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The gene variant for desmin rs1058261 may protect against combined cancer and cardiovascular death, the Tampere adult population cardiovascular risk study

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Abstract Desmin-containing intermediate filaments are a part of muscle cytoskeleton. We have previously reported that the wild-type cytosine/cytosine genotype of a common Desmin synonymous single nucleotide polymorphism (C > T) (rs1058261) associated with cardiovascular diseases in a cohort of subjects from the Tampere adult population cardiovascular risk study. We now examined whether rs1058261 also associates with early death by following the cohort of 801 subjects from the age of 50 up to the age of 65. Outcomes for death were collected from the National Statistics Centre. Linkage disequilibrium analysis and gene expression correlations for rs1058261 were done *in silico*. With follow-up, subjects with wild-type cytosine/cytosine genotype had higher incidence of cancer deaths (odds ratio [OR] 5.27, confidence interval [CI] 1.160–23.946, $P = .031$), combined deaths from cardiovascular diseases or cancers (OR 3.92, CI 1.453–10.596, $P = .007$), and "hard" acute cardiovascular disease events (early myocardial infarction and/or death) (OR 3.90, CI 1.287–11.855, $P = .016$) compared to subjects with the T-allele. The *in silico* results of linkage disequilibrium and gene expression analyses showed negative gene expression sizes associated with rs1058261, which theoretically decreases desmin expression. Our findings suggest that variation rs1058261 in Desmin may serve as a surrogate marker for other variations involved in decrease of deaths from combined cancer and cardiovascular disease.

Abbreviations: D = coefficient of linkage disequilibrium, DES = Desmin, LD = linkage disequilibrium, MI = myocardial infarction, SNP = single nucleotide polymorphism, SPEG = striated muscle enriched protein kinase, TAMRISK = Tampere adult population cardiovascular risk.

Keywords: cancer, death, desmin, genetic variation, intermediate filaments/genetics, vascular diseases

1. Introduction

Desmin (DES) belongs to the family of intermediate filament proteins that connect cell organelles by forming a cytoskeletal network. Desmin is muscle specific and is expressed in skeletal, cardiac, and smooth muscle cells,[1] including pericytes aligning neovessels in angiogenesis.[2] In carcinogenesis, increased numbers of desmin-positive pericytes are observed in late-stage tumors, consistent with increased angiogenesis and microvessel maturation.[3] Desmin is a useful marker for disease in atherogenesis, since it is expressed in mature contractile smooth muscle cells (SMCs), and absent in dedifferentiated SMCs of the synthetic type, which are typical in arterial wall repair.[4,5]

Desmin is encoded by the gene *DES* which has been found to have several rare mutations causing skeletal muscle weakness, cardiomyopathy, cardiac conduction disease, respiratory insufficiency, and smooth muscle defects.[1] In our previous study of a common single nucleotide polymorphism (SNP) *DES* rs1058261, the wild-type genotype cytosine/cytosine (CC) was associated with higher incidence of combined cardiovascular diseases compared to subjects with the T-allele.[6]

Rare mutations of desmin cause severe diseases. However, common SNPs of *DES* might prove even beneficial in terms of disease outcomes from cardiovascular diseases and cancer, which are responsible for over two-thirds of all premature deaths from non-communicable diseases worldwide.[7] "Hard" acute cardiovascular disease events may be defined as myocardial infarction (MI) by the age of 50 years and/or premature mortality due to cardiovascular disease. Premature mortality may be denoted as death before the age of 65 years.[7,8] We therefore wanted to study whether *DES* rs1058261 associates with "hard" cardiovascular events or early death.

2. Materials and Methods

2.1. Subjects

Tampere adult population cardiovascular risk study data was collected from periodic health examinations done in 2003 to 2006 for 50-year-old men and women living in Tampere, a city in

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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southern Finland with 220,000 inhabitants.[9] Health and disease, such as previously suffered MI, were assessed using a structured questionnaire. Buccal swabs for deoxyribonucleic acid (DNA) extraction and a permissions form to use periodic health examinations information and national registry data were collected by mail from the study subjects. Cases were subjects who had reported hypertension at the age of 50 years (as diagnosed by a physician) and for each case, at least 1 normotensive control subject with the same sex, and similar smoking habits, was chosen. All participants gave informed consent, and the Ethics Committee of the Tampere University Hospital approved the study.

2.2. Outcomes for death

For follow-up from the age of 50 years up to the age of 65 years, the original cases and controls were combined. Vital status was ascertained based on social security number and the cause of death from death certificates. This information was obtained up to 2018 from the National Statistics Centre (Statistics Finland). For the 801 subjects with successful genotyping, there were altogether 40 deaths of various causes (6 women, 34 men). International classification of diseases-10 codes were used to group cause specific mortality into the leading causes that were cancer (4 women, 9 men) (ICD10: C17, C24, C43, C50, C56, C71) and cardiovascular disease (0 women, 12 men) (ICD10: I21, I25, I71).

2.3. Genotyping

DNA was extracted from buccal swabs using a commercial kit (Qiagen Inc., Valencia, CA). A SNP at nucleotide position 828 (rs1058261) of the *DES* gene was chosen.[2] For DNA genotyping, polymerase chain reaction (PCR) was performed in a final volume of 5 µL containing 10 ng of sample DNA, 0.05 µL of custom SNP-specific Assay mix and 2.18 µL of Taqman Universal PCR Master Mix. The Assay mix used was C_11735969_20. Amplification proceeded for 40 cycles of 15 s at 95°C and 60 s at 60°C. Genotyping followed the Applied Biosystems (Pleasanton, CA) protocol. Automatic genotype call was performed after PCR, by scanning plates on the 7900 HT Fast Real-Time PCR, which provides the SDS2.3 software (Applied Biosystems), as described previously.[6]

2.4. Linkage disequilibrium (LD) analysis and gene expression correlations

The DES rs1058261 was uploaded into Variant Effect Predictor (http://www.ensembl.org/Tools/VEP)[10] and the LD Calculator was used to determine associated SNPs with a coefficient of linkage disequilibrium (D) threshold value of over 0.9999 in the populations Finnish in Finland and Gambian in Western Divisions in the Gambia. Gene expression correlations were obtained from the Genotype-Tissue Expression project data (https://www.gtexportal.org/home/).

2.5. Statistical analysis

Logistic regression, Chi-square test or Fisher's exact test were applied for the comparison of genotype groups. Kaplan–Meier survival analysis from combined cardiovascular and cancer deaths was also performed. Analyses were carried out using SPSS 23.0 for Windows (SPSS Inc., Chicago, IL).

3. Results

Clinical characteristics of case group of hypertensive subjects and controls at the age of 50 years have been previously described.[6] Samples were available and genotyping for rs1058261 was successful for 801 subjects: 336 cases and 465 controls (351 women and 450 men). With follow-up, the original cases and controls were combined to a cohort with genotype frequencies of CC: 414 (51.7%), cytosine/thymine: 328 (40.9%), and thymine/thymine: 59 (7.4%). The genotypes were in Hardy-Weinberg equilibrium (Chi-square = 0.29, $P > .05$).

When the subjects were followed up to the age of 65, those with genotype CC had higher incidence of combined cardiovascular or cancer death (4.8%), compared to T-allele carriers (1.3%) ($P = .004$) (Table 1). When incidence of cardiovascular or cancer death was adjusted by gender, the odds ratio (OR) for CC genotype was 3.92 ($P = .007$, confidence interval [CI] 1.453–10.596), compared to T-allele carriers. The Kaplan–Meier survival curve illustrates the better survival of subjects with the T-allele (upper curve) compared to those with the wild-type CC genotype (lower curve) (Fig. 1; $P = .005$).

When cancer death was analyzed alone, subjects with genotype CC had higher incidence (2.7%), compared to T-allele carriers (0.5%) ($P = .017$) (Table 1). When incidence of cancer death was adjusted by gender, the OR for CC genotype was 5.27 ($P = .031$, CI 1.160–23.946), compared to T-allele carriers.

When cardiovascular death or early MI were analyzed separately, subjects with different desmin genotypes did not differ (Table 1). However, when they were combined the subjects with genotype CC had higher incidence of such "hard" cardiovascular endpoints (3.9%), compared to T-allele carriers (1.0%) ($P = .011$). When incidence of cardiovascular death and/or early MI was adjusted by gender, the OR for CC genotype was 3.90 ($P = .016$, CI 1.287–11.855), compared to T-allele carriers.

In the population Finnish in Finland, high levels of LD with rs1058261 were found for *DES* regulatory region variants rs2070927 ($D = .999998$), rs2854886 ($D = .999979$), rs142933981 ($D = .999979$), and striated muscle enriched protein kinase (*SPEG*) regulatory region variant rs11691617

Table 1

Clinical characteristics of the study population stratified according to *DES* rs1058261 genotypes.

Genotype (n)	CC (414)	CT (328)	TT (59)	*P* value * CC versus CT versus TT	*P* value * CC versus (CT + TT)	*P* value * TT versus (CC + CT)	*P* value ** CC versus (CT + TT)
Combined cancer and cardiovascular death by the age of 65 years% (n)	4.8 (20)	1.2 (4)	1.7 (1)	**.017**	**.004**	.517	**.007**
Cancer death by the age of 65 yr % (n)	2.7 (11)	0.6 (2)	0.0 (0)	.054	**.017**	.309	**.031**
Cardiovascular death by the age of 65 yr % (n)	2.2 (9)	0.6 (2)	1.7 (1)	.221	.104	.905	.116
Myocardial infarction by the age of 50 yr % (n)	1.7 (7)	0.0 (0)	1.7 (1)	.062	.070	.464	.077
Cardiovascular death and/or early MI by the age of 65 yr% (n)	3.9 (16)	0.6 (2)	3.4 (2)	**.017**	**.011**	.651	**.016**

CC = cytosine/cytosine, CT = cytosine/thymine, TT = thymine/thymine.

* Chi-square test or Fisher's exact test. ** Logistic regression adjusted by gender. *P* values < .05 are in bold.

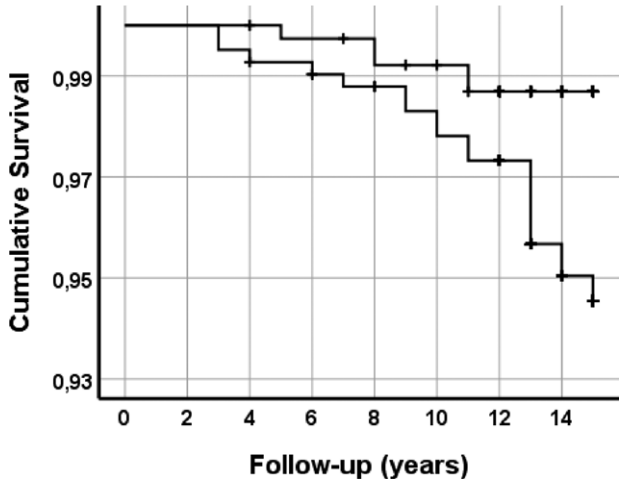

Figure 1. Kaplan–Meier survival analysis from combined cardiovascular and cancer deaths of subjects with the T-allele (upper curve) and those with the wild-type CC genotype (lower curve) (P = .005): The subjects were examined at baseline at 50 years of age and followed up to the age of 65 years. CC = cytosine/cytosine.

(D = 1.000000). In LD analysis of population Gambian in Western Divisions in the Gambia, high levels of LD with rs1058261 were found for *SPEG* regulatory region variant rs4674396 (D = .999936), and a regulatory region variant for both *DES* and *SPEG* rs907683 (D = .999931). For rs1058261 gene expression correlations (The Genotype-Tissue Expression project), the highest effect sizes (effect of the alternative allele relative to the reference allele) were obtained for muscular esophagus (–.52), skeletal muscle (–.49), left ventricle of the heart (–.42), aorta (–.42), tibial artery (–.42) and atrial appendage of the heart (–.40).

4. Discussion

The *DES* synonymous SNP rs1058261 (C > T) does not lead to amino acid change. However, we have previously reported that the wild type genotype CC associated with increase in cerebrovascular complications and cardiovascular diseases in the Tampere adult population cardiovascular risk study population.[6] Desmin is typically absent in dedifferentiated SMCs of the synthetic type, which are active in arterial wall repair.[5] The *in silico* gene expression correlations of the present study showed negative desmin gene expression sizes when rs1058261 T-allele was compared to the wild type genotype CC. The rs1058261 variation T may thus be associated with decreased expression of desmin, disturbing the cytoskeleton and promoting the dedifferentiated smooth muscle cells phenotype. We present evidence that variation in the synonymous rs1058261 is somehow beneficial, since combined early myocardial infarctions and/or premature cardiovascular deaths were decreased in the subjects with T-allele, consistent with our earlier findings.[6]

Actual human *DES* deleterious mutations may disturb the intermediate filament cytoskeletal network that binds together vital organelles resulting in loss of normal function and ultimately cell death.[11] Such mutations cause defects in skeletal-, cardiac-, and smooth muscle.[1]

The expression of desmin and its role in different cancers is not straightforward. The expression of desmin in colorectal cancer is associated with tumor stroma angiogenic microvessel pericytes,[3] which might suggest that the desmin-containing pericytes in the angiogenic component of cancer stroma are somehow involved. Whether rs1058261 (C > T) is somehow beneficial in decreasing the angiogenic component remains to be shown.

The *DES* rs1058261 synonymous codon has been proposed to be in LD with functional polymorphisms.[6] In LD analysis, *DES* rs1058261 was in high LD with *DES* regulatory region SNPs, which may affect transcription of the *DES* gene. In addition, high LD was found with regulatory region variants of *SPEG*, which codes a myosin light chain kinase in muscles. Mutations in *SPEG* have been associated with cardiomyopathy.[12] A close LD with *DES* rs1058261 was found also for rs907683, which is a regulatory region variant for both *DES* and *SPEG*, associated with resting heart rate and among genetic predictors of all-cause mortality.[13] These associations may offer an explanation how *DES* rs1058261 may associate with protection against death. A definite weakness of the present study is the relatively small population. However, possible genetic predisposition connected to *DES* genotype may be suspected since the cases were manifested at a premature age.

5. Conclusions

In conclusion, our findings suggest that variation rs1058261 in *DES* may serve as a surrogate marker for functional variations involved in decrease of deaths from combined cancer and cardiovascular disease. Because of the lack of direct causation, the potential for diagnostic or therapeutic approaches for this variation is likely to be reduced.

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