	0.000444	0.00281

Sensitivity analysis

We addressed the question of population stratification by a sensitivity analysis. The sensitivity analysis was performed in R version 2.10.0. For all Chi-Square tests a continuity correction was used leading to slightly different numeric results compared to the paper. The sensitivity analysis is based on the so-called inflation factor used in genome wide association studies (Biometrics, 55. p.997-1004, 1999), which assesses how much the average/median test statistic of single nucleotide polymorphisms, based on a Chi-Square distribution with one degree of freedom, deviates from the expectation. If the inflation factor is greater than 1, there is an indication that there might be population stratification. This inflation factor can be used to correct results from genome wide association studies by dividing the test statistic by the inflation factor, thereby assuring that a re-analysis is uninflated. We used this concept to determine how large the inflation factor could be in our study to still get significant results at a certain significance level (table 3).

For all groups an inflation of 1.1 is allowed to still achieve a significance of 0.01. An inflation factor of 1.1 is larger than the maximal inflation factor observed in the WTCCC study (Nature, 447. p.661-678, 2007). The maximal reported inflation factor for a genome wide association studies is 1.4 to our knowledge (BMC Proc, 3 Suppl 7. s.13, 2009) (NARAC

study). Note, that group 2 and group 3 do not reach the significance level of 10^{-3} but the combined sample is still significant and still exceeds the inflation factor 1.4 from the NARAC study. We have repeated the analysis with a permutation test in the individual groups and present these results in table 4.
In conclusion, there is no indication for a systematic error due to population stratification based on our sensitivity analysis.

Table 3. Sensitivity analysis for P-values of the study. For an assumed inflation factor the significance level would be precisely alpha for inflation factor > 1. For inflation factor = 1 the nominal p-value is greater than alpha.

	P-value	Alpha	Inflation factor
DN group 1	0.0005	0.050	3.11
DN group 2	0.0010	0.050	2.81
DN group 3	0.0070	0.050	1.89
All	<0.0001	0.050	4.45
DN group 1	0.0005	0.010	1.80
DN group 2	0.0010	0.010	1.63
DN group 3	0.0070	0.010	1.10
All	<0.0001	0.010	2.57
DN group 1	0.0005	0.001	1.10
DN group 2	0.0010	0.001	1.00
DN group 3	0.0070	0.001	1.00
All	<0.0001	0.001	1.58

Table 4. Sensitivity analysis for P-values using a permutation test. For an assumed inflation factor the significance level would be precisely alpha for inflation factor > 1. For inflation factor = 1 the nominal p-value is greater than alpha.

	P-value	Alpha	IF
DN group 1	0.0004	0.050	3.31
DN group 2	0.0007	0.050	3.03
DN group 3	0.0045	0.050	2.10
All	<0.0001	0.050	4.53
DN group 1	0.0004	0.010	1.92
DN group 2	0.0007	0.010	1.75
DN group 3	0.0045	0.010	1.22
All	<0.0001	0.010	2.63
DN group 1	0.0004	0.001	1.17
DN group 2	0.0007	0.001	1.07
DN group 3	0.0045	0.001	1.00
All	<0.0001	0.001	1.61