Histological Groups of Human Postpubertal Testicular Germ Cell Tumours Harbour Different Genetic Alterations

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Abstract. *Background: Testicular germ cell tumours are the most common malignancies in young males. Molecular biology studies of these tumours are often contradictory. Two histological groups, seminoma and non-seminoma, differ both morphologically and in malignant behaviour. Although a common cytogenetic feature is seen, namely the amplification of the 12p chromosomal region, the development mechanisms of less aggressive seminomas and more aggressive non-seminomas are unknown. Materials and Methods: Occurrence of structural genetic alterations was analyzed in 18 seminomas and 22 non-seminomas for genes involved in the malignant tumour phenotype: cadherin 1, Type 1, E-cadherin (Epithelial), CDH1; adenomatous polyposis coli, APC; NME/NM23 nucleoside diphosphate kinase 1, NME1; tumour protein P53, TP53; cyclin-dependent kinase inhibitor 2A, CDKN2A; retinoblastoma 1, RB1; RAD51 recombinase, RAD51; mutS homolog 2, MSH2; MutL homolog 1, MLH1; breast cancer 1, early onset, BRCA1; BCL2-Associated X Protein, BAX; ATP-Binding Cassette, Sub-Family G (WHITE), Member 2, ABCG2. Genetic alterations, loss of heterozygosity and microsatellite instability, were analyzed using restriction fragment or microsatellite repeat length polymorphisms. Results: A difference in genetic alteration occurrence between seminomas and non-seminomas was observed. Conclusion: Occurrence of genetic alterations correlates with clinical behaviour of these tumours and may indicate that such alterations could occur early in the development of seminomas and non-seminomas.*

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Testicular germ cell tumours (TGCT) are the most common malignancies in men aged between 20 and 40 years, representing 60% of all malignancies found in that age group (1). They are heterogeneous tumours histologically and clinically distinguished as seminomas and non-seminomas (2). Of all TGCTs, 50% are seminomas, 40% are non-seminomas, and 10% are combined tumours comprising of non-seminomatous histology with a seminoma component, and intermediary tumours which have seminomatous morphology but with a higher proliferation rate. Most non-seminomas are of mixed histology, having components classified as embryonal carcinoma, yolk sac tumour, choriocarcinoma and teratoma (3).

Compared to non-seminomas, seminomas occur later in life, progress less rapidly, are less likely to metastasize, are rarely chemoresistant and are curable in about 95% of cases, regardless of tumour stage. In non-seminomas, embryonal carcinoma content increases the risk for metastasis, choriocarcinoma is highly aggressive, and teratoma becomes chemoresistant requiring total surgical excision (4-6). Curability of non-seminomas decreases with tumour stage. Differences in chromosomal aberrations, gene expression and epigenetics were also reported (7-9).

Most postpubertal TGCTs develop from a precursor stage, intratubular germ cell neoplasia (ITGCN). Infantile TGCTs and spermatocytic seminomas do not develop from ITGCN and are mostly benign (10).

TGCTs developing from ITGCNs have the chromosomal 12p11.2-12p12.1 region amplified (9). In 80% of cases, it is found as an i(12p) chromosome. This region involves several genes considered important for TGCT development, such as Kirsten Rat Sarcoma viral oncogene homolog, *KRAS* and

cyclin D2, *CCND2* (11). Expression of these genes is linked to invasiveness and early disease onset (12). However, 12p amplification has not been reported in all ITGCN studies, hence its role in TGCT development seems to be linked to transition to malignancy (13, 14).

Despite numerous molecular biology studies, the reported results are often contradictory. Such issues span from the role of specific genes (15, 16), and the number and structure of chromosomes (17, 18) to the origin and development of tumour cells (3, 19). The importance of microsatellite instability (MSI), another important mechanism involved in tumour progression, on the outcome of these tumours is also unclear (20).

Although the presence of unaltered p53 protein is proven for TGCT, its structure and tumour suppressor activity in TGCT is not unambiguously confirmed (15, 21, 22), and similar questions remain for the role of p16, p21 (23), and E-cadherin (22, 24). The apoptosis mechanism in TGCT is another unresolved issue (25-27). Studies of chromosomal instability focusing on loss of heterozygosity (LOH) and copy number alteration have also reported contradictory results. While some confirm the differences between histologies found in TGCT (19, 28), or some common chromosomal aberrations among different histological types (7, 3), others have found no difference between the histologies of TGCT (29). The nature of these chromosomal aberrations is not always clear (29, 30) and similar uncertainty exists for the methylation profile of different TGCT histologies (31).

To gain information from the multiple genetic alterations found in tumours, a statistical approach to the genetic background of similar tumours, called tumour stratification or genetic profiling has been applied. A heterogeneous population of tumours is thereby divided into clinically and biologically meaningful subtypes with their own causes and clinical outcomes (32).

In order to shed more light on the genetic background of different TGCT histologies and the role genetic changes have in clinical behaviour of these tumours, we analyzed the occurrence of structural genetic aberrations (*LOH* and *MSI*) in a set of TGCTs. These were investigated *via* several genes involved in the malignant tumour phenotype: cadherin 1, type 1, E-cadherin (Epithelial), *CDH1*; adenomatous polyposis coli, *APC*; NME/NM23 nucleoside diphosphate kinase 1, *NME1* (cellular adhesion and proliferation); cyclin-dependent kinase inhibitor 2A, *CDKN2A*; retinoblastoma 1, *RB1*; tumour protein P53, *TP53* (cell cycle regulation); breast cancer 1, early onset, *BRCA1*; mutL homolog 1, *MLH1*; mutS homolog 2, *MSH2*; RAD51 recombinase, *RAD51* (DNA repair); BCL2-Associated X Protein, *BAX* (apoptosis); and ATP-Binding Cassette, Sub-Family G (WHITE), Member 2, *ABCG2* (xenobiotic transport and multidrug resistance). Descriptive statistics were then performed to determine whether such genetic differences are informative regarding the clinical behaviour and development of the studied tumour histologies.

Materials and Methods

Tumour tissue samples along with the corresponding samples of phenotypically normal testicular tissue were taken from 40 TGCT cases (18 seminomas, 22 non-seminomas) collected from the Sisters of Mercy Clinical Hospital Center and University Hospital Center, Zagreb, Croatia. The samples were formalin-fixed and paraffin-embedded. Clinical and pathological data for the 40 TGCTs according to the WHO 2004 classification are shown in Table I.

For each specimen, 20 μm paraffin-embedded sections were prepared for DNA extraction. In addition, a 4 μm section was stained with hematoxylin-eosin to identify the tumourous and normal tissue areas. These were removed separately from microscopic slides, transferred to microtubes and extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Selected microsatellite and restriction fragments length polymorphism genetic markers were amplified by polymerase chain reaction (PCR), put under enzymatic restriction when necessary and visualised on polyacrylamide gels. Gene markers and primers used for PCR amplification are given in Table II. PCR amplifications were carried out in an Eppendorf Mastercycler Personal thermocycler (Hamburg, Germany), with cycling times of 96˚C for 5 min (one cycle), and then 45 cycles of 96˚C for 30 s, approximately 57˚C for 45 s, and 72˚C for 30 + 1 s. The final step was incubation at 72˚C for 10 min. Amplified DNA fragments were analysed on silver-stained 15% polyacrylamide gels (33, 34).

The selection of gene markers was based on several criteria: the gene marker had to be either intragenic or close to the selected gene; the selected gene marker needed to be variable in the population to ensure enough heterozygous (informative) patients, or the pseudomonomorphic marker would be informative in the case of genetic instability; amplified markers did not exceed 300 base pairs to ensure successful amplification, which can be difficult due to genomic DNA degradation in paraffin-embedded samples (35).

LOH was considered to have occurred if one out of two alleles in heterozygous samples was missing or expression was significantly reduced in comparison to that of alleles from adjacent normal tissue. Samples were considered positive for MSI if an additional DNA band was observed, or the existing DNA band had shifted position compared to the DNA profile seen in adjacent histologically normal tissue.

Descriptive statistics on obtained data were performed using Minitab® 15.1.30.0, Minitab Inc., USA; Statistica ver.10, StatSoft Inc., USA; Simple Interactive Statistical Analysis software, Quantitative Skills, the Netherlands.

Results

For *BRCA1, MLH1, MSH2, RAD51, BAX* and *ABCG2* samples were informative in 80% (100% seminomas, 63.6% non-seminomas), 95% (100% seminomas, 90.9% non-seminomas), 85% (88.9% seminomas, 81.8% non-seminomas), 97.5% (100% seminomas, 95.4% non-seminomas), 100%, and 85% (77.8% seminomas, 90.9% non-seminomas) of the cases, respectively. For those genes, LOH was not observed. The

Table I. *Clinical, pathological and occurence of loss of heterozygosity data for 40 cases of testicular germ cell tumour.*

Patient no	Age, years	pTNM	Histology	Occurrence of LOH
1	26	pT1NXMX	ITGCN, S	*APC*
2	26	pT1NXMX	ITGCN, S	
3	37	pT1NXMX	S	
4	33	pT1NXMX	ITGCN, S	
5	31	pT1NXMX	ITGCN, S	
6	29	pT1NXMX	ITGCN, S	
7	39	pT1NXMX	ITGCN, S	
8	27	pT3NXMX	S	*APC, CDH1*
9	41	pT1NXMX	ITGCN, S	
10	48	pT1NXMX	S	
11	48	pT2NXMX	S	
12	34	pT1NXMX	ITGCN, S	*TP53* intron 6
13	60	pT1NXMX	ITGCN, S	
14	29	pT1NXMX	ITGCN, S	
15	60	pT1NXMX	S	*APC*
16	29	pT1NXMX	ITGCN, S	
17	28	pT1NXMX	ITGCN, S	
18	32	pT1NXMX	ITGCN, S	
19	37	pT1NXMX	EC	*TP53* exon 4
20	18	pT2NXMX	EC, IT, MT, S	*TP53* exon 4, *RB1*
21	24	pT1NXMX	EC, ITGCN, S	
22	22	pT2NXMX	EC, YST	
23	37	pT1NXMX	EC, ITGCN, S	
24	28	pT2NXMX	C, EC, IT, MT	
25	17	pT2NXMX	EC, MT	*TP53* exon 4, *RB1*
26	34	pT2NXMX	EC	
27	19	pT1NXMX	EC, ITGCN, MT, YST	
28	39	pT1NXMX	MT, YST	
29	21	pT2NXMX	EC, MT, YST	*TP53* exon 4, *CDH1*
30	23	pT2NXMX	EC, IT, MT	*APC*
31	22	pT1NXMX	MT, YST	*CDH1*, *CDKN2A*
32	25	pT3NXMX	EC	*TP53* intron 6
33	45	pT2NXMX	EC, ITGCN, S, YST	
34	NK	pT2NXMX	C, EC, ITGCN, S, YST	*TP53* intron 6, *CDKN2A*
35	23	pT2NXMX	EC, IT, ITGCN, MT, YST	
36	39	pT1NXMX	EC, ITGCN, S, YST	
37	24	pT2NXMX	EC, ITGCN, YST	*CDH1*
38	30	pT1NXMX	EC, ITGCN, YST	
39	36	pT1NXMX	EC, ITGCN, MT, YST	*TP53* exon 4
40	58	pT2NXMX	EC, ITGCN, YST	

C: Choriocarcinoma; EC: embryonal carcinoma; IT: immature teratoma; ITGCN: intratubular germ cell neoplasia; MT: mature teratoma; S: seminoma; YST: yolk sac tumour; NK: not known; pT1: tumour limited to testis and epididymis without vascular/lymphatic invasion, tumour may invade *tunica albuginea* but not *tunica vaginalis*; pT2: tumour limited to testis and epididymis with vascular/lymphatic invasion or tumour extending through *tunica albuginea* with involvement of *tunica vaginalis*; pT3: tumour invaded spermatic cord with or without vascular/lymphatic invasion; pNX: regional lymph nodes not assessed; MX: distant metastasis not assessed.

frequencies of LOH for *CDH1, APC, NME1, CDKN2A, RB1* and *TP53* are shown in Table III. None of the gene markers revealed MSI.

Occurrence of LOH was analysed with regard to the histological profile of the TGCTs studied, tumour stage and patient age. Selected results are additionally shown graphically for terms of simplicity.

LOH was 2.45-fold more frequent in non-seminomas than in seminomas (68.2% and 27.8%, respectively). Statistical significance was confirmed by proportion testing (p=0.011, Z=−2.54) and Fisher's exact test (p=0.025). No statistically significant difference was observed for LOH between two restriction sites of *TP53*, exon 4 *Bsh123GI* and intron 6 *MspI*.

There was marginal statistical significance between observed and expected occurrence of LOH in seminomas (Fisher's exact test, p=0.061), not seen in non-seminomas (p=0.387). Although the different LOH profiles for the histological groups are clear, marginal statistical significance in LOH for specific gene markers in both groups was observed only for exon 4 of *TP53* (p=0.0657). The occurrence of LOH observed for any given gene marker was either around 9% (mean=9.4±2.32916%) or 26% (mean=25.75±2.21736%), thus differentiating the histology groups (Figure 1). The difference in observed LOH frequencies confining to these two levels is not statistically significant, but the probability that all LOH observed falls within these two categories is p=0.0156.

There was a statistically significant difference in the occurrence of LOH between different TGCT histological types (p=0.005, Fisher's exact test). Comparing the frequency of observed LOH relative to the frequency of a particular histological type for different histologies, most LOH was observed within the teratoma and choriocarcinoma component of TGCTs (100%). Embryonal carcinoma had LOH in 65% of cases, while yolk sac tumour component exhibited LOH in 61.5% of cases. Seminoma had the least LOH, in 37.5% of the cases. Proportional testing of total LOH occurrence between histology components revealed a significant difference in the levels of LOH found in teratoma and choriocarcinoma compared to all other histological types (p=0.001, Figure 2).

Each TGCT histology was also examined for preferential LOH of certain gene markers (Figure 3). A marginally significant difference between observed and expected LOH was found for exon 4 of *TP53* (p=0.075). The most frequent LOH in embryonal carcinoma was for exon 4 of *TP53* (25%), in yolk sac tumour for *CDH1* (23%), in teratoma for exon 4 of *TP53* (40%), in choriocarcinoma for intron 6 of *TP53* and *CDKN2A* (50%), and in histologically-pure seminoma for *APC* (16.7%). In the seminoma component of combined tumours, LOH was evenly distributed among gene markers, with no pronounced maximum. The occurrence of observed

Table II. *A: Restriction fragments length polymorphism genetic markers and corresponding primers. B: Microsatellite genetic markers and corresponding primers.*

A

Genetic marker	Gene name	PCR product size (bp)	Restriction endonuclease used	Restriction sequence	Restriction fragment size (bp)	Primer sequence
APC exon 11	Adenomatous Polyposis Coli	133	RsaI	GT↓AC	48, 85	GGACTACAGGCCATTGCAGAA GGCTACATCTCCAAAAGTCAA
TP53 exon 4	Tumour Protein P53	247	Bsh1236I	CG↓CG	87, 160	GATGCTGTCCGCGGACGATAT CGTGCAAGTCACAGACTTGGC
TP53 intron 6	Tumour Protein P53	107	MspI	C↓CGG	44, 63	AGGTCTGGTTTGCAACTGGG GAGGTCAAATAAGCAGCAGG
RB1 intron 17	Retinoblastoma 1	190	XbaI	C↓CTAGA	15, 75	TCCCACCTCAGCCTCCTTAG GTAGGCCAAGAGTGGCAGCT

B

Genetic marker	Gene name	PCR product size (bp)	Repeat sequence	Primer sequence
CDH1 D16S752	Cadherin 1, Type 1, E-Cadherin (Epithelial)	102-126	$(GATA)_n$	AATTGACGGTATATCTATCTGTCTG GATTGGAGGAGGGTGATTCT
NME1 M2	NME/NM23 Nucleoside Diphosphate Kinase 1	110-120	$(CA)_n$	TATGAGTTCAACTACGCACG CTCGAGCACAGGAGCAGGTT
CDKN2A hMp16α-I1	Cyclin-Dependent Kinase Inhibitor 2A	190-210	$(A)_{23}$	CAATTACCACATTCTGCGCTT CAGGCAGAGAGCACTGTGAG
BRCA1 D17S855	Breast Cancer 1, Early Onset	143-155	$(CA)_n$	ACACAGACTTGTCCTACTGCC GGATGGCCTTTTAGAAAGTGG
MLH1 D3S1283	MutL Homolog 1	150-160	$(CA)_n$	GGCAGTACCACCTGTAGAAATG GAGTAACAGAGGCATCGTGTATTC
MSH2 BAT-26	MutS Homolog 2	107-122	$(A)_{26}$	TGACTACTTTTGACTTCAGCC AACCATTCAACATTTTTAACC
RAD51 D15S1006	RAD51 Recombinase	190-228	$(CA)_n$	AGGGAATACTTCAAAACTC CCACTTGGCTATGGTGAAT
BAX Exon 3 polyG	BCL2-Associated X Protein	91-184	$(G)_8$	TTCATCCAGGATCGAGCAGGGCG ACTCGCTCAGCTTCTTGGTG
ABCG2 D4S2929	ATP-Binding Cassette, Sub-Family G (WHITE), Member 2	105-133	$(CA)_n$	GGCCAGGAGTTCAAAA TGCAGCAAGTCCAACA

LOH for any given gene marker in the seminoma component of the combined tumours was 16.7%, which corresponds to the maximum observed level of LOH for pure seminoma. All tumours with LOH in *APC* were seminomas. Seventy-five percent of LOH in *CDH1* comprised of tumours with yolk sac tumour component. Almost 67% of LOH in intron 6 of *TP53* was found in tumours with embryonal carcinoma component, and all tumours with LOH in exon 4 of *TP53* had embryonal carcinoma component. Eighty percent of tumours with LOH in exon 4 of the *TP53* also had a teratoma component.

Seminoma components of the combined tumours had LOH in 66.66% of cases, 2.4-fold more frequently than histologically-pure seminomas which exhibited LOH in 27.77% of cases. Proportion testing revealed marginally significant differences in the occurrence of LOH between different seminoma histologies (p=0.088).

LOH was found in 24% of pT1 tumours (42.86% tumours with LOH), 46.15% pT2 tumours (42.86% tumours with LOH) and 100% of pT3 tumours (14.29% tumours with LOH). A marginally significant difference was found between observed and expected occurrence of LOH with regard to tumor stage (p=0.0592, Fisher's exact test).

The average age of patients with seminoma was 36.5±10.891 years, and for those with non-seminoma was 29.57±10.390. This difference was significant (p=0.0495, t-test). On average, non-seminomas were detected seven years earlier than seminomas.

The average age of patients with LOH was 28.46±11.413 years, and for patients with no LOH 34.92±10.42 years. The difference was marginally significant (p=0.085, t-test). On average, tumours with LOH were detected 6.5 years earlier than those without LOH.

Table III. *Occurrence of loss of heterozygosity in studied cases.*

Patient no.	Age	pTNM	Histology profile	LOH observed
1	26	pT1NXMX	ITGCN, S	*APC*
8	27	pT3NXMX	S	*APC, CDH1*
12	34	pT1NXMX	ITGCN, S	*TP53* intron 6
15	60	pT1NXMX	S	APC
19	37	pT1NXMX	EC	*TP53* exon 4
20	18	pT2NXMX	EC, IT, MT, S	*TP53* exon 4, *RB1*
25	17	pT2NXMX	EC, MT	*TP53* exon 4, *RB1*
29	21	pT2NXMX	EC, MT, YST	*TP53* exon 4, *CDH1*
30	23	pT2NXMX	EC, IT, MT	*APC*
31	22	pT1NXMX	MT, YST	*CDH1*, *CDKN2A*
32	25	pT3NXMX	EC	*TP53* intron 6
34	NK	pT2NXMX	C, EC, ITGCN, S, YST	*TP53* intron 6, *CDKN2A*
37	24	pT2NXMX	EC, ITGCN, YST	*CDH1*
39	36	pT1NXMX	EC, ITGCN, MT, YST	*TP53* exon 4

C: Choriocarcinoma; EC: embryonal carcinoma; IT: immature teratoma; ITGCN: intratubular germ cell neoplasia; MT: mature teratoma; S: seminoma; YST: yolk sac tumour.

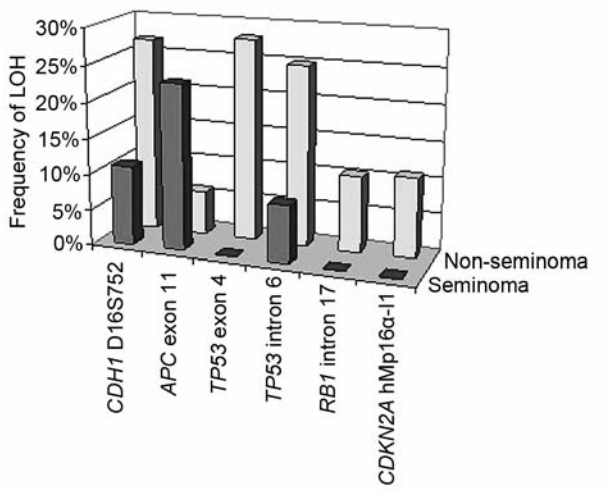

Figure 1. *Occurrence of loss of heterozygosity of gene markers in seminoma and non-seminoma.*

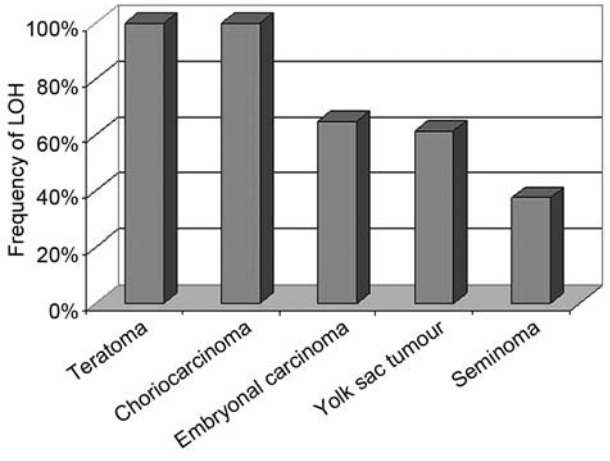

Figure 2. *Occurrence of loss of heterozygosity within different histological types.*

The average age of patients with pT1 tumours was 34.60±10.07 years, with pT2 tumours 30.08±13.30 years, and with pT3 tumours 26.0±1.41 years. The difference was not significant (p=0.3513, ANOVA).

Discussion

Genomic instability is one of the causes of acquiring new characteristics and phenotypic shift during tumour development (36). As a consequence of genomic instability, tumour cells are often aneuploidic. It seems aneuploidy arises after amplification of the chromosomal content (37). The same genomic changes have been observed in development of TGCTs (9).

Since we were unable to separate different histologies within non-seminomas, clear LOH found in a mixed tumour may indicate that it is an early event present in all histological types for that tumour. This implies that certain genetic changes are present in all histologies of the same mixed tumour. Since this is an early event, it further afffects genetic reprogramming leading to the development and differentiation of various histologies found in non-seminomas. However, for is found when the same reason we did not see LOH that might have been present in only one particular histological component, which could be characteristic for that histology.

In about half (43%) of the TGCTs studied, we found LOH in multiple genes that might act synergistically in tumourigenesis. In cases with multiple LOH, we found de-activation of *TP53* or a member of the pRb pathway, which are the most commonly altered genes in human cancers. A different LOH pattern comparing the two main TGCT histological groups, in accordance with previous studies (7, 38). Differences between observed and expected occurrence of LOH seen in seminomas was not seen in non-seminomas. This might be due to heterogenity of histologies comprising mixed non-seminomas. A marginally-significant difference between the two histological groups was found for only one genetic marker (exon 4 of *TP53*, p=0.0657), which is in accordance with previous studies, where statistical significant difference was found when using a frame of three locus-

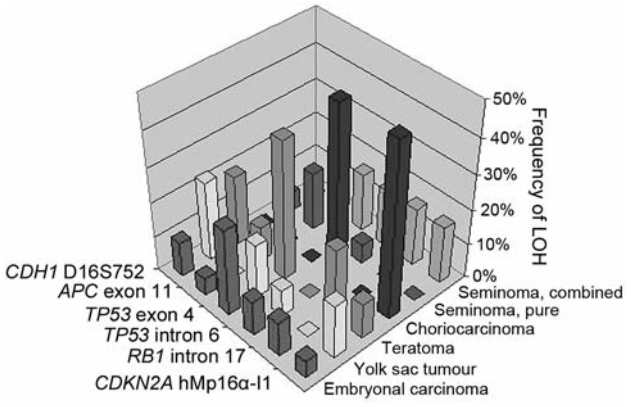

Figure 3. *Occurrence of loss of heterozygosity of gene markers according to histological type.*

specific markers, but was insignificant when adjusted for a larger number of single-locus tests (7). Higher occurrence of LOH in non-seminomas observed in our study may be responsible for their higher malignancy in comparison to seminomas. A similar relation is seen with the incidence of metastases, which are 2.4-fold more common with non-seminomas (3). A comparable level of LOH was also found in the seminoma component of the combined TGCTs in our study compared to the histologically-pure seminomas.

We found a correlation between the occurrence of LOH and the clinical outcome of TGCT, not seen in earlier studies (39). However, some authors found a link between LOH at 10q21.1~q22.2 and the tumour stage (7). With marginal significance, our study also found a higher occurrence of LOH in TGCTs of higher stage (a quarter of pT1 tumours, around half of pT2 and all of the pT3 tumours had LOH) and earlier onset of TGCT with LOH compared to those without LOH. Earlier onset of non-seminomas was also found. It may be due to a greater number of genetic alterations or slower progression of seminomas. Lack of any significant link between tumour stage and patient age does, however, indicate that earlier onset of non-seminomas is probably not caused by slower growth of seminomas.

The frequency of LOH in chromosomal regions with a higher LOH level was about twice as high compared to chromosomal regions with a low level of LOH, which was reported in a previous study as corresponding to allelic imbalance between cytogenetically aberrant and normal chromosomes, respectively. It has been suggested the lower LOH level might represent random, background LOH, whereas regions of higher LOH might point to a non-random loss of genes linked to differentiation of a particular histology (29). Since we found genetic markers with no LOH, and observed LOH to be confined to distinct levels, our results suggest LOH is a mechanism for phenotypic shift and is mostly non-random.

In our study, LOH of *TP53* was the most common genetic aberration (57.1% of total LOH). Loss of functional p53 was confirmed in previous studies, which found lack of p53 expression in almost half of TGCTs (40). The presence of normal p53 and its overexpression in TGCT therefore remains an unresolved issue, although some studies found p53 to be inactive in TGCT even when expressed (26, 31, 41).

Seminomas seem to have more LOH for genes involved in the Wingless/Integrase-1 (WNT) pathway (*APC*; *CDH1*), while non-seminomas exhibited greater LOH of genes involved in pRb and p53 pathways. This is in accordance with theoretical models showing faster developing tumours utilize different pathways for proliferation from slower ones (42). Our findings confirm previous studies finding that seminomas do not express E-cadherin and β-catenin from the WNT pathway, whereas their expression was found in non-seminomas (24). Some epigenetic studies do not confirm these findings (43).

Significant correlation between LOH and clinical outcome has been also found. Histologies with the highest relative LOH frequency are teratomas (100%), with a tendency for chemoresistance; choriocarcinomas (100%), the most aggressive histology with poor prognosis; and embryonal carcinomas (65%) with the most metastases found at the time of diagnosis (2). These results also indicate that LOH within TGCTs is non-random and might be responsible for the clinical behaviour of these tumours.

Preferential LOH of certain gene markers in different histologies of non-seminomas would support that conclusion, suggesting that genetic deletions underlie non-seminoma differentiation. LOH results in our study are not statistically significant when comparing between histological types and gene markers, but they might suggest genetic 're-programming' in TGCTs. This has also been found by previous studies (7).

Although showing a higher occurrence of genetic alterations, no preferential LOH of a particular gene marker was seen in the seminoma component of combined TGCTs. This might be due to the overall heterogeneity of histologies within combined non-seminomas, or may indicate lower levels of genetic alterations that are responsible for the seminoma-like phenotype.

Differentiation in TGCT therefore represents a mechanism for acquiring a phenotypic shift and does not reduce malignancy, which contradicts some studies (44). In confirmation of this, the highest levels of LOH were found in more differentiated histologies (teratomas and choriocarcinomas), with clinically worse outcome.

We found neither MSI nor LOH in genes related DNA damage response. This is in accordance with previous studies which found no MSI in most primary TGCTs (45,46). We

also did not find any LOH for 58.3% of the genes studied. This, together with clear levels of LOH for each gene marker affected, opposes the notion of random LOH in TGCT (29). Silenced but unaltered DNA damage response in TGCTs might be one of the reasons for their high sensitivity to therapy. Both metastases and primary tumours with MSI are resistant to chemotherapy (47).

This study showed the seminoma component of combined tumours has 2.4-fold more LOH than histologically pure seminoma, and genetic alterations found are the same as those in the other non-seminomatous histologies. We also found ITGCN in half of the non-seminomas studied. Fifty four percent of those tumours did not contain a seminoma component. This implies LOH seen in this study is an early event and had probably already occurred in the associated ITGCN. Similar results have been reported by others (7). Our results might indicate different development of seminomas and non-seminomas, with seminoma-like component found in combined TGCTs perhaps representing the most undifferentiated non-seminomatous histology.

The observed marginal statistical significances in results may be due to low sample numbers. As such, those were not totally disregarded, but require additional research.

Conflicts of Interest

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