Genomics for Paediatric Cancer

Morgan C[1]

[1]Swansea University Medical School. Swansea University, Singleton Park, Swansea. SA2 8PP

Abstract

In the UK, approximately 1,800 children are diagnosed with cancer every year. Paediatric cancers are very diverse and differ from adult tumours at both the histological and molecular level. The majority of childhood cancers are comprised of haematological malignancies, central nervous system tumours, embryonal tumours (retinoblastoma, neuroblastoma, nephroblastoma) and sarcomas. Whilst paediatric cancer is rare it is still one of the leading causes of death for children. Due to its rarity, the prevalence and variety of the predisposing mutations remains largely unknown. However, the advent of next generation sequencing over the past decade has seen our molecular understanding of childhood cancers advance significantly. This review gives an overview on the aetiology of paediatric cancer and how NGS is helping to advance our understanding of the molecular basis of paediatric cancers to improve diagnosis, prognosis and treatment of paediatric cancer patients.

Introduction

Childhood cancer is used to describe cancers that arise before the age of 15 years. Cancer is one of the leading causes of death for children. Up to 2% of paediatric cancers are documented in developing countries whereas in Europe and North America, childhood cancers give rise to less than 0.5% of all cancer cases. [1] In the UK alone there are approximately 1,800 children diagnosed with cancer every year which equates to 5 children every day having a cancer diagnosis; furthermore, around 230 children die each year from cancer – more than four children each week. [2]

Paediatric cancers are very diverse and differ from adult tumours at both the histological and molecular level. [3] The majority of childhood cancers are comprised of haematological malignancies, central nervous system tumours, embryonal tumours (retinoblastoma, neuroblastoma, nephroblastoma) and sarcomas (Figure 1). Carcinomas, which are the most common type of cancer in adults accounts for less than 5% of children's cancer.[4]

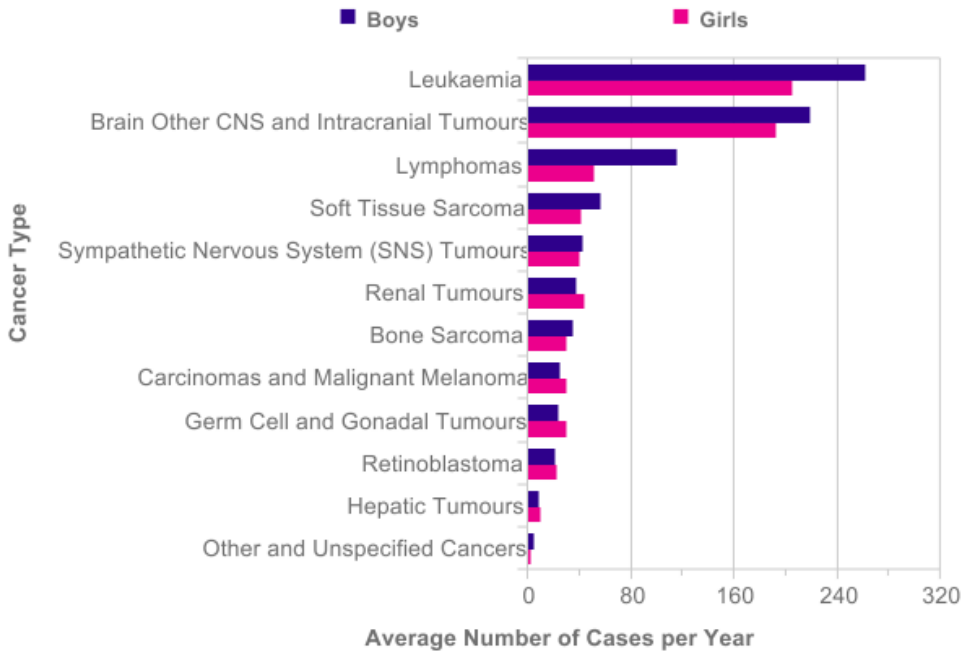

Figure 1. Average number of childhood cancer cases diagnosed per year in children aged 0-14 from 2006-2008 in the UK. [2]

Aetiology

The aetiology of childhood cancers is still unknown due to the rarity of the disease and many reports in the literature are contradictory. However, as with adult cancers; combinations of factors, both extrinsic and intrinsic, are believed to play a role.

Extrinsic risk factors

Exposure to high dose radiation and prior chemotherapy are the only two extrinsic factors accepted to cause cancer in children. Other extrinsic factors believed to contribute to paediatric cancers include lack of exposure to infections. Leukaemia, the most common form of childhood cancer, primarily arises as a result of acquired, rather than, genetic changes with Greaves [5] proposing that delayed exposure to childhood infections actually causes the immune system to function abnormally; giving rise to an increased risk of paediatric leukaemia. Maternal smoking and alcohol consumption are also

cited as playing a role. Derivatives of tobacco have been identified in placental tissue, foetal blood, the urine of newborns and also in the breast milk of mothers who smoke. [6] However, studies looking at the effect of maternal smoking and paediatric cancer are contradictory. One such study carried out by Stjernfeldt et al, (1986) [7] showed a dose-response relationship between the number of cigarettes smoked on a daily basis by the mother and the risk of cancer in the child. A more recent study has shown that whilst there is no association between childhood cancers and smoking mothers in general, there was an association between maternal smoking and retinoblastoma. [8] Rumrich et al, (2016) [9] carried out a meta-analysis using 62 studies and concluded maternal smoking is only associated with nervous system cancers. Finally, de Smith et al, (2017) [10] conducted a study on tobacco smoke exposure and the associated risk of childhood acute lymphoblastic leukaemia to determine if tobacco-smoke exposure influences leukaemogenic genomic deletions. They found total number of deletions was positively associated with tobacco smoke exposure in smoking mothers and also a dose response relationship with paternal smoking during pre-conception and deletion number. Parental smoking is not the only risk factor for childhood cancers. Scientist have also assessed the effect of parental alcohol consumption on childhood cancers. As with smoking, results are inconsistent or contradictory. For example, a study conducted by Ferreira et al, (2015) [11] suggests there is no association with maternal alcohol intake during pregnancy and increased risk of childhood cancers, whilst a study by Latino-Martel, et al (2010) [12] argues that maternal alcohol consumption is a risk factor. But why is there so much contradiction between these studies and extrinsic risk factors? The majority of the studies are case-control studies and parental interview which are susceptible to recall and selection bias.[13] It also needs to be remembered that even in the most common childhood cancers such as leukaemia and CNS tumours, where accumulation of sufficient data allows for meta-analysis to be conducted, bias can be introduced by researchers based on what is or is not reported and by the diversity of the clinical trials.

Intrinsic risk factors

It has been postulated that the risk of childhood cancer is linked to increasing birth weight (birth weights > than 4000g/ 8.8lbs. [14] Several studies have shown cancer incidence rises with increasing birth weight; with one such study reporting an association with increased risk of paediatric cancer and high birth weight is strongest in children under 2 years of age. [15] Increased birth weight has been associated with childhood cancers such as leukaemia, neuroblastoma, astrocytomas and Wilms tumour and one reason which has been put forward to explain this association is that of growth factor pathways/growth hormone exposure. One study has attributed the positive association of high birth weight and childhood cancer to high levels of circulating insulin-like growth factor-1 (*IGF-1*); suggesting that the transforming event occurs in utero and as a result, high levels of *IGF-1* may act on already genetically altered cells to provide them with a proliferative advantage. [14] Spector et al, (2015) [13] provide a more basic reasoning for the association between birth weight and paediatric cancer as potentially being down to the greater number of cells that could be at risk for carcinogenic transformation. However, the genetic reasoning behind this was not given. Another reason put forward by researchers is based on advancing parental age attributing to increased childhood cancer risk. A pooled case-controlled study of 17,672 cancer cases in children diagnosed between the ages of 0-14 showed maternal age was associated with a linear increase in the risk of childhood cancers with an 8% increase per 5-year increment of maternal age for cancers such as leukaemia, lymphoma, CNS, neuroblastoma, Wilms' tumour, bone tumours and soft tissue sarcomas. However, this increased risk was not seen for retinoblastoma, hepatoblastoma or germ cell tumours (Johnson et al, 2009). [16] A previous study by Yip & Czene (2006) [17] conducted a population-based cohort study (~4.3 million children) on the risk of parental age on five childhood cancers (leukaemia, retinoblastoma, non-Hodgkin's lymphoma, Wim's tumour and CNS). The study split the cohort into two diagnostic age groups; <5 and 5-14 years and concluded that advanced parental age was associated with an increased risk of early childhood cancer when children were 5 years of age or younger, with the oldest maternal age group (>40) exhibiting an elevated risk of retinoblastoma. The contradiction between the studies with regards to maternal age and retinoblastoma may come down to study design, environmental influences and sample sizes across the maternal age ranges; to name but a few confounding factors. Regardless of some contradictory studies, it is accepted that increasing parental age influences cancer risk in children. It is believed to occur due to increased mutation frequencies in paternal germ cells and increased frequencies of chromosomal aberrations during the maturation of maternal germ cells. Aging has also been hypothesised to change physiological parameters, such as estrogens levels, which may also induce the risk of childhood cancer.

Genetic Factors

The most obvious risk factor for paediatric cancer is an underlying genetic component. Research has progressed from Sanger sequencing, whereby a maximum of 96 (800bp) sequences could be analysed, to sequencing millions of DNA fragments; furthermore, we are now able to detect, not just point mutations, but also copy number changes, insertions, deletions and translocations. An additional and very important aspect of next generation sequencing (NGS) is the ability to detect variants in small subpopulations of cells, which would go undetected with Sanger sequencing due to the low copy number, yet may be important in terms of cancer recurrence or treatment resistance. [18] NGS can broadly be divided into whole genome sequencing (WGS) or whole exome sequencing (WES). As the names suggest, WGS involves sequencing the entire genome and identify variations in any part of an individuals' genome. WES is concerned with sequencing/analysing only the exons in an individuals' genome; in other words, the protein or RNA coding regions (Figure 2). The exome represents less than 2% of the entire genome, yet contains ~85% of all known disease-causing mutations. Thus, for many researchers and clinicians, WES is seen as the most cost effective and efficient method to employ. However, it can be argued that WES could miss regions of interest or variation outside the exome that could alter gene activity and protein production.

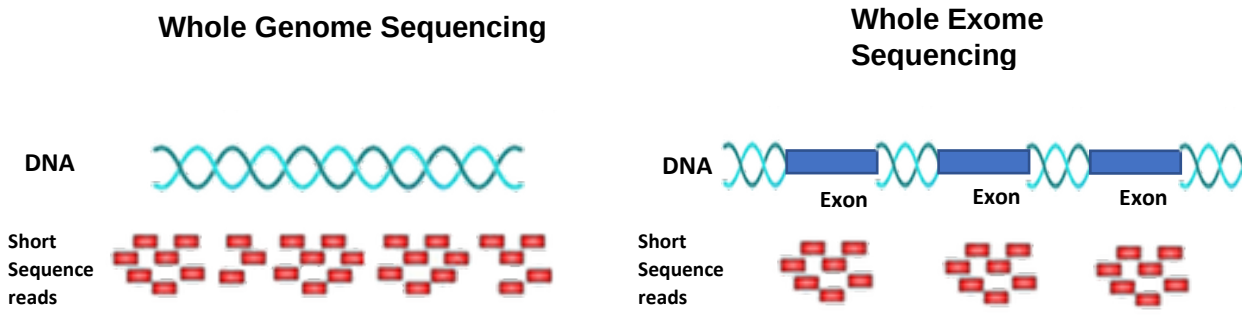

Figure 2. Whole Genome Sequencing involves sequencing the entire genome to ascertain variations in any part of an individuals' genome, whereas Whole Exome Sequencing involves sequencing only the coding regions of an individuals' genome (adapted from GenomixLab, 2019). [19]

Whilst the prevalence and variety of predisposing mutations in paediatric cancers remains largely unknown, [20] the advent of NGS over the past decade has seen our molecular understanding of childhood cancers advance significantly.

In 2010 the Paediatric Cancer Genome Project was launched and to date, the complete genomes of both tumour and normal cells from more than 800 patients have been sequenced with many of their findings published in the scientific literature. Two studies which demonstrate some of these findings are published by Huether et al, (2014) [21] and Grobner et al, (2018). [3] Studies on paediatric cancers have shown that genes involved in epigenetic regulation are mutated at a high frequency. Thus, Huether et al, sequenced 633 epigenetic regulatory genes from 1020 paediatric cancers, encompassing 21 different cancer sub-types arising in solid tumours, brain tumours and leukemias. From this study, they found 16 genes (e.g. *H3F3A, CREBBP, USP7*) most frequently mutated and a marked variation in the frequency of mutations across the three tumour classes; providing further understanding of the genetic/epigenetic variation in paediatric tumours. In the study by Grobner et al,

[3] 961 tumours representing 24 paediatric sub-types were sequenced. The authors found that the mutation rate in the paediatric tumours was 14 times lower than that of adult tumours, pathogenic germline variants were found in 7.6% of the cohort with most variants found in DNA repair genes from mismatch (*MSH2, MSH6, PMS2*) or double—stand break repair (*p53, BRCA2, CHEK2*) They also found 453 potential drug targets in 59 genes with further analysis suggesting that 52% of all primary paediatric tumours may harbour a potential drug target.

A further study, conducted by Ma et al, 2018, [22] sequenced the genomes of 1699 paediatric leukaemia's and solid tumours. The authors identified 142 "driver genes" (a gene whose mutations increase the growth of a cell) of which, only 45% matched those found in adult cancer studies - deletions occurring in *CDKN2A* were found at the highest frequency. An interesting finding of MA's study was that whilst the percentage of tumours with point mutations in driver genes was highly consistent between whole-genome sequencing (WGS) and whole-exome sequencing (WES), WGS can also detect copy number alterations, which are frequently driver events for paediatric cancers. However, when neuroblastomas were analysed by WGS, 72% of the tumours were found to have driver mutations compared to just 26% of those analysed by WES; highlighting that perhaps there is a need to perform WGS rather than WES when trying to understand the molecular landscape.

These studies represent just the tip of the iceberg in terms of "discovery" sequencing, but what does all this mean for clinicians? After all, discovery sequencing studies are not designed with the needs of the clinic in mind and do not assess the ability to analyse and interpret sequencing results at the patient-level that will ultimately inform approaches to patient diagnosis, treatment and care .[23] Furthermore, the mere genomic detection of variants is not adequate for a clinical setting. It is necessary to determine whether variants are benign, likely benign, pathogenic, likely pathogenic or of uncertain clinical significance. [18] WGS can unearth numerous genetic variations but how can we tell which variants are clinically relevant? Reliable interpretation of variants requires experience, validation and skilled bioinformaticians to analyse the NGS data. [24] In addition, the vast volume of sequence data generated (millions if not billions of reads per run) gives rise to bioinformatics challenges in terms of data storage, quality control, assembly of the data and annotation. [25]

However, studies carried out in the clinical setting testify to the advantages of NGS over more traditional methods such as Sanger. An example of one such study is that by Oberg et al, (2016) [26] who sought to determine the clinical utility of genomic sequencing in standard clinical practice for paediatric cancer and haematological disorders. The authors performed WES of patient-matched turmour-normal samples and RNA sequencing (RNA-seq) of tumour samples to identify variants, gene expression, fusion transcripts and CNVs (copy number variants). From their study, they found 38% of patients had a targetable genomic alteration and matched therapy, based on these genomic findings, was administered to 16% of the patients. Genomic sequencing also allowed the molecular diagnosis of 23 patients and contributed to the prognostic and pharmacogenomics recommendations of 32 patients. The authors also reported how genomic sequencing identified a *STAT5B* mutation in a 5-year old girl who had been diagnosed with T-cell acute lymphoblastic leukaemia, the identification of this *STAT5B* mutation helped inform and ultimately change the patients' diagnosis to gamma-delta T-cell lymphoma. Furthermore, germline variations which held clinical significance were found in 20% of patients; allowing them to be referred for genetic counselling, along with the additional consideration for future cancer screening in the patients and their families.

An article published in BMJ [27] reports that England will start offering WGS to all children in 2019 as a strategy to offer comprehensive and precise diagnosis in a move towards personalised medicine in a bid to reduce the use of unnecessary and harmful drugs (Figure 3).

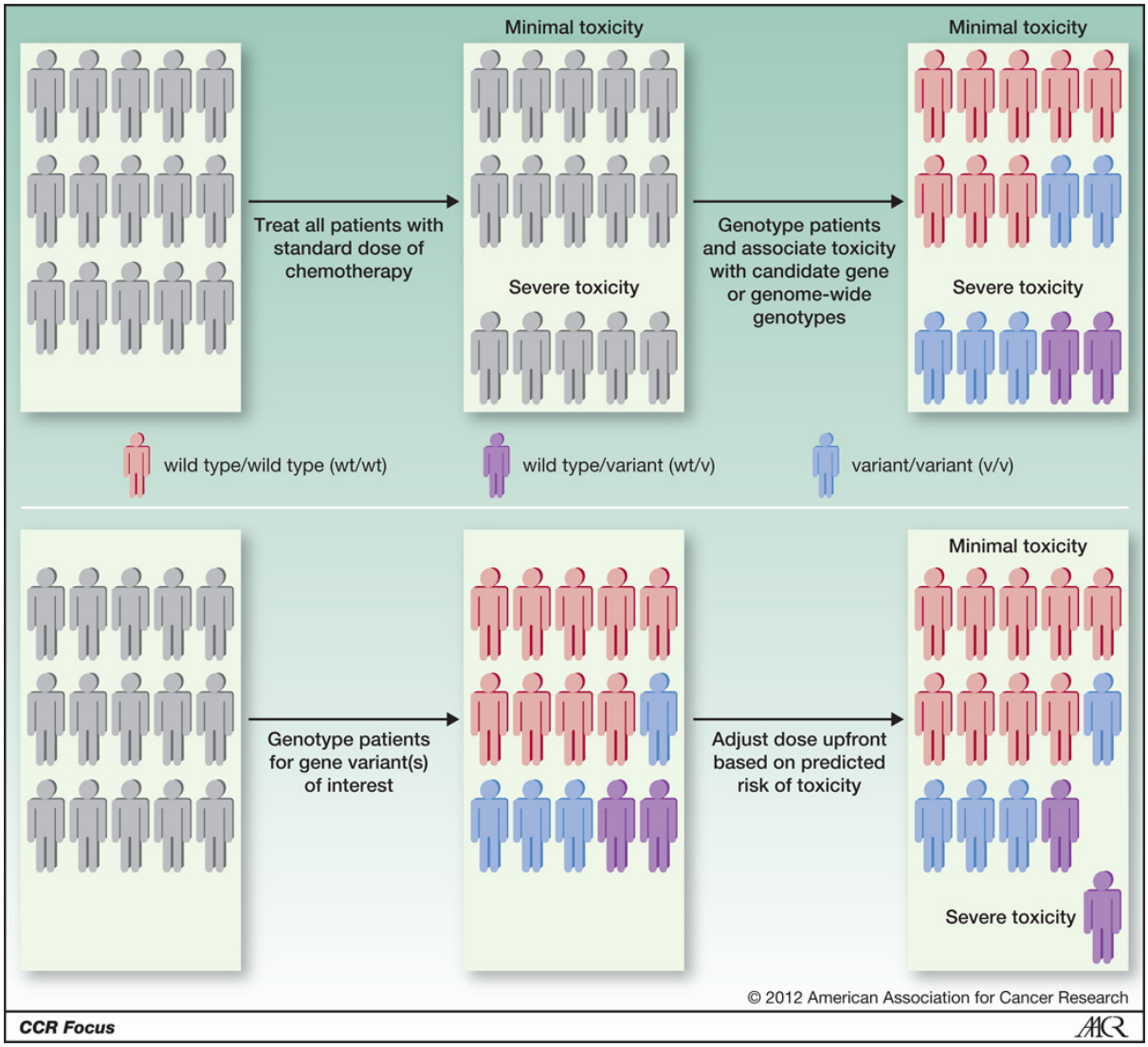

Figure 3. NGS is increasingly being used to develop personalised therapy to reduce treatment toxicity and non-responsiveness. [28]

Although it is still difficult to predict which child is at the greatest risk of chemotherapy-related toxicity and/or non-response WGS studies have been conducted that have shed light on genetic variants in genes not previously associated with drug toxicity or efficacy. [28] Yang et al, (2009) [29] conducted a study on paediatric acute lymphoblastic leukaemia to assess the effect of gene variants in response to therapy. WGS was employed to investigate the treatment response of 487 children. Whilst WGS identified 102 germline variants, 5 SNPs (single nucleotide polymorphisms) were found in *interlukin 15*, a cytokine shown *in vitro* to protect haematological cancers from glucocorticoid induced apoptosis. A more recent study conducted by Abaji et al, 2018, [30] conducted WES to identify genetic markers associated with vincristine-induced peripheral neuropathy (VIPN) in childhood acute lymphoblastic leukaemia. The authors found germline variants in *SYNE2*, *MRPL47* and *BAHD1* that were linked to increased risk of VIPN.

Conclusion

Paediatric cancer, whilst rare, it is still one of the leading causes of death for children and the molecular basis for these childhood cancer remains largely unknown. However, with research such as the Paediatric Cancer Genome Project, coupled with NGS, our understanding of paediatric cancer has made great strides – and with it, our ultimate goal of achieving more effective and individualised treatment regimens.

References

1. Sullivan R, Kowalczyk JR, Agarwal B, et al. New policies to address the global burden of childhood cancers. *Lancet Oncol* 2013;14(3):e125-35. doi: 10.1016/S1470-2045(13)70007-X [published Online First: 2013/02/20]
2. Cancer Research UK. Children's Cancer Statistics 2018 [Available from: https://www.cancerresearchuk.org/health-professional/cancer-statistics/childrens-cancers.
3. Gröbner SN, Worst BC, Weischenfeldt J, et al. The landscape of genomic alterations across childhood cancers. *Nature* 2018;555(7696):321-27. doi: 10.1038/nature25480 [published Online First: 2018/02/28]
4. Pritchard-Jones K, Pieters R, Reaman GH, et al. Sustaining innovation and improvement in the treatment of childhood cancer: lessons from high-income countries. *Lancet Oncol* 2013;14(3):e95-e103. doi: 10.1016/S1470-2045(13)70010-X [published Online First: 2013/02/20]
5. Greaves MF. Aetiology of acute leukaemia. *Lancet* 1997;349(9048):344-9.
6. Eden T. Aetiology of childhood leukaemia. *Cancer Treat Rev* 2010;36(4):286-97. doi: 10.1016/j.ctrv.2010.02.004 [published Online First: 2010/03/12]
7. Stjernfeldt M, Berglund K, Lindsten J, et al. Maternal smoking during pregnancy and risk of childhood cancer. *Lancet* 1986;1(8494):1350-2.
8. Stavrou EP, Baker DF, Bishop JF. Maternal smoking during pregnancy and childhood cancer in New South Wales: a record linkage investigation. *Cancer Causes Control* 2009;20(9):1551-8. doi: 10.1007/s10552-009-9400-5 [published Online First: 2009/07/16]
9. Rumrich IK, Viluksela M, Vähäkangas K, et al. Maternal Smoking and the Risk of Cancer in Early Life - A Meta-Analysis. *PLoS One* 2016;11(11):e0165040. doi: 10.1371/journal.pone.0165040 [published Online First: 2016/11/08]
10. de Smith AJ, Kaur M, Gonseth S, et al. Correlates of Prenatal and Early-Life Tobacco Smoke Exposure and Frequency of Common Gene Deletions in Childhood Acute Lymphoblastic Leukemia. *Cancer Res* 2017;77(7):1674-83. doi: 10.1158/0008-5472.CAN-16-2571 [published Online First: 2017/02/15]
11. Ferreira JD, Couto AC, Emerenciano M, et al. Maternal Alcohol Consumption during Pregnancy and Early Age Leukemia Risk in Brazil. *Biomed Res Int* 2015;2015:732495. doi: 10.1155/2015/732495 [published Online First: 2015/05/18]
12. Latino-Martel P, Chan DS, Druesne-Pecollo N, et al. Maternal alcohol consumption during pregnancy and risk of childhood leukemia: systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2010;19(5):1238-60. doi: 10.1158/1055-9965.EPI-09-1110
13. Spector LG, Pankratz N, Marcotte EL. Genetic and nongenetic risk factors for childhood cancer. *Pediatr Clin North Am* 2015;62(1):11-25. doi: 10.1016/j.pcl.2014.09.013 [published Online First: 2014/10/18]
14. Ross JA. High birthweight and cancer: evidence and implications. *Cancer Epidemiol Biomarkers Prev* 2006;15(1):1-2. doi: 10.1158/1055-9965.EPI-05-0923
15. Daling JR, Starzyk P, Olshan AF, et al. Birth weight and the incidence of childhood cancer. *J Natl Cancer Inst* 1984;72(5):1039-41.
16. Johnson KJ, Carozza SE, Chow EJ, et al. Parental age and risk of childhood cancer: a pooled analysis. *Epidemiology* 2009;20(4):475-83. doi: 10.1097/EDE.0b013e3181a5a332
17. Yip BH, Pawitan Y, Czene K. Parental age and risk of childhood cancers: a population-based cohort study from Sweden. *Int J Epidemiol* 2006;35(6):1495-503. doi: 10.1093/ije/dyl177 [published Online First: 2006/09/28]
18. Calabria I, Pedrola L, Berlanga P, et al. The new challenge in oncology: Next-generation sequencing and its application in precision medicine. *An Pediatr (Barc)* 2016;85(5):273.e1-73.e7. doi: 10.1016/j.anpedi.2016.05.006 [published Online First: 2016/10/31]

19. GenomixLab. Whole Genome Sequencing 2019 [Available from: http://www.genomixlab.com/human-genome-sequencing/#.XIZ1ViL7RhE.
20. Zhang J, Walsh MF, Wu G, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. *N Engl J Med* 2015;373(24):2336-46. doi: 10.1056/NEJMoa1508054 [published Online First: 2015/11/18]
21. Huether R, Dong L, Chen X, et al. The landscape of somatic mutations in epigenetic regulators across 1,000 paediatric cancer genomes. *Nat Commun* 2014;5:3630. doi: 10.1038/ncomms4630 [published Online First: 2014/04/08]
22. Ma X, Liu Y, Alexandrov LB, et al. Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours. *Nature* 2018;555(7696):371-76. doi: 10.1038/nature25795 [published Online First: 2018/02/28]
23. Forrest SJ, Geoerger B, Janeway KA. Precision medicine in pediatric oncology. *Curr Opin Pediatr* 2018;30(1):17-24. doi: 10.1097/MOP.0000000000000570
24. Di Resta C, Galbiati S, Carrera P, et al. Next-generation sequencing approach for the diagnosis of human diseases: open challenges and new opportunities. *EJIFCC* 2018;29(1):4-14. [published Online First: 2018/04/30]
25. Xuan J, Yu Y, Qing T, et al. Next-generation sequencing in the clinic: promises and challenges. *Cancer Lett* 2013;340(2):284-95. doi: 10.1016/j.canlet.2012.11.025 [published Online First: 2012/11/19]
26. Oberg JA, Glade Bender JL, Sulis ML, et al. Implementation of next generation sequencing into pediatric hematology-oncology practice: moving beyond actionable alterations. *Genome Med* 2016;8(1):133. doi: 10.1186/s13073-016-0389-6 [published Online First: 2016/12/23]
27. Wise J. Genome sequencing of children promises a new era in oncology. *BMJ* 2019;364:l105. doi: 10.1136/bmj.l105 [published Online First: 2019/01/08]
28. Pinto N, Cohn SL, Dolan ME. Using germline genomics to individualize pediatric cancer treatments. *Clin Cancer Res* 2012;18(10):2791-800. doi: 10.1158/1078-0432.CCR-11-1938
29. Yang JJ, Cheng C, Yang W, et al. Genome-wide interrogation of germline genetic variation associated with treatment response in childhood acute lymphoblastic leukemia. *JAMA* 2009;301(4):393-403. doi: 10.1001/jama.2009.7
30. Abaji R, Ceppi F, Patel S, et al. Genetic risk factors for VIPN in childhood acute lymphoblastic leukemia patients identified using whole-exome sequencing. *Pharmacogenomics* 2018;19(15):1181-93. doi: 10.2217/pgs-2018-0093 [published Online First: 2018/09/07]