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Testicular Cancer: Genes, Environment, Hormones

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Testicular cancer (TC) represents one of the most peculiar clinical challenges at present. In fact, currently treatments are so effective ensuring a 5 years disease-free survival rate in nearly 95% of patients. On the other hand however, TC represents the most frequent newly diagnosed form of cancer in men between the ages of 14 and 44 years, with an incidence ranging from <1 to 9.9 affected individuals per 100,000 males across countries, while the overall incidence is also increasing worldwide. Furthermore, cancer survivors show a 2% risk of developing cancer in the contralateral testis within 15 years of initial diagnosis. This complex and multifaceted scenario requires a great deal of effort to understand the clinical base of available evidence. It is now clear that genetic, environmental and hormonal risk factors concur and mutually influence both the development of the disease and its prognosis, in terms of response to treatment and the risk of recurrence. In this paper, the most recent issues describing the relative contribution of the aforementioned risk factors in TC development are discussed. In addition, particular attention is paid to the exposure to environmental chemical substances and thermal stress, whose role in cancer development and progression has recently been investigated at the molecular level.

Keywords: suscepibility genes, temperature, endocrine discruptors, disorders of sex development, GWAS

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INTRODUCTION

With an overall annual incidence of nearly 1% among all newly diagnosed cancers in males, testicular cancer (TC) represents the most common tumor in men at the age ranging between 14 and 44 years, which is considered to be the fully working/reproductive age (1, 2). Before the 1970s, the mortality rate for TC was extremely high due to the metastatic degeneration of the disease, whilst the only two treatments to contain the risk of relapse were the retroperitoneal lymph node dissection, associated or not with radiotherapy. Thereafter, the development of an effective chemotherapy changed the "rules of the game." In fact, the current multidisciplinary approach to the treatment of TC, comprising surgery and adjuvant chemo- or radiotherapy, results in a 5 years survival rate of >95%. As a consequence, TC is now considered as a model for a curable cancer (3).

In spite of these indisputable progresses, TC still presents multi-leveled challenges that should not allow our guard to be lowered:

- Epidemiological evidence: the annual incidence of TC has doubled over the past 40 years with an increasing trend over time, particularly in Caucasian males (4). Indeed, data from the African and Asian continents show an incidence lower than one case every 100,000 males, whilst Scandinavian countries report the highest rate of newly affected individuals worldwide (from 9.4 to 9.9 males every 100.000 males). This trend by ethnicity is further confirmed by data from United States where TC is found more frequently in white males compared to African Americans (1.2 vs. 6.9 affected individuals per 100.000 males, respectively) (5, 6).
- Clinical evidence: previous history of TC, even if properly treated and monitored, represents a major risk factor for a second contralateral cancer. The overall risk for a secondary TC is approximately 5% within 5 years from diagnosis, and in most cases presenting within 2 years from the first diagnosis (7). In this regard, primary testicular size and the degree of invasion of the rete testis are considered two major prognostic factors for relapse (8, 9).
- Therapeutic evidence: in spite of the aforementioned effectiveness, the treatment of TC itself is frequently associated with an increased risk of developing long lasting adverse effects like infertility, hypogonadism, metabolic/cardiovascular derangements, and osteoporosis, which actually represent the most relevant life threatening consequences of the TC therapy (10–12).

For all these reasons, the identification of pathogenic mechanisms and risk factors involved in testicular carcinogenesis still represent topics of extremely high clinical interest. Indeed, the probability of developing TC is the result of a combination of a number of factors that can be distinguished, in general terms, into genetic, environmental, and hormonal factors.

GENES

The fact that TC development relies on genetic factors is widely acknowledged. Despite the fact that 90% of males affected by TC have no previous familiar cases of this disease, population-based studies in the late 1990's-early 2000's showed that having a brother with an history of TC increases the risk of the disease from 8- to 10-fold, compared to the general male population. On the other hand, having a father affected by TC increases the relative risk for the male child from four- to 6-fold (13–15). In 2002, a pioneering study on a population-based registry, evaluating 9.6 million individuals from the nationwide Swedish Family-Cancer Database, attempted to distinguish between the respective genetic, pure-environmental and childhood-environmental contribution to the development of cancers, essentially based on epidemiologic considerations (16). Interestingly, TC resulted as one of the most associated neoplasms with genetic factors (25%), right after the thyroid (53%), and endocrine glands in general (28%). In addition, a recent study on a population-based registry, evaluating monozygotic and same-sex dizygotic twin individuals disclosed an esteemed familiar risk of heritability for TC of nearly 40% with a significant portion, however, attributable to shared environmental conditions (17).

In spite of the clear evidence supporting the genetic background in TC development, the availability of reliable studies providing qualitative and quantitative data about the genetic basis of familial TC still represents a major challenge. In 2006, a linkage study on 237 pedigreed families, with a history of one or more cases of TC, identified six regions of interest on chromosomes 2p23, 3p12, 3q26, 12p13-q21, 18q21-q23, and Xq27 as susceptibility loci. However, further widenings showed that no single locus accounted for the majority of the familial aggregation observed in TC, suggesting at the same time a major role of multiple susceptibility loci with singular weaker effects (18). To this regard, significant advances have been provided by the availability genome wide association studies (GWAS) that, since the mid-2000's, progressively increased the number of susceptibility loci with a predicted effect on TC development (19–24). In a recent GWAS and meta-analysis, comprising more than 5,500 cases and 19,000 controls from northern Europe, (25) identified and confirmed 44 independent TC risk loci (19 newly discovered and 25 previously reported). Interestingly, through a complex *in situ* chromosome conformation-capture analysis in TC cells, a tentative model of chromatin interactions between predisposition SNPs and target genes was performed, identifying three possible pathogenic mechanisms. In particular, 10 of the risk loci contained genes associated with the transcriptional regulation of cell development such as *GATA4* and *GATA1* genes. These are transcription factors involved in the specification and differentiation of postnatal testicular development, whose risk alleles polymorphisms have been previously associated with tumor progression (26–31). A significant association was also found for *PRDM14* and *DMRT1* genes, involved in germ cell specification-sex determination, and the *SALL4* gene through the disruption of the *POU5F1* binding motif (32–35), the latter associated with the maintenance of pluripotency in embryonic stem cells (36). In addition, five TC risk loci were associated with candidate genes with roles in microtubule and chromosomal assembly, particularly the *TEX14* gene, involved in kinetochore-microtubule assembly in the testicular germ cells (37–39), the *WDR73* gene, encoding a key protein for microtubule organization during interphase (40), and the microtubule assembly–related genes *PMF1*, *CENPE,* and *PCNT* (41–44). Furthermore, three TC risk loci subtended a major role of KIT–MAPK signaling, in agreement with recent evidence showing the *KIT* gene as a major somatic driver for TC development (24). In clinical terms, the 44 identified risk loci for TC accounted for 34% of the father-to-son familiar risk for TC development, whilst the top 1% genetic risk at a polygenic risk scores model had a relative risk of 14% and a 7% lifetime risk of developing TC (25). However, this pattern is likely to be widened, thanks to the increasing number of GWAS successively issued. A very recent meta-analysis of five available GWAS, including the X-chromosome, identified further 12 risk loci associated with TC, highlighting the possible involvement of additional cell pathways in TC, such as germ cell development and pluripotency through the *TFCP2L1* and *ZFP42* genes, the kinetochore function through the *ZWILCH* gene, the response to DNA damage through the

TIPIN gene and the mitochondrial function through the *TKTL1* and *LHPP* genes (45).

From this brief summary, it is clear that the pathogenesis of TC relies on a wide spectrum of genetic factors. Recently, a great deal of interest has been sparked by the role of gene copy number variations (CNVs) in cancer development and, particularly, in TC (46–48). In this regard, our group recently investigated the involvement of the *E2F1* gene CNVs as a TC risk factor (49). As a member of the E2F protein family, E2F1 is a transcription factor that regulates the transition of the cell cycle from the G1 phase to the S phase, through an interaction with the retinoblastoma tumor suppressor (RB) protein (50–52). Deregulation of E2F1-pRB binding increases the access of E2F1 to E2F1-binding target genes, containing the E2F-binding site, and this is thought to increase the susceptibility of tumor development (53). Importantly, experimental overexpression of E2F1 in carcinoma cell lines has been associated with increased cell proliferation through the mTOR signaling pathway (54). Interestingly, in our study group of the 261 patients with an history of testicular germ cell tumors and the 165 controls, we found duplications of the *E2F1* gene only in TC patients with a global prevalence of 6.5 percent. This was associated with the increased expression of the E2F1 protein only in tumor tissue specimens obtained from those patients harboring three copies of E2F1, whilst surrounding non tumor-tissue showed both lower E2F1 protein expression and downstream-mTOR phosphorylation (49). These results are highly suggestive of an involvement of E2F1 CNVs in TGCT susceptibility through the Akt/mTOR signaling pathway.

It should also be noted that several risk factors clinically associated with TC development, largely rely on genetic factors. Cryptorchidism, the failed descent of the testis in the scrotum through the inguinal canal during the embryonic life, affects 2–9% of boys born full term and is associated with an almost 9-fold increased risk of TC, compared to the general population (55, 56). The migration process of the embryonal testis can be functionally divided into two sequential phases: the trans-abdominal phase and the inguino-scrotal phase (57). Data from animal models disclosed that each phase is finely regulated by specific factors. In particular, the transabdominal migration of the testis depends primarily on insulin-like peptide 3 (INSL3) and its receptor, RXFP2 (58–60), whilst the inguino-scrotal phase largely depends on androgens signaling (61, 62). Genetic screening in cryptorchid boys showed, respectively, a 2 and 4% prevalence of mutations in the *INSL3* and *RXFP2* genes, more frequently in bilateral forms, whilst there is less agreement for a causative role of polymorphic variants (63–65). Interestingly, there is poor association between mutations of the *AR* gene and isolated cryptorchidism since the prevalence in cryptorchid males is generally lower than 2% (63, 66). In addition, expansion sites in the first exon of the *AR* gene, also known as poly CAG and GGN repeats, are acknowledged as a modulator of AR transactivation activity but their causative role in undescended testis is still under debate (67, 68).

With regards to cryptorchidism, one of the most relevant causes of this clinical condition is a chromosomal alteration such as Klinefelter syndrome (KS), affecting ∼1 in every 700 men (69). KS patients typically present with small testes, infertility, high levels of gonadotrophins and testosterone (T) at the lower levels of normality, whilst cryptorchidism presents nearly six times more frequently than in the general male population (69). The existing literature relating KS and TC, principally Leydig cell tumor, is quite abundant and mainly rely on case reports, however no conclusive association has been provided by the few available epidemiological studies on larger cohorts (63, 70–84). Hence, further studies are required to clearly identify the relative risk of TC associated to KS.

Other genetic causes of isolated cryptorchidism are ascribed to mutations of the AMH gene or its receptor in the persistent müllerian duct syndrome described below (85, 86). In addition, hypospadias, the urethral malformation during embryonal penis development, is also considered a risk factor for TC (87). In particular, hypospadias accounts for about 10% of familial clustering, whilst the estimated heritability of this disease ranges from 57 to 77% (88, 89).

ENVIRONMENT

The identification of direct environmental causes of TC development represents a problem with higher complexity. In fact, most of the acknowledged tumorigenic physical or chemical agents act indirectly through the disruption of the hormonal circuits regulating testis function, or by influencing the function of susceptibility genes (90). However, according to available literature, exclusive environmental risk factors for TC can be formally distinguished into four main classes: microbiological, mechanical, chemical, and physical.

Microbiological

Epidemiological data in 2002 estimated viral infections to be the causative role of ∼12% of cancers worldwide (91). In particular, the pathogenic role of infectious agents in testis tumors has been hypothesized since the late 1980s. Based on epidemiological similarities between Hodgkin's disease and TC, Algood et al. (92) investigated the possible causative role of early exposure to the Epstein-Barr virus (EBV), through the evaluation of antibodies to the EBV capsid antigen, in a small group of patients with an history of stage I germ cell tumors of the testes, receiving surveillance after orchiectomy (92). Interestingly, 80% of patients showed elevated titers for anti EBV antibodies compared to the control subjects, strongly linking cancer disease to previous viral exposure. In 1994 (93) further investigated the detection of EBV-DNA in testis specimens from patients with testicular germ cell tumors, including preinvasive carcinoma *in-situ*. A weak positivity for EBV DNA was detected in only six out of the 20 samples but none of the specimens showed a positive staining at either anti EBV-immunohistochemistry or *in situ*-hybridization techniques, ruling out a direct involvement of EBV and rather suggesting a putative growth-stimulating role of EBV-transformed lymphocytes infiltrating in testis stromal tissue (93). In 2013 Yousif et al. (94) aimed to quantify the possible association between viral infections and TC through a meta-analysis. Interestingly, serological markers of exposure to EBV, Cytomegalovirus, and Parvovirus B19 were associated with TC with pooled odd ratios (OR) of respectively, 4.80, 1.85, and 2.86.

Particularly for Human-immunodeficiency virus (HIV), authors first identified a pooled OR of 1.79 (94). This evidence was subsequently confirmed by several studies showing a relative risk ranging from 0.7 to 3.1 [reviewed in Hentrich and Pfister (95)]. However, as for EBV, a clear mechanistic model explaining the association between TC and HIV is currently under debate.

Mechanical

Despite being poorly acknowledged among typical risk factors for TC, mechanical, and particularly traumatic events on the testis, are considered a causal factor of this disease. Indeed, the experimental model of intra-testicular hematoma induced by injection of an autologous blood equivalent in rat testis was associated with a significant and long-lasting alteration of the testis structure, such as the reduction of the overall testis volume and the reduction of the seminiferous epithelium size. All these features resulted in altered testis function such as the under-representation of the germ cell population within the seminiferous tubule, altered sperm parameters, and a trend toward lower testosterone levels (96). Based on this profound alteration of the testis' functional architecture, the degeneration into cancer is rather intuitive, particularly in those situations of prolonged though subclinical testis trauma. In spite of this simple model, available evidence linking testis trauma to cancer are sparse and/or non-conclusive.

An interesting study from Dusek et al. (97) aimed to quantify the contribution and mutual interactions of very different types of potential risk factors for TC through the administration of standardized questionnaires to patients recruited in two Czech cancer centers, compared to healthy and age-matched controls (97). Interestingly, in addition to acknowledged risk factors like cryptorchidism and testis atrophy, a significant association was found for testicular trauma resulting with a nearly doubled risk for TC compared to the controls.

Another example of this model is represented by prolonged testis-micro traumas in patients practicing sports. In fact, testicular derangements such as testicular torsion, epididymitis, and testicular tumors are frequently observed by medical sport physicians (98). Coldman et al. (99) showed that cycling, particularly during teenage years, was associated with an almost doubled risk for TC even after correction for confounding factors like cryptorchidism or inguinal hernia. Also, horse-riding resulted in a nearly 3-fold greater risk for TC, which remained substantially unaltered after correction for confounding factors, whilst no significant association was reported for motorcycling or soccer (99). However, subsequent studies failed to identify a significant association between these sports with TC, suggesting further investigation (100, 101).

Chemical

Available data on chemicals exerting a direct role as a risk factor for TC mainly derive from occupational studies. Particular attention has generally been drawn by the exposure to heavy metals in extracting and processing plants. Heavy metals, most frequently absorbed as organometallic compounds, are known to accumulate in tissues, both disrupting their biological functions and representing long-term reservoirs, resulting in prolonged exposure to metal pollutants (102). In particular, transition series-metals like cadmium (Cd), mercury, and cobalt are acknowledged as carcinogens from several experimental studies performed in both animal and cell models (103–105). However, a direct association between Cd exposure and TC is still under ascertainment. In 2011, an epidemiological study for cancer incidence was performed in the Kempen area across the Dutch-Belgian border, featuring the very long activity of cadmium and zinc smelters. Compared to the control population, identified through regional population-based cancer registries, environmental exposure to Cd showed an increased risk for female lung cancer, male and female bladder cancer and prostate cancer but not TC (106). Similarly, another study focused on the north-east Belgium area investigated the ∼17 years incidence of cancers, finding an overall increased risk of doubling the 24-h urinary cadmium excretion, however no significant association with TC was documented (107). On the other hand, previous studies performed on metal workers in the Hannover region of Germany showed a nearly doubled risk of developing TC compared to the aged matched healthy controls, but no single chemical emerged at significant levels from the association analysis (108). In addition, Norwegian metal workers working with ferrosilicon and silicon furnaces showed a more than doubled incidence of TC compared with the estimated incidence in the general Norwegian population according to the age and historical period (109).

Another class of environmental chemicals associated with TC is pesticides, as depicted by epidemiological studies disclosing an increased incidence of TC in agricultural employees (110–112). However, two great meta-analysis in 1992 and 1998, respectively, failed to recognize a significant risk due to the exposure to pesticides in farmers (113, 114). To this regard, opportune distinctions should be made since substantial difference exists among countries in terms of chemicals, formulations, and regulatory principles (90). Furthermore, great differences in terms of toxicological effects of the different molecule classes is likely to exist in humans. In fact, organochlorines pesticides are supposed to act as endocrine disruptors (see below) whilst pyrethroids are likely to exert a direct effect on the cell cycle (115, 116).

Physical

Among those physical risk factors theoretically associated with an increased risk of TC, such as the exposure to ionizing radiations, ultraviolet light and electrical work, the most clinically valued is exposure heat stress. As depicted by the external location of male genitalia, the proper germ cell maturation within the seminiferous tubule is maintained at 2–8°C below the body core temperature (117). Systematic exposure of the testis to over-physiological temperatures has been associated with several, and generally reversible, testis derangements such as a reduced sperm count, motility, mitochondrial function, and even altered sperm membrane composition (118, 119). As for other environmental factors, the possible association between heat exposure and TC was investigated through occupational studies. Early studies in 1995 performed on TC patients and healthy age-matched

controls, revealed that occupational exposure to high or extreme temperatures was associated with an adjusted OR of respectively, 1,2 and 1,7, suggesting external temperature as an independent risk factor (120). A subsequent study in 2001 confirmed that the standardized incidence ratio for TC in fire fighters was 3,0 with no increased risk from any other cause of death (121). However, exposure to milder heat stress like showering and bathing was not associated with any significant risk for TC (122).

HORMONES

Exactly like other endocrine tissues in the body, the testis is both the target and the source of hormones strictly linked in a feedback-loop regulating pathway (123). In particular, the activity of the hypothalamus/pituitary/gonadal axis takes place from the early phases of embryo development, regulating testis descent and, the adequate location within the scrotal sac and the proper spermatogenic and endocrine functions, whose systemic effects are well-known (124). The early disruption of this hormonal circuit reverberates on testis function in adult life and represents a major risk factor for TC. Indeed, a tentative mechanistic model of the neoplastic transformation of germ cells has been developed by Rajpert-De Meyts et al. (125). This hypothesis is based on the strict similitude between primordial germ cells and gonocytes with tumor cells of the carcinoma *in situ* (CIS), verified at the molecular level by the shared expression of genes involved in pluripotency and proliferation, such as *NANOG*, *STELLAR, DPPA-5*, *GDF3*, *K-RAS,* and *CCND2* (126–129). In this context, the delayed development of germ cells, associated with long-term maintenance of embryonic genes, would represent a key event for the subsequent degeneration into cancer cells (130, 131). The disruption of the hormonal milieu of germ cells would then result in misleading signals altering the cell phase-switch toward mitosis and meiosis, with the consequent risk of a neoplastic transformation in adult life (125).

The most studied model of hormonal risk factor for TC is the disorders of sex development (DSD) in 46, XY males, frequently associated with androgen-insensibility syndrome (AIS), further distinguished into complete (CAIS), partial (PAIS), or mild (MAIS) forms (132). As can be guessed from the name of this pathological condition, its clinical characteristics range from a female phenotype of CAIS, in spite of an XY karyotype and normal androgen production, to severe under masculinization in PAIS, such as female external genitalia or hypospadias or micropenis, or male infertility and/or gynecomastia in MAIS. A general feature of the different forms of AIS is the altered function of the androgen receptor, resulting in a resistance to androgens as activating ligands. Genetic variants of the *AR* gene are commonly acknowledged as being causative of AIS. In particular, 95% of CAIS are associated with inactivating mutations of AR. In PAIS however, mutations of the *AR* gene are detected in $<$25% of patients whilst a complementary causative role has been ascribed to genetic variants of the protein and cofactors that concur to the AR signaling pathway, such as the deficiency 17β-hydroxysteroid dehydrogenase (17β-HSD), a key enzyme in steroidogenesis (133, 134). Also, persistent müllerian duct syndrome (PMDS) is a form of disorder of sex differentiation in 46, XY males caused by an inactivating mutation of the gene for AMH/MIS (45% of cases) or its type II receptor (39% of cases) (135). PMDS patients present genotypically and phenotypically as males with unilateral or bilateral cryptorchidism and/or an inguinal hernia at infancy (136). In general, DSD, and in turn AIS, are associated with and increased risk of TC with an estimated overall prevalence of nearly 5.5 percent, ranging from 0.8% in CAIS-associated DSD, 15% in PAIS to 17% in 17β-HSD deficiency (137, 138). Despite the fact that TC in PMDS has been described in several case reports, association studies on large cohorts are actually not available (139–145). Of note, testis retention in the abdomen represents an association with DSD and represents by itself a risk factor of TC as previously discussed. Interestingly, the association between TC and cryptorchidism has been documented in DSD from both AR mutation and PMDS. Thus, a major pathogenic role of DSD-associated testis retention in TC cannot be ruled out (146, 147).

Intriguingly, available data on derangements of the upstream hypothalamus/pituitary/gonadal axis showed an inconsistent association with TC. In particular, activating and inactivating mutations of the LR-receptor (LHR) gene are causative of DSD forms like isosexual precocious puberty in boys and Leydig cell hypoplasia, respectively. The latter presents variably from normal appearing female external genitalia to hypergonadotropic hypogonadism with microphallus and hypoplastic male external genitalia (148, 149). However, few available data documented TC only in patients with isosexual precocious puberty, particularly for testicular interstitial cell tumor observed in a 9 years old boy (with no available genetic screening at the time of the analysis), and two cases of activating mutations of the *LHR* gene reporting testicular seminoma in adult life (150–152).

TESTIS CANCER: SOMETHING IN BETWEEN GENES, ENVIRONMENT, AND HORMONES

As for the majority of oncological diseases, TC is the result of a complex interaction among the aforementioned genetic, environmental, and hormonal risk factors. To this regard, testicular dysgenesis syndrome (TDS) is probably the most reliable and clinically adherent model that describes the actual pathogenesis of TC (153).

The concept of TDS derives from the observation of the worldwide increasing incidence of a cluster of male urinary-genital alterations such as infertility, cryptorchidism, hypospadias and, indeed, TC. All these clinical conditions share the common feature of originating during fetal life, during which the impaired function of Sertoli and/or Leydig cells alters the proper testis function from germ line development to hormone production. The combination of these conditions results in a range of clinical consequences in adult age,

spanning from infertility, to disorders of sexual development and cryptorchidism that, in turn, are risk factors for TC (154). In addition to the already mentioned genetic factors, there is a general agreement according to which the failure of testis nourishing cells is due to environmental factors, such as the exposure to chemical pollutants with endocrine disrupting activities (155). This hypothesis was originally suggested by the wide geographical variation observed for TDS symptoms in different countries. The most striking evidence, suggesting the possible influence of anthropogenic factors, was surely the differential prevalence of TDS-related disorders observed in two nations at equal latitude and industrialization, respectively, Denmark, with "higher" prevalence of TDS symptoms, and Finland with "lower" prevalence of TDS symptoms (156–159).

The mechanistic hypothesis of the endocrine disruption in TDS relies on two main pathways. The first one is the estrogen hypothesis, consisting of the exposure to chemicals with estrogenic properties that exert a central disruption of the hypothalamus/pituitary/gonadal axis, reducing in turn T release from the testis. Diethylstilbestrol (DES) is a clear example of an endocrine disruptor with estrogen action. Being an estrogen receptor agonist, DES was frequently prescribed to pregnant women during the 50s−60s in order to relieve abortions and pregnancy-related complications. However, males born from DES-treated mothers showed an increased incidence of epididymal cysts, altered sperm parameters, cryptorchidism, and TC (160–162). Similar to DES, the common plastic additive bisphenol A (BPA) was also acknowledged as a partial estrogen agonist (163). Exposure to BPA in males has been associated with increased levels of prolactin, estradiol, and the sex hormone-binding globulin level (164). Furthermore, higher levels of BPA in semen were associated with signs of a direct impairment of spermatogenesis such as poor sperm count and function (165).

Another suggested mechanism of endocrine disruption in males is the anti-androgen activity, typically exerted by phthalates plasticizers (166). Data obtained in cell and animal models are highly suggestive of direct influence of phthalates compounds on the endocrine function of Leydig cells, particularly impairing T and INSL3 production (167–169). Accordingly, increased prevalence of reduced anogenital distance (AGD), cryptorchidism, hypospadias, and other genital-urinary disorders were observed in male subjects from mothers exposed to this class of chemicals (170).

The list of substances with acknowledged or possible endocrine disrupting effects is continuously increasing and the exposure to these environmental agents is currently considered the major causative factor of the increasing incidence of TC throughout the last decades. However, equal exposure to the same disruptor is not univocally associated with the same phenotype of TDS, highlighting the role of the genetic background in the establishment of the susceptibility to genital-urinary disorders, in general, and to TC in particular (171). An interesting example of how genetic and environmental factors interact, determining a clinical phenotype, has been provided by our group in a very recent investigation focused on the *E2F1* gene (172). As cited above, altered *E2F1* expression has been significantly associated to several testis disorders such as spermatogenic impairment, cryptorchidism, and TC, particular in those cases of increased gene expression related to supernumerary gene copy numbers (49). Interestingly, through the use of an engineered NTERA-2 cl.D1 cell model, cultured at over-physiological temperature, the *E2F1* gene expression was up-regulated in a temperature- and gene-copy number- dependent manner. Altogether, these results suggest that the clinical condition associated with abnormal E2F1 expression, due to copy number variation, can be worsened even more by other concomitant environmental conditions, such as heat stress or an history of cryptorchidism, with a likely impact on TC development.

TABLE 1 | Genetic factors and related mechanism associated to testis cancer.

Gene	Mechanism	References
GATA4, GATA1	Specification and differentiation of postnatal testicular development	(25–31)
PRDM14, DMRT1	Germ cell specification-sex determination	(25, 32–35)
SALL4	Disruption of POU5F1 binding motif; maintenance of pluripotency in embryonic stem cells	(25, 36)
TEX14, WDR73, PMF1, CENPE, and PCNT	Microtubule and chromosomal assembly; kinetochore-microtubule assembly; microtubule organization during interphase; microtubule assembly–related genes	(25, 37–44)
KIT	KIT–MAPK signaling	(25)
FCP2L1, ZFP42	Germ cell development and pluripotency	(45)
ZWILCH	Kinetochore function	(45)
TIPIN	Response to DNA damage	(45)
TKTL1, LHPP	Mitochondrial function	(45)
E2F1	Copy number variation	(49)
INSL3, RXFP2	Cryptorchidism	(55, 56, 63–65)
AR, 17β-HSD	Cryptorchidism; steroidogenesis; disorders of sex development	(55, 56, 63, 66–68, 133, 134, 137, 138)
AMH, AMH type II receptor	Cryptorchidism; disorders of sex development	(85, 86, 139–145)
LHR	Steroidogenesis	(150–152)

CONCLUSIONS AND FUTURE PERSPECTIVES

TC is the most prevalent tumor disease in male subjects at reproductive age, showing a progressive increase throughout the last four decades. The current model that better explains this trend, based on clinical and experimental evidence, relies on the increased exposure to environmental factors, particularly chemical pollutants with endocrine disrupting activity, that alters the major hormonal axis that drives testis development and function from gestational age. The susceptibility to these alterations further depends on genetic factors that strongly justify the strong familiarity of TC (**Table 1**).

A very recent field of investigation that aims to integrate genetic and environmental factors on the risk for TC is epigenetics, namely the inheritance of genetic factors that do not rely on the variation of the genetic sequence but rather on the regulation of gene expression trough DNA methylation and histone modification. Very recent investigations showed that DNA from tumor cells display significant hypomethylation compared to normal germ cells, evidence likely due to the over-expression of de-methylating factors that are generally suppressed after fetal germ cell development (173). It is a shared opinion that environmental factors largely govern the balance between methylating and de-methylating factors (174).

Finally, it should be noted that in spite of the high sensitivity of TC to chemotherapy, explaining the good prognosis of treatment, there is still a large population of patients suffering from drug resistance or inefficient treatment settings among chemotherapy, radiotherapy, or surveillance (175). In this regard, some pioneering studies have succeeded in identifying genetic markers of good responses or tolerability to therapeutic agents, thus improving the overall outcome of treatments. This is the case for the identification of genetic variants in the *SLC16A5* gene, which has been significantly associated to ototoxicity induced by cisplatin (176).

In conclusion, the availability of novel strategies of investigation are of paramount importance to clarify the key aspects of TC development, progression and therapy, in order to further improve the prevention and treatment of a highly curable disease with an unexplained increasing diffusion.

AUTHOR CONTRIBUTIONS

AG conceived the manuscript. IC, MG, and IŠ performed bibliographic research. CF supervised manuscript draft. LD wrote most of the manuscript.

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