Pathology & Oncology Research (2020) 26:2057–2066
https://doi.org/10.1007/s12253-019-00765-3

REVIEW

Review: Ewing Sarcoma Predisposition

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Received: 28 June 2019 / Accepted: 10 October 2019 / Published online: 26 October 2019

Abstract

Ewing sarcoma is a rare tumor developed in bone and soft tissues of children and teenagers. This entity is biologically led by a chromosomal translocation, typically including EWS and FLI1 genes. Little is known about Ewing sarcoma predisposition, although the role of environmental factors, ethnicity and certain polymorphisms on Ewing sarcoma susceptibility has been studied during the last few years. Its prevalence among cancer predisposition syndromes has also been thoroughly examined. This review summarizes the available evidence on predisposing factors involved in Ewing sarcoma susceptibility. On the basis of these data, an integrated approach of the most influential factors on Ewing sarcoma predisposition is proposed.

Keywords Ewing sarcoma · Cancer predisposition · Genetic susceptibility · Polymorphism

Abbreviations

ES	Ewing sarcoma
CNV	Copy number variations
CPS	Cancer predisposing syndromes
MSC	Mesenchymal Stem Cell
AACR	American Association of Cancer Research
LFS	Li-Fraumeni Syndrome
WGS	whole genome sequencing
WES	Whole exome sequencing
NGS	Next generation sequencing
RB1	RB transcriptional corepressor 1
BLM	Bloom Syndrome RecQ Like Helicase gene; Bloom syndrome gene
RET	RET Proto-Oncogene
GENESIS	Genetics of Ewing Sarcoma International study

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Introduction

Ewing sarcoma (ES) is an aggressive and rare tumor developed usually in bone, but sometimes in soft tissues as well [1], whose incidence is estimated to be 1.2 cases/million in U.S. [2]. White people have higher incidence than black and Asian people [3, 4], and there is a peak between 5 and 24 years old [1, 5–13].

Chromosomal translocation between TET and ETS genes is the best known and the most important molecular event in ES. Most of cases present a balanced reciprocal chromosomal translocation (t(11;22)(q24;q12)), which results in *EWS/FLI1* oncogenic gene fusion [14]. Fusion protein EWS/FLI1 acts as a pathogenic transcription factor and determines tumor development [15–22]. The chromatin remodeling event mediated by EWS/FLI1 leads to gene activation and repression [22]. GGAA microsatellite regions are the binding site of EWS/FLI1 [23–27]. The oncogenic transcription program mediated by EWS/FLI, up-regulates and down-regulates thousands of genes [15, 16].

Some copy number variations (CNV) (gain of chromosome 1q, 8, 12 and loss of 9p21 and 16q) [28–30] and gene mutations (in *STAG2, TP53* and *Rb1*genes) are recurrent in ES [31, 32], but not as unfailing as the chromosomal translocation. Thus, single nucleotide variants in genes commonly related to cancer have a minor role in ES. Interestingly, this fact coincides with the remarkable absence of ES among pediatric cancer predisposing syndromes (CPS). In the same line, the genes implicated in classic CPS have been rarely related to ES

predisposition. Additionally, no clear ES incidence clustering has been reported among families [33]. However, an increased risk of several cancers besides ES, among patients and their relatives (first, second and third relatives), has been described [34].

Ewing's sarcoma cell of origin is not well described and consensus about it is lacking. However, the Mesenchymal Stem Cell (MSC) has been proposed as the most acceptable possibility [35–40]. Amaral et al. described that MSC in ES patients did not carry either *EWSR1-FLI1* gene fusion, or other *EWSR1* gene rearrangements [41]. Therefore, this study did not support the presence of pre-malignant clones in the cell of origin, as happens with MLL rearranged pediatric leukemia, which could be present pre-birth [42]. Moreover, the debate about the existence of a microenvironment that promotes tumor development from MSC is still in course. Furthermore, the molecular steps that conditions tumor development until achieving a complete ES phenotype have not been described [43]. Therefore, ES origin cell uncertainty and the confusion about initial steps of tumor development, make the study of environmental and molecular events that conferring ES risk, difficult. In fact, not much is known about environment influences on ES susceptibility. Environment suspected contributions are also here reviewed.

Ethnic distribution and family cancer aggregation among ES patient and their relatives, have inspired the study of contributing polymorphisms to ES risk. Several collaborative groups are focused on the study of GGAA microsatellite polymorphic heterogeneity among ethnicities. A correlation between the number of GGAA repeats in concrete genomic regions and ES susceptibility and prognosis have been proposed. Other common polymorphisms (*CD86* rs1129055-A) and their role in ES risk have been studied as well. Intriguing results are available in this field and will be discussed below.

In summary, there is an intense discussion around the risk factors for ES development and predisposition. The lack of awareness about them, limits a directed screening to detect predisposed children. Thus, the following questions are still on the stand: Are *there predisposing gene mutations? If yes, which are them? Do genetic predisposing polymorphisms really exist in Ewing sarcoma? How much influence do they have? What do we know about environmental risk factors? Must we discard their role?* This fascinating field is reviewed. Fig. 1.

Ewing Sarcoma Predisposition

Are there Predisposing Gene Mutations?

ES is not part of cancer predisposition syndromes. Nevertheless, some of them develop sarcomas, an exceptionally, Ewing sarcoma family of tumors. During the past few years, a compilation guide of cancer predisposing syndromes has been published in *Clinical Cancer Research* journal. This was an effort developed under the shelter of American Association of Cancer Research (AACR) [44–59].

Some classic cancer predisposing syndromes deserve a special attention because of ES or other sarcomas were reported previously among them. Moreover, heterozygous rare variants in genes associated to recessive inheritance syndromes, can condition an increased cancer of risk. Relation between these mono-allelic variants and ES is also here considered. Common genes which require deeper investigation about their implication in ES predisposition are reviewed through next lines.

Ewing Sarcoma and Cancer Predisposition Syndromes

Li Fraumeni Syndrome (LFS)

Li-Fraumeni Syndrome (LFS) is an uncommon predisposing cancer disease transmitted by autosomal dominant inheritance. Mutations in *TP53* are responsible for most of cases. *CHEK2* and *POT1* mutations have also been implicated in LFS [60–68]. Association among soft-tissue sarcomas, breast cancer, and other neoplasm was firstly described by Li and Fraumeni in 1969 [69]. The most frequent tumors in LFS are soft tissue sarcomas, osteosarcoma, breast cancer, brain tumors, leukemia and adrenocortical carcinoma (#151623 OMIM) [70, 71]. In spite of the large spectrum of tumors described in Li-Fraumeni syndrome, ES has been rarely reported, and consequently, based on large cohorts, ES would not be considered part of Li-Fraumeni clinical spectrum [70, 71].

Curiously, in the current context of next generation sequencing technologies development, germline genetic variants from ES patients have been studied around the world. Personalized medicine projects in pediatric oncology studied somatic and germline variants in relapsed patients during these past years. These studies included ES patients, and found new mutations which probably predispose this and other pediatric cancers [72–79]. Moreover, St Jude Research Hospital led germline studies in pediatric oncology patients and published their results [80] and Brohl et al. reported germline sequencing results in a large Ewing sarcoma cohort in 2017 [81]. Therefore, the knowledge derived from all these studies did not come from Li-Fraumeni family cohorts but from ES patients whose *TP53* gene was sequenced in blood, and in most cases, without clinical suspicion of Li Fraumeni syndrome. From St Jude, Zhang et al. studied germline of 46 ES patients, and four of them carried *TP53* germline pathogenic variants (8,7%) [80]. In addition, Brohl et al. studied germline from 175 patients affected by ES and sequenced whole genomes or exomes (WGS/WES). They detected pathogenic or likely pathogenic germline mutations in 13.1% of

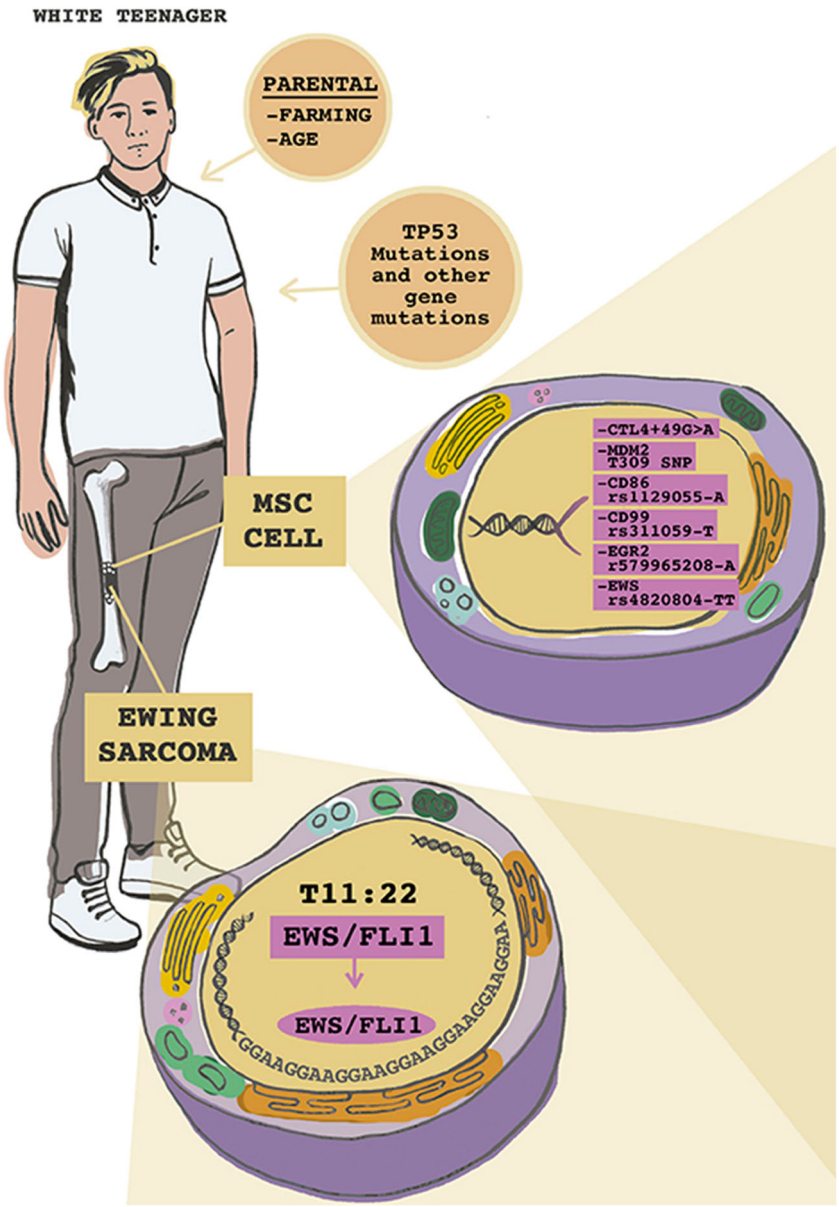

Fig. 1 multifactorial predisposition to Ewing sarcoma. Ewing sarcoma typically arises in bone and soft tissues of white teenagers and is less prevalent between other ethnicities and ages. Polymorphic variabilityin GGAA microsatellite repeats has been proposed responsible of this. Moreover, many other polymorphic variants among populations could bepredisposing. The presence of these variants or maybe their interaction may facilitate the Ewing sarcoma development from progenitor cell. Environmentand parental age might be conditioning offspring epigenomics. Epigenomic marks in specific genome regions could increase ES risk. TP53 and other genescould be mutated among few Ewing sarcoma patients and predispose to Ewing sarcoma family of tumors.

their cohort. Concretely, only one patient carried on a pathogenic variant in *TP53* (*TP53* p. R151C) [81]. The most relevant Personalized Medicine projects in pediatric oncology did not report *TP53, CHEK2* or *POT1* germline mutations among the studied Ewing sarcoma patients [72–79].

In conclusion, information coming from large cohorts of Li-Fraumeni families and recent data from NGS studies is controversial, so this field requires more research. Hence, although *TP53* mutations could be present in around 5–10% Ewing sarcoma tumors, their role in ES predisposition is not well characterized [82]. In fact, if some ES patients carried on deleterious *TP53* variants which may be predisposed to ES, why there are not any families affected by ES in successive generations? Why ES does not appear repeatedly among Li-Fraumeni patients? We must keep in mind that ES is a rare entity, exceptionally associated with *TP53* pathogenic variants. We should consider that few of them arrived healthy to reproductive ages and transmitted a genetic syndrome (whose penetrance is not complete, and its clinical story is very heterogeneous). Therefore, we cannot discard a predisposing role of *TP53* in ES.

Li-Fraumeni phenotype is modified by several genetic and epigenetic marks. In addition, some polymorphic variants in *TP53* or *MDM2* genes were proved to be important for LFS phenotype [83–91]. Based on this knowledge, Thurow et al. studied the influence of *TP53* Arg72Pro and *MDM2* T309G SNPs in ES risk, but independently of Li Fraumeni presence. They found a significant association between the G allele of *MDM2* T309G SNP and ES risk [92]. No associations regarding the Arg72Pro SNP were found in their work. *MDM2*

T309G SNP should be prospectively studied among ES patients, independently of *TP53, CHEK2* or *POT1* mutational status.

Studying *TP53* is mandatory when considering ES germline approximations. Although not enough scientific data associates *TP53* mutations with ES risk, the study of germline *TP53* deleterious variants should be translated to ES patients/parents for genetic counseling adapted to family risk.

Retinoblastoma Predisposition Syndrome

Hereditary retinoblastoma patients carry on a germline mutation in *Rb1* gene. This predisposes Retinoblastoma, but also, increases the risk of developing a second primary tumor [56]. *Rb1* is recurrently mutated among ES tumors [31, 32], and therefore, it justified the study of ES incidence among *Rb1* mutated carriers. The study of large patients cohorts have demonstrated that patients with *Rb1* mutations presented an increased risk of soft tissue sarcomas, even in not irradiated patients [93]. Leiomyosarcoma was proved the most frequent second primary sarcoma in these patients [93]. In addition, the risk of developing Osteosarcoma and other soft tissue sarcomas as second primary tumors is higher in *Rb1* mutation carriers. However, Ewing sarcoma was an exception in these series [94]. Therefore, *RB1* gene has not been related to Ewing sarcoma family of tumors predisposition up to now. It would not be routinely studied in germline among ES patients.

Bloom Syndrome

Bloom syndrome is an autosomal recessive disorder due to *BLM* (Bloom Syndrome RecQ Like Helicase gene; Bloom syndrome gene) mutations [47, 95]. Leukemia and lymphoma are the most frequent cancers in Bloom Syndrome. The cancer distribution is similar to the general population, but cancer occurs at younger ages. Sarcomas were as well described, but significant increased risk has not been demonstrated among patients [95]. Additionally, no data support an increased risk of cancer among heterozygous *BLM* mutated carriers [96]. However, Brohl et al. detected 1 pathogenic or probably pathogenic variant in *BLM* among sequenced Ewing patients [32]. More contrasted data are necessary on this variant. We consider important discard pathogenic variants in this gene when studying ES germline.

Fanconi Anemia

Fanconi anemia is an autosomic recessive disorder has been associated, until now, with 19 genes that encode Fanconi anemia complementation group proteins (*FANCA, FANCB, FANCC, BRCA2, FANCD2, FANCE, FANCF, FANCG, FANCI, BRIP1, FANCL, FANCM, PALB2, RAD51C, SLX4, ERCC4, RAD51, BRCA1, UBE2T*) [47, 97]. Besides, pathogenic variants in *FANCB* conditions X-linked recessive inheritance and *FANCR* mutations an autosomal dominant heredity. Fanconi Anemia predisposes to hematologic disorders through childhood and to solid cancers (mainly oral carcinomas) afterwards. Solid tumors have been reported in childhood only exceptionally [98]. ES is not a frequent cancer in Fanconi.

Interesting data derived from next generation sequencing studies revealed ES carriers of heterozygous mutations in Fanconi anemia genes. Parson et al. reported a *BRCA2* mutation in a patient affected by ES. Short stature, thrombocytopenia and mild anemia were present in this patient as well [75]. Brohl et al. reported pathogenic or likely pathogenic heterozigous germline variants in *BRCA1, FANCC, FANCM* genes [32]. More information is needed to draw definite conclusions on the implication of Fanconi genes in ES predisposition.

Constitutional Mismatch Repair Deficiency Syndrome (CMMRD)

Constitutional mismatch repair deficiency syndrome depends on biallelic mutations in any of the four mismatch repair genes *MSH2, MSH6, MLH1*, or *PMS2* [48]. This disease has many phenotypic similarities with NF1. The spectrum of CMMRD-associated childhood malignancies includes high-grade Glioma, Acute Myeloid Leukaemia and Rhabdomyosarcoma, all of them also described in NF1 [99–101]. Real impact in Ewing predisposition is not yet known [32, 80]. Zhang et al. and Brohl et al. reported one heterozygous germline variant in *PMS2* among ES patients. No other information on heterozygous carriers and ES risk has been previously described. More details about this field are required.

Multiple Endocrine Neoplasia Type 2A (MEN2A)

MEN2A syndrome is an inherited cancer syndrome, usually caused by an oncogenic RET protein activation. In contrary, *RET* mutation carriers do not develop ES, Zhang et al. and Brohl et al. detected the same germline *RET* variant (*RET* p.L790F) among Ewing patients [32, 80]. MEN2A patients develop Medullary Thyroid Carcinoma, Pheochromocytoma, and parathyroid hyperplasia but no other tumors.

Kawai et al. found tissue specificity for tumor development in a transgenic mice model expressing mutated *RET* Proto-Oncogene. Mice developed only tumors described in MEN2A patients. The study demonstrated failed RET dimerization in no affected tissues. Despite this knowledge, we cannot rule out that *RET* variant (*RET* p.L790F) could have an effect Ewing predisposition [102].

To conclude, ES has not been considered part of predisposing cancer syndromes. Nevertheless, next generation

sequencing studies are opening new questions that demand more attention. At least, pathogenic variants in *TP53* and Fanconi anemia genes should be studied prospectively in ES patients.

Do Genetic Predisposing Polymorphisms Really Exist in Ewing Sarcoma?

The relationship between polymorphic variants in genes implicated in ES biology and their role in susceptibility have been studied. Positive relation between some polymorphisms and Ewing sarcoma risk has been reported. CD99 is a cell surface molecule with critical relevance for the pathogenesis of ES. High expression of CD99 is a common and distinctive feature of ES cells [103]. The CD99 rs311059-T variant was significantly associated with ES onset in Italian pediatric patients (odds ratio [OR] =3.9 $p = 0.0029$) [103]. Furthermore, single nucleotide polymorphisms (SNPs) in *EWS* breaking region were studied, in order to analyze Es's susceptibility. The rs4820804-TT SNP was proposed as a candidate marker in ES risk. This polymorphism increases the chance of having a chromosome break, and thus, increases the chances for a translocation to occur [104].

Other gene polymorphisms were previously related to cancer risk, and based on that, several groups looked for their role in ES susceptibility. CD86 (B7–2) may affect cancer susceptibility by modulating T cell response. *CD86* rs1129055-A (*CD86* 1057G > A) allele has been associated to ES risk in Chinese population (odds ratio [OR] =2.12; $p = 0.021$) [105]. Additionally, *CTLA-4* + 49G > A gene variant has been strongly associated with Ewing's sarcoma and Osteosarcoma risk (for ES odds ratio [OR] = 1.36 $p = 0.000$) [106].

Genome-wide association studies have identified ES susceptibility variants in different loci during past years. Postel-Vinay et al. reported in 2012 an increased ES risk associated with 1p36.22, 10q21 and 15q15 loci. They found positive correlation between rs9430161 (upstream of *TARDBP*), rs224278 (upstream of *EGR2*) and rs4924410 polymorphism at 15q15 and ES risk. These major risk haplotypes were less prevalent in Africans [107]. *EGR2* has been proposed as a target gene for EWSR1-FLI1. In fact, *EGR2* knockdown inhibits proliferation, clonogenicity and spheroidal growth in vitro and induced regression of ES xenografts [108]. Based on this information, Grunewald et al., evidenced that the A-allele of rs79965208 in *EGR2* is significantly associated with ES risk [108]. The EWSR1-FLI1 oncogenic transcription factors binds DNA at GGAA motifs, and therefore, the number of GGAA motifs near *EGR2* may condition EWSR1-FLI1/*EGR2* interaction. Interestingly, the A-allele of rs79965208 in *EGR2* increases the number of consecutive GGAA motifs and thus the EWSR1-FLI1-dependent enhancer activity [108]. That might partially explain prevalence differences between populations (Table 1).

More recently, Machiella et al. performed a genome-wide study in ES cases and controls of European ancestry. They replicated the susceptibility loci reported by Postel-Vinay et al. at 1p36.22, 10q21.3 and 15q15.1 and identified new loci at 6p25.1, 20p11.22 and 20p11.23. The 20p11.22 locus is near *NKX2–2*. Interestingly, most loci reside near GGAA repeat sequences (binding site of EWS/FLI transcription factor). Therefore, these variants may condition the EWSR1-FLI1 binding on GGAA motifs [109].

Other important contributions about polymorphic GGAA motifs, were reported by COG-group. They studied polymorphic microsatellite regions GGAA in both *NROB1* and *CAV1* genes. Their results demonstrated that the *NR0B1* and *CAV1* GGAA microsatellites were highly polymorphic in both European and African populations. The *NR0B1* microsatellite was substantially more polymorphic in both populations, whose number of GGAA motifs ranged from 16 to 60 and 14–72 in Europeans and Africans, respectively. This study concluded that efficient occupancy of EWS/FLI and associated co-factors were more optimal across microsatellites containing 21–25 or 55–60 GGAA motifs next to *NR0B1* gene than other GGAA repeats [110, 111]. Therefore, polymorphic differences in genomic locations where EWS/FLI fusion protein binds, could explain the prevalence of particularities among populations.

On the other hand, *Alu* elements are a type of transposon (a type of SINE or Short INterspersed Element) and it was proposed that *Alu* elements are preferential sites for genetic recombination in cancer [112]. Due to *EWSR1-FLI1* importance in ES biology, it was hypothesized that polymorphism in *Alu* elements could have a role in ES susceptibility. Zucman-Rossi et al. looked for polymorphic differences in Alu repeats in *EWS* gene. They reported interethnic polymorphism differences in intron 6 of *EWS*. This intron (near the molecular common EWS breakpoint region), is at least 50 % smaller, due to diminished interspersed repeat sequences (*Alu* elements), in about 10% of the African population [113]. Large-scale studies on germline DNA from Ewing's sarcoma patients need to be performed for supporting this hypothesis [114].

What we Know about Environmental Risk Factors?

Environmental factors have probably a minor role in pediatric cancer predisposition, but we cannot discard their contribution. Epidemiological Ewing sarcoma studies suggested five external factors which occurred more often among Ewing sarcoma patients than in healthy population: taking anti-nausea medications by mother during pregnancy, umbilical and inguinal hernias, heart conditions, parental smoking, and father's occupation in farming. Nevertheless, none of them have been significantly more frequent in ES patients compared to either siblings or general population controls [33]. Following reports

Table 1 Genes or genetic variants related to Ewing susceptibility. Some well characterized genes and polymorphic variants may be related to Ewing susceptibility. Due to the fact that not enough information is available about these hypothesized contributing factors, collaborative groups should study these genes or polymorphic variants among all ES patients, in order to integrate more information

Genes or genetic variants related to Ewing susceptibility	Influence	Reference
TP53 gene mutations	Probable	•Zhang et al. [80] Brohl et al. [81]
MDM2 T309G SNP	Possible	•Thurow et al. [92]
BLM gene mutations	Improbable	•Brohl et al. [81]
Fanconi Anemia genes mutations	Doubtful	•Brohl et al. [81]
CMMRD genes mutations	Doubtful	•Zhang et al. [80] •Brohl et al. [81]
RET gene mutations	Improbable	•Zhang et al. [80] •Brohl et al. [81]
CD99 rs311059 -T SNP	Possible (in caucasian)	•Martinelli et al. [103]
EWS rs4820804-TT SNP	Possible	•Silva et al. [104]
CD86 rs1129055 -A SNP	Possible (in Chineses)	•Wang et al. [105]
CTLA-4 49G > A SNP	Possible	•Zhang et al. [106]
EGR2 rs79965208-A SNP	Possible	•Grünewald [108]

have suggested the association of Ewing's sarcoma and parental exposure to pesticides, solvents, and farming or agricultural occupation [115–118]. Holly et al. detected that ES risk was elevated in children whose fathers were engaged in agricultural occupations during the period from 6 months prior to the conception of subject up to the time of ES diagnosis (relative risk (RR) = 8.8, 95% confidence interval (CI) 1.8–42.7) and for children whose fathers had occupational exposure to herbicides, pesticides, or fertilizers (RR = 6.1, 95% CI 1.7–21.9, $p = 0.002$) [115]. Valery et al. have studied expositions that confer ES susceptibility in Australia. The meta-analysis results supported the hypothesis of an association between ES and parental occupation in farming [118].

A concrete *mutational sign* has not been described in ES tumors, and therefore, a clear environmental triggering is probably not present. However, environmental exposures may condition the individual epigenetic signature. For example, arsenic exposure is associated to DNA hypermethylation of several genes, including *CDKN2A, RASSF1A* and *PRSS3* (curiously *CDKN2A* is a gene commonly deleted in ES) [119]. These influences on the epigenome appear mainly during key periods, like first states of intrauterine life and the fetal period of gonadal sex determination. Therefore, during periods of extensive epigenetic reprogramming, epigenome is sensible to environmental influences [120–123]. Interestingly, some of the epigenetic marks established in germ cell lineage members (ovule and spermatozoon) could be transmitted to subsequent generations [120, 124]. Trans-generational inheritance of epigenetic marks supposes that the epigenetic reprogramming state during embryonic and fetal period do not remove these hereditable marks [120, 121]. Curiously, these inherited epimutations cluster in concrete genome regions [125]. Therefore, not only maternal environmental exposures during pregnancy, but also both paternal and maternal exposures several years before, may condition the offspring epigenome. Even expositions in forebear many years ago might collaborate in ES predisposition.

Ewing's sarcoma risk was also found associated with increasing both maternal and paternal age by Jonhson et al. [126] and it might be related with epigenetic marks. Epigenomics role in pediatric cancer risk has been understudied, and its interaction with genetics, age and cancer predisposition is unknown.

Other Molecular Events Have a Predisposing Role?

Copy number variations are commonly detected among ES tumors [28–30], but few studies have been focused on its effect on ES risk. Krepischi et al. detected rare deletions and duplications in germline of pediatric patients (none patient suffered Ewing sarcoma). They concluded that constitutive CNVs contribute to the etiology of pediatric cancer. Further studies including ES patients should be performed [127].

Discussion

In this work we have reviewed the main contributing factors to ES predisposition. An important body of work allows us to hypothesize a genetic contribution to ES susceptibility. Firstly, incidence's differences through ethnicities. The significant variations among ethnicities might be related to environmental factors, but their scarce role in pediatric cancer, and particularly in ES, suggest a remarkable genetic contribution. Secondly, the peak of ES incidence throughout adolescence also draws attention to genetic predisposition above

environmental repercussion. However, despite environmental contribution to ES predisposition is mild, it could explain, in part, some ES cases.

Since ES is not part of cancer predisposition syndromes and family aggregation is not frequently described, probably heritable genetic alterations are not highly damaging in this tumor. Nevertheless, increased cancer rates between ES patients and their relatives point out at least to a minor genetic contribution. In this sense, the presence of *MDM2* T309G and many other polymorphic variants have an effect on ES risk. Germline next generation sequencing studies have revealed pathogenic variants in *TP53*, Faconi anemia related genes as well as in mismatch repair genes suggesting that in a small percentage of ES patients, highly pathogenic variants could be predisposing this disease. Systematic studies analyzing all these genetic variants simultaneously are lacking, thus large prospective cohort studies are required.

The integrating analysis of genetic and environmental factors affecting parents and ancestors would be necessary to draw conclusions. Only collecting all this information through large international consortiums would help us to clarify ES predisposition. On this matter, GENESIS (Genetics of Ewing Sarcoma International study; AEPI10N5), a COG clinical trial, is working in this way [128], but many other efforts are necessary.

Acknowledgments Arash Javadinejad (Health Institute La Fe, Valencia, Spain): English review and editing. Loreto Sales Triguero: graphic design of Fig. 1.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

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