Retinoblastoma

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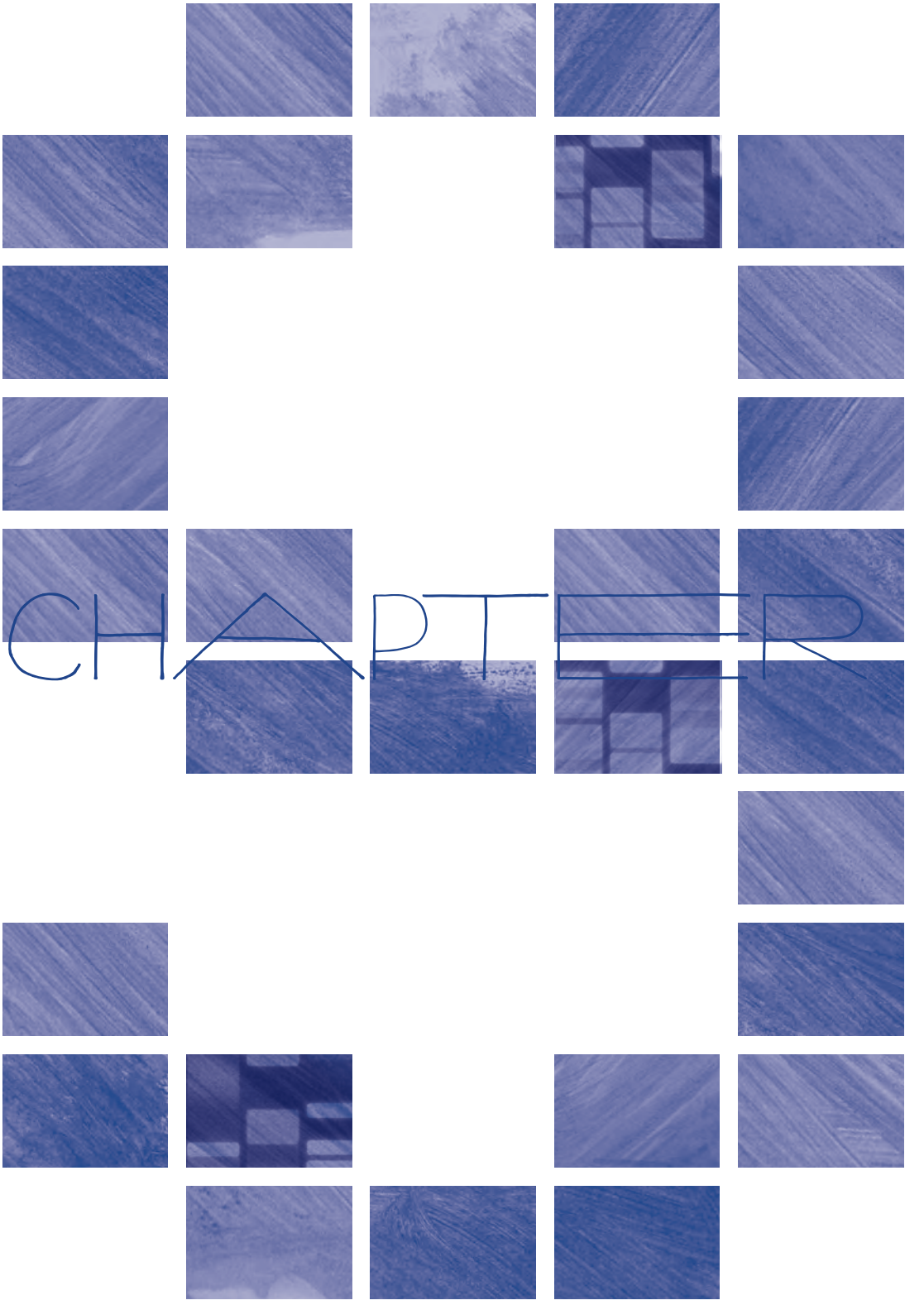
CHAPTER

SUMMARY AND GENERAL DISCUSSION

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This thesis reports on the molecular genetic and clinical aspects of retinoblastoma (Rb) and is centred on three main research subjects: 1) The spectrum of *RB1* mutations in the Netherlands, 2) Reproductive decisions of couples at increased risk for a child with Rb and 3) The possible association between in-vitro fertilisation (IVF) and Rb. In this chapter, the main findings are summarized and discussed, followed by recommendations for future research and clinical practice.

SUMMARY

Chapter 1 provides a general introduction on Rb and describes the aims and outline of this thesis.

In **Chapter 2** the *RB1* mutation spectrum detected in the Dutch National Retinoblastoma Register is described. From the 1173 Rb patients registered until January 2013, 529 patients from 433 unrelated families could be included. Our mutation detection methods revealed *RB1* mutations in 92% of bilateral and/or familial Rb patients and in 10% of non-familial unilateral cases. Overall an *RB1* germline mutation was detected in 187 (43%) of 433 non-related Rb cases, including 33 novel mutations. We concluded that the frequency of the type of mutations in the *RB1* gene in the unbiased national cohort is the same as the mutation spectrum described worldwide. Furthermore, our *RB1* mutation detection regimen achieves a high scanning sensitivity.

In **Chapter 3** we investigated whether specific *RB1* germline mutations are associated with greater risk of second primary tumours (SPT) in a large cohort of survivors of heritable Rb. We included 199 Rb patients with a documented *RB1* germline mutation diagnosed between 1905-2005. In total, 44 Rb patients developed an SPT after a median follow-up of 30.2 years (range, 1.33-76.0). There was no correlation between the different types of second malignancies diagnosed in these patients and the type of mutation or the region of the gene where the mutation was located. However, a significantly increased risk of SPT was observed among carriers of one of the 11 recurrent CGA>TGA nonsense *RB1* mutations (hazard ratio (HR) =3.53; [95% confidence interval (CI) =1.82-6.84]; *P*=.000), and there was a significantly lower risk for subjects with a low penetrance mutation (HR=.19; [95% CI=.05-.81]; *P*=.025).

Chapter 4 is a qualitative interview study of 14 couples with an increased risk for a child with Rb. In this study the impact of prospective risk on reproductive decisions, factors influencing these decisions, and the needs of couples with regard to reproductive counselling were explored. In most cases, the diagnosis of Rb influenced subsequent family planning. Prenatal diagnosis was used by two couples, while others refrained from having more children. Reproductive decisions

were influenced by the burden of the disease for the patient and family members, the impact of ophthalmological screening under anaesthesia, and couples' perceived risk, which did not always relate to their actual risk. Reproductive choices with regard to the number of children wanted changed over time. Our findings indicate topics to be discussed during genetic counselling of couples at increased risk for a child with Rb.

Chapter 5 is a cross-sectional questionnaire survey among 118 counselees visiting the clinical genetics department of the National Retinoblastoma Treatment Center in the Netherlands. The response rate was 69%. Of 43 respondents considering having children after becoming aware of their increased risk, Rb influenced reproductive behaviour for 25 (58%), of whom 14 had a recurrence risk of less than 3%. Twenty of these 25 decided against having more children and 5 used prenatal diagnosis. Eighteen of the 43 respondents did not use any of the alternative reproductive options (prenatal diagnosis, adoption or pre-implantation genetic diagnosis) and had (more) children, although half indicated having had doubts about their decisions. Multiple logistic regression showed that only perceived risk ($p = 0.003$) was significantly associated with Rb influencing reproductive behaviour. Of 17 respondents planning (more) children, 11 (65%) considered using one of the alternative reproductive options.

Chapter 6 investigates how often prenatal diagnosis (PND) has been performed for Rb in the Netherlands and compares this with uptake of PND for four other hereditary cancer syndromes: Von Hippel-Lindau disease (VHL), Li-Fraumeni syndrome (LFS), familial adenomatous polyposis (FAP), and hereditary breast ovarian cancer (HBOC). The number of independent mutation-positive families identified from the start of diagnostic testing until May 2013 and the number of PNDs performed for these syndromes within these families was assessed by sending a questionnaire to all nine DNA diagnostic laboratories in the Netherlands. We showed that uptake of PND for Rb was 11·8% (22/187 of mutation-positive families). This did not significantly differ from uptake of PND for VHL (6·5%, 7/92) or for LFS (4·9%, 2/41). But comparing uptake of PND for Rb to FAP (1·6%, 6/364) and HBOC (<0·2%, 6/>3000), did show a significantly higher uptake for Rb. The first PND for Rb was performed three years after the introduction of diagnostic DNA testing, while for the other cancer syndromes this was 10-15 years after the introduction of diagnostic testing. Uptake of PND for Rb has remained stable over the years, and uptake for the other syndromes showed an increase after 2009. So we concluded that uptake of PND for Rb was significantly higher than for FAP and HBOC, but not different from VHL and LFS. Early age of onset of cancer may be one of the factors that influence uptake of PND for cancer syndromes.

Chapter 7 describes a follow-up study on IVF and Rb. We collected information of infertility treatment of parents of virtually all 162 (98%) children diagnosed between January 1, 1995 and December 31, 2007. Seven children with Rb were conceived by IVF in this period. From nationwide estimates of numbers of live births conceived by IVF, the expected numbers of patients with retinoblastoma conceived by IVF was estimated in that period. The relative risk (RR) was calculated and we found a statistically significantly increased risk of retinoblastoma in children conceived by IVF in the period 1995-2007. However, the increased risk in the total study period was mostly based on the much stronger risk increase observed in a previous report for the period 1995 to 2002.
In **Chapter 8** the suggested association between IVF, retinoblastoma and tumour methylation characteristics is evaluated by investigating the *RB1* mutations in seven Rb tumours from children conceived by IVF or intracytoplasmic sperm injection (ICSI). For all tumours two causative *RB1* mutations were found. None of the tumours showed hypermethylation of the *RB1* promoter, demonstrating that an association between IVF or ICSI and retinoblastoma through this epigenetic mechanism is unlikely.

GENERAL DISCUSSION

RB1 mutations

The spectrum of RB1 mutations in patients from the Dutch National Rb register

Retinoblastoma is in the vast majority of cases initiated by loss of both alleles of the *RB1* gene in a retina precursor cell. The only exception currently known to this paradigm are tumours without *RB1* loss that harbour an amplification of MYC-N.[1] Knowledge of the presence of a heritable *RB1* mutation can help in risk management and reproductive decision making. Therefore it is important to screen all Rb patients for *RB1* mutations. In **Chapter 2** we describe all germline and tumour *RB1* mutations detected in the Dutch Rb cohort. With our current screening techniques we were able to detect 92% of germline mutations in bilateral and/or familial Rb patients.[2] Although this is a high percentage, still 8% of *RB1* mutations in heritable Rb cases remain undetected. An important challenge is to increase this percentage up to a 100%. There are several potential causes for missing mutations in *RB1* using current diagnostic screening techniques. First of all, mutations can be located in regions that cannot be detected with the methods used so far, like deep intronic mutations that create cryptic splice sites or promoter mutations in other regions than included in our test. Further testing of these regions (**Chapter 2**) did so far not detect mutations in our cohort of patients without *RB1* mutations. Second, for *de novo* mutations germline mosaicism is the major issue. For the latter group of patients deep sequencing was able to increase the percentage of germline mutations in sporadic unilateral patients from 13% to 18% and 96% to 97% in bilateral cases according to a recent study.[3] In the near future we will add next generation sequencing as a diagnostic tool for *RB1* mutation scanning and expect to increase our mutation detection rate. As pointed out in **Chapter 2**, theoretically another gene could be involved in a very small subset of Rb patients, although Rb is generally seen as a monogenetic disease. Whole exome sequencing of Rb familial patients without a detectable *RB1* mutation, if all additional options for *RB1* testing in these patients have failed to detect a mutation, may be able to discover mutations in a gene other than *RB1* as a cause of Rb. The Rb protein belongs to the family of pocket proteins, together with p107 (*RBL1*) and p130 (*RBL2*), which regulate the cell cycle by binding to E2F transcription factors. We tested *RBL1* and *RBL2* for mutations in heritable Rb patients without a detected *RB1* mutation, but did not find a mutation in these genes. Another explanation could be changes in epigenetic regulatory elements in non-*RB1* mutated tumours. There is a growing body of evidence that epigenetics play a part in retinoblastoma tumour formation. E.g. for the proto-oncogene SYK (spleen tyrosine kinase, widely expressed in hematopoietic cells and involved in mediating diverse cellular responses, including proliferation, differentiation, and phagocytosis) higher expression was seen in human retinoblastoma tissue and little or no expression in normal retina.[4] SYK

seemed to be required for Rb tumour cell survival. Furthermore, Zhang and co-workers found somatic mutations in the BCOR gene in 13% of retinoblastoma tumours. BCOR associates with proteins that repress gene expression epigenetically, so loss of BCOR may contribute to epigenetic changes in retinoblastoma tumours, potentially by increasing SYK expression. Several other studies have found micro-RNAs (miRNAs) to be involved in Rb tumorigenesis.[5] And recently a study described three Rb tumours without a detectable *RB1* mutation showing focal chromothripsis spanning the *RB1* locus, although the investigators were unable to rule out hypermethylation of the *RB1* promoter as a cause of tumour formation.[6] All these epigenetic phenomena may point to an alternative route of inactivation of the *RB1* gene and initiation of Rb.
In **Chapter 2** the frequency of the type of mutations in the *RB1* gene in our unbiased national cohort is described. We found this to be the same as the mutation spectrum described worldwide.
Germline nonsense mutations were detected in 37% (69 out of a total of 187) of cases.[2] Of these 69 nonsense mutations, 40 were one of the 11 well known recurrent *RB1* CGA>TGA nonsense mutations occurring in CpG dinucleotides, which are hotspots for spontaneous mutation by deamination of 5-methylcytosine within these CpGs.

Second primary tumours and RB1 mutations

Patients with heritable Rb have a high increased risk of second primary tumours (SPT) and this risk increases with use of radiation as treatment for Rb, especially when administered below the age of one year.[7,8] Types of SPT include soft tissue and bone sarcoma, melanoma and epithelial cancers like lung cancer and bladder cancer. The study by Marees et al. showed a cumulative risk 40 years after Rb of 28.0% (95% CI = 21.0% to 35.0%) and in another study the cumulative risk was 48.3% (95% CI = 38.1% to 59.7%) 50 years after Rb diagnosis.[9,10] If a genotype-phenotype correlation can be established for SPT-risk for individual Rb patients this could lead to knowledge about biologic mechanisms and eventually therapy for SPT and influence the screening recommendations for heritable Rb patients. In **Chapter 3** we described the SPTs of 199 heritable Rb patients with a documented germline *RB1* mutation. The most notable finding was an increased SPT risk for carriers of a recurrent nonsense mutation in *RB1*.[11] A possible explanation for the higher risk for SPT may be that some nonsense mutations escape nonsense mediated decay (NMD) in certain tissues and thus lead to expression of a truncated protein. This truncated protein may have a differential effect between these *RB1* mutations and other truncating mutations. As described above, the *RB1* gene is involved in several epigenetic processes, so another option may be an epigenetic effect where loss of a 5-methylcytosine within the gene may affect the chromatin structure and/or expression of the gene and thereby increasing

the chance of transformation of a cell. Our findings need confirmation by future studies through collaboration with other centres to enlarge the power.

Reproductive decisions and retinoblastoma

Reproductive behaviour of couples with an increased risk for a child with Rb and factors of influence on reproductive decision-making

To gain more insight into reproductive behaviour of couples at increased risk for a child with Rb and factors influencing behaviour, we conducted two studies. The first study (**Chapter 4**) concerned a qualitative interview study with 14 couples at increased risk of a child with Rb, to explore impact of prospective risk on reproductive decisions, factors influencing these decisions, and the needs of couples with regard to reproductive counselling. We found that reproductive decisions were influenced by the burden of the disease for the patient and family members, the impact of ophthalmological screening under anaesthesia, and couples' perceived risk, not always relating to their actual risk.[12]

The second study was a cross-sectional questionnaire survey among 81 individuals with an increased risk of a child with Rb to investigate the reproductive decision-making process, to examine past reproductive behaviour and to determine factors of influence on reproductive behaviour (**Chapter 5**).

The results show that 44% of the 81 respondents from all four risk groups had had doubts about their reproductive decisions as a result of Rb, while 38% had changed their minds about their decision whether or not to have any (or more) children.[13] So for many couples at increased risk of having a child with Rb, having children is not self-evident. Of the 81 respondents 43 had considered having children after becoming aware of their increased recurrence risk. Twenty-five (58%) of these 43 respondents reported that they had changed their reproductive behaviour because of Rb, showing that Rb had a great impact on reproductive choices. Of these 25 respondents who changed their reproductive behaviour, 20 (80%) decided against having any (or more) children, including 11 respondents with a recurrence risk of less than 3%. Five couples out of the 25 respondents changing their behaviour had used chorionic villi sampling to determine whether the foetus was affected, including three healthy couples with a child affected by hereditary Rb and a recurrence risk of 2-3%. Eighteen (42%) of the 43 respondents who had considered having children after becoming aware of their increased risk, decided to have a (subsequent) child and did not choose any of the alternative reproductive options.

It has been shown that several factors, such as the perceived burden and familiarity with the disorder, can be of influence on reproductive decisions of couples at increased risk of a child with a genetic disorder.[14,15] With regard to Rb, factors associated with reproductive decisions in univariate analysis were type of treatment and perceived consequences of Rb. The latter included factors such

as the risk of passing Rb on to offspring, the risk of a child with impaired vision or blindness and fear or worries about developing second primary tumours later in life. Treatment with bilateral enucleation, chemotherapy and/or radiotherapy was more of influence on reproductive decisions than treatment with just unilateral enucleation and/or local therapy. We found the only factor significantly associated with influence of Rb on reproductive behaviour in multiple logistic regression was perceived risk. This is in agreement with several other studies concluding that people's perception of the genetic risk, more than the magnitude of the actual risk, is of influence on reproductive decisions.[14,16-18]

One of the aims of genetic counselling is to correct misperceptions of risk and increase understanding of the genetic risk information, to enable informed decision-making.[19] However, risk perception is a complex process and is influenced by many factors. Personal beliefs and expectations about risk prior to counselling, psychological impact of the family history and emotional aspects also influence risk perception.[17,20] Some parents tend to relate to their risk as a two-way option: it will or will not happen, rather than a probabilistic figure, provided by genetic counselling.[21,22]

Risk communication in cancer and reproductive genetics has been subject of many studies and is known to be crucial for interpretation and decision making of counselees. In genetic counselling of individuals with an increased risk of a child with Rb, it seems important to give special attention to the interpretation of the objective risk.

Another finding from our study was that many individuals changed their minds over time with regard to having (more) children or not, so easy access to a follow-up genetic counselling session may be important. When treatment of Rb is centred on a national level, patients with Rb can be offered lifelong follow-up by a multidisciplinary team, including a clinical geneticist. This ensures easy access to the clinical geneticist in different phases of life of the Rb patient and renewed counselling of parents of an Rb patient when their views change over time.

Uptake of prenatal diagnosis for Rb and other hereditary cancer syndromes

The uptake of prenatal diagnosis (PND) for Rb was investigated and compared to four other hereditary cancer syndromes (**Chapter 6**): Von Hippel-Lindau disease (VHL), Li-Fraumeni syndrome (LFS), familial adenomatous polyposis (FAP), and hereditary breast ovarian cancer (HBOC). PND was done significantly more often for Rb than for FAP and HBOC. Uptake of PND was not significantly different between Rb and VHL, and Rb and LFS. Early onset, high penetrance, lack of preventive surgery and perceived burden of disease may explain these differences. We also found that PND for Rb started many years before it was used for the other hereditary cancer syndromes. Just three years after DNA diagnostic testing for Rb became available, the first PND was performed. For the other four cancer

syndromes PND was not started until 10-15 years after beginning of DNA testing. An important difference between Rb and the other cancer syndromes is that expression of Rb is at a very early age. This means that the diagnosis in a future child carrying the disease causing mutation will be soon after birth. For most other syndromes, the possible diagnosis may not be until 20 years later, leaving the future parents room for hope of better treatment options. Furthermore, parents of an affected child with Rb will still be in the reproductive age, so they have the option for PND in a subsequent pregnancy. It is also very well possible that genetic counsellors, especially in the 1990s, were more likely to discuss PND as an option with future parents affected with Rb, than with carriers of *VHL* or *BRCA1/2* mutations. Of importance may also be that in the beginning of *BRCA1/2* testing, most counselees were women in their 30s and 40s and their families were already completed at the time of diagnosis. The children of these women are now in the reproductive age and presymptomatic *BRCA1/2* testing is actively pursued, including discussion about reproductive implications of mutation carrier status. This in accordance with an increase of uptake of PND for *BRCA1/2* **(Chapter 6)** and also with an increase for preimplantation genetic diagnosis (PGD) for *BRCA1/2* over the past few years, while uptake for Rb has been stable over the years.[23]

IVF and retinoblastoma

As mentioned in the introduction, around 2,5% of live born children are born after IVF or ICSI in the Netherlands. In 2003, our study group reported an observed increased incidence of Rb in Dutch children conceived by IVF born between 1997 and 2001.[24] Other, IVF register-based, studies did not find an increased incidence of Rb after IVF, but these studies will likely have lacked power to detect a potential increase.[25,26] **Chapter 7** describes the follow-up study on IVF and Rb. Information was collected about infertility treatment of all parents of Rb patients born between 1995-2007. We found that the relative risk (RR) for Rb was significantly increased in children conceived by IVF in the period 1995-2007, but this was mostly based on the much stronger risk increase observed in the period 1995 to 2002.[27] Because the association between *RB1* and IVF is suggested to lay in disturbance of epigenetic hypermethylation mechanisms, we examined retinoblastoma tumours of seven children conceived by IVF or ICSI for hypermethylation of the *RB1* promoter. In none of the seven tumours hypermethylation of the promoter as a means of *RB1* silencing was found. This demonstrates that an association between IVF or ICSI and retinoblastoma through this epigenetic mechanism is highly unlikely. Furthermore, of the total of nine Rb patients conceived by ICSI or IVF three were bilaterally affected and had a *de novo RB1* germline mutation, whereas the other six patients were unilaterally affected and no germline mutation could be detected. The mutations in the Rb tumours originate thus, at least for the first hit, at different moments in time. For

the patients with heritable Rb, the first hit is constitutional and must have been present in the fertilized oocyte (or in one of the cells of the multicell embryo in the one case of germline mosaicism). The second hit occurs somatically in the retinal cells. In the cases without a constitutional *RB1* mutation, the first and second hits arise in a single somatic retinal cell. A common cause for both heritable and non-heritable Rb originating in the IVF/ICSI procedure seems therefore unlikely.
A recent study from France on the risk of Rb after fertility treatment did not find an increased risk, but did show an increased risk for women for whom time to pregnancy exceeded 24 months.[28] A study from Sweden investigated cancer risk in children and young adults conceived by IVF. They found a slight increase: overall cancer risk estimate 1.42 (95% CI: 1.09-1.87).[29] No increased risk was seen for Rb. Our findings of an increased risk in the 1995-2002 period may most likely be due to chance clustering. Although it may be too early to completely refute the association between Rb and IVF/ICSI, the absolute risk of Rb after IVF/ICSI is low.

RECOMMENDATIONS FOR FUTURE RESEARCH

RB1 mutations and SPT

To gain more insight in the relation between SPT, *RB1* mutations and tumour formation we are now planning collaborative studies with several large Rb centres. Furthermore, we will investigate tumour tissue of both Rb tumours and SPTs of *RB1* mutation carriers and perform tumour exome studies to look for other genetic and epigenetic factors influencing tumour risk. Collaboration with scientists working with mouse models for Rb is crucial to confirm theories on tumour development and test new therapy options.
The ultimate goal for future research is to be able to prevent Rb and SPT or, as next best option, to be able to treat these tumours with as little damage as possible. This may be feasible when we improve personalized genetic risk for each patient with heritable Rb. In the end, this could lead to preventive treatment or influence screening recommendations and decrease morbidity and mortality from Rb and SPTs.
At the moment no evidence based surveillance program for SPTs in heritable Rb patients exists. Our research group has started a prospective multicenter study to evaluate the potential benefit of annual craniofacial MRI for early detection of SPT before symptoms arise in irradiated hereditary retinoblastoma patients 8–18 years old. Whether this will lead to reduced morbidity and mortality is yet to be determined. A pilot study on annual whole-body MRI in heritable Rb patients suggested that SPTs were detected, but with modest sensitivity (66.7%).[30] Another subject for future investigations is the long-term effect of treatment of Rb with chemotherapy on SPT risk and overall health.

Reproductive decisions

In future studies it will be of interest to compare intended reproductive behaviour of our cohort with actual decisions and to investigate prospective reproductive decisions of new couples at increased risk for a child with Rb. It will be important to get more insight in the underlying motives of couples that have opted for PND as a reproductive option. Besides, a comparison between couples opting for PND and those opting for PGD should be included. This information can be useful to improve care for families with a genetic predisposition for cancer.

IVF

Lastly, it is still important to continue collecting information on fertility treatment of parents of Rb children, to be able to completely refute the association of Rb and IVF/ICSI. Furthermore, large scale long-term studies of follow-up of all children conceived by IVF are needed.

RECOMMENDATIONS FOR CLINICAL PRACTICE

Specialized treatment centre

First of all, because Rb is such a rare disease, treatment of Rb should be in a specialized centre with a multidisciplinary team. In the Netherlands the care for Rb patients has been regulated at a national level: all patients are treated in the National Retinoblastoma Treatment Center. Several other countries also have a national referral centre.[31-34] This multidisciplinary team needs to be centred around dedicated ophthalmologists and should further include a paediatric oncologist, and adult oncologist, a clinical geneticist and molecular geneticist, a specialized radiologist and pathologist, radiotherapist, psychologist and specialized nurses. Close collaboration of all members of the team is important and will assure timely referral to a member of the team when indicated.

RB1 mutation scanning

Scanning of the *RB1* gene for mutations is essential, irrespective of laterality and family history, both in the germline and in tumour tissue when available. This can aid in choice of therapy and surveillance, and in reproductive decisions of the patient and/or the parents of a patient. A flow-chart to guide the different steps in DNA-testing and guide ophthalmological screening of family members at risk is included (**Figure 1**).

Follow-up

When the Rb treatment is completed, regular contact with a member of the team is advised. Patients should be invited to lifelong follow-up in ophthalmology, the frequency depending on age, heritability and type of treatment. Patients treated

with chemotherapy, radiation and all heritable patients should also be included in life-long oncology follow-up. At the moment, the main purpose of oncology follow-up is adequate and prompt evaluation of complaints that may be an indication of an SPT. Annual craniofacial MRI for SPT can be considered for all irradiated heritable patients, and should be part of a research study to evaluate the use of this type of surveillance.

Recommendations for genetic counselling

First of all, it is important that patients treated for Rb and their parents have continued access to genetic counselling, also after the initial diagnosis and treatment.

The following issues are important to consider when discussing family planning with couples at increased risk for a child with Rb:

— Perceived Rb risk for offspring, as in the interpretation of the objective risk by the counselee.
— Perceived consequences of Rb for child and parents, including the risk of passing Rb on to offspring, the risk of a child with impaired vision or blindness and fear or worries about developing second primary tumours later in life.
— The perceived impact of extensive treatment of Rb patients, including the negative experience with the sequellae of Rb treatment of affected individuals.
— The perceived burden of ophthalmological screening under anaesthesia for children at risk.

All patients with heritable Rb and/or their parents and the physicians caring for them should be informed about their increased risk for SPT. Patients and/or parents of Rb patients should be aware of the potential factors that could further increase their risk of cancer such as smoking and excessive UV exposure. When possible, diagnostic or treatment exposure of X-rays should be avoided. Patients and/or their parents should be encouraged to contact a physician when unexplained complaints arise.

Participation in future research

It is advised to note in the medical files of all newly diagnosed Rb patients whether the child was conceived with the use of fertility treatment.

All patients and/or their parents should be invited to participate in research by asking informed consent for including anonymized clinical and genetic information in ongoing and future studies.

Figure 1. Flowcharts for DNA diagnostic testing of patients with Rb and ophthalmological screening of family members

UL = unilateral

BIL = bilateral

OS = ophthalmological screening

EUA = examination under anaesthesia

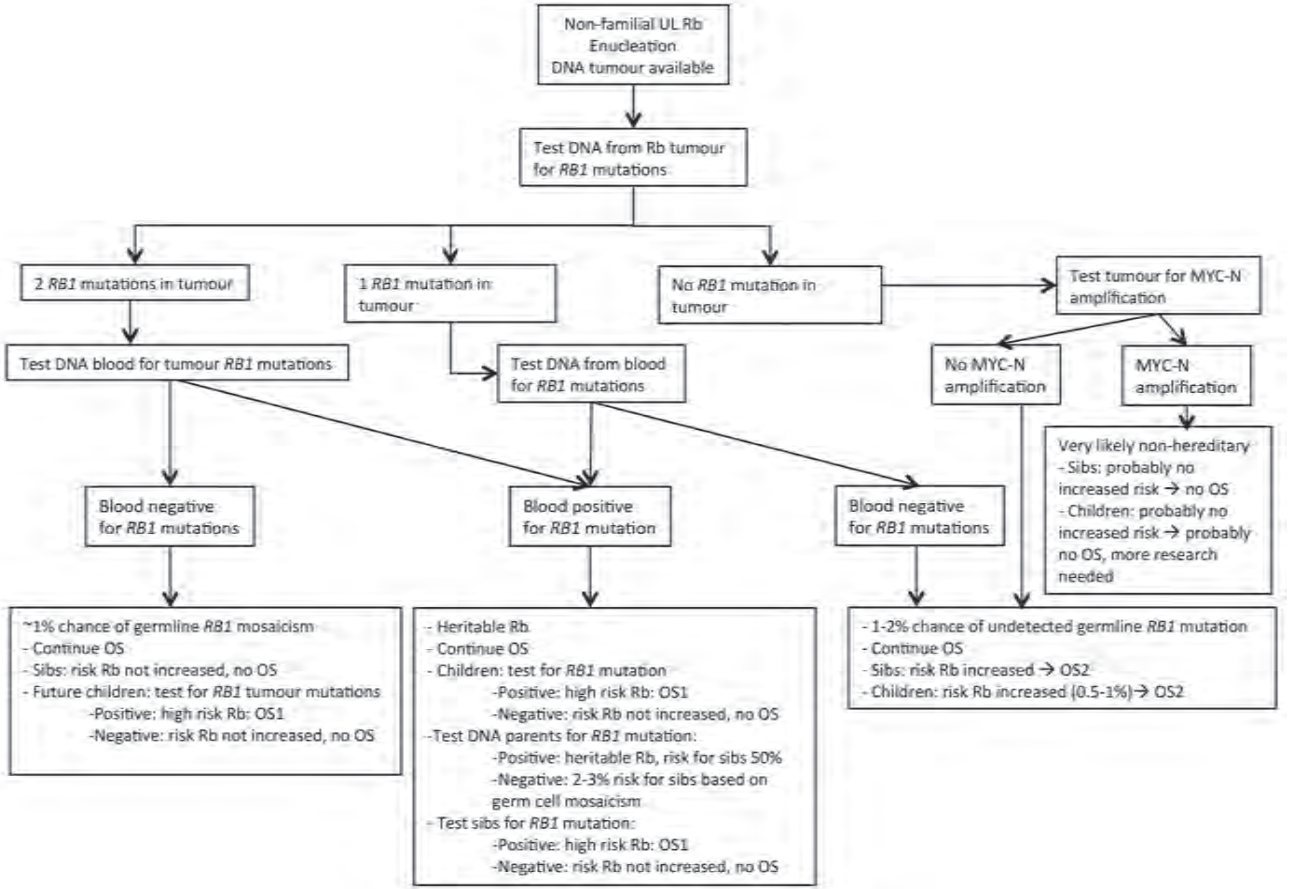
— OS: OS schedule for children affected by Rb is determined by the treating ophthalmologist

— OS1: OS for children who are carrier of an RB1 mutation or who have a 50% risk of being carrier of an undetected RB1 mutation:
  - Start as soon as possible after birth until the age of 3 months ophthalmological examination without anaesthesia
    - Every 2-4 weeks

— If no Rb has been detected, start EUA from the age of 3 months:
  - 3 months – 6 months: every 4 weeks
  - 6 months – 1 year: every 4 – 8 weeks
  - 1 – 2 years: every 3 months
  - 2 – 3 years: every 4 months
  - Until the age of 4 years: ophthalmological examination every 6 months without anaesthesia
  - If Rb has been detected, the OS schedule is determined by the treating ophthalmologist

— OS2: OS for children with an increased risk for Rb of less than 3%:
  - Start 2-3 weeks after birth until the age of 6 months: ophthalmological examination without anaesthesia
    - Every 6 weeks

— If no Rb has been detected, start EUA from the age of 6 months:
  - 6 months - 1 year: every 2 months
  - 1 – 2 years: every 3 months
  - 2 – 3 years: every 4 months
  - Until the age of 4 years: ophthalmological examination every 6 months without anaesthesia



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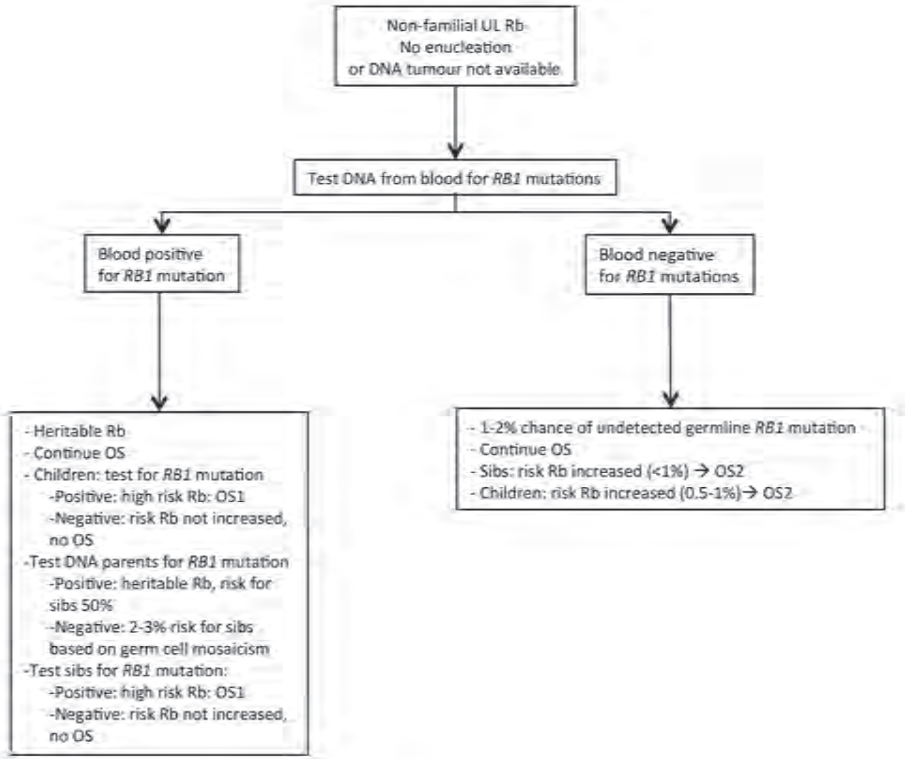

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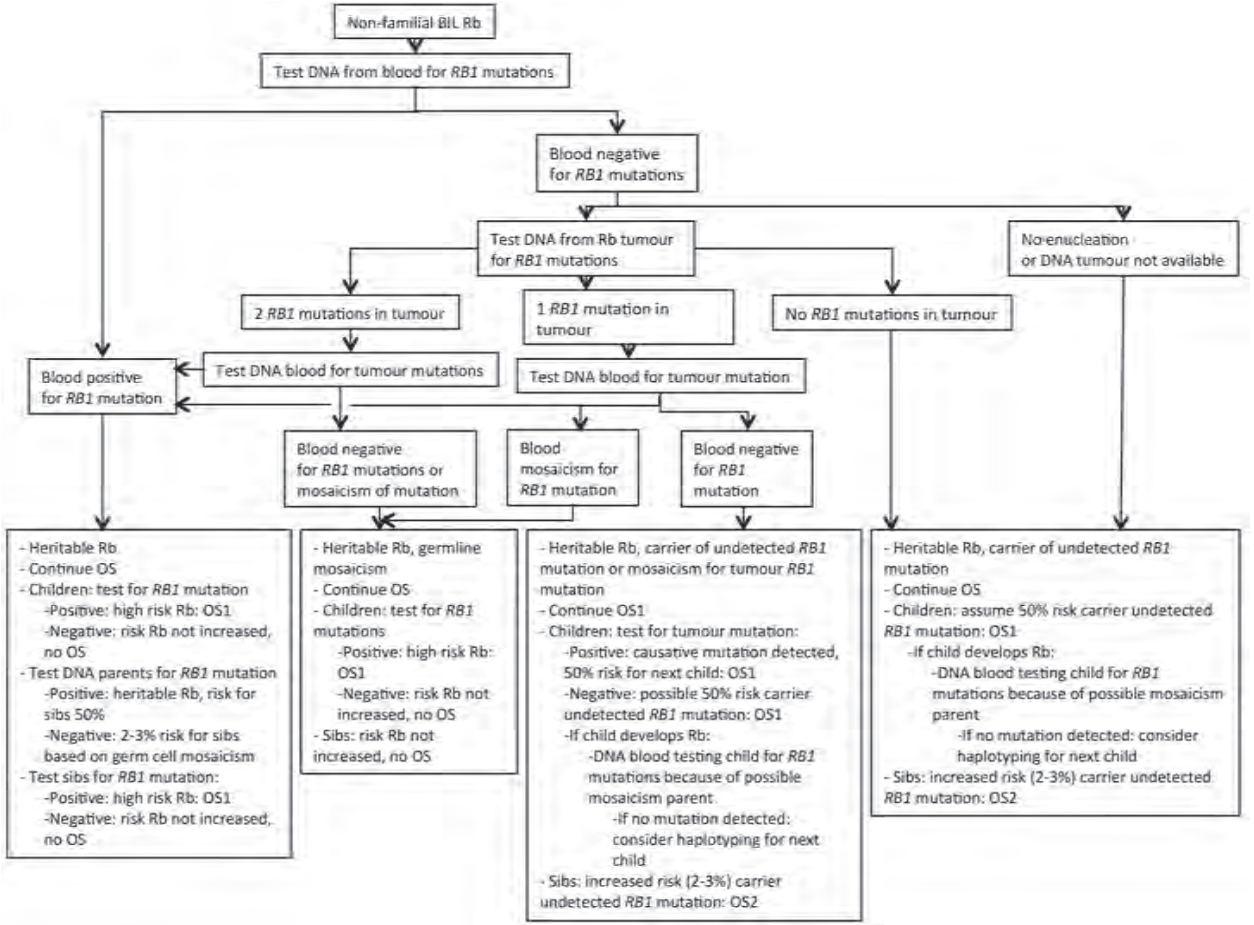

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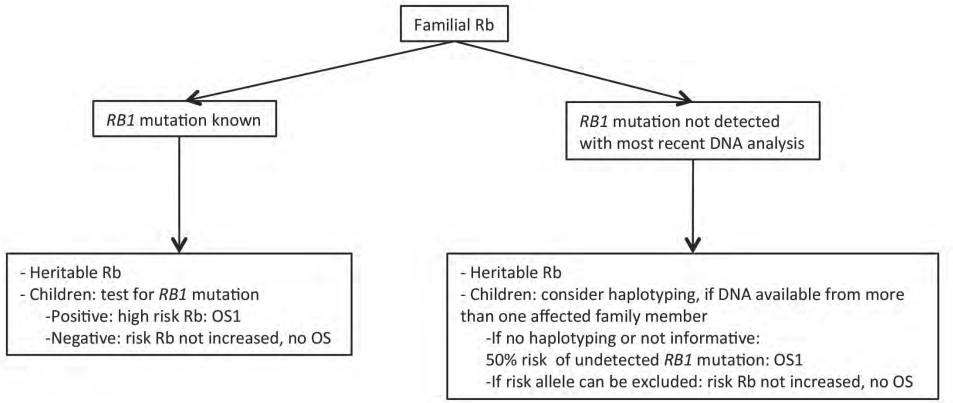

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