



Genetic predisposition and chromosome instability in neuroblastoma

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Abstract

Neuroblastoma (NB) is a pediatric tumor of embryonic origin. About 1–2% of all NBs are familial cases, and genetic predisposition is suspected for the remaining cases. During the last decade, genome-wide association studies (GWAS) and high-throughput sequencing approaches have been used to identify associations among common and rare genetic variants and NB risk. Substantial data has been produced by large patient cohorts that implicate various genes in NB tumorigenesis, such as *CASC15*, *BARD1*, *CHEK2*, *LMO1*, *LIN28B*, *AXIN2*, *BRCA1*, *TP53*, *SMARCA4*, and *CDK1NB*. NB, as well as other pediatric cancers, has few recurrent mutations but several copy number variations (CNVs). Almost all NBs show both numerical and structural CNVs. The proportion between numerical and structural CNVs differs between localized and metastatic tumors, with a greater prevalence of structural CNVs in metastatic NB. This genomic chaos frequently identified in NBs suggests that chromosome instability (CIN) could be one of the major actors in NB oncogenesis. Interestingly, many NB-predisposing variants occur in genes involved in the control of genome stability, mitosis, and normal chromosome separation. Here, we discuss the relationship between genetic predisposition and CIN in NB.

Keywords Neuroblastoma · Chromosome instability · Allelic variance · Mutation · SNP

Abbreviations

CIN	Chromosome instability
GWAS	Genome-wide association studies
NGS	Next generation sequencing
SNP	Single nucleotide polymorphisms
NCCs	Neural crest cells
CNVs	Copy number variations
HSCR	Hirschsprung disease
CCHS	Congenital central hypoventilation syndrome
lncRNA	Long noncoding RNA
m-CGH	Microarray-comparative genomic hybridization

1 Introduction

Neuroblastoma (NB) is a pediatric tumor that shows great biomolecular and clinical heterogeneity. This tumor is also classified as embryonic tumor, as there is extensive evidence supporting its origin from neural crest cells (NCCs) during the fetal life [1]. The tumor may originate as localized or disseminated disease in a range of ages, from birth to 16 years. Clinical stages of NB have been divided as the following: localized stage 1, 2, or 3; disseminated stage 4; and disseminated stage 4S occurring in patients younger than 1 year of age. The overall 5-year survival rate is lower than 40%. Luksch et al. [2] provide an extensive review of NB. Over the past decades, the discovery of genomic aberrations such as *MYCN* amplification, 17q gain, and 11q and 1p36 deletion have greatly improved NB risk stratification and supported oncologists in choosing effective and personalized treatment regimens [3]. More recently, it was shown that gene expression-based signatures can identify children with higher risk disease who would benefit from new and more aggressive therapeutic approaches [4–6]. Patients will thus benefit from more appropriate and effective therapeutic choices if new genetic markers are included in the current NB treatment protocols.

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2 Neuroblastoma predispositions

The contribution of genetic predisposition is particularly important in pediatric cancers. Indeed, about 10% of cases are associated with pathogenic germline mutations in cancer genes [7, 8]. In the last 10 years, through linkage scans of families with the disease, genome-wide association studies (GWAS), and next generation sequencing [3] of sporadic cases, we have gained a more comprehensive understanding of the heritability of NB.

2.1 Familial neuroblastoma

Familial NB usually occurs at a young age and is more likely than sporadic tumors to present with multiple primary tumor sites. Only 1–2% of all NB cases belong to the familial type, which is inherited in an autosomal dominant manner.

The first gene to be implicated in familial NB tumorigenesis was *PHOX2B* [9, 10]. In 2004, the association of NB with other congenital malformations of neural crest origin, such as Hirschsprung disease (HSCR) and/or congenital central hypoventilation syndrome (CCHS) [11] determined by *PHOX2B* mutations [12], led researchers to identify *PHOX2B* as a disease-causing gene in hereditary NB. This gene encodes a transcription factor required for neural crest differentiation into noradrenergic neurons [13]. It has been estimated that mutations in the *PHOX2B* gene account for approximately 10% of heritable disease [14, 15] and have also been observed in up to 2% of sporadic NBs [16, 17].

A robust correlation genotype-phenotype for *PHOX2B* mutations has been reported. Non-polyalanine repeat mutations that are usually missense mutations associated with the development of neural crest tumors, including NB, frequently in association with HSCR and CCHS [17]. *PHOX2B* pathogenic mutations associated exclusively with NB predisposition are primary missense and frameshift mutations, which are clustered in two gene regions: 200–300 bp and 600–714 bp from the translation start codon, respectively [18]. Functional data suggest that reduced *PHOX2B* dosage, due to heterozygous deletion or dominant-negative mutations, blocks differentiation of sympathetic neuronal precursors generating a cell population more susceptible to secondary transforming events [19, 20]. Another experimental study demonstrated that NB-associated *PHOX2B* mutations impair *MSX1* inhibition, leading to both Delta-Notch pathway upregulation and *NOTCH3* overexpression [21, 22]. Recently, an analysis of the NB super-enhancer landscape has demonstrated that *PHOX2B* governs a regulatory circuit that confers a sympathetic noradrenergic identity to tumor [23]. Together, these findings strongly suggest that defective precursor cell differentiation due to genomic alterations of *PHOX2B* can induce malignant transformation of precursor cells.

In 2007, a screening of a highly informative family with recurrent NB identified 2p (containing *ALK*) as a predisposition locus [24]. After 1 year, three independent research groups identified four distinct germline missense mutations in the tyrosine kinase *ALK* domain, which were present in the most of familial NB cases evaluated [25–27]. These three studies, in addition to the George et al. paper, reported that 9.6% of the sporadic NB cases investigated had somatic single-nucleotide variants in *ALK* [28]. Later, a large study including 1596 sporadic NB primary tumors showed that point mutations or copy number alterations of *ALK* can be found in 8% of cases [29]. All together, these studies have established *ALK* as the most frequently somatically mutated gene in sporadic NBs, in addition to its role of familial NB predisposition gene. Among the mutations predisposing to familial NB, the R1275Q *ALK* mutation showed an almost complete penetrance in families and was one of the most activating mutations tolerated in the germline [29]. In contrast, the G1128A mutation was found to be more weakly activating and correlates with an approximate 25% likelihood of developing NB [29]. Regarding the somatically acquired *ALK* mutations, F1174* and F1245* are reported to be the most frequent. Both mutations have been also found in germline DNA of NB patients with serious neurocognitive defects and brain stem abnormalities, underlining the connection between genotype and phenotype and the critical role played in ordinary neurodevelopment [30].

ALK is currently considered a promising therapeutic target. Indeed, functional studies show that knockdown of *ALK* results in growth inhibition in all NB cell lines with *ALK* mutations and some with wild-type *ALK*. However, despite encouraging results in preclinical studies, the first clinical trial evaluating Crizotinib, an *ALK* inhibitor, found the drug was only effective for a small fraction of *ALK*-mutant NB patients [31]. Alternative small molecule inhibitors are presently being studied, and further attempts are being made to recognize and bypass resistance processes [32]. Interestingly, a possible mechanism of resistance appears to be due to upregulation of *BORIS* gene, which promotes chromatin interactions in *ALK*-mutated, *MYCN*-amplified NB cells that in turn promotes resistance to *ALK* inhibition [33].

Despite significant advancement in understanding the genetic factors of family predisposition to NB over the past two decades, roughly 15% of family cases remain unresolved. Beyond *ALK* and *PHOX2B*, no other mutated gene has been associated with familial NB. De Mariano and colleagues have recently found that *GALNT14* mutated in two second-degree cousins with NB [34], but no other mutations have been yet identified in any other NB family. Other evidences for a hereditary NB predisposition locus at chromosome 16p12-13 [35], 4p16 [36], and 1p [37, 38] have been reported. Together, the genetic data obtained so far indicate that an oligogenic mode of inheritance might explain the existence

of different NB loci genetically interacting to cause and/or modify the disease-phenotype [39].

The discovery of predisposing germline mutations has impacted clinical management of familial NB. Indeed, for patients with a family history of NB, genetic consultation and testing is now suggested and along with abdominal ultrasounds and chest x-rays along with urine metanephrines every 3 months until 6 years of age even in patients not carrying *ALK* and *PHOX2B* mutations [40].

2.2 Sporadic neuroblastoma

Literature evidences of paucity of somatic alterations in NBs [41–43] suggest that heritable genetic risk variants may have a relevant role in NB carcinogenesis. Due to the rarity of the disease, studying genetic susceptibility in NB is challenging. Whole-exome sequencing approaches have identified rare genetic variants that are associated with cancer predisposition in patients with NB who lack the classic clinical criteria for a cancer predisposition syndrome. Candidate gene studies and GWAS were also used to explore the contribution of prevalent genetic variations. The list of susceptibility genes and disease predisposing variants is reported in Table 1.

2.3 Rare inherited genetic susceptibility

Two independent groups have reported that diverse genes are enriched in rare, potentially pathogenic, germline variants in children with NB [41, 43]. The first study, published in 2013 [43], analyzed by whole-exome sequencing 220 high-risk tumors and found that in *ALK*, *CHEK2*, *PINK1*, *TP53*, *PALB2*, and *BARD1*, rare, possibly pathogenic variants of germline were significantly enriched whereas the second study by Lasorsa et al. [41] identified *BARD1*, *CHEK2*, and *AXIN2* enriched in functional variants in 52 NB patients (48 high-risk and 4 intermediate risk) (Table 1). It is intriguing to note, the nonsense variant rs587781948, included in ClinVar, has been found in two patients in these two different gene-sequence projects (Table 1).

Germline DNA from 100 NB patients was sequenced (whole-exome or whole-genome) as part of a larger study of 1120 pediatric patients with cancer [8]. Two cases of NB had an *ALK* mutation, and three separate cases had a mutation in either *APC*, *BRCA2*, or *SDHB*. Other two large germline sequencing studies of pediatric cancers, including 19 [44] and 59 [7] NB cases, respectively, identified pathogenic germline variation in *ALK*, *TP53*, *SMARCA4*, *BRCA1*, *LZTR1*, and *SDHB* (Table 1). The association of rare variants in *TP53* with NB has been discovered by GWAS. Indeed, analysis of 10,290 individuals across three independent case-control cohorts identified two rare SNPs, rs78378222, and rs35850753, very strongly associated with NB predisposition [45]. Interestingly, most of the rare germline variants reported are

located in genes crucial for DNA repair and maintenance of genomic integrity. However, despite these relevant studies, the complete spectrum of rare germline variants predisposing to NB remains to be defined.

Among the rare risk variants, it is also necessary to include those associated with syndromic diseases in which recurrence of NB onset is also observed: These comprise *NF1* in neurofibromatosis type 1 [46], *PTPN11* in Noonan syndrome [47], *HRAS* in Costello syndrome [48], *TP53* in Li Fraumeni syndrome [49], *EZH2* in Weaver syndrome [50], *SDHB* in familial paraganglioma/pheochromocytoma [51], *CDKN1C* in Beckwith-Wiedemann syndrome [52], and *MSX1* in Wolf-Hirschhorn Syndrome [53].

2.4 Common inherited genetic susceptibility

GWAS is a high-throughput approach to genotype hundreds of thousands of SNPs to identify associations between common single nucleotide polymorphisms (SNPs) and disease risk. Over the last 10 years, the use of this approach has allowed us to elucidate the genetic basis of predisposition to NB. The first GWAS of NB, comprised of 1752 European American NB cases and 4171 cancer-free control subjects [54], identified a susceptibility locus at chromosome 6p22 in a newly identified long noncoding RNA (lncRNA) annotated as *CASC15* gene [55] conferring risk for NB development, specifically the more aggressive high-risk subset (Table 1). This association has been also observed in cohorts from the UK [54], Italy [56], and China [57]. Subsequent studies have demonstrated that the more advanced disease is correlated with reduced expression of the truncated isoform *CASC15-S* [55] and that the loss of another lncRNA, *NBAT-1* (*CASC14*), also found at the 6p22 susceptibility locus, causes proliferation and invasion [58]. Finally, the interaction of *CASC15* and *NBAT1* has been demonstrated to favor differentiation through their regulatory interactions with important cancer-associated *SOX9* and *USP36* genes located on chromosome 17q, a region often gained in high-risk NB [59].

A second GWAS analysis restricted to high-risk patients found six predisposing SNPs in *BARD1* at chromosome 2q35 [60] (Table 1). The association at this locus replicated in a cohort from the UK [60] as well as African Americans [61], Italians [56], Chinese [62], and Spanish [63]. A recent work has evaluated the known genetic association with different NB tumor sites and found that SNPs in *BARD1* (previously associated with high-risk NB) were found to be strongly associated with predisposition for origin at adrenal gland [64]. This association was also observed in an independent Chinese study [62].

BARD1 is the binding partner of *BRCA1* but can independently act as both tumor suppressor and oncogenic gene [65]. In NB, a potential functional risk allele of the rs17489363 SNP within the promoter region correlates with decreased

Table 1 Germline variants associated with sporadic NB

Chromosome	ID variant	Candidate gene	Clinical sub-group
Common genetic variants			
6p22	rs6939340	CASC15/NBAT-1	High-risk
2q35	rs6435862 and rs17489363	BARD1	High-risk
11p15	rs2168101	LMO1	High-risk
11p11.2	rs11037575	HSD17B12	Low-risk/high-risk*
1q23	rs1027702	DUSP12	Low-risk
6q16	rs17065417	LIN28B	None
6q16	rs4336470	HACE1	None
2q34	rs1033069	SPAG16	None
8p21	rs1059111	NEFL	None
3q25	rs6441201	MLF1/RSRC1	None
4p16	rs3796727	CPZ	None
12p13	rs34330	CDKN1B	None
1p13.2	rs2153977	SLC16A1	None
4p16.2	rs11944652	MSX1	None
11q22.2	rs10895322	MMP20	11q deletion
3p21.31	rs80059929	KIF15	MYCN-amplified
Rare genetic variants			
2p23.1	NRC	ALK	None
2q35	rs587781948	BARD1	None
22q12.1	NRC	CHEK2	None
17q24.1	NRC	AXIN2	None
17p13.1	rs35850753	TP53	None
5q22.2	NRC	APC	None
13q13.1	NRC	BRCA2	None
1p36.13	NRC	SDHB	None
19p13.2	NRC	SMARCA4	None
22q11.21	NRC	LZTR1	None
17q21.31	NRC	BRCA1	None
Copy number variations			
1q21.1	hg18:147.3–147.4 Mb	NBPF23	None
16p11.2	hg18:29.65–30.20 Mb	SEZ6L2/PRRT2	None

NRC, No recurrent variants

*There are controversial results in literature (See Nguyen le et al. 2011 and Capasso et al. 2013)

expression of the full-length form of *BARD1* that has onco-suppressor functions; indeed, its decreased expression is associated with advanced tumors and with increased proliferation and invasion capacity of NB cells. Conversely, another risk SNP (rs6435862) within intron 1 is associated with increased expression of the oncogenic isoform *BARD1β* [66] which can induce cell growth and stabilize the Aurora family of kinases in NB cell lines, suggesting a possible action mechanism and potential therapeutic strategy. These findings support evidences that more than one disease-contributing *BARD1* variant may exist.

A third NB risk locus was identified at chromosome 11p15.4 close to *LMO1* by using an expanded GWAS in

Caucasian children from the USA, the UK, and Italy [67] (Table 1). The association was subsequently identified in a Chinese population [68]. The minor genotype of the variant rs110419 associated with increased risk of aggressive NB forms and *LMO1* expression. The *LMO* family of genes had earlier been involved in the development of leukemia and breast cancer [69]. Additional functional investigations have established the role of *LMO1* in promoting tumorigenesis of aggressive phenotype NB [67, 70] whereas a fine mapping of 11p15.4 locus has identified the causal SNP rs2168101 resided in a super-enhancer element that ablated a canonical GATA transcription factor binding site [71].

By increasing the case-control sample size, we were able to identify two new genome-wide significant independent signals at chromosome 6q16 within the genes *LIN28B* and *HACE1* [72] (Table 1), which had both been previously implicated in cancer as an oncogene and a tumor suppressor, respectively. Both signals associated with NB in Caucasian children from the USA [72] and Italy [56], and as well as in children of African American [72] or Chinese [73] descent. Functional investigations at this locus showed that risk genotype of rs17065417 SNP within *LIN28B* correlated with increased *LIN28B*, decreased let-7 microRNA family, and increased *MYCN* expression. Furthermore, *LIN28B* knockdown inhibited growth in NB cellular models carrying the risk allele at rs17065417 suggesting the oncogenic functions of *LIN28B* in NB [72]. Additional mechanistic studies have explained how *LIN28B* promotes malignant transformation in NB [74–77].

All of the above-described studies have both identified genetic risk loci for NB and have unraveled novel biological processes underlying this devastating disease which can have implications in the development of new therapeutic strategies, highlighting thus the relevance on performing GWAS. Moreover, it is now established that there is no evidence of epistasis among the NB-associated variants but a cumulative effect of risk variants on NB risk and development of high-risk phenotype [3, 56, 78].

The more recent NB GWAS identified novel variants at *RSRC1/MLF1* (3q25) and *CPZ* (4p16) in 2101 cases and 4202 controls of European ancestry and replicated in an African American cohort and two other Caucasian cohorts from Italy and the UK [79] (Table 1). The signal at 3q25 was replicated in Chinese children [80]. In-depth functional analyses are needed to define the biological role of the found variants and the associated genes in the development of NB.

Other loci have been identified by using alternative re-analysis of GWAS and replicated in independent case-control subjects with different ethnic origin such as *HSD17B12* at 11p11.2 [56, 81, 82], *DUSP12* at 1q23.3 [56, 81] *SPAG16* at 2q34 [83], *NEFL* at 8p21.2 [84], and *CDK1NB* at 12p13.1 [85]. The NB risk locus 5q11 (*DDX4/IL31RA*) (Table 1), reported in Nguyen le et al. [81], failed to be replicated in other independent case-control studies [56, 82].

Genome-wide approaches have been also used to demonstrate interactions between germline disease predisposing variants and somatically acquired genomic aberrations. Indeed, SNPs in *MMP20* [86] and *KIF15* [87] have been associated with NB risk only in patients with 11q-deleted and *MYCN*-amplified NBs, respectively (Table 1).

Another interesting aspect related to NB genetics has been brought to light by our group. We have provided evidence that inter-individual susceptibility to diverse pathological conditions can reveal a common genetic architecture. We have shown that genomic regions (2q35, 3q25.32, and 4p16.2)

containing risk SNPs were cross-associated with NB and congenital heart diseases that, similar to NB, originate from abnormal neural crests formation [88]. Interestingly, some of these shared susceptibility loci regulated the expression of relevant genes involved in neural crest cells formation and developmental processes (such as *BARD1*, *MSX1*, and *SHOX2*) and were enriched in several epigenetic markers from NB and fetal heart cell lines [88]. Moreover, by a cross-associated analysis of melanoma and NB GWAS results, we have identified a genome-wide signal at locus 1p13.2 (rs2153977) in a NB specific enhancer that interacts with *SLC16A1* [89], demonstrating for the first time that neural crest derived tumors share disease predisposing variants. Further functional investigations demonstrated that rs2153977-T protective allele correlated with decreased *SLC16A1* expression and altered a binding site of T-box proteins [89] which are involved in neural crest development.

2.5 Rare and common inherited copy number variations

Copy number variations (CNVs) have been also involved in predisposition to NB. Two genome-wide analyses have revealed a common deletion at 1q21.1, containing a gene belonging to the NB breakpoint family (*NBPF23*), and a rare microdeletion 16p11.2, containing causal candidate genes *SEZ6L2* and *PRRT2*, [90] associated with NB development (Table 1). By a genomic re-analysis of 40 pediatric patients enrolled from 2016 to 2018, we have recently identified *SLFN11* deletion, *SOX4* duplication, and *PARK2* partial deletion in three NB patients [91]. These findings suggested that chromosome abnormalities can be among the genetic factors predisposing to NB. However, additional genome-wide analyses are needed to further define the spectrum of CNVs associated with NB development.

3 Chromosomal instability in neuroblastoma

Since oncogenes and tumor suppressor genes were discovered, the development of cancers has been closely associated to mutations of these genes. The participation of both oncogenes and tumor suppressor genes in tumorigenesis of adult cancers has been well-established. In the last decade, the advent of genome-wide sequencing has shown that pediatric cancers have few recurrent mutations [7], making it difficult to link mutations of oncogenes or tumor suppressor genes to the pediatric cancer oncogenesis. In particular, NB is one of the most puzzling pediatric tumors. This tumor occurs in a range of age between 0 and 16 years. NB is classified as an embryonic tumor because there are several evidences supporting it originates during embryonic and fetal life [92, 93].

Today, a huge amount of genome data of NB cells is available [94]. The first consistent observation was made by Franke et al. [95] that described the loss of the terminal region of chromosome 1p in both human NB cell lines and in tumor samples. The persistent loss of chromosome 1p in NB that was observed in about 30–40% of cases raised the suspicion that 1p region contained one NB tumor suppressor gene. Few years later, it was evident that the NB tumor suppressor gene was not located at chromosome 1p. In 1984, Manfred Schwab et al. [96] discovered the *MYCN* oncogene amplification in both cell lines and tumor samples. Furthermore, Brodner et al. [97] were able to observe the close relation between *MYCN* gene amplification and worst patient outcome [98]. However, *MYCN* amplification was only found in about 20% of cases. Afterward, deletion of chromosomes 11q [99] and 14q [100] were also detected in some NB. An improvement in the study of NB genome was subsequently made using the microarray technology. The introduction and development of nanotechnologies applied to biological systems allowed us to better understand the intimate abnormalities in the NB genome. Several studies have been carried out using the microarray-comparative genomic hybridization (m-CGH) [94, 101]. The m-CGH allowed us to evaluate the entire genome in a whole. The analysis of more than 1000 NB tumor samples from different institutions has shown that numerical CNVs are largely present in localized NB, whereas structural CNVs are major represented in advanced metastatic NB [102, 103].

CNVs greatly influence tumor development. The presence of CNVs in a tumor cell suggests that the genome is unstable and can be prone to several replication errors or abnormal mitosis. This condition is known as chromosome instability (CIN). CIN is a hallmark of human tumors, and in the last few years, several genes associated with CIN have been discovered [104]. CIN has been also observed during the early phases of human normal embryogenesis [105]. Thus, it is possible to speculate that CIN in NB cells is facilitated by pre-existing genomic instability.

There is moreover evidence that NB origins from NCCs, a group of cells, located just over the neural tube [1]. NCCs migrate towards their final destination just few days after egg fertilization, and they reach their final destination within 56 days. NCCs undergo to several modifications in their morphology and function during embryogenesis. Their highly migratory and invasive features permit them to reach distant body sites. NCCs can assume the specification of several cell lineages, including peripheral neurons and the adrenal medulla. Indeed, one of the most frequent sites of NB origin is the adrenal medulla.

In children, NB can present with either localized or metastatic disease. Patients with stage 1, 2, or 3 NB have tumor cells with several numerical CNVs. Many of these patients are diagnosed in their first year of life and have a good prognosis,

whereas patients with a later onset have a poor prognosis. Taken together, these evidences suggest that numerical CNVs are associated with a better prognosis. The special stage 4S, which includes patients younger than 1 year with metastatic disease, has tumor cells with primarily numerical CNVs, but few structural CNVs [106]. A completely different situation is observed in NB cells of tumor of patients older than 1 year and having stage 4 disease. The tumor cells of stage 4 have several structural CNVs and less numerical CNVs. The chromosomes show a great complexity of damages including chromosome deletion, chromothripsis, double minutes bodies, and rearrangements. This feature indicates that CIN is very relevant in this clinical stage. The chromosome defects have been one of the first abnormalities observed in human cancer cells. It is suggested that CIN drives intratumoral heterogeneity, one of the most common features of NB. Recurrent mutations of single genes are infrequent in primary NB with activating mutations in *ALK* and inactivating mutations in *ATRX*, and *TERT* rearrangements being the most frequent [41, 43]. So, the oncogenesis of NB remains still unsolved. Recently, the role of CIN has been reconsidered the mayor player responsible of NB growth and development.

A lot of evidences suggest that CIN is the major player in the NB oncogenesis [107]. It is plausible that CIN initiates in the early phase of embryonic life just during the migration of NCCs. The CIN could be triggered by the malfunction of proteins dedicated to maintain the genome identity. Indeed, several proteins codified by NB susceptibility genes are involved in chromosomal segregation, centrosome segregation, DNA repair, and spindle apparatus machinery such as *TP53*, *BRCA1*, *BARD1*, *CHECK2*, *CDKN1B*, *APC*, and *KIF15* (Table 1).

The so-called *in situ* NB described by Ikea et al. [108] and observed in infant autopsies has proven that NB is already present during embryonal development and suggests CIN is associated to NB development. NB tumors can also be present in infants, just after the birth. Actually, patients younger than 1 year of age that onset with either localized stage 1 or 2, or metastatic tumor stage 4S show a good outcome. Genome analysis of these tumors shows several numerical but few structural CNVs [106]. Conversely, tumors of patients older than 1 year of age and at any stage show several structural CNVs. It is widely accepted that structural CNVs have a severe negative effect on genome and are associated with the more aggressive NB phenotype [102, 109]. Furthermore, a common feature of the tumor of advanced stages is the chromothripsis of chromosome 5, suggesting that CIN is very active in the NB of stage 4. Finally, there is the peculiar stage 4S in which we can argue that CIN is at low level inducing the production of entire chromosome extra-copies that cannot contribute to the tumor aggressiveness and allow the spontaneous regression of the tumor.

4 Conclusions

To improve clinical courses and outcomes for children with NB, it is extremely important to fully understand the disease etiology. Genomic analyses of NBs suggest a higher than expected prevalence of pathological variants in genes involved in molecular mechanisms of carcinogenesis or associated with known syndromes of cancer predisposition. Because of the paucity of recurrent mutations in NB, additional mechanisms to explain the oncogenesis of NB should be evaluated. The CIN is considered background of any tumor, and up to now it is unclear if the CIN is an initial event or the result of gene mutations. Although diverse genes have been identified in association with CIN, this association has yet to be investigated in NB. One possible cause of CIN in NB could be the deleterious effect of inherited risk variants on genes that code for proteins involved in chromosomal segregation, centrosome segregation, DNA repair, and spindle apparatus machinery.

In the future, the therapies proposed to the patient will be based mainly on genetic predisposition and a comprehensive understanding of the underlying mechanisms of NB oncogenesis will be mandatory. Thus, promising NB research should aim to carry out studies capable of incorporating the germinal and somatic genetic component to identify the most effective prognostic tumor markers and most efficacious therapy.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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