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A survey of etiologic hypotheses among testicular cancer researchers

Andreas Stang^{1,2}, Britton Trabert³, Carsten Rusner⁴, Charles Poole⁵, Kristian Almstrup⁶, Ewa Rajpert-De Meyts⁶, and Katherine A. McGlynn³

¹Zentrum für Klinische Epidemiologie, Institut für Medizinische Informatik, Biometrie und Epidemiologie, Universitätsklinikum Essen, Essen, Germany

²School of Public Health, Department of Epidemiology, Boston University, Boston, USA

³Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD 20852-7234, USA

⁴Institut für Klinische Epidemiologie, Medizinische Fakultät, Martin-Luther-Universität Halle-Wittenberg, Halle, Germany

⁵Department of Epidemiology, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC 27599-7435, USA

⁶University Department of Growth and Reproduction, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark

Abstract

Basic research results can provide new ideas and hypotheses to be examined in epidemiological studies. We conducted a survey among testicular cancer researchers on hypotheses concerning the etiology of this malignancy. All researchers on the mailing list of Copenhagen Testis Cancer Workshops and corresponding authors of PubMed-indexed articles identified by the search term “testicular cancer” and published within 10 years (in total 2750 recipients) were invited to respond to an e-mail based survey. Participants of the 8th Copenhagen Testis Cancer Workshop in May 2014 were subsequently asked to rate the plausibility of the suggested etiologic hypotheses on a scale of 1 (very implausible) to 10 (very plausible). This report describes the methodology of the survey, the score distributions by individual hypotheses, hypothesis group and the participants’ major research fields, and discuss the hypotheses that scored as most plausible. We also present plans for improving the survey that may be repeated at a next international meeting of experts in testicular cancer.

Corresponding author: Prof. Dr. med. Andreas Stang, MPH; Zentrum für Klinische Epidemiologie, Institut für Medizinische Informatik, Biometrie und Epidemiologie, Universitätsklinikum Essen, Hufelandstr. 55, 45147 Essen, Germany; Phone +49 201 92239-290, Fax +49-201-92239-333, andreas.stang@uk-essen.de.

Disclosure Statement

The authors have nothing to disclose.

Author contributions

AS, BT, KAM, and CR designed the survey; KA and ERM implemented the protocol and collected data from participants. Statistical analyses were conducted by AS and CP. AS wrote the first draft of the manuscript. All authors reviewed, edited and approved the final manuscript.

Overall 52 out of 99 (53%) registered participants of the 8th Copenhagen Testis Cancer Workshop submitted the plausibility rating form. Fourteen out of 27 hypotheses were related to exposures during pregnancy. Hypotheses with the highest mean plausibility ratings were either related to prenatal exposures or exposures that might have an effect during pregnancy and in post-natal life.

The results of the survey may be helpful for triggering more specific etiologic hypotheses that include factors related to endocrine disruption, DNA damage, inflammation, and nutrition during pregnancy. The survey results may stimulate a multidisciplinary discussion about new etiologic hypotheses of testicular cancer.

Introduction

Despite many previous etiologic studies of testicular cancer, the number of established risk factors for testicular cancer is limited. Well-known risk factors include a family history of testicular cancer, subfertility, cryptorchidism, hypospadias, a personal history of testicular cancer and adult height (Møller et al., 1996; Dieckmann et al., 2008; McGlynn & Cook, 2009; McGlynn & Trabert, 2012; Lip et al., 2013; Banks et al., 2013).

Many basic research studies have provided insights into the pathogenesis of testicular cancer. Results of these studies could provide new ideas and hypotheses that could be examined in epidemiological studies. However the translation of hypotheses from basic studies into formulation of epidemiological studies has been very limited. Since 1980, internationally well-known experts on testicular cancer from many fields (medical oncologists, urologists, laboratory researchers, geneticists, epidemiologists, and others) regularly come together in Denmark, for a meeting entitled “Copenhagen Workshop on Carcinoma in situ (CIS) Testis and Germ Cell Cancer” (referred to subsequently in this article as ‘Copenhagen Testis Cancer Workshop’) that includes virtually all areas of research related to testicular cancer. Although relatively small in terms of participant numbers, this workshop series is an ideal venue to conduct such a survey, because of the focus of these meetings on the pathogenesis of testicular cancer and the mix of basic researchers, epidemiologists, and clinicians among the participants (Rajpert-De Meyts & Skakkebaek, 2011).

Within the field of breast cancer, consensus approaches on several topics have been organized on an ongoing basis by major conferences. For example, the San Antonio Breast Cancer Symposium and the annual meeting of the American Society of Clinical Oncology (ASCO) have examined major breast cancer questions. Perhaps most notable, the biennial European Congress, also known as the St. Gallen conference, is ranked among the most important in the world in breast cancer. One reason for this is that on the last day of the congress, fifty well known specialists in the world (medical oncologists, surgeons, radiotherapy specialists, researchers) vote on the most important topics relating to breast cancer treatment. The results of the panel are then published after the meeting (Goldhirsch et al., 2013).

Surveys among experts in the field of testicular cancer diagnosis, treatment, or prognosis are limited, but do exist. For example, Shetty et al. recently performed a survey on the relevance

and management of testicular microlithiasis in the UK (Shetty, Bailey, & Freeman, 2014). Inspired by these approaches, we set up a survey among testicular cancer researchers who were invited to participate or attended the previous (7th) Copenhagen Testis Cancer Workshop held in October 2010.

The aims of this report are to (1) describe the methodology, as an adaptation of St. Gallen, (2) list and group the hypotheses raised in this inaugural survey, (3) describe the score distributions by individual hypothesis, hypothesis group and the participants' major research fields, (4) review available literature on the plausibility of the most highly rated hypotheses and (5) develop plans for improving the survey that may be repeated using an email approach or at a next international meeting of experts in testicular cancer.

Material and Methods

Survey step 1

All researchers on the mailing list of the previous (7th) Copenhagen Testis Cancer Workshop in 2010 and corresponding authors of PubMed-indexed articles identified by the search term "testicular cancer" and published within the last 10 years (in total 2750 recipients) were invited to respond to an e-mail based survey. The questionnaire asked respondents to identify findings from research that raise new hypotheses concerning potential risk factors for testicular cancer in a broad sense (i.e. infantile tumors, tumors of young adults, tumors of older adults, any histology). Genetic susceptibility hypotheses were explicitly excluded from the survey.

The first invitational email with a short questionnaire (sent on 11th December 2013) included an "unsubscribe" option so that people who were not willing to participate would not get any reminder emails. Those researchers who did not use the "unsubscribe" option received a maximum of two reminder emails sent on 5th March 2014 (2735 recipients) and 25th April 2014 (2723 recipients). Based on the number of recipients with an e-mail client that allows loading of images, on average 16% of the recipients opened the emails. Loading of images is however turned off by default in many newer e-mail clients like Outlook and Google Gmail. In addition, at the first day of the 2014 meeting, participants were reminded to fill in the self-administered survey questionnaire. Participants were asked to provide their major research field (clinical medicine, epidemiology, basic research, other).

Survey step 2

At the second day of the meeting in Copenhagen, the assembled list of proposed etiologic hypotheses was distributed to each participant. Participants were asked to identify their major research field and to rate the plausibility of the suggested etiologic hypotheses on a scale of 1 (very implausible) to 10 (very plausible). A subset of 20 out of 47 hypotheses was not eligible for the plausibility rating for several reasons: 9 hypotheses had been previously studied, 4 hypotheses were related to pathogenetic mechanisms that did not give any clues to etiologic factors, 3 hypotheses were proposals for specific study designs, 1 hypothesis was related solely to genetic susceptibility, and 3 respondents submitted survey forms but did not postulate any hypotheses. In this report, we include the proposed study designs as these

proposals may also trigger the discussion about the etiology of testicular cancer. We present literature reviews on hypotheses that had a mean rating score of > 5 . After the 8th Copenhagen Testis Cancer Workshop, a two-hour meeting took place to further discuss the results of the survey. A summary of this meeting is provided in the discussion section of the manuscript.

Statistical Methods

Besides the plausibility of hypotheses, individual rater characteristics may influence the plausibility rating. To assess these characteristics, we calculated the mean plausibility value across all rated hypotheses per participant as a proxy.

Results

Overall 52 of 99 registered participants (53%) of the 8th Copenhagen Testis Cancer Workshop submitted the plausibility rating form at the meeting. Three surveys were excluded as they did not rate the plausibility of any hypotheses leaving 49 completed surveys for the analysis. One survey participant evaluated only 5 out of 27 hypotheses and was excluded. Survey participants self-reported their major working field as 1) basic research (N=23) including five participants who additionally stated that they are also medical doctors, 2) clinical medicine (N=13), or 3) epidemiology (N=10). In addition, two participants self-reported they were doctoral students and did not specify their major research field.

The majority of proposed etiological hypotheses (14 out of 27) were related to exposures during pregnancy. In addition, 2 hypotheses were related to exposures among the ancestors and 3 hypotheses were related to factors that may act during pregnancy and thereafter. The majority of hypotheses (18 out of 27) were accompanied by proposed mechanisms or evidence. For 8 hypotheses, no mechanism or evidence was given and one hypothesis was based on a case report only. Some of the hypotheses overlapped with others. For example, sugar consumption during pregnancy was independently raised by two respondents. In addition, in-utero exposure to low dose DNA damaging agents was raised two times with slightly different connotations. Some hypotheses were rather unspecific including a hypothesis concerning nutrition during pregnancy and a hypothesis concerning exposure to/impact of inflammatory processes. One participant rated 9 hypotheses with a value of zero although the pre-specified scale had a range of 1-10. We interpreted this rating as “very implausible” (value of 1). Hypotheses with the highest mean plausibility ratings were either related to prenatal exposures or exposures that might have an effect during pregnancy and in post-natal life. Among these, exposures to DNA damaging factors and to endocrine disruptors were rated as the most plausible hypotheses (Table 1).

Fig. 1 displays the distribution of average plausibility scores per participant which is unimodal and has a mean of 4.3 (standard deviation: 1.2) with a range of 1.6 to 7.4. Fig. 2 reveals that individual rater characteristics were associated with the self-reported major research field (labelled: field). In comparison to clinical researchers (mean score: 3.8), basic researchers (mean score: 4.5) and epidemiologists (mean score: 4.8) tended to provide higher average plausibility scores than clinicians. A linear regression analysis revealed that

basic researchers and epidemiologists rated on average 0.70 units (standard error [SE] 0.37) and 0.92 units (SE 0.45) on the scale of 1-10 higher than clinicians.

Figures 3a-3d display the mean plausibility scores by hypothesis and major research field of the rater. Among 15 out of 27 hypotheses, epidemiologists gave the highest plausibility ratings on average whereas clinicians tended to give the lowest plausibility ratings on average.

Discussion

Our study had a mixture of approaches: a quantitative plausibility rating and a qualitative approach experience with a survey among participants from several research areas. We made several observations while conducting the survey. First, whereas the pre-congress response to the mailed survey was low (17 hypotheses), participants at the workshop in Copenhagen provided another 30 hypotheses. Most participants were interested in the results of the plausibility rating presented on the third day of the meeting. One basic researcher reported that he/she had never previously thought about testicular cancer etiology.

The mean values of plausibility ratings of the participants depended on the self-reported major research field with clinicians providing the lowest mean plausibility ratings. It is believed that plausibility judgment involves some assessment based on consistency with prior knowledge that determines how plausible people judge hypotheses or statements (Connell & Keane, 2004). Therefore, it is no surprise to observe that average plausibility ratings depended on the major research field of the participants as each field carries its own prior knowledge. In addition, plausibility judgments as all judgments are prone to several biases (Kahnemann, 2011).

Although an extensive review of all hypotheses suggested is outside the realm of the current report, brief summaries of the background of the hypotheses with mean ratings >5 are provided below.

***In utero* DNA damage through radiation or cyclophosphamide**

The proposed mechanism associated with the hypothesis was stimulated by results of murine experiments. These studies have reported increased incidence of testicular germ cell tumors (TGCT) in genetically predisposed 129.MOLF (L1) congenic mice after *in utero* exposure to radiation during days 10-11 (Shetty, Comish, Weng, Matin, & Meistrich, 2012). The results suggest that radiation exposure of humans at gestational weeks 5-6 might influence risk of TGCT. Similarly, exposure to cyclophosphamide during gestation increased the incidence of TGCT in the male offspring of 129.MOF (L1) congenic mice, suggesting that DNA damaging chemotherapeutic agents *in utero* may increase risk of TGCT in humans (Comish et al., 2014). Further, exposure of C18-4 spermatogonial cells to bleomycin, etoposide, cisplatin or 4-hydroperoxycyclophosphamide (4OOH-CPA), a pre-activated analog of cyclophosphamide, affects telomeres indicating that telomere dysfunction could contribute to infertility and developmental defects (Liu, Hales, & Robaire, 2014).

A literature review found no human studies that evaluated the association between *in utero* exposure to radiation or cyclophosphamide and risk of TGCT. Studies of radiation exposure in humans have been confined to occupational exposure among adult males (Yousif, Blettner, Hammer, & Zeeb, 2010) or to therapeutic exposure to cyclophosphamide or alkylating agents.

The hypotheses on in-utero DNA damage will not be easy to evaluate by epidemiologic studies as the prevalence of *in utero* exposure to radiation or cyclophosphamide is very low. Furthermore, murine tumors closely resemble the infantile types of TGCT, thus extrapolation to TGCT among human adult males is unclear.

Maternal nutrition including fat consumption and elevated insulin levels

A growing body of research indicates that excess body fat and increased insulin levels may be directly related to cancer risk (Taubes, 2012). Biological mechanisms may provide metabolic links, including insulin resistance and reduced glucose tolerance, increased activation of the growth hormone/IGF-I axis, alterations in sex-steroid synthesis and bioavailability, and low-grade chronic inflammation. A 'Western' lifestyle is characterized by low levels of physical activity, and a diet rich in energy-dense food with a high percentage of nutritional energy from (saturated) fats, sugars and other refined carbohydrates. Furthermore, the 'Western' diet is characterized by a high proportion of dietary protein of animal origin (Dossus & Kaaks, 2008). Previous studies on risk factors for testicular cancer did not stress this 'Western' lifestyle/nutritional pattern and focused primarily on dietary patterns during early life and adulthood but not on maternal diet (McGlynn & Trabert, 2012). Studies investigating the risk of congenital anomalies related to TGCT, (cryptorchidism and hypospadias), in association with maternal diet and gestational diabetes have provided contradictory results (Pierik et al., 2004; Virtanen et al., 2006; Giordano et al., 2008; Trabert et al., 2014). Furthermore, it remains unclear how epigenetic events in male germ cells triggered by maternal diet and gestational diabetes would influence the etiology of TGCT (Godmann et al., 2009).

Exposure to endocrine disrupting chemicals

A number of studies have evaluated associations between select endocrine disrupting chemicals (EDCs), principally organochlorine compounds, and the conditions of the Testicular Dysgenesis Syndrome (TDS). While support has been found for a link between EDCs and TGCT, studies of EDCs and cryptorchidism or hypospadias have been less convincing (reviewed in Cook et al., 2011). The current evidence suggests that p,p'-dichlorodiphenyldichloroethylene (DDE) and chlordane-based pesticides are positively associated with TGCT risk (reviewed in McGlynn & Trabert, 2012), however these associations are primarily based on studies measuring EDC concentrations in men, rather than maternal concentrations during pregnancy. A study from Sweden that examined maternal concentrations of DDE and chlordanes at the time of the sons diagnoses, however, found no associations with either compound (Hardell et al., 2003). The current weight of the evidence does not support an association between maternal levels of organochlorine compounds and cryptorchidism or hypospadias (reviewed in McGlynn & Trabert, 2012; Trabert et al., 2012). Most EDCs, however, have yet to be evaluated. EDC research will

need to assess these chemicals in the most appropriate specimen to ensure accurate exposure assessment of EDCs during pregnancy (i.e., serum for lipophilic compounds, serial urine samples for plasticizers and other EDCs with short half-lives, as well as placenta). To our knowledge there are currently no studies evaluating placental concentrations of EDCs and risk of any TDS condition. Further, effect modifications of the EDC-TDS associations by potential genetic factors or inflammatory processes have not been evaluated to date.

Other maternal exposures, such as smoking during pregnancy, have been associated with impaired sperm production, however, associations with testicular cancer, cryptorchidism or hypospadias have been weak or null (Virtanen et al., 2012). *In utero* exposure to antiepileptic drugs or aromatase inhibitors are exceedingly rare exposures and have not been studied to date.

Summary of the two-hour post-workshop meeting

After the conclusion of the Workshop, interested participants attended a 2-hour meeting to critically discuss the proposed hypotheses and to imbed these hypotheses into new epidemiologic outlines. In summary, this meeting revealed: first, a majority of proposed hypotheses in our survey were related to factors operating during pregnancy. As pathways of migration, differentiation and sex determination are most likely involved in the etiology of testicular cancer, the study of placental function and its influence on testicular pathology may be informative. Second, due to the presumed temporal relation between potentially harmful *in utero* exposures (especially 1st trimester) and occurrence of invasive testicular cancer at ages 15-44 years and the low incidence of testicular cancer, prospective cohort studies that address *in utero* risk factors are statistically inefficient. Case-control studies at the time of testicular cancer diagnosis do not enable a reliable measurement of exposures during the first trimester of the mothers of cases. Therefore, an alternative may be the focus on intermediate outcomes associated with 1st trimester exposures including pathologies of the testes such as testicular hypotrophy, and congenital anomalies such as cryptorchidism, hypospadias and inguinal hernia. The study of all TDS conditions in concert (cryptorchidism, hypospadias, subfertility and TGCT) may also permit the insights from condition to inform the study of the other conditions.

Two proposals that focused only on epidemiologic study designs were submitted in the survey and discussed at the 2-hour meeting. As these proposals did not contain etiologic hypotheses, they were not evaluated by the meeting participants. A cohort study that only enrolled adolescents or men with prior cryptorchidism, prior hypospadias, low sperm count, low sperm quality or infertility, (i.e. men with TDS), would allow the study of other risk factors as some of these patients will develop testicular cancer. Alternatively, a cohort study that only included men with a history of testicular cancer might enable the study of factors that are related to the occurrence of a contralateral testicular cancer. In this regard, some cohorts of testicular cancer survivors have already been established (Beard et al., 2013; Brabrand et al., 2012; van Leeuwen et al., 2014).

Study of hypogonadotropic hypogonadism, e.g. Kallmann syndrome, would be of interest as it is associated with cryptorchidism but not with TGCT (Ginsburg, 1997). Similarly, a study

of children undergoing organ transplantation and immunosuppression may provide insights as there may be at increased risk of TGCT as the immune system is involved in the control of cancer. Given the rarity of these conditions, studies of this nature appear to be only feasible if they are conducted as multicenter and/or multinational studies.

Genome-wide association studies (GWAS) have already provided clues to genetic susceptibility to testicular cancer.. At present, GWAS studies have only focused on index persons but not on their relatives. A number of studies in different countries have biospecimens of first-degree relatives of affected men so a GWAS of these samples might prove informative.

It is widely believed that the development of testicular germ cell cancer among adolescents and young men (age 15-44 years, type II germ cell cancers (Oosterhuis & Looijenga, 2005)) depends on several hits. For example, Brody recently used the multistage model for carcinogenesis model to fit the age-specific incidence rate of invasive testicular cancers (Brody, 2011). He found that the age-specific incidence is best described by a multi-hit model with four mutations. It is conceivable that some hits occur during embryogenesis and fetal period and some occur during childhood, adolescence and early adulthood.

Limitations

Several factors limit our results. First, only 99 scientists joined the 8th Copenhagen Testis Cancer Workshop and this sample is unlikely a representative sample of all researchers in the field of testicular cancer etiology. Second, our study suffered from nonresponse and it is unclear whether nonresponse bias was a factor. Third, participants of the survey had only limited time to think about the hypotheses and to rate the suggested hypotheses at the Workshop itself. In the discussion of the survey results on the day after the workshop, participants indicated that they might have rated hypotheses differently if they were provided more background and more time to consider them. Fourth, scientists may have been reluctant to provide etiologic hypotheses in order to protect their “favorite” hypotheses. Lastly, not many surveys have been conducted within the field of testicular cancer before and researchers might therefore be reluctant to participate as they are unfamiliar with the process.

Conclusions

In conclusion, the survey provided a good opportunity to collect new hypotheses concerning the etiology of testicular cancer. The scientific community, as represented by participants in a small but focused testis cancer meeting, rated highly the hypotheses related to *in utero* exposures although some of the proposed hypotheses suffered from lack of specificity and others suffered from lack of proposed biological mechanisms. The results of the survey, however, may be helpful for triggering more specific etiologic hypotheses that include factors related to endocrine disruption, DNA damage, inflammation, and nutrition during pregnancy. The survey and its results may be helpful to stimulate a multidisciplinary discussion about new etiologic hypotheses of testicular cancer.

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Figure 1.

Average plausibility scoring of 27 etiologic hypotheses among participants of the survey
Plausibility of hypotheses was rated on a scale of 1 (very implausible) to 10 (very plausible).

Figure 2.

Association between major research fields of participants and individual rater characteristics (that is average plausibility score of the rater)

The average plausibility score of the participant is considered to be an individual rater characteristic; the whiskers are drawn to the most extreme points in the group that lie within the fences. The upper fence is defined as the third quartile (represented by the upper edge of the box) plus 1.5 times the interquartile range (IQR); the lower fence is defined as the first quartile (represented by the lower edge of the box) minus 1.5 times the interquartile range; the horizontal lines within boxes indicate means and the dots indicate medians. Plausibility of hypotheses was rated on a scale of 1 (very implausible) to 10 (very plausible).

Figure 3a.

Nutritional factors during pregnancy: average plausibility scores for each hypothesis and subgroup of major research fields of raters

Dots indicate mean values, whiskers indicate mean $\pm$ one standard deviation (SD); labels of the hypotheses are taken from Table 1; plausibility of hypotheses was rated on a scale of 1 (very implausible) to 10 (very plausible)

Figure 3b.

Other exposures during pregnancy: average plausibility scores for each hypothesis and subgroup of major research fields of raters

Dots indicate mean values, whiskers indicate mean $\pm$ one standard deviation (SD); labels of the hypotheses are taken from Table 1; plausibility of hypotheses was rated on a scale of 1 (very implausible) to 10 (very plausible)

Figure 3c.

Exposures related to index persons: average plausibility scores for each hypothesis and subgroup of major research fields of raters

Dots indicate mean values, whiskers indicate mean $\pm$ one standard deviation (SD); labels of the hypotheses are taken from Table 1; plausibility of hypotheses was rated on a scale of 1 (very implausible) to 10 (very plausible)

Figure 3d.

Preconceptional factors among ancestors and exposures related to both, mothers and index persons: average plausibility scores for each hypothesis and subgroup of major research fields of raters

Dots indicate mean values, whiskers indicate mean $\pm$ one standard deviation (SD); labels of the hypotheses are taken from Table 1; plausibility of hypotheses was rated on a scale of 1 (very implausible) to 10 (very plausible)

Table 1

Etiologic hypotheses and plausibility ratings (in descending order of the mean plausibility rating within hypothesis group)
Plausibility of hypotheses was rated on a scale of 1 (very implausible) and 10 (very plausible).

Mean rating (SD)	Short title of the hypothesis	Hypothesis	Proposed mechanism or evidence
Preconceptional factors among ancestors			
4.1 (2.0)	FATHER	Sedentary occupations, age, smoking and alcohol consumption, chemicals like acrylamide	carcinoma <i>in situ</i> of the testis originate from DNA damage in the sufferer's father or paternal grandfather
3.3 (2.0)	PAT-SMOKE	Preconceptional paternal smoking	paternal smoking increases the risk of some cancers in the off-spring
Nutritional factors during pregnancy			
5.7 (2.4)	NUTRITION	Nutrition during pregnancy	not provided
5.4 (2.2)	INSULIN	High insulin level	the incidence of testicular cancer in their off-springs is lower, when women starved during pregnancy
5.2 (2.2)	FAT	High fat & trans-fat animal meat (cattle) consumption, fried foods, and processed foods with high sugar content	this diet may lead to low-level inflammation, disruption of numerous lipid-regulated signaling pathways, including arachidonic acid- and prostaglandin-regulated pathways in the testis
4.1 (2.2)	FOLATE	Folate deficiency	the relative frequency of spina bifida occulta is increased among testicular cancer patients
3.9 (2.4)	SUGAR	Sugar consumption (also perinatal)	components of sugar as fructose have known toxic effects
3.6(2.1)	ANTIOXIDANTS	Increased exposure to antioxidants	antioxidants can have a pro oxidant effect that can result in DNA damage of the germ line
Other exposures during pregnancy			
6.1 (2.4)	EDA	Exposure to endocrine disrupting agents in the placenta	not provided
5.6(2.5)	SYNERGISM	Synergism between endocrine disruptors and exposure to/impact of inflammatory processes	not provided
5.5 (2.5)	DNA DAMAGE	Exposures as radiation, cyclophosphamide and environmental pollutants, e.g. acrolein	these exposures may damage DNA. DNA damaging agents in utero among mice (129 teratoma model) have shown to be associated with an increased risk of teratoma (see Shetty et al., 2012; Comish et al., 2014)
5.5 (2.5)	RADIATION, CPA	Exposures to low doses radiation or cyclophosphamide at the time when primordial germ cells are undergoing extensive epigenetic changes	these exposures may damage DNA (see above) as seen by mouse models
5.0 (2.1)	SEPSIS-INFECT	Sepsis or infection pre-eclampsia, or exposure to medications (e.g. serotonin inhibitors) (also postnatal)	these factors may have an impact on activin or the TGF beta superfamily pathway signaling
4.9 (2.3)	INFLAMM	Exposure to/impact of inflammatory processes	not provided
4.8 (2.2)	ANALGESICS	Mild analgesics	antiandrogenic effects in animal experiments; birth cohort studies showed an increased risk of cryptorchidism (see Kristensen et al., Human Reproduction 2011)
3.8 (2.2)	OCHRATOXIN	Ochratoxin exposure	not provided

Mean rating (SD)	Short title of the hypothesis	Hypothesis	Proposed mechanism or evidence
Exposures related to index persons			
4.4 (2.2)	OBESITY	Plentiful nutrition in childhood; childhood obesity	it causes early adolescence with rapid growth of germ cell numbers causing mutations to occur in susceptible individuals and the development of ITGCN/CIS shortly after puberty
4.3 (1.9)	INFECT	Deprivation from exposure to infectious agents during early childhood	this deprivation is known to be associated with "immune deviation", i.e. atopic diseases which may enhance the risk of testicular germ cell tumors
3.8 (2.3)	MICROBIOME	Microbiome of the testis	not provided
3.6(2.1)	SUN	Sun exposure	not provided
3.2 (1.8)	HSV	HSV 1 & 2 serological status	
3.2 (2.0)	SEDENTARY	Sedentary lifestyle +/- seasonal affective disorders	sedentary lifestyle may cause increased scrotal temperature
2.5 (1.9)	LAPTOP	Frequent use of laptop computers balanced on the legs and therefore close to the scrotum	Scrotal hyperthermia is a documented risk factor for infertility and infertility is associated with germ cell tumors; laptops are also a source of non- ionizing radiation
2.0 (1.3)	BATTERY	Close contact to dry battery as a cause of seminoma	case report
Exposures related to both, mothers and index persons			
5.4 (2.4)	XENOESTR	Transplacental, early life and prepubertal exposure to xenoestrogens in food and air or exposure to aromatase inhibitors (cotinine if mothers smoke during pregnancy or intake of antiepileptic drugs)	not provided
4.6(2.1)	DERMAL ABS	Dermally absorbed chemicals or pharmaceuticals among men with atopy or pregnant women with atopic dermatitis or filaggrin gene mutations	women with atopic dermatitis or filaggrin gene mutations are more frequently exposed to dermally absorbed chemicals or pharmaceuticals; their sons may have high exposure to these compounds; men with loss of function of filaggrin mutations have higher concentrations of phthalate metabolites; atopy and cryptorchidism are associated in infertility clinics; patients with testicular cancer have a higher prevalence of atopy; testicular cancer patients with atopy have lower testosterone and estradiol levels and higher LH levels than health controls (see Joensen et al., 2014)
3.1 (2.2)	SEASON	Season of birth	there is increasing evidence from date of birth studies in causation of multiple sclerosis and schizophrenia; stage of germ cell migration may be influenced by season