The influence of genetic variation on late toxicities in childhood cancer survivors: a review

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*Both authors contributed equally

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ABSTRACT

**Introduction:** The variability in late toxicities among childhood cancer survivors (CCS) is only partially explained by treatment and baseline patient characteristics. Inter-individual variability in the association between treatment exposure and risk of late toxicity suggests that genetic variation possibly modifies this association. We reviewed the available literature on genetic susceptibility of late toxicity after childhood cancer treatment related to components of metabolic syndrome, bone mineral density, gonadal impairment and hearing impairment.

**Methods:** A systematic literature search was performed, using Embase, Cochrane Library, Google Scholar, MEDLINE, and Web of Science databases. Eligible publications included all English language reports of candidate gene studies and genome wide association studies (GWAS) that aimed to identify genetic risk factors associated with the four late toxicities, defined as toxicity present after end of treatment.

**Results:** Twenty-seven articles were identified, including 26 candidate gene studies: metabolic syndrome (n=6); BMD (n=6); gonadal impairment (n=2); hearing impairment (n=12) and one GWAS (metabolic syndrome). Eighty percent of the genetic studies on late toxicity after childhood cancer had relatively small sample sizes (n<200), leading to insufficient power, and lacked adjustment for multiple comparisons. Only four (4/27=15%) candidate gene studies had their findings validated in independent replication cohorts as part of their own report.

**Conclusion:** Genetic susceptibility associations are not consistent or not replicated and therefore, currently no evidence-based recommendations can be made for hearing impairment, gonadal impairment, bone mineral density impairment and metabolic syndrome in CCS. To advance knowledge related to genetic variation influencing late toxicities among CCS, future studies need adequate power, independent cohorts for replication, harmonization of disease outcomes and sample collections, and (international) collaboration.
INTRODUCTION

Survival rates after childhood cancer now approach 80% in developed countries as a result of enhanced stratification, more effective treatment and optimized supportive care. The increasing number of childhood cancer survivors (CCS) has led to the growing awareness of chronic health effects resulting from treatment for childhood cancer. Examples of long-term consequences include hearing impairment, gonadal impairment and cardiotoxicity. The inter-individual variability in the number and magnitude of health problems in similarly treated CCS suggests that genetic variation modifies the association between treatment and risk of late toxicity.

To identify such genetic variants two common approaches have been applied: a candidate gene approach, and more recently, the genome wide association study (GWAS) approach. Candidate gene studies focus on associations between genetic variation within pre-specified genes of interest and specific outcomes, while GWASs are hypothesis-free searches that can identify novel single-nucleotide polymorphisms (SNPs) that potentially modify the risk of a late toxicity.

After completion of the Human Genome Project (HGP) in 2003 and the International HapMap project, GWASs have discovered many thousands of genetic variants associated with a variety of diseases, which catalyzed research on genetic variation underlying late toxicity among cancer survivors. Except for cardiotoxicity, the resulting number of genetic variation studies in CCS have not produced unambiguous evidence in this field. The lack of strong evidence has impeded translation into clinical practice, such as patient counseling or dose-reduction trials. In contrast, genotyping of childhood cancer patients in order to risk-adapt treatment based on risk models predicting susceptibility to specific (direct and late) toxicities is expected to become standard of care. A comprehensive review of genetic aspects of acute toxicity was recently published. However, a recent overview of genetic susceptibility studies concerning late toxicities in CCS is not yet available.

An international collaboration is currently working on the identification of genetic determinants associated with hearing impairment and female gonadal impairment, in a large cohort of CCS (European Union’s Seventh Framework programme project PanCareLIFE). In the current study, we summarize the results of a systematic literature search and evaluate the results and quality of available literature on genetic susceptibility of these two late toxicities (hearing impairment and female gonadal impairment) and three hormone-related late toxicities (male gonadal impairment, metabolic risk factors and bone mineral density impairment).
METHODS

Search strategy
To provide an overview of the established genetic susceptibility factors associated with late toxicities in childhood cancer survivors, we identified relevant articles, published up until September 2017, by systematically searching Embase, Cochrane, Google Scholar, MEDLINE and Web of science. Details of the full search strategy for each database are included in Appendix I. The computer-based searches were conducted by a medical information specialist at the university medical library in the Erasmus Medical Center.

Definitions
The majority (>80%) of the cohort in every article had to be diagnosed with cancer ≤21 years of age. As we were specifically interested in ‘late’ toxicity, defined as toxicity still apparent at follow-up after end of treatment, we only included studies that evaluated metabolic risk factors, bone mineral density, gonadal impairment or hearing impairment in CCS present after end of treatment regardless of follow-up time. Definition of endpoints used by the authors were extracted from the corresponding papers and assembled in tables.

Study selection
Two independent investigators (EC and ALFvdK) reviewed all titles and abstracts, and independently selected potentially eligible studies. Case series, case reports, abstracts or reviews were excluded. Only studies published in English were selected for the analysis. Disagreements were resolved through consensus. Full text papers were retrieved to assess fulfilment of the selection criteria (Figure 1). Cross reference check was performed to identify additional studies that were potentially overlooked during the initial search. Authors were contacted to clarify or supplement their results where necessary.

RESULTS & DISCUSSION

The search strategy yielded 2,762 unique records (Figure 1). After screening titles and abstracts, 156 articles were selected for detailed evaluation of full texts. After full-text review, 56 articles remained that reported on late toxicities. For the purpose of the current review we focused on gene-association studies of metabolic syndrome, low bone mineral density, and gonadal impairment and hearing impairment. As a result, 27 articles were considered in this review, including seven studies on metabolic syndrome (six candidate gene studies and one GWAS), six candidate gene studies of low bone mineral density, two candidate gene studies of gonadal impairment, and 12 candidate gene studies of hearing impairment (Table 1-4).
Of the candidate gene studies, 50% (13/26) had less than 100 participants while 80% (21/26) had less than 200 participants. Only two included a cohort of more than 500 CCS (n=532 and n=600)\textsuperscript{9,10}. Only six of the candidate studies (23%) adjusted for multiple testing to reduce the chance of type I error (false positive results), which would take into account the multiple models tested\textsuperscript{11-16}. One candidate study investigated both metabolic syndrome and bone mineral density\textsuperscript{9}. Where possible, the multivariable analysis of the combined results of the discovery and replication cohort are reported (Tables 1-4). Where applicable, the adjusted p-value corrected for multiple testing was reported.

\textbf{Figure 1.} Flowchart study selection process Review Genetics of Late Effects
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Cohort size (cases/control)*</th>
<th>Country of origin; ethnicity</th>
<th>Gender (% males)</th>
<th>Tumor type</th>
<th>Treatment</th>
<th>Replication</th>
<th>Definition endpoint</th>
<th>Studied no of SNPs (adj for multiple testing)</th>
<th>Gene / region</th>
<th>Variant</th>
<th>Effect allele / genotype</th>
<th>Multivariate analysis adjust for:</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson et al. 2015</td>
<td>GWAS</td>
<td>1996 (723/1273)</td>
<td>USA; 86.5% white, 12.5% black</td>
<td>51</td>
<td>Solid and hematological</td>
<td>CRT</td>
<td>Yes</td>
<td>Obesity BMI &gt; 30 kg/m²</td>
<td>2</td>
<td>SOX11</td>
<td>rs4971486</td>
<td>G</td>
<td>race, age at follow-up, age at diagnosis</td>
<td>2.01</td>
<td>(1.50–2.71)</td>
</tr>
<tr>
<td>Sawicka-Zkowska et al.2013</td>
<td>Cand. gene</td>
<td>74 Poland; 100% Caucasian</td>
<td>ALL and lymphoma</td>
<td>61</td>
<td>2.2% CRT</td>
<td>No (no replication in another CCS cohort)</td>
<td>1</td>
<td>LEPR rs1137101</td>
<td>leptin levels (linear)</td>
<td>NA</td>
<td></td>
<td></td>
<td>GG</td>
<td>total BMD SDS, spinal BMD SDS, lean mass SDS, cranial radiotherapy</td>
<td>NA</td>
</tr>
<tr>
<td>Van Waas et al. 2013</td>
<td>Cand. gene</td>
<td>532 Netherlands; 100% Caucasian</td>
<td>Solid and hematological</td>
<td>55</td>
<td>1.6% CRT</td>
<td>No</td>
<td>7 (no multiple testing)</td>
<td>ATP2B1 rs2681472</td>
<td>Hypertension: blood pressure ≥ 140/90 mmHg; MetS: two of the following: blood pressure ≥ 140/90 mmHg; BMI ≥ 30 kg/m²; self-reported prevalence of diabetes or medication; serum total cholesterol ≥ 5.2 mmol/l or medication</td>
<td>AQP2B1</td>
<td>rs2681492</td>
<td>GA vs AA</td>
<td>Age, gender, educational level, follow-up time, abdominal and cranial irradiation</td>
<td>None significant</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

**Table 1. Overview of studies on the influence of genetic variation on components of metabolic syndrome in CCS**
### Table 1. Overview of studies on the influence of genetic variation on components of metabolic syndrome in CCS (continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Cohort size (cases/control)*</th>
<th>Country of origin; ethnicity</th>
<th>Gender (% males)</th>
<th>Tumor type</th>
<th>Treatment</th>
<th>Replication</th>
<th>Definition endpoint</th>
<th>Gene / region</th>
<th>Variant</th>
<th>Effect allele/ genotype</th>
<th>Multivariate analysis adjust for:</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surapolchai et al. 2011</td>
<td>Cand. gene</td>
<td>131 (IR 40/91 and IGT 10/121)</td>
<td>Thailand; (ethnicity not specified)</td>
<td>59</td>
<td>ALL</td>
<td>low/standard/high ALL risk stratification</td>
<td>No</td>
<td>Impaired glucose tolerance: fasting plasma glucose level of 101 to 126 mg/dL and a 2-hour plasma glucose level of 160 to 200 mg/dL; Insulin resistance: whole body insulin sensitivity index &lt; 2.7</td>
<td>TCF7L2</td>
<td>rs12233580</td>
<td>AG vs GG</td>
<td>age at follow-up</td>
<td>5.28 (1.06-26.40)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Skoczen et al. 2011</td>
<td>Cand. gene</td>
<td>77 (24/53)</td>
<td>Poland; (ethnicity not specified)</td>
<td>55</td>
<td>ALL</td>
<td>BFM/New York treatment regimens</td>
<td>No</td>
<td>BMI (≥ 85th percentile)</td>
<td>LEPR</td>
<td>n.s.</td>
<td>n.s.</td>
<td>NA</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>Skoczen et al. 2011</td>
<td>Cand. gene</td>
<td>191 Polish ALL CCS (40/151)</td>
<td>Poland; (ethnicity not specified)</td>
<td>48</td>
<td>ALL</td>
<td>BFM/New York treatment regimens</td>
<td>No</td>
<td>BMI (≥ 85th percentile)</td>
<td>FTO</td>
<td>rs99929609</td>
<td>AA</td>
<td>Stratification for treated with CRT (≥ 20 Gy) yes/no</td>
<td>0.24 (0.08-0.7)</td>
<td>0.016</td>
</tr>
<tr>
<td>Rosset et al. 2004</td>
<td>Cand. gene</td>
<td>600 (278/322)</td>
<td>USA; non-hispanics</td>
<td>51</td>
<td>ALL</td>
<td>20 Gy CRT (females)</td>
<td>No</td>
<td>BMI ≥ 25 kg/m²</td>
<td>LEPR</td>
<td>GINQ2334g</td>
<td>Ag/Gln and Gln/Gln</td>
<td>Stratification for treated with CRT ≥ 20 Gy in females, adjusted for age at diagnosis</td>
<td>6.1 (2.1-22) (effect only in females)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**Abbreviations:** CRT: cranial radiotherapy; HT: hypertension; IGT: impaired glucose tolerance; MS: metabolic syndrome (defined by blood pressure ≥140/90 mmHg, BMI ≥ 30 kg/m², self-reported prevalence of diabetes or serum total cholesterol ≥ 5.2 mmol/l); N/A: not applicable; NA: not available

*indicated is the cohort size (cases and controls), as defined by the authors of the original article

P-values in bold are considered statistically significant by the authors of the original article. 
Ethnic race is stated if reported in original article.

Where applicable, the multivariable analysis of the combined results of the discovery and replication cohort are reported. If no replication cohort was included, multivariable analysis of the discovery cohort is reported, or univariate analysis is of the discovery if multivariable analysis was missing.

Where applicable, the adjusted p-value corrected for multiple testing was reported.
Metabolic syndrome components

The prevalence of components of the metabolic syndrome, including obesity, hypertension, dyslipidemia and type 2 diabetes (or specifically hyperglycemia or hyperinsulinemia), has been reported to be higher in CCS compared to the general population. Six candidate gene studies and one GWAS investigated polymorphisms associated with different aspects of metabolic syndrome. The polymorphisms in the candidate gene studies had been identified previously in GWASs performed in the general population or were based on the genes coding for hormones (or its receptor) associated with obesity. No studies addressed the genetic susceptibility of dyslipidemia. The only variants that had been investigated in multiple independent cohorts (Table 1) were variants within the gene coding for the leptin receptor (LEPR) which were evaluated because of their hypothesized functional contribution to obesity.

Leptin, a hormone secreted in adipocytes, has a key role in increasing satiety and energy homeostasis. Leptin insensitivity has been reported to be associated with obesity, leading to the hypothesis that obesity in CCS may be influenced by a carrier status of polymorphisms in the leptin receptor (LEPR). Only one of the three independent candidate gene studies in CCS that investigated the leptin pathway found a statistically significant correlation between a polymorphism in LEPR (GlnQ223Arg) and higher odds of being obese. The effect was sex-dependent and after stratification on sex, it was only significant in females (n=294, OR 2.4, 95% CI 1.3-4.8) and not in males (n=306). In addition, in the female subgroup, a significant interaction with cranial radiation (>20 Gy) was observed, suggesting that the impact of the polymorphism is especially prominent in female survivors who were treated with cranial irradiation. The impact of cranial irradiation can for a large part be attributed to the subsequent increased risk for growth hormone deficiency. The association between the GlnQ223Arg LEPR polymorphism and obesity has not been validated in the other two candidate gene studies, although cranial radiotherapy did amplify the association with leptin levels. These two studies were small (77 and 74 survivors, respectively) as compared to the study by Ross (600 survivors), which suggests that this inconsistency may be due to lack of power, especially considering the possible need for stratification for sex, which both studies did not carry out. Alternatively, this discrepancy in results could be due to a false positive result in the initial study by Ross et al, which did not include an independent replication cohort.

Using a candidate gene approach based on polymorphisms identified in GWASs in the general population, the association of seven polymorphisms (rs2681472, rs2681492, rs987237, rs7826222, rs864745, rs758597, and rs2943641) with respect to hypertension, waist circumference, diabetes and metabolic syndrome (defined as blood pressure ≥140/90 mmHg; BMI ≥30 kg/m²; self-reported prevalence of diabetes, or serum total cholesterol ≥5.2 mmol/l) was investigated. None of these SNPs were associated with the development of any single parameter of metabolic syndrome among CCS, including the presence of diabetes, and adjustment for cranial and abdominal radiotherapy did not change these
results. In contrast, cranial and abdominal radiotherapy were strongly associated with the presence of, or components of, metabolic syndrome. This may suggest that the impact of treatment, mainly radiotherapy, is more dominant than the influence of the tested variants on the components of metabolic syndrome.

The most recent genetic study was a GWAS in CCS of the St. Jude Lifetime Cohort, performed to identify genetic variants associated with obesity\textsuperscript{24}. In this GWAS, the cohort was stratified on cranial radiation exposure. Next, 70% of the strata was used as discovery cohort and 30% as replication cohort. Neither strata showed polymorphisms in the LEPR gene to be associated with obesity\textsuperscript{24}. Polymorphisms in regions near or within the SOX11 and CDH18 genes, regulators of neuronal growth, repair, and connectivity\textsuperscript{25,26} increased the risk of obesity among cranial radiated CCS\textsuperscript{24}. On the other hand, a polymorphism in FAM155A, thought to disrupt the hypothalamic-pituitary axis\textsuperscript{24,27}, decreased the likelihood of obesity in cranial radiated CCS. These findings have not yet been investigated in independent cohorts. Nevertheless, it is important to stress that the observed genetic variation will only partly explain the total variation in obesity, as other environmental factors such as cancer treatment and lifestyle are of major importance. In this GWAS, the pseudo $R^2$ (a measure for the amount of variability explained) in the cranial radiated strata was 0.174 for the clinical risk factors model, and 0.303 for the clinical risk factors combined with the SNPs model\textsuperscript{24}. Despite a significant increase, it also shows that this complex human trait deserves further research to understand its pathophysiological mechanism and its genetic components. Although a polymorphism in the LEPR gene would be a logical genetic determinant of metabolic risk factors, the evidence to date for the association is limited.

Gonadal impairment

Two candidate gene approach studies examined gonadal impairment; one in female and one in male CCS, and neither included a replication cohort.

The candidate gene study in female CCS explored the association between genetic variation and gonadal impairment based on high or low AMH levels\textsuperscript{11} (Table 2). Seven polymorphisms, each in a different gene, were evaluated. The polymorphisms had previously been identified in GWASs as associated with age at natural menopause in the general population\textsuperscript{28,29}. In this study in CCS, females with a heterozygous genotype for rs1172822 in the BRSK1 gene had higher odds of having a low AMH value (OR=3.15, 95% CI 1.35-7.32, $p=0.008$). A modifying effect of the SNPs on the impact of treatment was not specifically evaluated, but the OR was adjusted for alkylating agents score and abdominal radiotherapy. BRSK1 is expressed in the human forebrain and to a lesser extent in mammalian ovaries. Overexpression of the BRSK1 gene has been hypothesized to disturb hypothalamic-pituitary-ovary axis regulation by affecting the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus\textsuperscript{30} or to influence cell-cycle progression since it is essential for centriole duplication.
Table 2. Overview of studies on the influence of genetic variation on gonadal impairment in CCS

<table>
<thead>
<tr>
<th>Study</th>
<th>CoHORT size (cases/controls)*</th>
<th>Country of origin; ethnicity</th>
<th>Gender (% males)</th>
<th>Tumor type</th>
<th>Treatment</th>
<th>Replication</th>
<th>Definition endpoint</th>
<th>Studied no of SNPs (adj for multiple testing)</th>
<th>Gene/region</th>
<th>Variant</th>
<th>Effect allele/ genotype</th>
<th>Multivariate analysis adjust for: OR P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van Dorp et al. 2013</td>
<td>Cand. gen 176 (61/115)</td>
<td>Dutch; 100% Caucasian</td>
<td>0</td>
<td>Solid and hematological</td>
<td>Miscellaneous, with and without alkylating agents and abdominal radiation</td>
<td>No</td>
<td>AMH level below/ above 1 µg/L</td>
<td>7 (multiple testing)</td>
<td>IGF2R</td>
<td>rs9457827 CT</td>
<td>Age at measurement, AAD score and abdominal radiotherapy</td>
<td>0.75 (0.24-2.40) 0.633</td>
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<td></td>
<td>MCM8</td>
<td>rs236114 CT</td>
<td>0.96 (0.44-2.11) 0.919</td>
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<td></td>
<td>ARHGF7</td>
<td>rs7333181 GA</td>
<td>1.14 (0.46-2.83) 0.777</td>
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<td></td>
<td>PCSK1</td>
<td>rs271924 TT</td>
<td>1.40 (0.40-4.91) 0.602</td>
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<td></td>
<td></td>
<td>TNF</td>
<td>rs909253 GG</td>
<td>1.46 (0.47-4.49) 0.510</td>
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<td></td>
<td>BRSK1</td>
<td>rs1172822 CT</td>
<td>3.15 (1.35-7.32) 0.008</td>
<td></td>
</tr>
<tr>
<td>Romerius et al. 2011</td>
<td>Cand. gene 127 (23/104)</td>
<td>Sweden; 100% Caucasian</td>
<td>100</td>
<td>Miscellaneous, with and without alkylating agents and radiation</td>
<td>No</td>
<td>azoospermia: no sperms found in 40 microscopic fields of semen sediment at 400x magnification; 51 (no multiple testing)</td>
<td>ER Alpha rs12207396 AG vs GG</td>
<td>only univariate analyses, but stratified on high risk group (high-doses alkylating agents or radiotherapy)</td>
<td>8.8 (2.1-36) 0.004</td>
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<td></td>
<td>ER Alpha</td>
<td>rs9340958 CT vs CC</td>
<td>16 (2.1-100) 0.008</td>
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<td></td>
<td>ER Alpha</td>
<td>rs9340978 AG vs GG</td>
<td>8.1 (1.1-56) 0.091</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: RT: radiotherapy; TBI: total body irradiation

*indicated is the cohort size (cases and controls), as defined by the authors of the original article.
P-values in bold are considered statistically significant by the authors of the original article. Ethnic race is stated if reported in original article.

Where applicable, the multivariable analysis of the combined results of the discovery and replication cohort are reported. If no replication cohort was included, multivariable analysis of the discovery cohort is reported, or univariate analysis of the discovery if multivariable analysis was missing.

Where applicable, the adjusted p-value corrected for multiple testing was reported.
In male non-CCS, estrogen receptor deficiencies and polymorphisms are associated with infertility, although the exact mechanism remains to be elucidated. The only genetic study addressing estrogen receptor polymorphisms in 127 CCS examined 51 SNPs. This study did not adjust for multiple comparison and had no replication cohort, increasing the risk of type 1 errors. Only SNPs in the estrogen receptor α gene were associated with increased risk of developing azoospermia, and this effect was stronger in the subgroup treated with high cumulative doses of alkylating agents/cisplatin or lower doses with additional radiotherapy. Other polymorphisms, coding for androgen receptors and estrogen receptor β, were not found to be associated with infertility in these CCS.

In both male and female CCS only one candidate gene study has been performed to evaluate the genetic component of variation in long-term gonadal impairment. This variation needs further investigation, preferably in large GWASs with a replication cohort.

**Bone mineral density impairment**

Genetic variation in low bone mineral density (BMD) in CCS has been studied in six candidate gene studies (Table 3), of which one candidate gene study included up to 100 SNPs and adjusted for multiple comparisons. The most recently published study included a replication cohort, which failed to corroborate any of the earlier associations from the discovery cohort.

The CRHR1 gene has previously been found to be associated with impaired lung function in asthma patients and it has been suggested that CRHR1 gene variants may also explain differences in susceptibility to exogenous corticosteroid therapy, thereby influencing lung function, but also BMD. The G allele of a polymorphism (rs1876828) in the CRHR1 gene was associated with lower BMD in male survivors of acute lymphoblastic leukemia (ALL) (p=0.02), while, in contrast, a non-significant higher BMD was observed in female ALL survivors (p=0.09). As previously indicated for obesity, stratification by gender can be valuable, which again stresses the need for adequately sized cohorts.

Te Winkel and colleagues investigated 69 and 83 ALL survivors for respectively two and seven polymorphisms of six candidate genes and published this in two articles that in previous studies had shown an association between BMD impairment in the general population. ALL survivors who were carriers of the vitamin D receptor (VDR) 5'-end haplotype had an increased risk for lower lumbar spine BMD. Similarly, the MTHFR gene T-allele (rs1801133) was also identified as a risk factor for lower total body BMD. These studies also showed that carrier status of both VDR and MTHFR polymorphisms were associated with low BMD at diagnosis, before any treatment had been administered. However, the subsequent rate of BMD decline during treatment did not differ between carriers and non-carriers. Also, parameters of body composition were not different between carriers and non-carriers of the MTHFR and MTRR polymorphisms at diagnosis, nor during treatment or
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Cohort size</th>
<th>Country of origin; ethnicity</th>
<th>Tumor type</th>
<th>Treatment</th>
<th>Replication</th>
<th>Definition endpoint</th>
<th>Studied no of SNPs (adj for multiple testing)</th>
<th>Gene/region</th>
<th>Variant</th>
<th>Effect allele/ genotype</th>
<th>Multivariate analysis adjust for:</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Den Hoed et al. 2016</td>
<td>Cand. gene 334</td>
<td>Netherlands; caucasian</td>
<td>ALL, AML, lymphoma, brain tumor, renal tumor, sarcoma, neuroblastoma</td>
<td>59</td>
<td>Glucocorticoid</td>
<td>No</td>
<td>Lumbar spine bone mineral density (standard deviation score)</td>
<td>12</td>
<td>VDR</td>
<td>rs4516035</td>
<td>Height</td>
<td>NA</td>
<td>0.02</td>
</tr>
<tr>
<td>Park et al. 2016</td>
<td>Cand. gene 59</td>
<td>USA (73% white; 27% other)</td>
<td>ALL</td>
<td>52</td>
<td>Glucocorticoid</td>
<td>No</td>
<td>Lumbar spine bone mineral density (z-scores)</td>
<td>100</td>
<td>RAPGEF5</td>
<td>rs6416639</td>
<td>Ref allele homozygote</td>
<td>age, gender, height, BMD, Z-score, height, Tanner stage and vitamin D level measured at baseline</td>
<td>NA (lower BMD)</td>
</tr>
<tr>
<td>Sawicka-Zkowska et al. 2013</td>
<td>Cand. gene 74</td>
<td>Poland; caucasian</td>
<td>ALL</td>
<td>61</td>
<td>Glucocorticoid</td>
<td>No</td>
<td>Total bone mineral density (standard deviation score)</td>
<td>1</td>
<td>LEPR (Q223R)</td>
<td>rs1137101</td>
<td>GG</td>
<td>No</td>
<td>NA</td>
</tr>
<tr>
<td>Te Winkel et al. 2011</td>
<td>Cand. gene 83</td>
<td>Netherlands</td>
<td>ALL</td>
<td>57</td>
<td>Glucocorticoid</td>
<td>No</td>
<td>Bone mineral density total body (standard deviation score)</td>
<td>2</td>
<td>MTHFR</td>
<td>rs1801133</td>
<td>T</td>
<td>No</td>
<td>NA (lower BMD)</td>
</tr>
<tr>
<td>Te Winkel et al. 2010</td>
<td>Cand. gene 69</td>
<td>Netherlands</td>
<td>ALL</td>
<td>57</td>
<td>Glucocorticoid</td>
<td>No</td>
<td>Bone mineral density lumbar spine (standard deviation score)</td>
<td>7</td>
<td>VDR 5'-end (haplotype 3, bAT)</td>
<td>rs4616035</td>
<td>G</td>
<td>No</td>
<td>NA (lower BMD)</td>
</tr>
<tr>
<td>Jones et al. 2008</td>
<td>Cand. gene 309</td>
<td>USA (87% white, 12% black, 1% other)</td>
<td>ALL</td>
<td>51</td>
<td>Glucocorticoid</td>
<td>No</td>
<td>Bone mineral density (z-scores)</td>
<td>9</td>
<td>CRHR1</td>
<td>rs1876828</td>
<td>G</td>
<td>Ethnicity, weight, treatment</td>
<td>NA (lower BMD)</td>
</tr>
</tbody>
</table>

**Abbreviations:** CRT: cranial radiotherapy; N/A: not applicable; NA: not available

P-values in bold are considered statistically significant by the authors of the original article. Ethnic race is stated if reported in the original article.

Where applicable, the multivariable analysis of the combined results of the discovery and replication cohort are reported. If no replication cohort was included, multivariable analysis of the discovery cohort is reported, or univariate analysis of the discovery if multivariable analysis was missing.

Where applicable, the adjusted p-value corrected for multiple testing was reported.
after treatment. This suggests that while genetic variation may play a role in BMD variation, it does not modify the effect of treatment on BMD in ALL patients.

### Hearing impairment

Hearing impairment is commonly observed after treatment of CCS with the platinum agents cisplatin and carboplatin, or after cranial radiation. The effect of these treatments could be modified by genetic polymorphisms.

Twelve candidate gene studies have been performed, none of which included a discovery cohort larger than 250 subjects (Table 4). Only three of the studies included an independent replication cohort and to date, no GWAS has been published on CCS after completion of treatment.

In a study by Ross et al, in 53 CCS subjects almost 2,000 SNPs in 220 key pharmacogenetic genes involved in the absorption, distribution, metabolism and elimination of drugs were genotyped. This study included an independent replication cohort of 109 CCS. They identified **COMT** and **TPMT** as genetic determinants of variation in hearing impairment between CCS. Catechol O-methyltransferase (**COMT**) is involved in the metabolism of catechol drugs and is highly expressed on hair cells of the mouse. However, its role in auditory function remains unclear. Thiopurine S-methyltransferase (**TPMT**), involved in the metabolism of thiopurine drugs, has not yet been linked to cisplatin metabolism in the general population, although it has been demonstrated in murine inner ear cells to play a role in cisplatin metabolism and detoxification. Four additional studies aimed to replicate the previously identified associations of hearing impairment with **COMT** and **TPMT**. While one study confirmed these associations, the other three did not.

In the study by Yang et al, nearly all patients (91%) received the otoprotectant amifostine and all had cranial radiotherapy, both of which might mask genetic susceptibility. However, in their small underpowered cohort of 41 survivors who did not receive amifostine or cranial radiation, the association between **TMPT** and hearing impairment are in line with the other studies. This highlights the importance of a homogenous population with a large sample size, in order to avoid type 2 errors.

Polymorphisms in the low density lipoprotein-related protein 2, or megalin (**LRP2**) gene, which is expressed in the marginal cells of the stria vascularis in the inner ear, have been
postulated to predispose to cisplatin-induced hearing impairment. Three studies investigated the association between the LRP2 gene polymorphism (rs2075252) and hearing impairment, of which one study showed that the prevalence of hearing impairment was higher in CCS who carried the A allele of this polymorphism\textsuperscript{15,16,55}. However, this study did not include a replication cohort.

Another variant in this gene (rs2228171) was investigated in 68 CCS and was found to be significant, but has not been replicated in subsequent studies\textsuperscript{15}.

The association between hearing impairment and GSTT1 and GSTP1 loci, members of the glutathione S-transferases (GSTs) superfamily, was first described in survivors of adult cancer\textsuperscript{56}. GSTs are known to play an important role in cell protection by scavenging free radicals caused by cisplatin by conjugating it with glutathione\textsuperscript{57,58}. In CCS, the association between cisplatin-induced hearing impairment and polymorphisms in the GST gene family (GSTP1, GSTT1, GSTM1, GSTM3, GSTZ1) was investigated in four studies\textsuperscript{15,16,57,59}. One study of 39 survivors identified the GSTM*B allele to be associated with a lower risk of hearing impairment (OR: 0.11, 95% CI not given, p-value: 0.02)\textsuperscript{57} and a larger study of 86 medulloblastoma survivors found that survivors with the GSTP1 AG or the GG genotype had a greater risk of hearing impairment (OR 4.0, 95% CI: 1.2-13.6, p=0.03) than survivors with the AA genotype\textsuperscript{59}. However, the latter finding may be false positive since the study by Ross et al\textsuperscript{16}, in 162 subjects had 99.9% power to detect a similar effect at p≤0.05, but did not find a significant association between the GSTP1 genotype and hearing impairment.

While no GWAS examining hearing impairment has been performed in CCS after completion of therapy, one GWAS in 238 subjects reported on susceptibility to cisplatin-induced hearing impairment measured during childhood cancer treatment\textsuperscript{60}. This study identified one significant SNP in the ACYP2 gene\textsuperscript{60}, which codes for an acylphosphatase that can influence Ca\textsuperscript{2+} homeostasis in the cochlea and is involved in hair cell development\textsuperscript{61}. This finding was replicated in an independent cohort of 156 CCS after treatment, although pooling of the results from both studies was needed to reach statistical significance\textsuperscript{62}. This stresses the need not only for replication in independent studies, but also for adequately sized studies. The replication indicates there is no difference in genetic susceptibility in the cohorts with hearing impairment measured during or after treatment, which is in line with current knowledge concerning the irreversibility of hearing impairment. However, recent data suggests that in some survivors, cisplatin-induced hearing impairment manifests later in life, suggesting that some cases of cisplatin-induced hearing impairment might be missed if hearing function is only measured during treatment\textsuperscript{63}. Up until now, no GWAS has been published to study the effect of genetic variation on hearing impairment in long-term CCS.

In summary, the following genes were associated with hearing impairment in at least two independent sets of CCS subjects: COMT (rs4646316 and rs9332377, five reports, two significant\textsuperscript{16,51}), TPMT (rs12201199, rs1142345, rs1800460, five reports, two significant\textsuperscript{16,51}) and ACYP2 (rs1872328, two reports, two significant\textsuperscript{13,62}). Although large cohorts and
replication cohorts are requirements for solid genetic research, many studies on hearing impairment do not meet these criteria. The functional significance is not fully understood for all SNPs and the clinical implication of polymorphisms in $TPMT$ in hearing impairment has only been recently demonstrated in murine inner ear cells\textsuperscript{49}. The functional significance of polymorphisms in $COMT$ in hearing impairment is still unclear.

**FUTURE DIRECTIONS**

Among childhood cancer survivors the heterogeneity of late toxicities is broad, even in survivors who have been treated with the same protocols. This suggests a role for genetic variation. However, the evidence for an association between genetic variation and late toxicities after childhood cancer is largely insufficient or inconclusive to date, with few exceptions such as the reported associations between $ACYP2$ and hearing impairment. The inconclusive evidence is mainly due to a lack of well-designed, adequately powered studies. To date, in the reported late effects, only one GWAS has been performed. Especially in candidate gene studies, a) cohorts are small, b) replication cohorts are often lacking, c) the definitions used for biological endpoints are inconsistent across studies, and d) there are differences in study design across studies which hinders comparability. The lack of consistent associations across studies can be largely explained by methodological factors. In addition, variations in biological factors play an important role, since most of the outcomes studied are known to have multi-factorial etiologies, which include differences in genetic background, environment, behavioral factor, as well as co-morbidity. Moreover, clinical feasibility to collect data in a sufficiently powered and homogeneous cohort may play a role. Future research studies in this field could therefore benefit from considering the following principles.

Firstly, future studies need to include adequately sized cohorts in order to have sufficient power to identify low risk variants, which are the expected risk variants in common traits such as the evaluated late toxicities (i.e., common disease, common variant hypothesis). Several studies highlight the need for stratification or sub-analyses\textsuperscript{10,37}, which again require larger study populations. Power calculations and adjustment for multiple testing are essential tools to minimize type 1 and 2 errors. GWASs are becoming more popular and are evaluating hundreds of thousands to millions of SNP markers at the same time and require a multiple testing adjustment to $p<5\times10^{-8}$. Therefore large sample sizes are required to achieve sufficient statistical power\textsuperscript{64}. The number of SNPs to be included can increase exponentially when the sample size increases and studies with larger sample sizes are able to detect smaller associations as a result of higher power\textsuperscript{65}. This highlights the need for international collaboration to assure sufficient sample sizes to identify genetic associations. Moreover, a large sample size is important as the focus of genetic studies in late
<table>
<thead>
<tr>
<th>Study</th>
<th>Method</th>
<th>Cohort size (cases/controls)</th>
<th>Country of origin; ethnicity</th>
<th>Gender (% males)</th>
<th>Tumor type</th>
<th>Treatment</th>
<th>Replication</th>
<th>Definition endpoint</th>
<th>no of SNPs (adj for multiple testing)</th>
<th>Gene/region</th>
<th>Variant</th>
<th>Effect allele/genotype</th>
<th>Multivariate analysis adjust for:</th>
<th>OR</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiesen et al. 2017</td>
<td>Cand. gene</td>
<td>116 UK; 88% white, 5% Asian, 3% African</td>
<td>Medulloblastoma, hepatoblastoma, osteosarcoma, rhabdomyosarcoma, and other solid tumors</td>
<td>64</td>
<td>Cisplatin alone, combined cisplatin and carboplatin, or carboplatin after cisplatin; CRT (34%); vincristine (54%)</td>
<td>No</td>
<td>CTCAE and Chang</td>
<td>6 (multiple testing)</td>
<td>ACYP2 rs1872328 AA</td>
<td>NA</td>
<td>0.027</td>
<td>0.027</td>
<td></td>
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<tr>
<td>Brown et al. 2015</td>
<td>Cand. gene</td>
<td>71 (26/45) USA; 42% non-Hispanic white, 35% Hispanic, 24% other</td>
<td>Osteosarcoma or supratentorial primitive neuroectodermal tumor</td>
<td>73</td>
<td>Cisplatin and CRT; amifostine (39%)</td>
<td>No</td>
<td>Chang</td>
<td>1 (no multiple testing)</td>
<td>ACYP2 rs1872328 A</td>
<td>No</td>
<td>1.06 (0.66-2.21)</td>
<td>0.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haglaitner et al. 2014</td>
<td>Cand. gene</td>
<td>110 (42/68) Netherlands</td>
<td>Osteosarcoma</td>
<td>50</td>
<td>Cisplatin; no CRT; vincristine (45%); no ototoxicants</td>
<td>No</td>
<td>CTCAE, SIOP Boston</td>
<td>5 (no multiple testing)</td>
<td>TPMT rs12201199 A</td>
<td>0.96 (0.30-3.08)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Overview of studies on the influence of genetic variation on hearing impairment in CCS
<table>
<thead>
<tr>
<th>Study population</th>
<th>Analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study</strong></td>
<td><strong>Method</strong></td>
</tr>
<tr>
<td>Yang et al. 2013</td>
<td>Cand. gene</td>
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<td></td>
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<tr>
<td>Rednam et al. 2013</td>
<td>Cand. gene</td>
</tr>
<tr>
<td>Pussegoda et al. 2013</td>
<td>Cand. gene</td>
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<tr>
<td>Choeyprasert et al. 2013</td>
<td>Cand. gene</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Cohort size (cases/ controls)*</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Ross et al. 2009</td>
<td>53 (33/20)</td>
</tr>
<tr>
<td>Riedemann et al. 2008</td>
<td>50 (25/25)</td>
</tr>
<tr>
<td>Knoll et al. 2006</td>
<td>11</td>
</tr>
<tr>
<td>Peters et al. 2000</td>
<td>39 (20/19)</td>
</tr>
</tbody>
</table>

**Abbreviations**: CRT: cranial radiotherapy; N/A: not applicable; NA: not available
indicated is the cohort size (cases and controls), as defined by the authors of the original article.
P-values in bold are considered statistically significant by the authors of the original article. Ethnic race is stated if reported in original article. Where applicable, the multivariable analysis of the combined results of the discovery and replication cohort are reported. If no replication cohort was included, multivariable analysis of the discovery cohort is reported, or univariate analysis of the discovery if multivariable analysis was missing.
Where applicable, the adjusted p-value corrected for multiple testing was reported.
toxicities after cancer often is on an interaction between treatment and a polymorphism, and interaction studies require even larger power than regular association studies.

Secondly, future genetic studies will benefit from inclusion of independent replication cohorts, as is common practice in the GWAS field, to strengthen the study design and avoid type I errors. Yet again this needs international collaboration to replicate findings in independent studies.

Thirdly, to ensure that the genetic difference observed between cohorts is related to the disease or condition under study and to rule-out spurious associations, inclusion of cohorts with similar genetic backgrounds (similar ethnicity) is preferred. For study situations, where this is not feasible by design, several methods have been developed to correct for ancestrally distinct populations, such as principal components analysis, based on the variance of the studied genotypes. To date, most genetic studies have been performed in Caucasians. Genetic analyses in all ethnicities are required to avoid disparities in addressing knowledge gaps related to genetic susceptibility to late treatment effects.

In addition, to increase the chance of replication of results, harmonization by consistent definitions of outcomes and evaluation of possible confounders are necessary. Also, sufficient understanding of the molecular mechanisms underlying the disease or condition is important to adequately define cases and controls. In this regard, the proper selection of cases and controls has been extensively discussed within genetic epidemiology.

Next, it is essential that collection, processing, storage and retrieval of bio-specimens is conducted under quality control programs using standard operating procedures to guarantee low inter-sample variance and high quality of the samples. Within international collaborations, the establishment of an international biobank could be of value. Biobanks require high ethical practice standards, but offer research and researchers the possibility of cross-collaboration and synergy between different fields which is needed to further advance genetic research.

Finally, genetic technology is continuously improving, resulting in even bigger datasets with higher genetic resolution. Yet, the same principles as described above apply and with even more necessity given the even larger number of genetic variants tested. With the increasing availability of commercially available arrays and increasing affordability of large-scale GWAS, performance, coverage and imputation quality should be considered when choosing an array. While whole genome sequencing and whole exome sequencing have gained considerable attention in genetic epidemiology, and are gaining ground in the diagnostic phase of childhood cancer, none of these approaches have yet been taken in the evaluation of genetic susceptibility to late effects in CCS.

Up until now, evidence-based guidelines for CCS concerning genetic susceptibility testing have only been developed for cardiotoxicity. However, these guidelines are not implemented in clinical practice yet. For other late toxicities after childhood cancer the currently available literature is not robust enough, as yet, to inform reliable prediction.
models. However, genotyping childhood cancer patients in order to risk-adapt treatment based on risk models predicting susceptibility to specific late toxicities is likely to become standard of care. International collaboration is critical to advance knowledge of specific genetic risk factors in order to guide the development of scientifically rigorous prediction models. Currently, we are investigating the genetic susceptibility of hearing impairment and female gonadal impairment in an international consortium (European Union’s Seventh Framework programme project PanCareLIFE) with replication planned in independent cohorts from North America\textsuperscript{69}.

**CONCLUSIONS**

With growing knowledge of genetic determinants of late-effects and the continuation in decreasing genotyping costs, more personalized treatment protocols may become possible in the future. The criteria of 1) adequately sized cohorts and 2) the inclusion of independent replication cohorts are mandatory for well-founded research in genetic variability. International collaboration can ensure adherence to these criteria and thus be beneficial for the quality of research.
REFERENCES


