Genetic Variants of *DICE1/INTS6* in German Prostate Cancer Families with Linkage to 13q14

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Key Words

Prostate cancer-susceptibility · *DICE1/INTS6* · 13q14.3

Abstract

Introduction: Prostate cancer is the most frequent malignancy found to occur in Caucasian men, but its genetic basis remains elusive. A prostate cancer-susceptibility locus has been identified on chromosome 13q14. The tumour suppressor gene *deleted in cancer cells 1 (DICE1/INTS6)* is located within this interval on 13q14.3. **Materials and Methods:** We performed mutation analysis of the *DICE1/INTS6* gene in thirteen German prostate cancer families. **Results and Conclusion:** None of the patients harboured *DICE1* mutations, and similar frequencies of the previously identified 13 bp deletion polymorphism in the *DICE1* promoter were observed in the familial prostate cancer patients as compared with sporadic prostate cancer patients and controls. However, in one family with three affected brothers, the variations c.1215A>C (p.T405T) in exon 10 and c.2568A>G (p.S856S) in exon 17 were detected in a heterozygous pattern. In sporadic prostate cancer patients, variant c.2568A>G (p.S856S) was detected in 10/325 (3.08%) compared with 5/207 (2.42%) control samples ($p > 0.05$). We conclude that *DICE1* appears to be involved in prostate cancer progression rather than in the initiation of prostate cancer.

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Introduction

Prostate cancer is the most frequent malignancy found to occur in Caucasian men, but its genetic basis has remained elusive. A combined genome-wide linkage scan for prostate cancer-susceptibility genes identified linkage at 13q14 [1]. The gene deleted in cancer cells 1 (*DICE1*; integrator complex subunit 6 *INTS6*; OMIM 604331) is located within this interval [2]. *DICE1* was found to be frequently deleted in prostate cancer specimens [3, 4]. A *DICE1* missense mutation has been previously detected in prostate cancer cell line LNCap and reduced *DICE1* expression appears to be associated with CpG promoter hypermethylation in prostate cancer cell lines [4, 5]. All these suggest that the *DICE1/INTS6* gene could be a putative prostate cancer-associated gene on 13q14.

Material and Methods

A cohort of 13 German families with suggested familial prostate cancer was included. In this cohort of 13 families, prostate cancer was recognised in at least 3 patients in 12 families. In 9 families, the disease was diagnosed in at least one patient before the age of 60 years, and in the remaining 4 families in at least one patient before the age of 70 [6]. In 6 families, patients with other types of cancer were reported (table 1): skin cancer (family 0239),

Fig. 1. Germline variants of *DICE1/INTS6*. Sequencing profile obtained from exon 10 (left panel) and exon 17 (right panel) of *DICE1/INTS6* (a). Blood DNA from prostate cancer patient 01 of family 0136 shows heterozygosity for c.1215A>C and c.2568A>G in exons 10 and 17, respectively (upper panel). Variation c.2568A>G creates a novel *TaqI* cleavage site in exon 17 (b). Heterozygosity for c.2568A>G occurs in the three affected brothers (lanes 01, 02, 08) of family 0136, but not in control individuals (lanes C). DNA size marker (100 bp ladder, Invitrogen) is shown in lane M. The novel fragment generated by *TaqI* cleavage is indicated by an arrow on the left.

Table 1. Summary of prostate cancer families and *DICE1/INTS6* gene sequence analysis

Family-ID	No. of generations	No. of affected members	LOD at D13S326	NPL at D13S326	<i>DICE1/INTS6</i> coding region	<i>DICE1/INTS6</i> 13 bp ^a
239	1	3 + 1 ^b	0.361	2.216	∅	++
136	1	3	0.341	2.037	c.1215A>C:p.T405T c.2568A>G:p.S856S	+Δ
159	2	3 + 3 ^b	0.170	1.316	∅	+Δ
065	3	4	0.142	0.756	∅	++
115	2	3 + 1 ^b	0.126	0.529	∅	+Δ
363	3	5	0.120	0.442	∅	ΔΔ
021	2	3	0.118	1.318	∅	+Δ
101	1	3	0.117	0.801	∅	++
145	2	4	0.117	1.309	∅	++
178	2 + 2	4 + 4 ^b	0.115	0.724	∅	++
002	2	4 + 1 ^b	0.108	0.556	∅	++
116	1	2	0.105	1.303	∅	+Δ
005	1	4 + 1 ^b	0.102	0.612	∅	++

^a 13 bp tandem repeat (+) and its deletion variant (Δ) located in the *DICE1* promoter. ^b Additional family members affected by other types of cancer.

kidney cancer (family 0159), gastrointestinal carcinoma, thyroid and lung cancer and leukemia (family 0178), and neuroblastoma (family 0101). Indeed, the occurrence of other types of cancers in the families agreed well with previous findings, which show allelic deletion of region 13q14, including *DICE1/INTS6* not only in sporadic prostate cancer but also in sporadic lung and esophageal carcinomas [2, 7].

Mutation analysis of the *DICE1/INTS6* gene was performed on DNA obtained from blood of family members affected by prostate cancer from each of the 13 families, using genomic sequencing. The coding sequence and adjacent splice sites of exons 1–18 of the *DICE1* gene were directly sequenced with exon-specific primers as described previously [8]. Sequence analysis was performed as described [4]. Sequence variant c.2568A>G (p.S856S) was analyzed by exon 17 amplification and cleavage with *TaqI* (New England Biolabs GmbH, Frankfurt, Germany). Statistical analysis was performed by applying 95% confidence intervals (CI) with binominal distribution. The 13 bp tandem repeat polymorphism of the *DICE1* promoter was analyzed as described previously [4].

Results and Discussion

We detected no *DICE1/INTS6* mutation in any of these patients (table 1). Similar frequencies of the previously identified 13 bp deletion polymorphism in the *DICE1* promoter were observed in the familial prostate cancer patients as compared with sporadic prostate cancer patients and controls [4]. In one family (0136) with three affected brothers, the variants c.1215A>C (p.T405T) in exon 10 and c.2568A>G (p.S856S) in exon 17 were detected in a heterozygous pattern (fig. 1). These variant alleles segregate with the 13 bp deletion polymorphism in the *DICE1* promoter in this family as shown by haplotype analysis using microsatellite marker D13S284 known to co-localise with the *DICE1* gene [8]. The variant alleles in exon 10 and 17 have been reported earlier only in a hepatocellular carcinoma cell line [5]. In sporadic prostate cancer patients, variant c.2568A>G (p.S856S) was detect-

Table 2. Frequency of c.2568A>G

Specimen	Frequency
Controls	5/207 (2.42%) (0.79–5.46)*
Sporadic prostate carcinoma	10/325 (3.08%) (1.49–5.52)*
Prostate cancer families	1/13 (7.69%) (1.95–36.03)*

* 95% confidence intervals with binominal distribution.

ed in 10/325 (3.08%) compared with 5/207 (2.42%) control samples without statistical significance (table 2). These results do not support a role of *DICE1/INTS6* in prostate cancer susceptibility in this cohort.

However, the primary mechanism for hereditary and familial prostate cancer predisposition in this gene may not involve sequence variations in the coding region [9, 10]. *DICE1* inactivation (e.g.,) is associated with progression rather than the initiation of prostate cancer [11]. It might, therefore, be useful in the differentiation of prostate carcinomas needing therapy versus those that may be surveilled only.

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Electronic-Database Information

<http://www.ncbi.nlm.nih.gov/>.
<http://AtlasGeneticsOncology.org/Genes/INTS6ID40287ch13q14.html>.

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