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HERITABLE *TP53*-RELATED CANCER SYNDROME IN SWEDEN: CHARACTERISATION OF GENOTYPE-PHENOTYPE CORRELATION AND SURVEILLANCE

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Heritable *TP53*-related cancer syndrome in Sweden:
Characterisation of genotype-phenotype correlation and surveillance
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To the families who generously participated in the studies.

ملخص مبسط للأطروحة

ينتج مرض السرطان عن تراكم الضرر في المادة الوراثية للخلايا، ويصاب ما يقرب من ثلث جميع الأشخاص بمرض السرطان. إن الخطر يزداد مع تقدم العمر ويتأثر بأنماط الحياة المختلفة والعوامل البيئية. في حوالي 10٪ من جميع مرضى السرطان، هناك ميل وراثي فطري للإصابة بالسرطان، والذي يمكن أن يزيد أيضًا من خطر الإصابة به في وقت مبكر من الحياة. غالبًا ما تكون مخاطر الإصابة بالسرطان المتزايدة بشدة بسبب التلف الخلقي في جينات السرطان المحددة. يمكن أن تكون هذه الأضرار، التي يطلق عليها المتغيرات الجينية المرتبطة بالمرض، إما وراثية أو تنشأ تلقائيًا في الفرد فيما يتعلق بالتخصيب. لدينا آليات مدمجة في الخلايا للحماية من تطور السرطان. أحد أهمها يسمى "حارس الجينوم" - الجين.TP53 إذا كان حراس الجينوم أنفسهم معاقين منذ الولادة، فما هي العواقب؟ يُطلق على متلازمة خطر الإصابة بالسرطان النادرة اسم متلازمة Li-Fraumeni (LFS) وهي ناتجة عن تلف خلقي في جين TP53 على وجه الخصوص. يتعرض حامل TP53 لخطر كبير جدا (70- 100٪) للإصابة بالسرطان في مرحلة ما من حياته. والأكثر شيوعًا هو سرطان الثدي وأورام الدماغ وسرطان الغدة الكظرية وسرطان العظام والأنسجة الرخوة (ساركوما) وأيضًا في سن مبكرة. يبدو أن بعض العائلات معرضة بشكل متزايد لخطر الإصابة بأورام الطفولة، بينما يظهر البعض الآخر ظهور السرطان في وقت لاحق، بالإضافة إلى أن هناك عائلات مصابة بشكل أساسي بسرطان الثدي. يبدو أن الآفات الخلقية المختلفة في جين TP53 مرتبطة بمخاطر السرطان المختلفة في العائلات المختلفة. وقد أدى هذا إلى إدخال بشكل واسع مصطلح "متلازمة السرطان المرتبطة بـ TP53 (hTP53rc)".كان جزء مهم من عمل الأطروحة هو تقييم الروابط بين الآفات المختلفة في جين TP53 فيما يتعلق بالأورام التي حدثت في العائلات، بهدف تصميم برنامج مراقبة مُكيف لكل عائلة. بالإضافة إلى ذلك، يكون المرضى الذين يعانون من hTP53rcأكثر عرضة لتطوير سرطانات جديدة إذا تلقوا العلاج الإشعاعي للورم الأول. لذلك، من المهم تكييف علاج السرطان بناءً على حالة حامل TP53 .

في **الدراسة الأولى**، تميزت جميع ناقلات TP53 السويدية المعروفة فيما يتعلق بالتنوع الجيني (genotyp) وأنواع الأورام التي تحدث في العائلات (fenotyp). تم تحديد ما مجموعه 188 حاملًا في 91 عائلة بإجمالي 47 نوعًا مختلفًا من المتغيرات الجينية. بعد إعادة التصنيف لتحديد متغيرات TP53 المرتبطة بالمرض فقط وفقًا للمعايير الحديثة، بقي 176 حاملًا في 83 عائلة فيها 42 متغيرًا جينيًا مختلفًا. من بين هؤلاء، أظهر ما يزيد قليلاً عن 60٪ من العائلات خطرًا أكبر للإصابة بالسرطان، بينما كان أقل من 40٪ منهم مصابًا بسرطان الثدي بشكل أساسي. في 40٪ من العائلات المصابة بسرطان الثدي، تم تحديد متغير TP53 يسمى c.542G>A/p.R181H. يتم تقييمه على أنه ما يسمى بصيغة المتغير السويدي، أي متغير موروث في العديد من العائلات من سلف سويدي مشترك. وبالتالي يبدو أن هذا البديل يؤدي في المقام الأول إلى زيادة خطر الإصابة بسرطان الثدي. ومع ذلك، هناك حاجة إلى مزيد من البحث لتأكيد ما إذا كان متغيرا سويديًا.

كان الهدف الآخر للأطروحة هو تطوير برنامج لمراقبة حاملات *TP53* من أجل التمكن من اكتشاف سرطان محتمل مبكر وبالتالي تقليل الحاجة إلى العلاج الإشعاعي أيضًا. بالنسبة للأفراد الذين يعانون من h*TP53*rc في جميع أنحاء العالم، غالبًا ما تتضمن الفحوصات اليوم نوعًا من فحص الجسم بالكامل باستخدام كاميرا مغناطيسية (MR-HK). في عام 2016، بدأت دراسة *TP53* السويدية - SVEP53 - بهدف، من بين أمور أخرى، تقييم برنامج لمراقبة بما في ذلك فحص الجسم بالكاملMR-HK. كجزء من العمل البحثي، تم تصميم الإرشادات السويدية التي توصي بأن يتم تقديم فحوصات لجميع حاملي *TP53* في السويد في إطار SVEP53. نوع الدراسة يعني الفرصة لتقييم برنامج للمراقبة بشكل منهجي. SVEP53 هي دراسة وطنية ومتاحة في المستشفيات الجامعية في أوميو وأوبسالا وستوكهولم ولينشوبينغ وغوتنبرغ ومالمو / لوند.

تصف **الدراسة الثانية** برنامج المراقبة السويدي في إطار SVEP53 وتقارنه مع برنامج المراقبة الأول الذي تم إنشاؤه في كندا في عام 2011. والفرق الأكبر هو أنه في كندا يتم تقديم اختبارات للأطفال باستخدام فحص كامل للجسم، بينما نقدم في SVEP53 بدلاً من ذلك برنامجا متكيفا مع الموجات فوق الصوتية للبطن للأطفال. في حالة عدم وجود مؤشر خاص، يتم اللجوء الى فحص الجسم الكامل MR-HK في السويد فقط لحاملي *TP53* البالغين.

في **الدراسة الثالثة**، قمنا بتقييم نتيجة أول اختبار MR-HK. تم فحص ما مجموعه 61 حاملا بالغا خاليا من الأعراض. بالنسبة لـ 31٪ (61/19) من المشاركين، كان يلزم إجراء مزيد من التحقق بعد فحص الجسم الكامل MR-HK بسبب التغييرات الواضحة. هذا هو نفس المستوى مثل الدراسات الأخرى في MR-HK. في 19/16 (84٪) من المشاركين، وُجد أن التغييرات حميدة، بينما تم تشخيص 19/3 (16٪) بسرطان غير معروف سابقًا. الاستنتاج الأكثر شيوعًا هو الحصول على نتائج حميدة أكثر من النتائج الخبيثة، ولكن يمكن اعتبار نسبة 16٪ من الثلث (31٪) الذين احتاجوا لمزيد من الفحص مصابون بالسرطان. وبسبب صعوبة التعامل بشكل صحيح مع جميع النتائج والنتائج الثانوية في هذه المجموعة عالية المخاطر، نوصي باتباع نهج متعدد التخصصات.

نريد أيضًا، في إطار SVEP53، تقييم كيفية تجربة هؤلاء الأفراد المعرضين لمخاطر عالية للمتابعة مع MR-HK السنوي من خلال تحليل الفوائد / العوائق المتصورة، والقلق الخاص بالسرطان (مقياس قلق السرطان، CWS)، والصحة العامة المتصورة (المسح القصير المكون من 36 عنصرًا، SF-36) عند المشاركين في البحث ومتابعة لمدة عام واحد.

تم تقديم هذا في **الدراسة الرابعة**. تم إجراء مقارنات بين حاملي *TP53* الذين تم تشخيص اصابتهم بالسرطان والذين ليس لديهم سرطان سابق. تم مشاركة ما مجموعه 60 حاملًا بالغًا من *TP53* وكان لدى 60/32 (53٪) تشخيص سابق بالسرطان. تشير نتائجنا إلى أن برامج التحكم مع MR-HK يُنظر إليه على أنه مفيد من قبل

حاملي *TP53*، ولا يسبب قلقًا اضافيا خاصة للمصابين بالسرطان، بغض النظر عما إذا كان لدى الشخص تشخيص سابق بإصابته بالسرطان أم لا.

الهدف العام لجميع الدراسات الفرعية ضمن الأطروحة هو تحسين العلاجات السريرية لحاملي *TP53*. أدى العمل في الأطروحة إلى بدء وتنفيذ اجراء علاجات جديدة في ست مستشفيات جامعية، حيث يتواصل الخبراء ذوو الكفاءات المختلفة مع بعضهم البعض لزيادة المعرفة وتحسين الرعاية ورعاية مرضى السرطان لجميع العائلات مع LFS/h*TP53*rc.

POPULÄRVETENSKAPLIG SAMMANFATTNING AV AVHANDLINGEN

Cancer orsakas av en ansamling av skador i cellernas genetiska material och ungefär en tredjedel av alla insjuknar i en cancersjukdom. Risken ökar med stigande ålder och påverkas av olika livsstils- och miljöfaktorer. Hos ungefär 10 % av alla cancerpatienter finns en medfödd genetisk benägenhet att utveckla cancer som då också kan öka risken att insjukna tidigare i livet. Kraftigt förhöjda cancerrisker beror ofta på medfödda skador i specifika cancergener. Dessa skador, så kallade sjukdomsassocierade genvarianter, kan antingen ärvas eller uppstå spontant hos en individ i samband med befruktningen. Vi har inbyggda mekanismer i cellerna som ska skydda mot cancerutveckling. En av de viktigaste kallas för "genomets väktare" – genen *TP53*. Om själva genomets väktare är satt ur spel från födelsen, vilka blir då konsekvenserna? Ett sällsynt cancerrisksyndrom kallas Li-Fraumenisyndrom (LFS) och beror på medfödda skador i just *TP53*-genen. En *TP53*-bärare har en extremt hög risk (70–100 %) att utveckla cancer någon gång under livet. Vanligast är bröstcancer, hjärntumörer, binjurebarkscancer samt cancer i skelett- och mjukdelar (sarkom), och dessutom i unga år. Vissa familjer verkar ha en ökad risk för barntumörer, medan andra uppvisar en senare debut av cancer och dessutom förekommer familjer med främst bröstcancer. Olika medfödda skador i *TP53*-genen verkar vara kopplade till olika cancerrisker i olika familjer. Detta har lett till att ett vidare syndrombegrepp har införts, "heritable *TP53*-related cancer (h*TP53*rc) syndrome". En viktig del av avhandlingsarbetet har varit att utvärdera samband mellan olika skador i *TP53*-genen i relation till vilka tumörer som förekommit i familjerna, med målet att utforma ett anpassat kontrollprogram för varje familj. Dessutom är patienter med h*TP53*rc känsligare för att utveckla nya cancrar om de får strålbehandling för sin första tumör. Därför är det viktigt att anpassa cancerbehandling utifrån *TP53*-bärarskap.

I **studie I** karakteriseras samtliga kända svenska *TP53*-bärare avseende genvariant (genotyp) och tumörtyper (fenotyp) som förekommer i familjerna. Totalt identifierades 188 bärare i 91 familjer med totalt 47 olika genvarianter. Efter reklassificering för att avgränsa mot enbart sjukdomsassocierade *TP53*-varianter enligt de senaste kriterierna, kvarstod 176 bärare i 83 familjer med 42 olika genvarianter. Av dessa var det drygt 60 % av familjerna som uppvisade en bredare tumörrisk medan knappt 40 % hade framför allt bröstcancer. I 40 % av bröstcancerfamiljerna identifierades en *TP53*-variant benämnd c.542G>A/p.R181H. Den bedöms som en potentiell så kallad svensk foundervariant, det vill säga en variant som nedärvs till flera familjer från en gemensam svensk förfader. Denna variant tycks alltså främst leda till ökad bröstcancerrisk. Dock behövs ytterligare forskning för att bekräfta om det rör sig om en svensk founder.

Ytterligare ett syfte med avhandlingen var att utveckla ett kontrollprogram för *TP53*-bärare för att kunna upptäcka en eventuell cancer tidigt och därmed möjligen också minska behovet av strålbehandling. För individer med h*TP53*rc världen över, innefattar kontrollerna idag ofta någon typ av helkroppsundersökning med magnetkamera (MR-HK). 2016 startade en svensk *TP53*-studie - SVEP53 - med syfte att bland annat utvärdera ett kontrollprogram inkluderande MR-HK. Som en del av forskningsarbetet utformades de svenska riktlinjerna som rekommenderar att alla *TP53*-bärare i Sverige erbjuds kontroller inom ramen för den Svenska *TP53*-studien, SVEP53. Studieformatet innebär en möjlighet att systematiskt utvärdera kontrollprogrammet. SVEP53 är en nationell studie och finns vid universitetssjukhusen i Umeå, Uppsala, Stockholm, Linköping, Göteborg och Malmö/Lund. I **studie II** beskrivs det svenska kontrollprogrammet inom ramen för SVEP53 och jämför det med det första kontrollprogrammet som etablerades i Kanada 2011. Den största skillnaden är att man i Kanada erbjuder barn undersökningar med MR-

HK medan vi inom SVEP53 istället erbjuder ett anpassat program med ultraljud av buken för barn. Om det inte föreligger någon särskild indikation så erbjuds MR-HK i Sverige enbart till vuxna *TP53*-bärare.

I **studie III** utvärderar vi utfallet av den första HK-MR-undersökningen. Totalt undersöktes 61 symptomfria vuxna bärare. För 31 % (19/61) av deltagarna krävdes vidare utredning efter MR-HK på grund av påvisade förändringar. Detta ligger på samma nivå som andra studier på MR-HK. Hos 16/19 (84 %) deltagare visade det sig vara godartade förändringar medan 3/19 (16 %) diagnostiserades med en dittills okänd cancer. Slutsatsen är att det är vanligare med godartade bifynd än elakartade fynd, men att 16 % av den tredjedel (31 %) som behövde undersökas vidare identifierades med cancer får anses vara en hög andel. På grund av komplexiteten i att korrekt handlägga alla fynd och bifynd i denna högriskgrupp rekommenderar vi ett multidisciplinärt omhändertagande.

Vi vill också inom ramen för SVEP53 utvärdera hur dessa högriskindivider upplever uppföljning med årlig MR-HK genom att analysera upplevd nytta/hinder (benefits/barriers), cancerspecifik oro (Cancer Worry Scale, CWS), samt generell upplevd hälsa (The 36-item Short Form Health Survey, SF-36) vid inklusion och vid ettårsuppföljning. Detta presenteras i **studie IV**. Jämförelser gjordes mellan de *TP53*-bärare som haft en cancerdiagnos och de utan tidigare cancer. Totalt inkluderades 60 vuxna *TP53*-bärare där 32/60 (53 %) hade en tidigare cancerdiagnos. Våra resultat tyder på att kontrollprogram med MR-HK upplevs som fördelaktigt av *TP53*-bärare, och orsakar inte ökad cancerspecifik oro, oavsett om man haft en tidigare cancerdiagnos eller inte.

Det övergripande målet med samtliga delstudier inom avhandlingen är att förbättra den kliniska hanteringen av *TP53*-bärare. Arbetet med avhandlingen har lett till uppstart och implementering av nya arbetsflöden i sex universitetssjukhus, där experter med olika kompetenser har kontakt med varandra för att öka kunskapen om, och förbättra omhändertagandet och cancervården för alla familjer med LFS/h*TP53*rc.

POPULAR SCIENCE SUMMARY OF THE THESIS

Cancer is caused by accumulation of damage in the genetic material of the cells, and around one-third of us will get cancer. The risk increases with rising age, and is affected by different life-styles and environmental factors. About 10% of all cancer patients have a congenital genetic disposition to develop cancer, which might also increase the risk of being affected earlier in life. Considerably increased cancer risks are often caused by congenital damage in specific cancer genes. Those damages, so called disease-associated gene variants, may either be inherited or develop spontaneously in an individual at the time of conception. We have built-in cellular mechanisms to protect us from developing cancer. One of the most important is the *TP53* gene, also referred to as "the guardian of the genome". If the guardian of the genome itself is put out of play from birth, what would the consequences be? A rare cancer risk predisposition syndrome called the Li-Fraumeni syndrome (LFS) is caused by congenital damages in the *TP53* gene. A g*TP53* carrier has an extremely high life-time risk (70-100%) to develop cancer at some point in life, and up to 50% of all carriers will have developed a tumour by the age of 30 years. The most common cancer types are breast cancer, brain tumour, adrenocortical cancer and cancers in the bones and soft tissues (sarcomas), often at young ages. Notably, some families seem to have an increased risk of tumours in childhood, while others present a later cancer onset, and yet other families seem to be more prone to develop mainly breast cancer. This has led to the introduction of a wider syndrome definition, "heritable *TP53*-related cancer (h*TP53*rc) syndrome", embracing a tumour presentation beyond the classical LFS.

An important part of the work with this thesis has been the evaluation of correlations between different types of alterations in the *TP53* gene in relation to the tumour types in the different families, with the ultimate aim to develop an adapted surveillance program for each family. In addition, patients with h*TP53*rc are more prone to develop new cancers if they receive radiation therapy for their first tumour. Therefore, it is important to customise cancer treatment with regards to being a g*TP53* carrier. In **Paper I**, all known Swedish g*TP53* carriers have been characterised regarding the gene variants (genotype) and tumour types (phenotype) in the families. In total, 188 carriers in 91 families were identified, carrying 47 different gene variants. After reclassification to define only disease-associated *TP53* variants in accordance with the latest criteria, 176 carriers in 83 families with 42 different gene variants remained. Roughly 60% of the families presented a wider cancer risk, while about 40% had mainly breast cancer. Furthermore, in 40% of the "breast cancer families", a specific *TP53* variant named c.542G>A/p.R181H was identified. This variant could potentially be a so-called Swedish founder variant, meaning that it is a variant that is inherited among several families, originating from a mutual ancestor. This variant seems to be mainly associated with an increased risk of breast cancer. However, more research is needed to confirm if this is a Swedish founder.

Another purpose with the thesis was to develop and evaluate a surveillance program for g*TP53* carriers to detect cancer early, and therefore possibly also decrease the need of radiation therapy. For individuals with h*TP53*rc world-wide, these follow-up protocols often include some kind of whole-body MR imaging (WB-MRI). As a part of the research project, the Swedish recommendations were developed where all g*TP53* carriers in Sweden are offered surveillance within the Swedish *TP53* Study, SWEP53. The study format enables the possibility to systematically evaluate the surveillance program. SWEP53 is a national study and is conducted at the university hospitals in Umeå, Uppsala, Stockholm, Linköping/Jönköping, Gothenburg and Malmö/Lund. In **Paper II**, the Swedish surveillance program within SWEP53 is described and compared to the first surveillance program, established in Canada in 2011. The main difference between the

programs is that children in Canada are offered WB-MRI, while the Swedish protocol instead offers an adapted surveillance including ultrasound of the abdomen. Unless there is a specific reason, WB-MRI is only offered to adult g*TP53* carriers in Sweden.

In **Paper III**, we evaluate the outcome of the first WB-MRI examination. In total, 61 asymptomatic adult carriers were evaluated. In 31% (19/61) of the participants, further examinations were needed after the first WB-MRI due to imaging findings. This is in line with other studies on WB-MRI. In 16/19 (84%) of the participants, the findings were benign, but the remaining 3/19 (16%), were diagnosed with a previously unknown cancer. The conclusion is that it is more common with benign imaging findings than malignant, but in 16% of the one-third that needed further work-up, cancer was diagnosed which is considered to be a high proportion. Because of the complexity to correctly handle all the findings and incidental findings in this high-risk group, we recommend a multidisciplinary approach to ensure the best possible care.

Within SWEP53, we also wanted to evaluate the psychosocial aspects regarding how these high-risk individuals perceives annual surveillance with WB-MRI by analysing perceived benefits/barriers to participation, cancer-specific worry (The Cancer Worry Scale, CWS) and general perceived health (The 36-item Short Form Health Survey, SF-36). Comparisons were made both at study inclusion and at year one, and also between g*TP53* carriers *with* and *without* previous cancer. These results are presented in **Paper IV**. In total, 60 adult carriers were included of whom 32/60 (53%) had a previous cancer. Our results indicate that surveillance with WB-MRI is perceived as beneficial among g*TP53* carriers, and does not cause an increase in cancer-specific worry regardless of previous cancer.

The overall aim of all the studies within the thesis is to improve the clinical handling of g*TP53* carriers. This has led to the initiation and implementation of new work-flows where experts with various areas of expertise have contact with each other to increase the knowledge, and to improve the clinical handling and cancer care for all families with LFS/h*TP53*rc.

ABSTRACT

Around 25% of all cancers are considered as familial and are caused by an inherited susceptibility to develop certain tumours. But only 5-10% are hereditary and caused by known high risk cancer genes associated with specific cancer risk syndromes. One of the most pronounced is the heritable *TP53* related cancer (h*TP53*rc) syndrome, commonly referred to as the Li-Fraumeni syndrome.

In 1969, two physicians, Li and Fraumeni, identified and described a new hereditary cancer predisposition syndrome. Twenty years later, germline pathological variants of the *TP53* gene – also referred to as "the guardian of the genome", was identified to be the cause. The Li-Fraumeni syndrome (LFS) is characterised by an extreme life-time risk of cancer in germline *TP53* (g*TP53*) carriers, up to a 100% by the age of 70. The most commonly occurring cancers are (early onset) breast cancer, CNS tumours, adrenocortical tumours, and sarcomas. In addition, individuals with a g*TP53* variant are also more prone to develop other types of cancers in comparison with the general population. Since the first descriptions of LFS with childhood tumours, a broader phenotype has emerged, with families prone to mainly breast cancer, or mainly adult-onset cancers. Therefore, the term "heritable *TP53* related cancer syndrome (h*TP53*rc) has been proposed to include the wide phenotypic range, not always explained by the genotype.

This thesis aims to explore the genotype-phenotype correlations in the Swedish g*TP53* cohort (**Paper I**), and to evaluate an extended clinical handling including surveillance within a prospective study, The Swedish *TP53* Study (SWEP53, **Papers II-IV**).

Within **Paper I**, we identified all g*TP53* carriers in Sweden since testing for the gene started. This retrospective national characterisation identified 188 carriers in 91 families harbouring 47 different g*TP53* variants. After reclassification according to the latest *TP53*-specific criteria from 2021, 42 of the g*TP53* variants were clinically actionable (class 4 or 5) and identified in the remaining 83 families and 176 carriers. These families fulfilled one of four different phenotypic characteristics; *classical LFS* (13 families), *Chompret criteria* (37 families), *hereditary breast cancer* (29 families), or *other* (4 families). The most commonly occurring g*TP53* variant c.542G>A/p.R181H was identified in 18 families and were considered as a potential Swedish founder variant, mainly associated with hereditary breast cancer. However, this variant need further evaluations to explore its founder potential.

The prospective SWEP53 study is the base for **Papers II-IV**. In **Paper II**, we describe the outline of the Swedish surveillance program including (for adults) yearly whole-body MRI (WB-MRI), surveillance for children including abdominal ultrasound, the collection of cell-free DNA, and the psychosocial evaluation of surveillance participation. In this study, comparisons are done with the pivotal first protocol of WB-MRI surveillance, the 'Toronto protocol' from 2011. A notable difference is the surveillance protocol for children, , as children below the age of 15 years were neither carrier tested nor offered annual WB-MRI in SWEP53. **Paper II** also adds data to the first Swedish registry on g*TP53* carriers established in **Paper I**, collecting information on the different g*TP53* variants, pedigree data, ages of tumour onset, and tumour types.

An evaluation of the consequences of WB-MRI surveillance in terms of radiological findings, anatomical distribution, and the need for further work-up, was performed within **Paper III**. A total of 61 asymptomatic g*TP53* carriers performed a baseline WB-MRI and 19 (19/61=31%) individuals had in total 30 radiological lesions that needed further work-

up. Three of the 19 (3/19=16%) participants were diagnosed with a malignant disease, which is considered to be a high proportion. Notably, all three were asymptomatic at baseline imaging. Because of the complexity to correctly handle all the findings and incidental findings in this high-risk group, we recommend a multidisciplinary approach to ensure the best possible care.

With **Paper IV**, we also wanted to evaluate the psychosocial aspects of a surveillance strategy with WB-MRI. There are few available quantitative studies within this area. We measured the perceived benefits and barriers to surveillance, cancer worry scale (CWS) and perceived overall health (The 36-Item Short Form Health Survey, SF-36) by comparing participants *with* and *without* previous cancer. They were evaluated both at baseline WB-MRI and at the one-year surveillance. In general, g*TP53* carriers *with* previous cancer reported higher levels of cancer worry than those *without* previous cancer, but, notably, at one year of surveillance the cancer worry was not increased. Both groups reported few barriers and scored high on the benefits. In conclusion, surveillance with WB-MRI did not increase cancer-specific worry, and was perceived as feasible by the participants regardless of previous cancer.

To summarise, the work presented in this thesis adds to the knowledge of the rare h*TP53*rc syndrome, both in the understanding of genotype and phenotype presentations, and in clinical handling in terms of genetic counselling and surveillance. It has also contributed to the implementation of new work-flows for g*TP53* carriers within the health care system to improve the overall care of this families.

LIST OF SCIENTIFIC PAPERS

I. Liu Y.[#], **Omran M.***[#], Sun Zhang A., Stenmark-Askmalm M., Rosén A., Hallbeck A-L, Poluha A., Persson F., Helgadottir T. H., Tham E., and Bajalica-Lagercrantz S. *Characterisation of heritable TP53-related cancer syndrome in Sweden – a retrospective nationwide study of genotype-phenotype correlations in 91 families*. *Manuscript in preparation*.

II. **Omran M.***, Blomqvist L., Brandberg Y., Pal N., Kogner P., Stahlbom AK., Tham E., Bajalica-Lagercrantz S. *Whole-body MRI within a surveillance program for carriers with clinically actionable germline TP53 variants - the Swedish constitutional TP53 study SWEP53*. *Hereditary cancer in clinical practice.* 2020;18(1):1–1.

III. **Omran M.***, Tham E., Brandberg Y., Ahlström H., Lundgren C., Paulsson-Karlsson Y., Kuchinskaya E., Silander G., Rosén A., Persson F., Leonhardt H., Stenmark-Askmalm M., Berg J., van Westen D., Bajalica-Lagercrantz S. and Blomqvist L. *Whole-Body MRI Surveillance-Baseline Findings in the Swedish Multicentre Hereditary TP53-related Cancer Syndrome Study (SWEP53)*. *Cancers (Basel).* 2022;14(2):380–.

IV. **Omran M.***, Johansson H., Lundgren C., Silander G., Stenmark-Askmalm M., Loman N., Baan A., Adra J., Kuchinskaya E., Blomqvist L., Tham E., Bajalica-Lagercrantz S., and Brandberg Y. *Whole-body MRI surveillance in TP53 carriers is perceived as beneficial with no increase in cancer worry regardless of previous cancer – data from the SWEP53 study*. *Cancer.* 2023; 1- 10.

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#Shared first-authorship, authors contributed equally.

CONTENTS

LIST OF ABBREVIATIONS

ACMG/AMP	American College of Medical Genetics and Genomics/Association for Molecular Pathology
AYA	adolescents and young adults (aged 15-39)
BRCA1/2	breast cancer gene 1/2 - the gene
cfDNA	cell-free DNA
CHIP	clonal haematopoiesis of indeterminate potential
CWS	Cancer Worry Scale
DCIS	ductal carcinoma in situ (pre-invasive breast cancer)
DNE	dominant-negative effect
ESMO	European Society for Medical Oncology
ERN	European Reference Network
GENTURIS	Genetic Tumour Risk Syndromes
gnomAD	The Genome Aggregation Database
GOF	gain-of-function
g*TP53* variant	germline *TP53* variant
HBC	hereditary breast cancer
HRQoL	Health-related Quality of Life
h*TP53*rc	heritable *TP53*-related cancer syndrome
LFS	Li-Fraumeni syndrome
LP/PV	likely pathogenic/pathogenic variant
LOF	loss-of-function
MDM2	mouse double minute 2 homolog
NGS	next-generation sequencing
OMIM	Online Mendelian Inheritance in Man
ONCO-RADS	The Oncologically Relevant Findings Reporting and Data System
PGT	preimplantation genetic testing
SF-36	The 36-item Short Form Health Survey
SNP	single-nucleotide polymorphism
SWEP53	the Swedish constitutional *TP53* Study
TME	tumour microenvironment
TP53	human tumour protein 53 – the protein
TP53	human tumour protein 53 – the gene
TSG	tumour suppressor gene
US	ultrasound
VAF	variant allele frequency
WB-MRI	whole-body magnetic resonance imaging

DEFINITIONS

| Allele	In humans, an allele is one of two versions of a DNA sequence at a given location (locus). By inheriting one allele from each parent, an individual can be homozygous for that allele if the alleles are the same, or heterozygous when the alleles differ.
Duplication	Extra copies of genetic material, ranging from a few bases to whole chromosomes.
Genetic variant	*De novo* variant: A congenital pathologic/likely pathologic variant of a gene (previously called mutation) occurring *de novo* in a person, either in the gametes or in early embryonic development. **Deletion:** a genetic change where DNA is missing. It might be a single base leading to frameshift, but can range to large parts of the DNA as in a whole gene. **Driver variant:** a specific genetic alteration driving the tumour development. **Frameshift variant:** the insertion or deletion of base pairs that are not multiples of three (not coding for a specific amino acid), leading to a disruption of the reading frame of the DNA sequence. This commonly results in a premature stop codon and a truncated protein. **Germline variant:** a congenital pathologic/likely pathologic variant of a gene (previously called mutation) occurring either *de novo* in a person, or that is inherited from one of the parents. **Likely pathogenic variant:** A genetic alteration that is likely (>95%) to be disease-causing. Clinically actionable, class 4. **Missense variant:** a genetic alteration in which a single base pair substitution alters the genetic code, producing an amino acid that is different from the usual amino acid at that position. As an example: the Brazilian founder variant c.1010G>A/p.Arg337His/p.R337H → at codon 337, the amino acid R (arginine) has been replaced by H (histidine) due to a change of the DNA nucleotide G (guanine) → A (adenine), producing a protein with the amino acid histidine instead of arginine at position 337. **Nonsense variant:** premature termination of a protein, commonly leading to partial/complete inactivation and subsequently a loss or change of protein function. **Pathologic variant:** A genetic alteration that is likely (>99%) to be disease-causing. Clinically actionable, class 5.

	Sporadic variant: disease-causing variant of a gene (previously called mutation), occurring within tumour cells, not germline and thus is not inherited. **Variant of uncertain significance:** a genetic alteration that is >5% and <95% likely to be disease-causing (class 3), therefore of uncertain significance regarding clinical actionability.
gnomAD	The Genome Aggregation Database as defined by their own website, "is a resource developed by an international coalition of investigators, with the goal of aggregating and harmonising both exome and genome sequencing data from a wide variety of large-scale sequencing projects, and making summary data available for the wider scientific community." gnomAD is supposed to reflect a "normal population".
Grounded theory	An iterative qualitative research method where data collection and data analysis occur at the same time to provide information of the other. Ideas/concepts are tagged into categories, serving as the base for hypotheses or new theories. This hypothetical-deductive model is used in several of the psychosocial interview studies mentioned in the 'background'.
Heritability	Heritability is an estimation of how much the variability of a certain trait that is due to genetics. A heritability of zero implies that all variability is due to environmental factors, as opposed to a heritability of one which is completely due to genetic influence. Different cancer types have different degrees of heritability.
Mendelian inheritance	How traits are inherited from parent to offspring. There are four basic types of Mendelian inheritance: autosomal dominant, autosomal recessive, X-linked recessive and X-linked dominant. In cancer predisposition syndromes, the pattern is usually autosomal dominant.
OMIM	Online Mendelian Inheritance in Man is a continuously updated catalogue of human genes, genetic disorders and traits, with a focus on the genotype-phenotype relationship. Each OMIM entry is given a unique six-digit identifier; for LFS it is #151623, for *TP53* it is *191170.
Penetrance	The likelihood of a genetic trait to be expressed in the phenotype. Incomplete penetrance is most common. As an example, the penetrance of breast cancer due to a germline pathological variant in BRCA1 is up to 80% during the carrier's lifetime. Incomplete penetrance might be due to genetic variation, environmental, or lifestyle factors.
Splicing	When the non-coding DNA regions (introns) are "cut out" of the mRNA transcript, leading to an mRNA with only exons serving as a template for a protein.

1. BACKGROUND

1.1. SPORADIC CANCER, FAMILIAL, AND HEREDITARY CANCER RISK SYNDROME

Cancer is a genetic disease, affecting many of us during lifetime, both directly as patients but also as relatives and friends. Most cancers are sporadic, referring to a single case of cancer within a family, or a cancer that occurs in line with the common presentation within the general population. These cancers occur as a result of somatic genetic alterations in the tumour. Around 25% of all cancer cases are familial. A familial cancer is typically described as when ≥2 family members within the same family branch are affected by the same tumour type. A familial presentation can therefore be due to genetic factors, but also to shared exposure of environmental agents such as smoking, diet, alcohol and infections. A hereditary cancer risk syndrome is defined as when an individual has an elevated risk of developing cancer due to one or more germline disease-causing variants. These variants can occur *de novo* within the person's germ cells, or are inherited either from one of the parents. Typically, cancer develops earlier than compared with the normal population and the pathological variant is inherited in an autosomal dominant fashion. About 5-10% of all human cancers are considered to be caused by inherited specific genetic alterations, and defined as hereditary cancer risk syndromes (Nagy *et al.*, 2004; Lu *et al.*, 2014). To date, over 100 different cancer predisposing genes with germline variants have been described (Rahman, 2014), where rare variants are more often associated with a higher cancer risk than common variants (Figure 1).
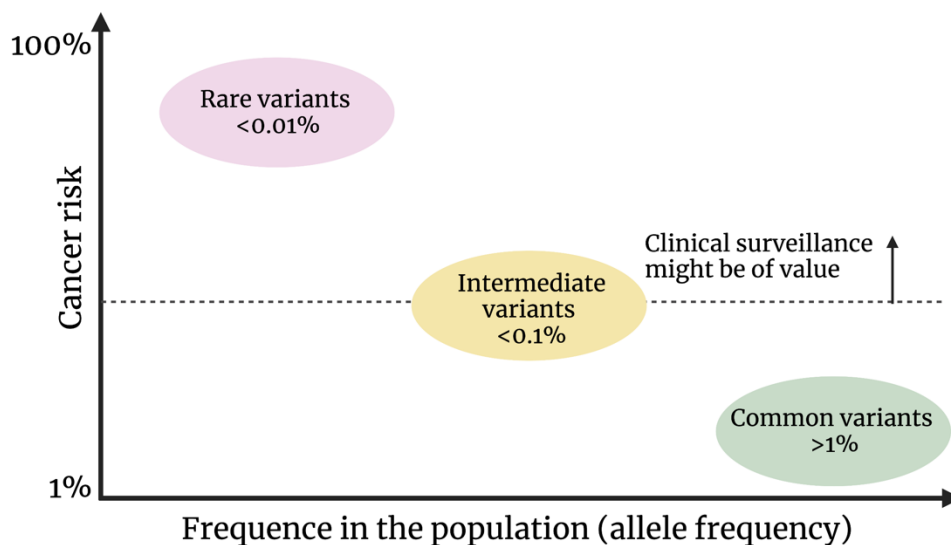
Figure 1. The relation between allele frequency in the population and cancer risk. Rare variants are very uncommon in the population, but can be associated with very high cancer risks in comparison with intermediate and common variants in the population. Courtesy of S. Bajalica-Lagercrantz.

1.1.1. The heritability of different cancers

The cumulative incidence of cancer is about 30%, and heritability accounts for one third of all cancers. Different cancers have different heritability, where excess familial risks have been found in prostate, melanoma, breast, ovarian and uterine cancer (Mucci *et al.*, 2016) without identification of monogenic high-risk genes. For breast cancer, around 10% is considered to be caused by germline pathogenic variants. The most common heritable disease-causing variants are *BRCA1* and *BRCA2*, responsible for roughly 5% of breast cancer cases (Tung *et al.*, 2016). In a meta-analysis, Blondeaux and colleagues estimate the

incidence of g*TP53* in breast cancer to range between 5% in highly selected cohorts (such as young age of onset and positive familial history) to 0.01% in unselected patients (Blondeaux *et al.*, 2023).

1.1.2. The two-hit model and the multistep process

A hereditary cancer risk syndrome should be suspected when there are several family members with the same type of cancer/associated tumours (such as breast and ovarian cancer), certain rare cancers, in the case of early onset, more than one primary tumour, and synchronous cancer in paired organs. Sometimes, other non-cancer traits may be present such as pneumothorax and fibrofolliculoma in Birt-Hogg-Dubé syndrome (Vincent *et al.*, 2003), or macrocephaly in Cowden syndrome (Starink *et al.*, 1986). The biological explanation for these characteristics could be explained by two theories: the two-hit model and the multistep process of cancer development. The two-hit hypothesis proposed in 1971 by Knudson in a statistical study of retinoblastoma, states that the development of cancer requires at least two mutational events in a tumour suppressor gene (Knudson, 1971). These events occur in both alleles, and in the case of a hereditary cancer risk syndrome, an individual is born with a pathological variant (PV) "to start with" (Figure 2). Therefore, as time passes and when the second hit occurs, cancer is manifested at earlier stages, are more likely to present bilaterally within paired organs or as multiple primary tumours than in the cases of sporadic cancers.
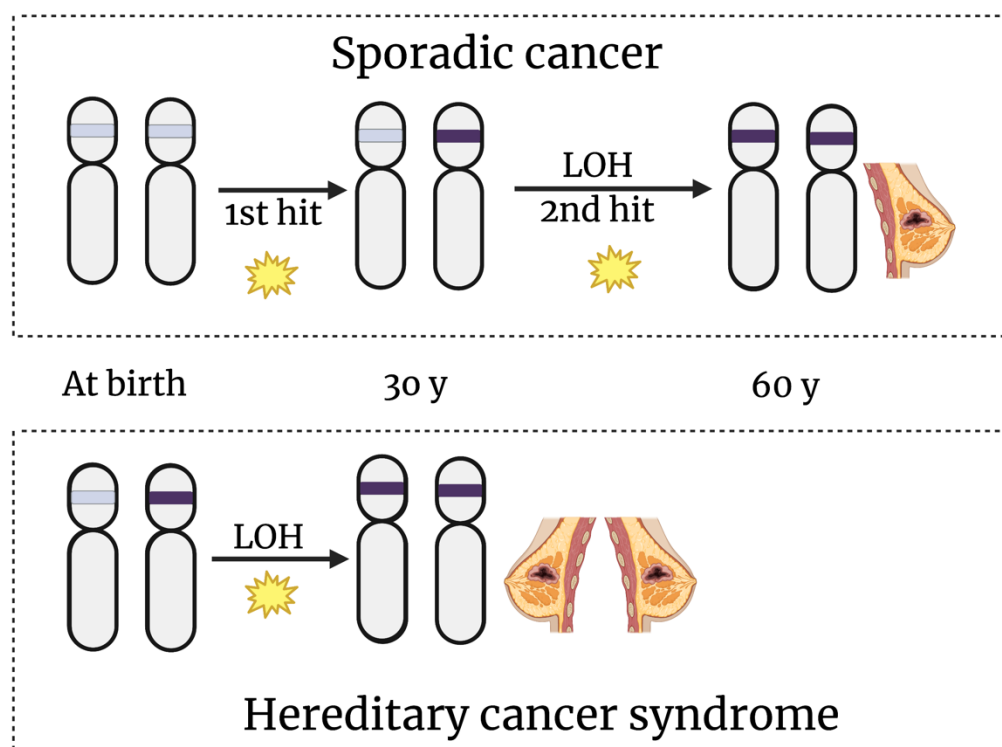
Figure 2. The two-hit model. *In comparison with sporadic cancers, tumours arising due to hereditary cancer syndromes occur at an earlier age and are more often bilateral than sporadic forms. Here exemplified with breast cancer. Gray band = wild-type tumour suppressor gene (TSG). Purple band = pathological variant of a TSG. LOH = loss of heterozygosity.*

In addition, cancer is a multistep process with accumulating specific genetic events over several years. A known disease model is colorectal cancer, where the subsequent transformation of normal mucosa to a manifest cancer requires several (around 5-7) sequential genetic variants to happen (Fearon and Vogelstein, 1990) (Figure 3). A germline disease-causing variant in crucial genes such as the adenomatous polyposis coli (APC)

tumour suppressor gene results in familial adenomatous polyposis syndrome (FAP) and therefore earlier onset of colon cancer as well as multiple tumours are manifested (Miyoshi *et al.*, 1992).
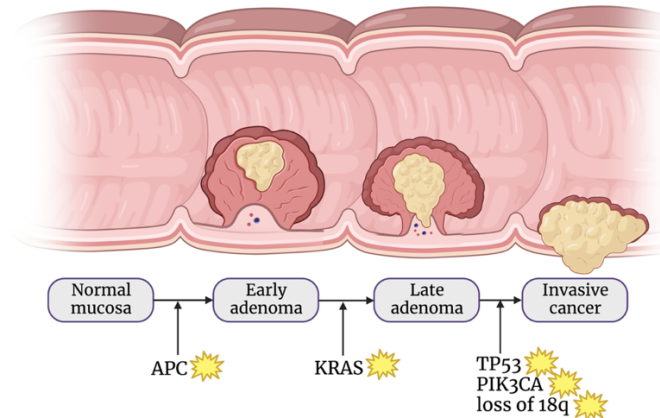
Figure 3. A "Vogelgram" describing the multistep carcinogenic process. *This model was proposed by Fearon and Vogelstein in 1990, indicating crucial genes that, when subsequently disrupted, contribute to the tumourigenesis of colorectal cancer. APC, KRAS, TP53 and PIK3CA refers to the disrupted proteins and loss of 18q refers to the loss of the long arm of chromosome 18.*

1.2.   THE HALLMARKS OF CANCER

The poet John Donne argued in 1624 that "No man is an island entire of itself; every man is a piece of the continent, a part of the main" (Donne, 1975). The same could be argued for our individual cells, dependent on each other in order to thrive and maintain homeostasis. There are several traits a cell must acquire or lose in order to transform into a malignant cell. Those traits are summarised in the most cited paper(s) in the field of tumour biology by Hanahan and Weinberg, referred to as "The Hallmarks of Cancer". The original paper (Hanahan and Weinberg, 2000) described six hallmarks. Eleven years later, two enabling characteristics were added (Hanahan and Weinberg, 2011), and in 2022 another two emerging hallmarks and two enabling characteristics joined the model (Hanahan, 2022) (Figure 4).
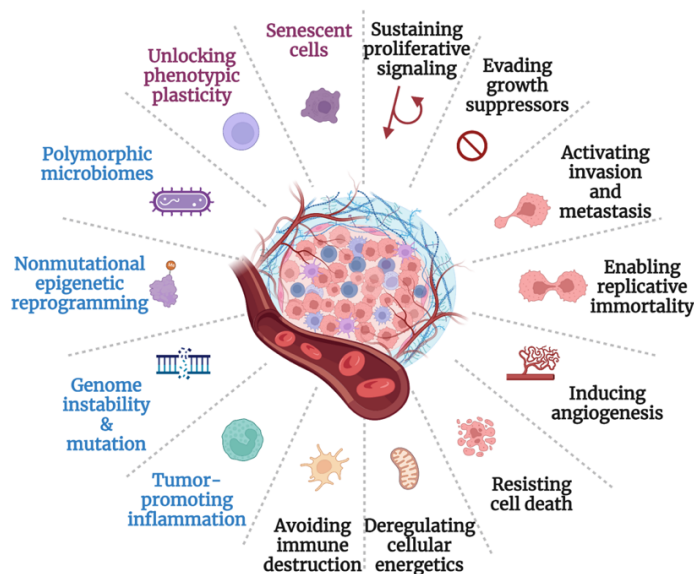
Figure 4. The Hallmarks of Cancer as proposed by Hanahan, 2022. *In black, the eight hallmarks of cancer, in plum the two emerging hallmarks, and in blue, the four enabling characteristics. Permission to reprint a version of the original figure has been obtained from the publisher.*

The eight hallmarks are defined as the ability to:

- *Sustain proliferative signalling*: the cancer cells become independent of exogenous growth stimulation, having a continuous proliferation. One mechanism is activation of the H-Ras oncogene.
- *Evade growth suppressors:* evading naturally occurring growth suppressing signals from nearby cells and the extracellular matrix. As an example, disruption of the Rb pathway leading to a cell proceeding from G1 to the S phase in the cell cycle instead of stopping.
- *Avoid immune destruction:* by escaping the continuous surveillance by the immune system, malignant cells and tumours can proliferate and invade other tissues.
- *Enable replicative immortality:* cells contain an intrinsic and independent program limiting their replicative ability regardless of signals outside the cell to around 60-70 cycles. Telomere maintenance, by switching on telomerase is one way to ensure replicative immortality beyond these cycles.
- *Activation of invasion and metastasis:* the tumour cells leaving their designated tissue of origin to migrate and thereafter invade another tissue is the main reason behind cancer death. To exemplify, adherence between the cell to its surroundings involves the loss/altered function of E-cadherin, seen in epithelial cancers, while the activation of extracellular proteases is required for metastasis.
- *Induce or access vasculature:* cells require to be within 100 μM from a capillary blood vessel to ensure access to nutrients and oxygen. Tumour cells seem to balance between induction and inhibition of angiogenesis such as increased expression of the inducer VEGF and/or down-regulation of the inhibitor thrombospondin-1.
- *Resist cell death:* resistance to apoptosis, most commonly by genetic alterations in the *TP53* gene.
- *Deregulate cellular energetics:* under normal aerobic conditions, the cell process glucose through the "usual" citric acid cycle and oxidative phosphorylation in the mitochondria. Another process, "aerobic glycolysis" is used by cancer cells regardless of the amounts of oxygen through glycolysis and lactic acid fermentation in the cytosol instead of the oxygen-consuming mitochondria. This yields less energy for the cell, but the glycolytic intermediates can be used to generate other molecules like nucleosides and amino acids.

The two emerging hallmarks are:

- *Unlocking phenotypic plasticity:* the enabling of different disruptions of cellular differentiation such as dedifferentiation, blocked differentiation and transdifferentiation/metaplasia.
- *Senescent cells:* contributes to tumour development by affecting nearby cancer cells and other cells in the tumour microenvironment (TME) through paracrine mechanisms.

The four enabling characteristics are:

- *Nonmutational epigenetic reprogramming:* in its physiological form, nonmutational epigenetic reprogramming is involved in embryonic development and long-term memory by changes in histones, chromatin structure and triggering of gene expression switches. In cancer development, these mechanisms can occur in the TME, playing a role in intratumoural heterogeneity and the regulation of stromal cells in the TME.

- *Polymorphic microbiomes:* both cancer-protective and tumour promoting microbiome can affect the tumour development, both directly (such mutagenesis of the colonic epithelium due to DNA damage from bacterial toxins) and indirectly by modulating the adaptive immune system.
- *Genome instability and mutation:* malignant cells can increase their sensibility to mutagenic agents, and the compromise of the usual surveillance as with *TP53*, this leads to an accumulation of genetic alterations.
- *Tumour-promoting inflammation:* inflammation can contribute to hallmark functions by providing molecules into the extracellular matrix, thereby facilitating angiogenesis, replicative immortality and so on.

Cancer is a process with accumulating genetic alterations, allowing the tumour cell to grow and disrespect the usual inhibitory safety functions. One could argue that larger animals alongside with a longer life-span, might be more cancer prone than their smaller, short-lived cousins because of the accumulations of genetic alterations occurring in the many proliferating cells over a longer life-span. Interestingly, some large animals like elephants are known to be less cancer prone than smaller animals, an observation known as Peto's paradox (Peto *et al.*, 1975) (Caulin and Maley, 2011). There might be several possible explanations to this paradox such as telomere length, a better immune surveillance, and more copies of tumour suppressor genes (TSG). With regards to TSG, humans have two alleles of *TP53* in comparison with 40 alleles in elephants. Interestingly, a study found that lymphocytes from elephants have an increased apoptotic response to DNA damage from irradiation when compared with human lymphocytes, which might be explained by the many *TP53* alleles (Abegglen *et al.*, 2015).

1.3. *TP53* – THE GUARDIAN OF THE GENOME

The TP53 protein was independently discovered by two groups in 1979 (Lane and Crawford, 1979; Linzer and Levine, 1979) (Figure 5). In 1992, the *TP53* was coined as "the guardian of the genome" due to its many and essential roles in maintaining the integrity of the genome (Lane, 1992). Since then, more and more evidence regarding its fundamental role not only in cell cycle regulation, but also in various other areas has emerged. *TP53* has been found in many different species, and dates back to at least 700 million years ago (Nedelcu and Tan, 2007).
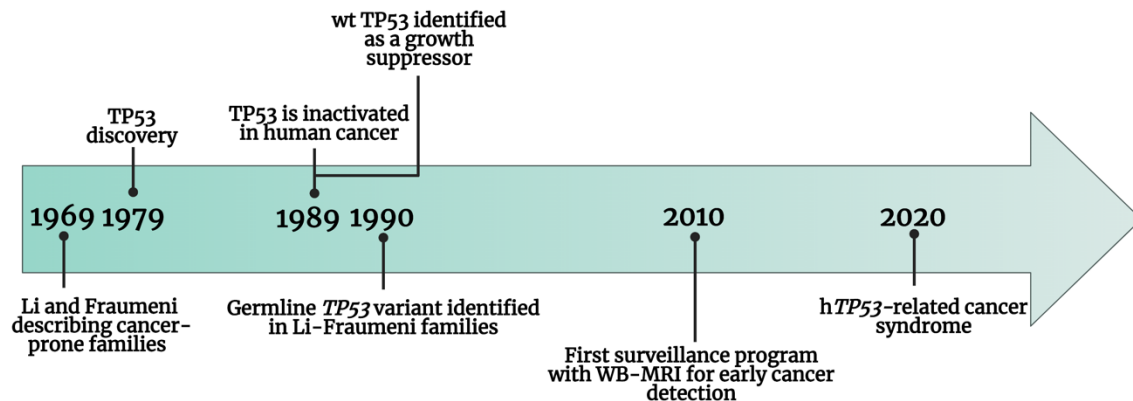

Figure 5. Time line for discoveries of TP53 and their connection to the clinical aspects of the Li-Fraumeni syndrome. The physicians Li and Fraumeni described the Li-Fraumeni syndrome in 1969 (Li and Fraumeni, 1969). The TP53 *protein was discovered in 1979 (Lane and Crawford, 1979; Linzer and Levine, 1979). In 1989, it was demonstrated that it is inactivated in human cancers (Baker et al., 1989) and acts as growth suppressor (Takahashi et al., 1989). A year later, germline TP53 variants were identified in LFS families (Malkin et al., 1990). In 2010, the first surveillance program with whole-body MRI (WB-MRI) was published (Villani et al., 2011). By 2020, the wider description of "heritableTP53-related cancer syndrome" was proposed to enclose other phenotypes than the classical LFS (Frebourg et al., 2020).*

The *TP53* gene is located on the short arm of chromosome 17 at p13.1 (Miller *et al.*, 1986) encoding for its protein TP53 consisting of 393 amino acids in 11 exons. In its active form, the protein monomers forms a tetramer that is built up as a dimer of dimers, more thoroughly presented in 1.4. The tetramer's architecture is important to ensure correct DNA-binding and its function as a transcription factor (Römer *et al.*, 2006). The wild-type *TP53* is regarded to be a tumour suppressor. Nonetheless, mutant forms of the protein can act as an oncogene and are thereby driving the malignant transformation in spite of an intact wild-type allele, through various gain-of-functions (GOFs) (Soussi and Wiman, 2015), thereby undergoing an "oncogenic shift". This shift implies many and different major mechanisms exerted by the oncogenic variant TP53 such as; increased genomic instability by disruption of the normal spindle checkpoint control, leading to an accumulation of cells with polyploid genomes and inactivation of the TP53-related proteins TP63 and TP73; resistance to anti-apoptopic signals including an increased resistance to chemotherapy and irradiation; enhanced ability of cell migration and invasion; interactions with the transforming growth factor β (TGF-β) pathway and thereby promoting tumour progression; and effects on the cellular metabolism (Oren and Rotter, 2010) (Soussi and Wiman, 2015). *TP53* is thought to have a role in both physiological events, and as a tumour suppressor gene (TSG) having a part in many different tumour inhibiting processes (Figure 6).
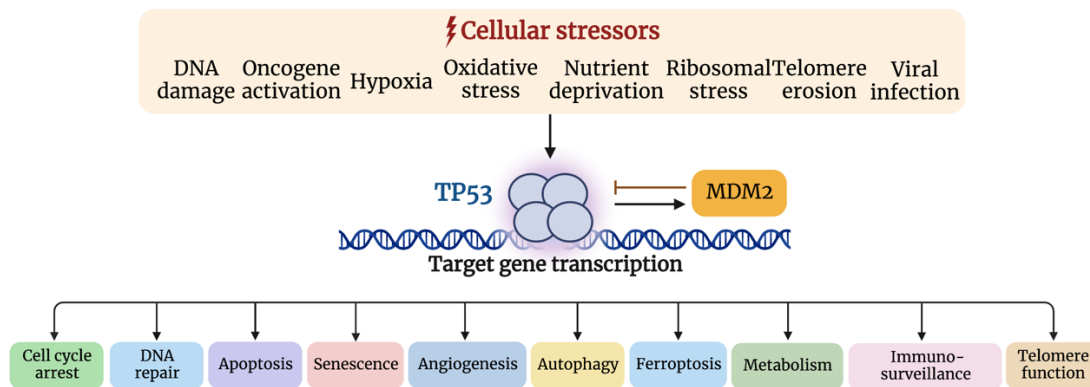
Figure 6. TP53 as a transcription factor, in response to various cellular stressors. *Wild type TP53 can be activated by different cellular stressors, and through DNA-binding to various target genes, TP53 is involved in the regulation of several cellular pathways. TP53 has therefore been referred to as "the guardian of the genome".*

The *TP53*'s key role in cancer development is highlighted by the fact that somatic genetic alterations in the *TP53* gene are found in 42% among 12 of the most common sporadic human cancers (Kandoth *et al.*, 2013). More than 80% of all variants in *TP53*, regardless being somatic or in germline, are missense variants leading to a mutant protein accumulating in the nucleus of the tumour cell (Soussi and Wiman, 2015). Moreover, a third of all *TP53* missense variants in sporadic cancers belong to five so-called mutational hotspots in the DNA-binding domain (Petitjean *et al.*, 2007) (Giacomelli *et al.*, 2018). These hotspot variants occur also in the germline, with the addition of seven others found in persons fulfilling the classical LFS criteria (Table 1) (Kratz *et al.*, 2021). These seven include two nonsense variants (p.C275* and p.R342*) (Figure 7). Up until now, over 300 different germline *TP53* (g*TP53*) variants have been reported (De Andrade *et al.*, 2022) (The *TP53* Database (R20, July 2019): https://tp53.isb-cgc.org).
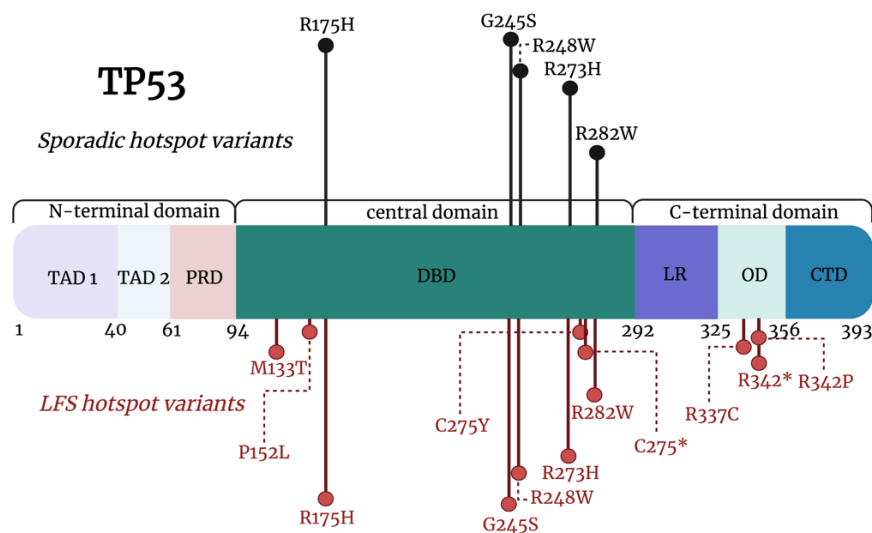
Figure 7. Linear schematic representation of the TP53 protein and outline of the hotspot variants. *The protein is viewed from N- to C-terminal with the domains indicated in different colours. Above, the hotspot variants identified in sporadic tumours are indicated in black, and below, the germline variants identified in LFS-families are shown in red. CTD = carboxyl terminus domain, regulatory domain enhancing the binding to DNA. DBD = DNA-binding domain. LR = linker region. PRD = proline rich domain, promoting p53-programmed cell death and growth suppression. OD = oligomerization domain. TAD = transactivation domain, TAD 1 is linked to transcriptional activity, and TAD 2 to MDM2 binding. Amino acid abbreviations: C = cysteine, G = glycine, H = histidine, L = leucine, M = methionine, P = proline, R = arginine, S = serine, T = threonine, W = tryptophan, Y = tyrosine. * = stop codon. (Petitjean et al., 2007; Giacomelli et al., 2018; Kratz et al., 2021)*

1.3.1. *TP53* in human cancer development

A normal cell has low steady state levels of inactivated TP53 (Vogelstein *et al.*, 2000), thought to maintain the physiological processes mentioned above (Evan and Junttila, 2009). *TP53* is activated in response to different types of DNA damage secondary to UV radiation, hypoxia, oxidative stress, chemotherapy, nutrient deprivation, and oncogenic signalling. Rising levels of TP53 in the cell induce either cell cycle arrest or apoptosis. Notably, the half-life of the wild-type TP53 protein is about 20 minutes. The rising levels will also induce TP53's own regulatory inhibition, most notably the mouse double minute 2 protein (MDM2). MDM2 binds to the TP53 molecules, thereby inactivating them, with or without subsequent ubiquitination and proteasome degradation of TP53 (Shangary and Shaomeng, 2009) (Shi and Gu, 2012). The short half-life enables the cells to address threats such as UV-radiation and hypoxia quickly as it can double the levels of accessible TP53 within 20 minutes instead of being required to activate a whole new synthesis through transcription.

1.3.1.1. *Genetic modifiers in gTP53 carriers*

In persons with g*TP53* variants, studies have shown that carriers have slightly shorter telomeres in comparison with controls from the population (Pinto *et al.*, 2009) and in comparisons between cancer affected carriers with healthy carriers and controls (Tabori *et al.*, 2007), possibly explaining earlier age of cancer onset. Modifications of the negative inhibitor MDM2 have also been found to affect g*TP53* carriers, possibly leading to an earlier age of cancer diagnosis especially for the SNP309 T>G variant in MDM2 (Tabori *et al.*, 2007) (Bougeard *et al.*, 2006) (Ruijs *et al.*, 2007). This variant leads to an increased expression of MDM2 and thereby increased inhibition of TP53.

1.3.1.2. *Physiological role of TP53*

An interesting thought has been raised among scientists (Soussi, 2010) regarding the "original" role of *TP53* – since it is highly conserved among species; when the human life expectancy 10 000 years ago was much lower, around 30 years, people did not die due to cancer which normally occurs later on in life. *TP53* has been described to have a role in various physiological functions such as ageing, development, stem-cell regulation, endurance during exercise, fecundity, sun tanning, neurodegeneration, ischaemia, ribosomal syndromes and diabetes (Vousden and Ryan, 2009) (Figure 8).
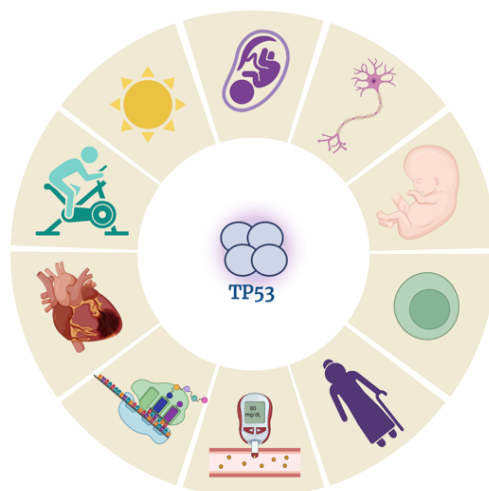

Figure 8. The physiological roles of TP53. The TP53 have various physiological roles, and is involved in a wide variety of cellular pathways. Thus, it has a role in various functions such as fecundity, neurodegeneration, development, stem-cell regulation ageing, diabetes, ribosomal syndromes, ischaemia, endurance during exercise, and sun tanning.

1.4. CLASSIFICATION OF GERMLINE *TP53* VARIANTS

All human variants are classified according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) criteria for interpretation of germline variants, addressed as disease-causing (class 4 and 5) or not (class 1-2, interpreted as commonly found in the population) or of uncertain significance (class 3) (Richards *et al.*, 2015). The disease-causing class 4 and 5 are often referred to as mutations, however, this terminology is disappearing in favour of the concept of variants. As new information becomes available, a variant previously annotated as "class 3" might be down-graded to a class 1 or 2 (or up-graded to 4 or 5, thus clinically actionable). Sources for annotation may come from population data, computational and predictive data, functional analyses, segregation data, or allelic data supporting different levels of evidence of the annotation. For a variant to be classified as "likely pathogenic", class 4 or "likely benign", class 2, the certainty must be ≥95% (Plon *et al.*, 2008).
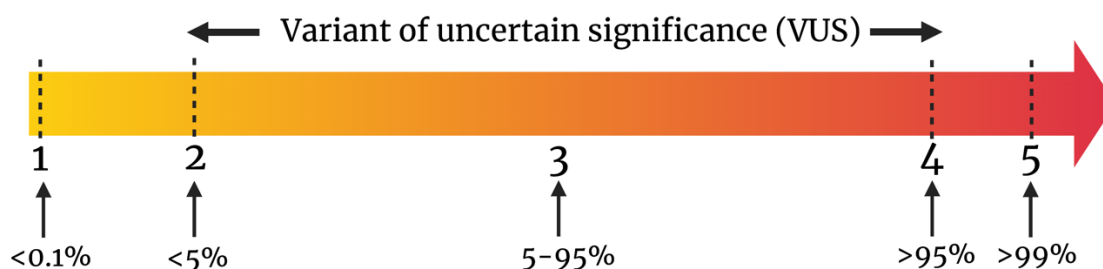
Figure 9. Schematic presentation of proportions of certainty in variant classification and their likelihood of being disease-causing. *As an example, for a class 3 variant, often referred to as a variant of uncertain significance (VUS), the likelihood of being disease causing can vary between 5-95%. Courtesy of S. Bajalica-Lagercrantz.*

Missense variants with a single amino-acid substitution are the most common variant type in both somatic and germline *TP53* variants (Malkin, 2011), often (around 80%) located within the DNA-binding domain. Variants will most often lead to either a wrongly folded protein, or disturbances in the DNA-binding capacity (Kastenhuber and Lowe, 2017; Sabapathy and Lane, 2018). Interestingly, an alternative explanation for the location of these missense variants (and especially the five hotspot variants indicated in black in Figure 7) may be due to the mutability of the DNA sequence at this particular location in the DNA-binding domain. All five hotspot codons contain methylated CpG dinucleotides, commonly leading to a C>T substitution (Giacomelli *et al.*, 2018). Certain missense variants can also cause a so-called dominant-negative effect (DNE) when the variant protein interacts with wild-type (wt) TP53 monomers, forming so called hetero-tetramers (Figure 10) and thereby inhibiting the normal function of the wt tetramer (Boettcher *et al.*, 2019) (Figure 10). Variants leading to a DNE or a loss-of-function (LOF) were associated with an earlier age of both first and second primary cancer, when compared to other types of variants (De Andrade *et al.*, 2021). Also, g*TP53* missense variants within the oligomerisation domain (resulting in monomeric conformation) has shown a complete penetrance when compared to multimeric proteins, resulting in a significantly lower survival (Fischer *et al.*, 2018).
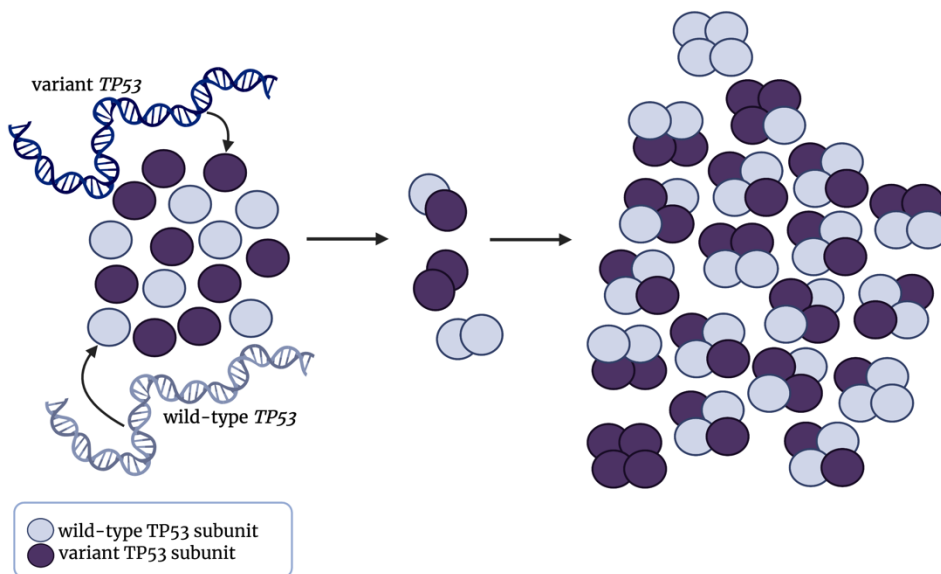
Figure 10. The tetramerisation of TP53 and the rationale behind the dominant-negative effect. After translation, the wild-type and variant TP53 monomers initially dimerises and thereafter forms a "dimer of dimer's" into tetramers prior to DNA-binding. This also illustrates the dominant-negative mechanism, as a variant subunit (purple) might bind to a wt subunit (grey) and through heteromerisation inhibits the otherwise normally functioning wt tetramers. The hetero-tetramers might become incapable of DNA-binding.

To summarise, variants in *TP53* might lead to three (not necessarily mutually exclusive) outcomes; 1) the tumour suppression function is lost in the affected allele, and when a "second hit" affects the wt allele, the cell is deprived of any normally functioning TP53; 2) dominant-negative effects (DNE) by the variant TP53 through interaction with the wt protein and 3) various types of gain-of-functions (GOFs) (Oren and Rotter, 2010) (Figure 11). A true GOF is separate from DNE, as a DNE "requires" a wtTP53 that the variant TP53 interacts with. In contrast, GOF are activities of TP53 variants that are expressed in the absence of normally functioning wtTP53.
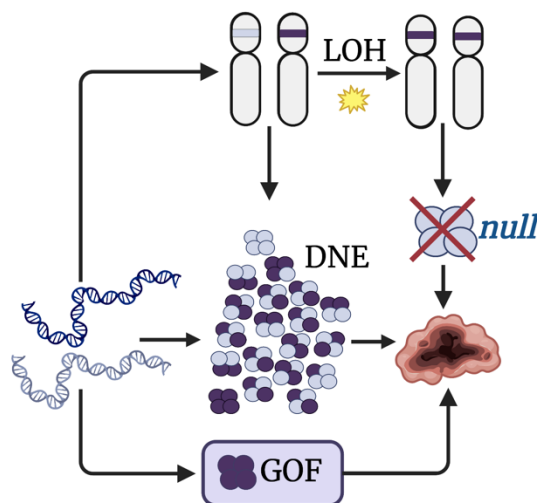
Figure 11. Schematic overview of different mechanisms of inactivation of TP53. A variant TP53 may cause tumourigenesis through different mechanisms, such as loss of heterozygosity (LOH), dominant-negative effect (DNE) and gain-of-function (GOF).

1.5. IS IT A TRUE GERMLINE *TP53* VARIANT?

TP53 is often included in gene panels (a selection of genes associated with the specific cancer that is analysed), from which it may be necessary to pursue further testing when a

germline alteration is suspected. This warrants the need to understand and interpret the findings from next-generation sequencing (NGS) in order to distinguish between a germline or a somatic *TP53* variant. This has implications both for the patient as it may guide into different treatment strategies, and, in case of germline variants for the patient's family with regards to genetic counselling and preventive strategies. Ideally, the variant allele frequency (VAF) in a blood test is 50% in a germline situation. Generally, frequencies between 30-70% are usually interpreted as a heterozygous pathologic germline variant. When the VAF is low (<30%), possible explanations could be 1) somatic (clonal haematopoiesis of indeterminate potential (CHIP, Figure 12a), 2) somatic mosaicism (due to post-zygotic *de novo* variant) 3) a true germline variant, but with technical issues 4) *TP53* variants from a few cells circulating in the blood and arising from a tumour (cell-free DNA, cfDNA), although less commonly and poses more of a theoretical argument (Batalini *et al.*, 2019). When it comes to mosaicism (Figure 12b), the patient presents with a somatic *TP53* variant. Depending on the timing of the post-zygotic variant during the embryonic development, the individual may carry the pathological variant in some cell lineages, with or without affection of the gonads. If the post-zygotic variant arises early, the person will have a generalised mosaicism. A somatic alteration occurring later, after the differentiation into different germ layers, leads to a more localised mosaicism (Chen *et al.*, 2022).

In the case of a low VAF, *TP53* variants from a blood test (representing leukocytes and therefore mesodermal origin), testing should be obtained from another cell type, for example fibroblasts in a skin punch biopsy (ecto-/mesodermal origin) or eyebrows (ectodermal origin) to confirm mosaicism. The endoderm gives rise to several internal organs and the gastrointestinal tract and is not easily tested, but it could be rational to assume that a finding of a pathologic variant of both ecto- and mesodermal origin reflects the same in the endoderm (Batalini *et al.*, 2019). Consequently, the carrier should be offered surveillance in accordance with g*TP53* variant carriers as we do not know how the mosaicism is expressed in different tissues.
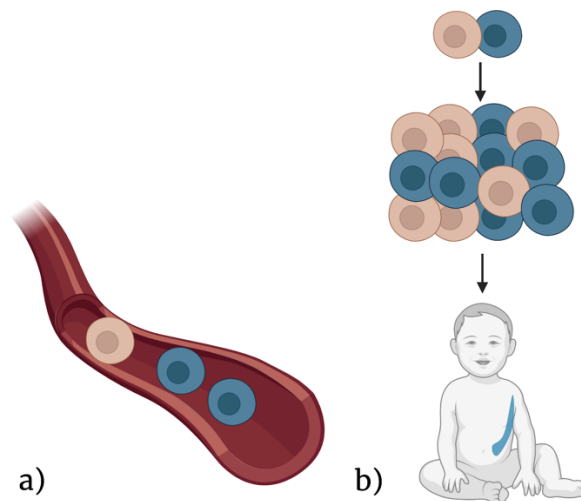
Figure 12. Clonal haematopoiesis of indeterminate potential (CHIP) a) and mosaicism in different tissues b). For CHIP, a clonal hematopoiesis has occurred within a distinct subpopulation of blood cells where a variant TP53 is expressed. Since germline analysis is often performed on blood, this could wrongly be interpreted as a true germline variant. In the case of mosaicism, a somatic TP53 variant can occur after fertilisation, leading to wt or variant TP53 in different tissues. These tissues carrying the variant TP53 can therefore have an increased of malignant transformation. In case of gonadal transformation, the offspring could potentially have hTP53rc.

CHIP arises when a single hematopoietic stem cell carrying a pathologic variant of *TP53* undergoes clonal expansion thereby contributing to a high proportion of cells with a detectable variant. Factors contributing to CHIP are older age (usually from 70 years, but

may be detected from 30 years of age), and also previous chemotherapy or irradiation, and tobacco use (Weber-Lassalle *et al.*, 2018; Batalini *et al.*, 2019). To verify that it is in fact a true germline variant, a biopsy from a tissue without lymphocytes such as skin, nail clippings or hair follicles is taken (Frebourg *et al.*, 2020).

1.6. GENETIC TESTING AND ITS IMPLICATIONS

When a hereditary cancer predisposition is suspected, genetic testing might be carried out after careful genetic counselling. This test is performed by analysing certain known genes, often as part of a gene panel usually on DNA from peripheral blood from a patient diagnosed with cancer. If a pathologic variant is found, further testing can be offered to healthy relatives (*cascade testing* as more and more relatives will be eligible for testing when finding new carriers). The testing of a cancer patient could also be a treatment predictive and/or enable prevention. The predictive implication means that we can offer risk-reducing surgeries and surveillance for early cancer detection. Treatment prediction includes both oncological and surgical adjustments as a part of the treatment stratification. If the family pedigree strongly suggests a hereditary effect, but without any (known) pathologic variants to be found, the term "familial cancer" is used, and, depending on the life-time risk of developing cancer as calculated by empiric data (such as CanRisk (Carver *et al.*, 2021) for breast and ovarian cancer risk), surveillance might be offered to individuals at risk "despite" the lack of a verified pathological variant. In these cases, a general proposal is usually to offer surveillance starting 5-10 years before the first diagnosis in the family and up to 10 years after the oldest family member with the specific cancer. The use of next generation sequencing (NGS) is increasing in clinical practise. This *mainstream testing* presents new challenges as more and more patients will likely have a previously unknown germline variant, requiring more actions from the treating physician.

It is also of importance to identify and address potential psychosocial consequences of the genetic testing, and to be able to offer psychosocial support when needed. Questions likely to arise include the testing of children, especially in relation to a family phenotype where adult tumour onset seem to be more characteristic than childhood tumours. Furthermore, testing should be put in perspective of the possible surveillance and risk-reducing measures, and also in the light of respecting a child's autonomy, as well as the consequences arising if one child is a carrier but its sibling is not.

In addition to preventive measures such as risk-reducing surgery, there is also the earliest possible prevention, taking place already at the family planning phase in the form of preimplantation genetic testing (PGT), in line with guideline recommendations from the European Society for Medical Oncology (ESMO) (Paluch-Shimon *et al.*, 2016). This is available in some parts of Sweden. PGT may be performed when a couple wishes to ensure that the germline *TP53* variant would not be passed on to the offspring. This is done by *in vitro* fertilisation, and testing for the specific variant on the early pre-embryo stage, comprising of only a few cells. If a non-carrier embryo is identified, it will then be implanted in the uterus (Practice Bulletin No. 162: Prenatal Diagnostic Testing for Genetic Disorders, 2016).

In summary, genetic screening and potential cascade carrier testing are valuable, given the possibility to offer prevention, early detection, and therefore potentially a more favourable prognosis for individuals with h*TP53*rc and other cancer predisposition syndromes. In the case of a negative genetic testing but in a family with a familial presentation, individualised surveillance programs might still be offered for early cancer detection.

1.7. CLINICAL CHALLENGES OF GERMLINE *TP53*

1.7.1. From LFS to h*TP53*rc

In 1969, two American physicians, Drs Li and Fraumeni, reported an unusual presentation of seemingly rare childhood cancers, rhabdomyosarcomas in children whose family members were prone to develop not only many cancer types, but also at an earlier age compared with the normal population (Li and Fraumeni, 1969). By 1990, germline *TP53* variants were identified as the cause of this rare syndrome (OMIM #151623)(Malkin *et al.*, 1990; Pirollo *et al.*, 1990). Since then, different phenotypic variations between families have been identified, where some are more prone to childhood tumours, while others are mainly at risk for tumours in adulthood or only an elevated risk for breast cancer. Therefore, twenty years later, the European Reference Network for the rare Genetic Tumour Risk Syndromes (ERN GENTURIS) recognised the need to broaden the description into h*TP53*rc - heritable *TP53*-related cancer syndrome also proposing clinical guidelines (Frebourg *et al.*, 2020). The prevalence of disease-causing g*TP53* variants has been estimated to be 1:4500 (Andrade *et al.*, 2019), but this study calculated the prevalence in gnomAD without further confirmation of the patient's phenotype or characterisation of penetrance. Another estimate is 1:5000 – 1: 20 000 (Olfson *et al.*, 2015). The penetrance of a disease-causing variant in *TP53* is almost complete (80-95%) by the age of 70 in classical LFS (Nagy *et al.*, 2004; Amadou *et al.*, 2018) with some exceptions such as the Brazilian founder variant R337H that corresponds to about 10% of penetrance and predisposes to adrenocortical carcinomas in children below 10 years of age (Figueiredo *et al.*, 2006). Different phenotypic presentations have been proposed by different authors. A recent publication propose a model of "LFS spectrum", incorporating h*TP53*rc with a likely pathogenic/pathogenic variant (LP/PV) disease-causing variant as well as "phenotypic LFS" where no LP/PV is found (Kratz *et al.*, 2021).

1.7.2. Genotype-phenotype aspects including genetic counselling

Family trees like the ones seen in Figure 13 yields a high suspicion of an underlying hereditary cancer syndrome. These particular phenotypic presentations fulfil the criteria for g*TP53* testing. An important clinical and phenotypical distinction is the fact that families harbouring the same variant may manifest varying different outcomes with regards to cancer spectra, both with respect to adult versus childhood tumour onset. Today, the underlying mechanisms behind the differences between these variations in families are not completely understood, except in maybe for the specific so-called "Brazilian founder variant", presented below.
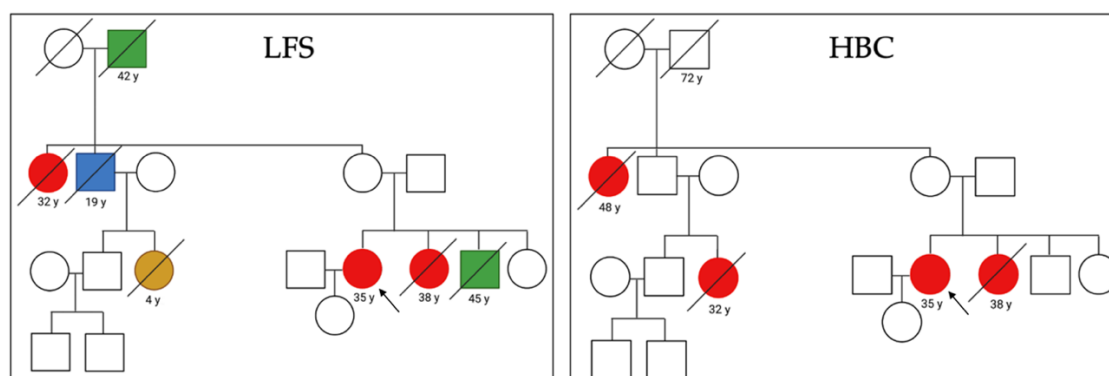
Figure 13. Two pedigrees with different phenotypes of h*TP53*rc. *To the left, a classic LFS presentation and to the right a pattern of hereditary breast cancer (HBC). Females are indicated with circles and male with squares. Numbers state age (y) of tumour onset. Individuals in red indicate breast cancer, green for CNS tumours, sarcoma is indicated with blue and adrenocortical carcinoma with yellow. Probands are indicated with an arrow.*

These phenotypically different presentations make the genetic counselling of these families challenging. There are several attempts ongoing to provide tools to improve genetic counselling and clinical management of these patients and families by creating prediction tools based on dominant-negative effect or for example the protein conformation impact of germline missense *TP53* variants predicting the likelihood of developing hereditary breast cancer (HBC) or LFS (Liu *et al.*, 2021) (Ben-Cohen *et al.*, 2022). However, it is important to underline that these models have not been validated in a prospective clinical setting and can therefore not yet be used to stratify the surveillance program. Furthermore, several risk estimating studies have been done to illustrate the different life-time risks of cancers within LFS, but it is important to consider the risk of ascertainment bias in these studies.

1.7.3. Brazilian founder variant

A founder variant is a genetic trait that occurs at a high frequency in a specific population due to a common ancestor. In Southeast/Southern Brazil, an enrichment of a specific *TP53* variant named c.1010G>A/R337H occurs in up to 0.3% of the population in this region. After a thorough genetic mapping detecting the same series of single-nucleotide polymorphisms (SNPs) near the *TP53* variant in unrelated individuals (Garritano *et al.*, 2008), this variant was referred to as "The Brazilian founder variant" (Gislaine *et al.*, 2013; Seidinger *et al.*, 2020). This variant is found in exon 10, in the tetramerisation domain. The phenotypic presentation of R337H ranges from asymptomatic carriers to classical LFS tumour spectra, but has a strong association with adrenocortical tumours, which has led to neonatal population screening in these areas (Gislaine *et al.*, 2013). An explanation of the wide phenotypic appearance has been found in the extended haplotype. For example, nonsense variant of tumour suppressor gene *XAF1* in a subset of R337H-carriers may contribute to an increased risk of sarcoma and other cancers (Paskulin *et al.*, 2015; Pinto *et al.*, 2020).

1.8. TUMOUR SPECTRUM WITHIN LFS

1.8.1.1. Overview

The classic Li-Fraumeni syndrome (LFS) is characterised by very early onset female breast cancer (before 31 years), sarcomas, brain tumours and adrenocortical carcinomas (Varley, 2003). The life time risk of cancer development has been estimated to 70-100% (Figure 14). Female carriers of g*TP53* pathologic variants appear to have a generally higher cancer risk, not only due to the increased risk for breast cancer, but studies show that they tend to carry an overall higher cancer incidence compared to male carriers (Chompret *et al.*, 2000; Wu *et al.*, 2006; Mai *et al.*, 2016; Amadou *et al.*, 2018). Furthermore, carriers of g*TP53* pathologic variants are at risk of developing cancer at substantially younger ages than non-carriers, in general half the age of the sporadic counterpart (Mai *et al.*, 2016; Amadou *et al.*, 2018).
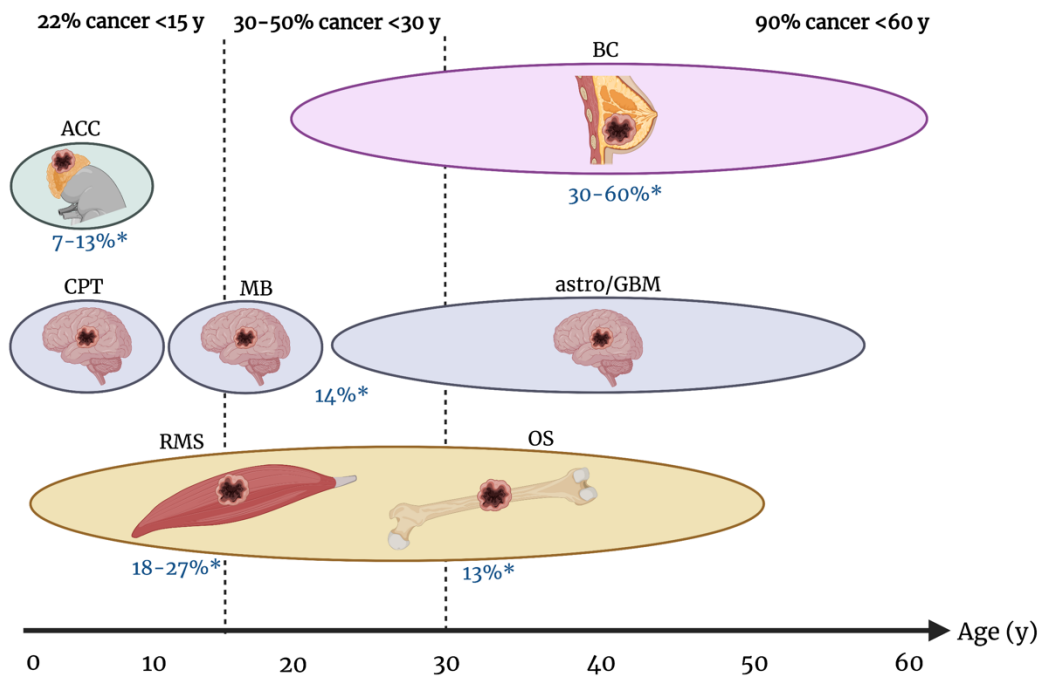
Figure 14. The classic LFS tumour spectrum and ages of onset. *The range of ages of onset is shown for the core tumours; breast cancer (BC), adrenocortical carcinoma (ACC), brain tumour, and sarcoma (rhabdomyosarcoma (RMS) and osteosarcoma (OS)). The childhood phase (0-15 years) comprise 22% of all cancers with mainly ACC, choroid plexus carcinoma (CPT), RMS and medulloblastoma (MB). In adults, the most common brain tumour types are astrocytoma (astro) and glioblastoma (GBM). Female breast cancer accounts for one-third of all diagnoses. *Indicating the proportion of gTP53 carriers with this certain tumour diagnosis, the ranges are suggestive of the wide phenotype distribution depending on the studied cohorts (Olivier et al., 2003; Bougeard et al., 2015; Mai et al., 2016; Amadou et al., 2018).*

Previously, childhood leukaemia has been listed as a core tumour of LFS, but this is no longer a part of the syndrome core tumours. A special type of B-cell acute lymphoblastic leukaemia (B-ALL) in children, namely hypodiploid, (reduced number of chromosomes than normal) has been linked to g*TP53* variants and found in 2% of a cohort with 3 801 genetically sequenced patients, of which 22/49 non-silent rare and coding variants were classified as pathogenic. The reported patients had a higher risk of secondary tumour development after 5 years (25% vs 0.7% in non-carriers) (Qian *et al.*, 2018).

1.8.1.2. Breast cancer

Breast cancer is the most common cancer type accounting for 30% of all cancers in individuals with h*TP53*rc (Olivier *et al.*, 2003). HER2 amplifications are more common (34-83% in contrast to sporadic cases of 15-20%) in individuals with h*TP53*rc with early onset breast cancer (Melhem-Bertrandt *et al.*, 2012) (Wilson *et al.*, 2010) (Blondeaux *et al.*, 2023). Germline *TP53* PV accounts for 5-8% of very early onset breast cancer, before the age of 30 years (Schon and Tischkowitz, 2018). The risk starts to increase by 20 years of age, with a median of 32-33 years (Khincha *et al.*, 2019) (Bougeard *et al.*, 2015). Thus, the clinical breast surveillance is initiated by the age of 20. Some researchers conclude that by 60 years of age, the cumulative breast cancer risk is 85% in female carriers (Mai *et al.*, 2016), but other findings indicate that the breast cancer risk after 60 years declines and reaches population levels. These findings could also be a reflection of relatively few carriers with intact breast tissue at >60 years of age (De Andrade *et al.*, 2021). In contrast to *BRCA2* families, male breast cancer is not reported to be of higher prevalence in patients with h*TP53*rc (Tai *et al.*, 2007; Bougeard *et al.*, 2015). The data on treatment outcomes in g*TP53* carriers in comparison with patients with sporadic breast cancer is scarce. A recent study

on 41 carriers and 82 matched controls did not present any differences in 5 year recurrence-free survival or in breast-cancer specific survival (Petry *et al.*, 2023).

1.8.1.3. CNS tumour

Various types of *CNS tumours* accounts for 14% of all cancers among carriers (Olivier *et al.*, 2003), with a high rate of the rare choroid plexus tumours among children (CPT). CPT is one of the tumours that should raise suspicion of LFS, as a g*TP53* variant is found in 40% of PCTs (Uri *et al.*, 2010). The cumulative risk of CNS tumours is 6% by age 70 for women and 19% for men (Mai *et al.*, 2016), and have a biphasic occurrence in line with sporadic brain tumours. In early childhood (0-5 years), the most common CNS tumour type is CPT, followed by medulloblastoma and astrocytoma/glioblastoma in young adults and throughout adulthood (Orr *et al.*, 2020). In a cohort of 265 families based on data from the IARC *TP53* database, 26% of children and 4% of adults with a g*TP53* variant had a CNS tumour (Olivier *et al.*, 2003), which is in line with another large cohort of 322 carriers reported reporting the same proportions (Bougeard *et al.*, 2015).

1.8.1.4. Sarcoma

Sarcoma represent around 25% of all cancer diagnoses in h*TP53*rc. Except for Ewing sarcoma, GIST, angiosarcomas and desmoid tumours, nearly all types of both soft-tissue and bone sarcomas have been reported at increased levels in families with h*TP53*rc, with a median age of onset at 15 years. Especially in the cases of rare tumours such as rhabdomyosarcoma of embryonal anaplastic subtype, suspicion of g*TP53* should be raised (Olivier *et al.*, 2003; Ognjanovic *et al.*, 2012). For soft-tissue sarcomas, the life time risk by 70 years of age is 22% for men and 15% for women, contrasting to the risk of osteosarcoma which is 5% for women and 11% for men (Mai *et al.*, 2016). More specifically, in the case of rhabdomyosarcomas, most cases associated with g*TP53* PV (95.6%) manifest before 50 years of age, and often as early as before age 5. Corresponding numbers for the population are an occurrence of 38.3% before age 50. Early sarcoma is more associated with missense variants within the DNA-binding domain of *TP53*, in contrast to leiomyosarcoma occurring after age 20 that are more often associated with *TP53* null variants and variants outside of the DNA-binding domain (Ognjanovic *et al.*, 2012).

1.8.1.5. Adrenocortical carcinoma

Adrenocortical carcinoma (ACC) is rare, and the diagnosis should therefore always raise suspicions regarding a h*TP53*rc as germline PV in *TP53* have been found in 50-80% of paediatric ACC (Wasserman *et al.*, 2012). ACC may be non-functional (presented as an abdominal mass) or functional, giving rise to hypercortisolaemia and/or virilisation. There are no differences in symptom presentation in g*TP53* carriers in comparison with sporadic tumours but g*TP53* carriers might have a more favourable treatment response compared with wt tumours (Brenna *et al.*, 2020). There is a female dominance of roughly 1.6:1 (Michalkiewicz *et al.*, 2004), in line with sporadic cases. In comparison, g*TP53* carriers tend to have a more unimodal age of diagnoses in early childhood with 68% below 4 years old and 92% before age 18, in comparison with sporadic cases showing a bimodal distribution peaking early in childhood and another peak in midlife (Wasserman *et al.*, 2012).

In Southeast/Southern Brazil, the incidence of paediatric ACCs is 10-15 times higher than otherwise reported (around 0.3% in new-borns) (Gislaine *et al.*, 2013; Costa *et al.*, 2019) due to the Brazilian founder R337H. This specific founder variant is associated with a lower penetrance of 9.9% in comparison with other g*TP53* PV (Latronico, 2001; Raul *et al.*, 2001; Figueiredo *et al.*, 2006).

1.8.1.6. Multiple primary tumours

An early retrospective study comprising data on 200 g*TP53* carriers collected between 1968-1986, reported a 15% prevalence of a secondary primary tumour, where 4% had a third, and 2% with a fourth primary tumour (Hisada *et al.*, 1998). A recent exploration of 322 carriers (data from 1993-2013) identified multiple primary tumours in 43%, of whom the majority was metachronous disease. In a subset of 64 individuals receiving radiotherapy, 30% developed secondary tumours within the radiation field (Bougeard *et al.*, 2015).

1.9. CRITERIA FOR *TP53* SCREENING

The first criteria for germline *TP53* screening were established by Li and Fraumeni in 1988 (Table 1) (Li *et al.*, 1988). Notably, 70% of all patients fulfilling the testing criteria for classical LFS will have a detectable g*TP53* variant (Olivier *et al.*, 2002). In comparison, the so-called Chompret criteria have a higher sensitivity to find *de novo* variants but has a lower specificity. A clinically actionable g*TP53* PV is therefore found in 29% of tested patients fulfilling the Chompret criteria (Bougeard *et al.*, 2008). Importantly, women older than 46 years old and without a personal or familial history, suggestive of a possible hereditary cause, should not be tested for *TP53* variants. With increasing age of breast cancer onset, the likelihood of identification of a clinically actionable *TP53* variant is lower, especially in the absence of HER2+ disease, and with increasing occurrence of CHIP (Evans *et al.*, 2020). Incidental findings of uncertain *TP53* variants may have large unnecessary consequences for the family, thereby causing unmotivated stress.

In 2015, an updated version of the previous Chompret criteria for testing of g*TP53* PVs was established (Bougeard *et al.*, 2015) and is currently being used (Table 1).

Table 1. Criteria for *TP53* genetic testing

Classical LFS criteria (Li *et al,* 1988)	
	• A proband with a sarcoma diagnosed before age 45 years AND
	• A first-degree relative with any cancer diagnosed before age 45 years AND
	• A first- or second-degree relative with any cancer diagnosed before age 45 years or a sarcoma diagnosed at any age.
2015 version of the Chompret criteria (Bougeard *et al,* 2015)	
Familial presentation	Proband with tumour belonging to LFS tumour spectrum (premenopausal breast cancer, STS, CNS tumour, ACC) before 46 years old, AND at least one first- or second degree relative with LFS tumour (except breast cancer if proband has breast cancer) before 56 years old or with multiple tumours.
Multiple primary tumours	Proband with multiple tumours (except multiple breast tumours), two of which belong to LFS tumour spectrum and first of which occurred before 46 years old.
Rare tumours	Patient with ACC, CPT, or rhabdomyosarcoma of embryonal anaplastic subtype, irrespective of family history.
Early-onset breast cancer	Breast cancer before 31 years old.
Criteria for testing of hereditary breast cancer (Swedish guidelines)	
	• One case of breast cancer diagnosed under 40 years of age or triple negative breast cancer diagnosed under 60 years of age; OR
	• Two cases of breast cancer diagnosed of which at least one under 50 years of age; OR
	• Three cases of breast cancer diagnosed of which at least one under 60 years of age; OR
	• breast and ovarian cancer; OR
	• male breast cancer.
ACC = adrenocortical cancer, CNS = central nervous system, CPT = choroid plexus tumour, STS = soft tissue sarcoma.	

As stated above in Table 1, patients with ACC, CPT or rhabdomyosarcoma of embryonal anaplastic subtype or breast cancer before 31 years old should be tested regardless of family history. This is because of the unusually high incidence of germline *TP53* variants in these cancer forms. The criteria for testing of suspected hereditary breast cancer are included, as g*TP53* sometimes manifest with a "hereditary breast cancer" phenotype only in some families. For children with ACC or choroid plexus carcinoma, a germline disease-causing variant in *TP53* is found in 50-80% (Bougeard *et al.*, 2015). In a study of 15 children with the anaplastic subtype of rhabdomyosarcoma, 11/15 patients (73%) had a germline variant of whom 4/11 (36%) did not have a family history consistent with the LFS cancer spectrum (Hettmer *et al.*, 2014). A review from 2017 concluded that 3.8-7.7% of women with breast cancer <31 years old harboured a g*TP53* variant without family history (Fortuno *et al.*, 2018). Reasons behind the occurrence of cancers without previous family history might be explained by *de novo* variants (Gonzalez *et al.*, 2009; Renaux-Petel *et al.*, 2018) and incomplete penetrance of the variants such as the 'Brazilian founder mutation'.

Around 20% (7-20%) of all verified PV in *TP53* are classified as *de novo* (Gonzalez *et al.*, 2009) (Renaux-Petel *et al.*, 2018).

1.10. IMPLICATIONS FOR TREATMENT STRATIFICATION

1.10.1.1. Radiotherapy - risks for secondary tumours

One of *TP53's* most important roles is to induce cell cycle arrest as a response to DNA damage, in order to either initiate reparation or to induce apoptosis. When the function of the protein is compromised, one of the consequences is an increased risk of secondary primary tumours after DNA damaging treatments such as radio- and chemotherapy, which has been shown in mice (Balmain *et al.*, 1994; Kasper *et al.*, 2018). In humans, limited case reports and case series predominantly on adjuvant radiotherapy for breast cancer, has shown that 16-34% are in risk of developing secondary cancer after irradiation, most commonly sarcoma (Henry *et al.*, 2012) (Salmon *et al.*, 2007) (Heymann *et al.*, 2010; Petry *et al.*, 2020). Various other tumours arising in the radiotherapy field have been described, such as colon cancer and small cell lung cancer (Limacher *et al.*, 2001) (Ferrarini *et al.*, 2011). These findings are in contrast to the normal population with an average risk of 0.2% and after 10 years (Rubino *et al.*, 2003), underlining the need to limit the use of radiotherapy for g*TP53* carriers. In comparison, individuals with h*TP53*rc might exhibit a secondary tumour onset as early as 2-3 years after irradiation. These findings highlight the need to discuss in a multidisciplinary team if adjuvant radiotherapy should be offered at all, or if it is more suitable to offer ipsilateral mastectomy and contralateral risk-reducing mastectomy in the case of breast cancer treatment. This argumentation is in line with guideline recommendations from 2020, where irradiation of the intact breast is advised against, and irradiation following mastectomy should only be performed in cases of a high risk of locoregional recurrence (Tung *et al.*, 2020).

With regards to other types of secondary tumours, case series on children treated with radiotherapy after adrenocortical carcinoma reports sarcomas within the radiation field in 3/7 patients (Varley *et al.*, 1999).

1.10.1.2. Risk-reducing mastectomy (RRM)

Compared with sporadic breast cancer, g*TP53* carriers have a higher risk of both ipsilateral breast cancer recurrence when treated with breast-conserving therapy (21.1% vs 3.8% after 6.7 years) and contralateral breast cancer (17.9% vs 3.6% HR 0.7, 10-year cumulative risk) (Guo *et al.*, 2022). With the use of mastectomy instead of breast-conserving therapy, no differences were found. In g*TP53* carriers below 36 years old, the 10-year cumulative risk of contralateral breast cancer was even higher, 53% (95% CI 29.6–80.6) and the 20-year cumulative risk was 82% (95% CI 49.9–98.8) (Hyder *et al.*, 2020). In another cohort with women of all ages and a previous breast cancer, of those who did not undergo contralateral RRM, 21/51 (49%) developed contralateral breast cancer with a median time of six years. The same publication reported that out of 15 women who completed bilateral RRM without a prior breast cancer diagnosis, none developed breast cancer but two women were diagnosed with ductal carcinoma in situ (DCIS) in the surgical specimen (Siegel *et al.*, 2022).

1.10.1.3. Chemotherapy

In line with the avoidance of radiotherapy, non-DNA-damaging chemotherapy should be preferred if appropriate to lower the risk of subsequent cancers (Frebourg *et al.*, 2020). At the present time, carrying a g*TP53* variant has not been considered to affect the outcome of the oncologic treatment. Nevertheless, a newly published study including 50 g*TP53* carriers with breast cancer found that among the 25 carriers who received neoadjuvant

therapy, they were more likely to achieve pathological complete response to a carboplatin-based treatment in comparison to an anthracycline-/or taxane-based regimen. Of note, the g*TP53* carriers in this study were also found to have a lower recurrence free survival and overall survival compared with non-carriers (Sheng *et al.*, 2020).

## 1.11. CURRENT GUIDELINES WITHIN SWEP53 IN SWEDEN

1.11.1.1. Surveillance

As of today, the current Swedish guidelines recommend all h*TP53*rc carriers with a clinically actionable *TP53* variant to undergo surveillance within the national clinical study named the Swedish *TP53* Study (SWEP53) (Table 2). This enables a homogenisation of the follow-up in Sweden and allows a structured evaluation of the surveillance protocol as well as an establishment of a biobank for future analyses.

Table 2. SWEP53: Inclusion and exclusion criteria

Inclusion criteria
• Adults ≥18 years old with a verified clinically actionable germline *TP53* variant.
• Youths aged 15-18 years old may choose to be included either in the adult or the child protocol for surveillance.
• Children 0-15 years old at risk (50%, *i.e.*, having one parent being a g*TP53* carrier).
Exclusion criteria
• Contraindications to MRI.
• Co-morbidities that preclude treatment of a cancer found in the surveillance program.

The SWEP53 adult protocol is largely in line with European guidelines published by ERN GENTURIS (Frebourg *et al.*, 2020) while the childhood protocol have several differences such as the recommendations for WB-MRI. The SWEP53 protocol was launched in 2016 and involved all units for hereditary cancer in Sweden. Study inclusion started at the Stockholm site (Karolinska University Hospital), and by 2020, inclusion had been initiated at all six centres in Umeå, Uppsala, Stockholm, Linköping, Gothenburg, and Malmö/Lund. The surveillance protocols are illustrated in Figure 15 a) for adults and b) for children.
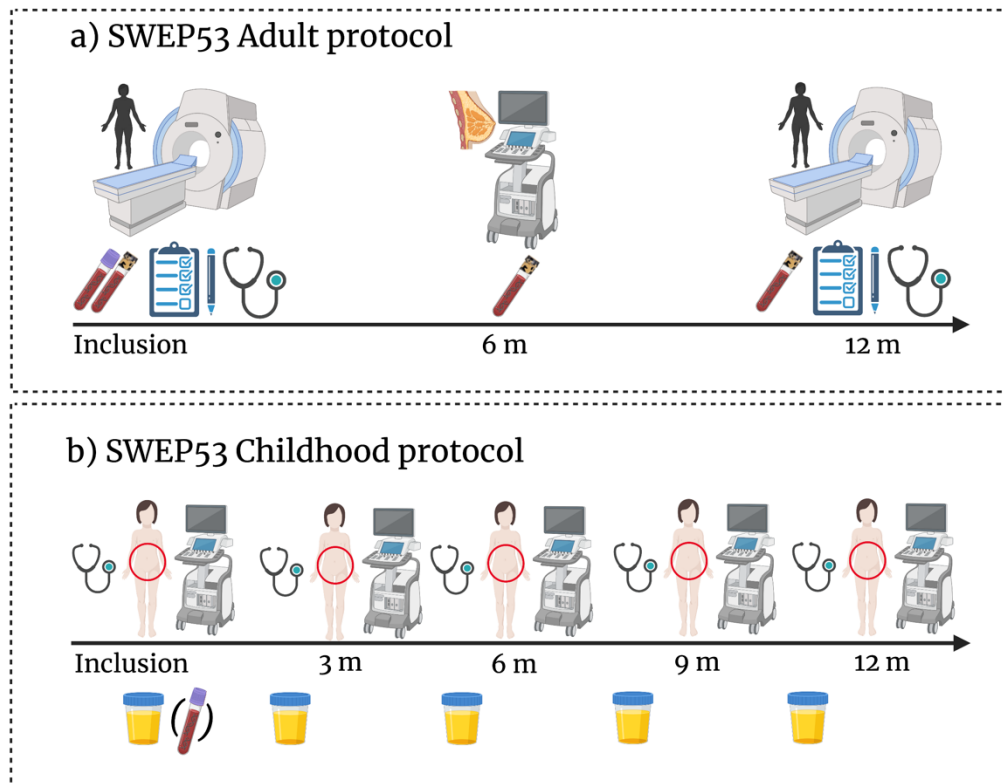
Figure 15. The full SWEP53 study outline for a) adults, and b) children. *a) For adults, the program includes four parts; registration in the national registry, surveillance program, psychosocial evaluation, and biobanking. At inclusion, the participants fill out a form on general clinical information such as weight and ongoing medication. A dedicated blood sample is taken for biobanking of DNA. The adult surveillance protocol is offered from ≥18 years and occasionally from 15 years old. It includes yearly physical examination and imaging with WB-MRI, brain-MRI and, in women that have not performed risk-reducing mastectomy, breast-MRI. The adults fill out annual psychosocial questionnaires and leaves blood samples for collection of cfDNA yearly and, in cases of further work-up, after the previous imaging. For women whom have not undergone risk-reducing mastectomy, a breast ultrasound is performed six months after the WB-MRI. b) For enrolment in SWEP53 during childhood (up to 18 years), the child does not have to be a verified gTP53 carrier, as they can be included at 50% carrier risk. Every three months (m), the children undergo clinical check-up by a paediatric oncologist, and perfume an abdominal ultrasound. With a six week "shift", urine is collected for corticosteroid analysis. There is also a possibility to opt for biobanking of DNA (at inclusion).*

1.11.1.2. Risk-reducing mastectomy

In agreement with the ERN GENTURIS recommendations (Frebourg *et al.*, 2020), risk-reducing mastectomy is offered to female g*TP53* carriers in Sweden from age 20 due to the high life-time risk of developing breast cancer. This is always preceded by discussions in a multidisciplinary team involving clinical geneticists, clinical oncologists, breast surgeons, plastic surgeons, as well as a psycho-oncological health care team. All patients are offered separate consultations with the different professions during their decision-making to ensure a high patient involvement.

1.11.1.3. Clinical examination

Within the SWEP53 protocol, an extended clinical examination was performed annually as a part of the surveillance program. The examination includes a visual status of the skin, a neurological examination including a status of the cranial nerves, auscultation of heart and lungs, and palpation of the abdomen, lymph nodes, and breasts. A separate protocol was developed to ensure a homogenisation of the check-ups at the different study sites.

1.11.2. Whole-body magnetic resonance imaging

1.11.2.1. Surveillance and further work-up

Surveillance with WB-MRI is recommended not only in individuals with h*TP53*rc, but also with various other syndromes such as constitutional mismatch repair deficiency syndrome, hereditary paraganglioma and pheochromocytoma syndromes, and patients with neurofibromatosis (Petralia *et al.*, 2021). Because of the high risk of secondary radiation-induced malignancies, non-ionising imaging modalities such as magnetic resonance imaging (MRI) and ultrasound (US) are preferred in g*TP53* carriers. Despite this, in 2015, a Brazilian group presented data on (18)F-Fluorodeoxyglucose positron emission tomography/computed tomography (18)F-FDG-PET/CT as a baseline scan in asymptomatic variant carriers, enrolled in clinical surveillance as recommended and based on familial presentation. They reported six lesions in 30 asymptomatic individuals requiring further work-up, resulting in three advanced cancers and three benign lesions (Nogueira *et al.*, 2015). There is no comparison of cancer detection between WB-MRI and PET/CT or CT alone in patients with h*TP53*rc. Most studies are based on non-ionising modalities considering the inherited specific sensitivity to ionising irradiation in this patient group (Rubino *et al.*, 2003; Heymann *et al.*, 2010; Petry *et al.*, 2020).

A meta-analysis on baseline whole-body MRI (WB-MRI) in 578 patients from 13 cohorts in six countries found a total of a 7% rate of primary, new non-disseminated cancers (Ballinger, Best*, et al.*, 2017). Six of the trials in the meta-analysis have published their results so far in independent papers (Villani *et al.*, 2016; Mai *et al.*, 2017; Ruijs *et al.*, 2017; Saya *et al.*, 2017; Bojadzieva *et al.*, 2018) (Ballinger, Ferris*, et al.*, 2017; Paixão *et al.*, 2018). It is important to address the level of further work-ups after a whole-body scan, and to inform the patient of its likelihood. In the previously mentioned meta-analysis, "the false positive" rate in detecting cancer with this modality was as high as 42.5%, but that included suspected lesions that were eventually found to be benign, recurrences of pre-existing cancers, and newly diagnosed metastatic cancers. Commonly reported benign findings in these studies include liver lesions such as haemangiomas and benign bone lesions. This high rate of false positives stresses the need of information given by the physician to the screened individual to prepare for the likelihood of recommendations to undergo further examinations especially after the first baseline examination. A presentation of published studies on surveillance with WB-MRI is presented in Figure 16.

Two publications have focused on surveillance with WB-MRI for children. In both reports, the children were verified g*TP53* carriers. The analyses were performed retrospectively on 10 and 31 children, respectively (Anupindi *et al.*, 2015) (Tewattanarat *et al.*, 2022). Both cohorts reported few further-workups after imaging. Notably, Tewattanarat and colleagues stressed the importance of developing a structured reporting system, double-reading for image interpretation, and optimising the WB-MRI protocol both technically and with regards to definitions of criteria to identify "hot signs" of potential malignancy.
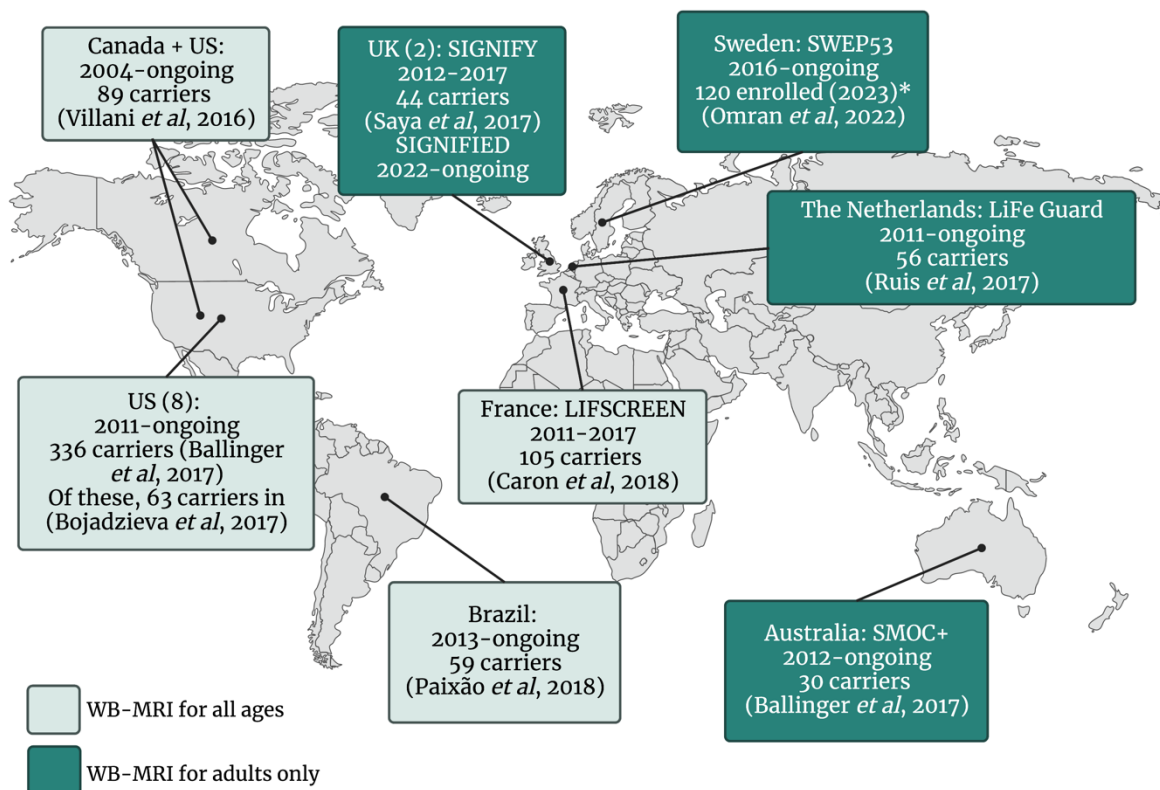
Figure 16. An overview of published studies with WB-MRI for gTP53 carriers. *Structured surveillance protocols for gTP53 carriers have been reported in eight countries, of which four are European, and are ongoing in all countries except for France. Dark green background indicates WB-MRI only for adults over 18 years old, light green indicate WB-MRI for all ages. For the Swedish SWEP53 Study, the general rule is WB-MRI only for adult carriers, if no clinical indications for younger persons states otherwise. WB-MRI is offered for carriers over 18 years old, and in some cases, from 15 years and onwards. *Within SWEP53, 68 adult carriers had been enrolled at the time of publication in 2022. The total of 120 enrolled individuals in 2023 indicate both adults (92 carriers) and children (28) by March 2023, where 12/28 children were confirmed carriers and the others at 50% carrier risk at the time of inclusion. For the LIFSCREEN study in France, criteria were >5 and <71 years old. Different age criteria apply for the different studies in the US.*

1.11.2.2. Use of contrast enhancement

In SWEP53, the protocol suggests the use of intravenous contrast enhancement with Gadolinium chelate (Gd) as part of the breast-MRI imaging in women that have not undergone risk-reducing mastectomy (RRM). In these women, Gd is simultaneously utilised to optimise imaging of the brain. No contrast enhancement is used for men or women after RRM. It is known that Gd is deposited in the form of chelates or Gd in small amounts in the brain, liver, skin, and bone. The long-term clinical impact of these deposits is not fully known and remains to be further studied (Guo *et al.*, 2018). Regardless of this, to ensure minimal possible risks due to surveillance of healthy individuals, the use of contrast enhancement should be limited to avoid unpredictable side effects.

1.11.2.3. ONCO-RADS

A suggestion of a standardisation of the MRI protocol and its interpretations have been proposed, including the use of the "Oncologically relevant findings Reporting and Data system" (ONCO-RADS). ONCO-RADS emphasise structured reports where each abnormal finding is assigned to one of seven regions (head, neck, chest, abdomen, pelvis, limbs and bones) followed by an assessment category ranging from 1-5 (Table 3). According to ONCO-RADS, categories 3-5 in high-risk individuals requires further examinations, in contrast to categories 4-5 in the general population. Limitations for WB-MRI as a cancer screening tool includes limited detection of lung and prostate cancer, and

the authors suggests evaluation of ONCO-RADS in prospective studies to evaluate the frequency of malignant findings within the categories in different patient cohorts (Petralia *et al.*, 2021).

ONCO-RADS category	Explanation
1	Normal
2	Benign finding highly likely
3	Benign finding likely
4	Malignant finding likely
5	Malignant finding highly likely
Other	Annotation of anatomic variation and other findings important for the individual's health.

Actionable in general population.

Actionable in high-risk population.

Table 3. Standardisation of MRI findings by ONCO-RADS. *The different categories within ONCO-RADS and actionable findings in different populations. ONCO-RADS classification indicated that lower threshold for actionable findings (red) should be used in patients with an increased cancer risk compared to the general population (blue) (Petralia et al 2021).*

1.11.3. Psychosocial aspects

1.11.3.1. Presymptomatic testing – uptake and psychosocial consequences

An early study from the Netherlands in 2010 (Lammens, Aaronson*, et al.*, 2010) was performed to evaluate the uptake and psychosocial consequences of presymptomatic testing, notably before the first publication of the "Toronto protocol" offering surveillance with WB-MRI (Villani *et al.*, 2011). The authors concluded that despite the lack of surveillance programs, 55% (65/119) underwent genetic testing, reporting that they wanted to have information on their cancer risk, estimations of the children's cancer risk and plans for regular surveillance were the motivations. Furthermore, carriers were not more worried in comparison with non-carriers. A subpopulation of both carriers and non-carriers expressed more cancer worry, but this was not associated with having undergone genetic testing or previous cancer history, but rather with gender (women), a high perceived risk of developing cancer, and a perceived lack of social support (Lammens, Aaronson*, et al.*, 2010). Four years after the first publication on the Toronto protocol (Villani *et al.*, 2011), a study across five sites in Canada and the US reported an uptake of g*TP53* testing of 92% (159/172 families), where uptake was significantly higher in the persons with a suspicion of cancer than in the healthy individuals at risk (95% vs 79%). In a subset of 39 families, interviews were performed. Among these, three different decision-styles emerged; automatic, considered and deliberated. Reasons to undergo testing were "promoting the health of the child" including surveillance, and a need to know. Perceived disadvantages were psychosocial concerns such as treating the child differently, and threats to privacy, including insurance problems and discrimination by future employers. Regardless of their decision-style, no one regretted their decision (Alderfer *et al.*, 2015). Another study among adolescents and young adults (AYAs, aged 15-39) identified three main motivations for undergoing presymptomatic or diagnostic testing; most commonly to reduce uncertainty and plan for the future, followed by finding a cancer cause, and to protect family across generations. The psychosocial reactions to the positive genetic test were also influenced by the access to surveillance with WB-MRI, where questions regarding the utility of knowing was put in contrast to not being able to manage the risks (Forbes Shepherd *et al.*, 2020).

The decision to undergo presymptomatic genetic testing (and also decisions regarding prenatal screening) is further affected both by personal cancer diagnoses, '*embodied*

knowledge', but also through being a part of a family members experience of disease and treatment, *'empathic knowledge'* (Werner-Lin *et al.*, 2022). Furthermore, it is not uncommon for several family members to have simultaneous diseases and losses, with limited possibilities to have a space for recovery (Oppenheim *et al.*, 2001).

Published studies specifically looking into non-carriers within g*TP53* families seem to be lacking, but it is reasonable to think that there might be some kind of feelings in alignment with the so called 'survivors' guilt'. This has been illustrated in an interview study on 12 young members of LFS families, where two out of three siblings tested positive. The carriers reported that this probably brought them closer together, while the non-carrying sibling reported feelings of guilt (Alderfer *et al.*, 2017). In extent, parents might treat siblings differently based on their carrier status, but this need to be further explored within future studies.

1.11.3.2. Surveillance with whole-body MRI

Studies have shown mainly a benefit among g*TP53* carriers of taking part of a surveillance program including WB-MRIs despite more worry and anxiety connected to living with the variant itself, and the anxiety related to the scans and awaiting the results (Mcbride *et al.*, 2017; Bancroft *et al.*, 2020; Rippinger *et al.*, 2020). Most of the mentioned studies have been descriptive and used questionnaires with/without interviews, except for one using only semi-structured phone interviews (Ross *et al.*, 2017). A matched case-control study compared 44 g*TP53* carriers with 44 non-carriers at study enrolment, after MRI, and at 12, 26 and 52 weeks after the scan results using questionnaires. The authors conclude an overall higher mean score for cancer-related worry in the carriers compared with non-carriers, but this was not negatively impacted by the WB-MRI screening. In carriers with additional work-up due to a finding on the scan, the levels of anxiety were not higher compared to carriers with a normal scan (Bancroft *et al.*, 2020). Another study with 46 patients at 13 different centres in Germany found that there was a need for a standardised surveillance protocol and a main point of contact to increase adherence to the surveillance (Rippinger *et al.*, 2020). A strictly qualitative study from the US, focusing on the embodied risk of being a carrier, concluded after analysing deep interviews with surveillance participants that WB-MRI introduced a permanently altered view of their bodies. Besides being time-consuming and uncomfortable, WB-MRI became an ongoing reminder of persistent, embodied risk leading to that the body had to be in constant surveillance. This shift of personal control into the hands of technology could lead to feelings of disembodiment. The same study, consisting of 66 interviews within 45 families, concluded that participants consider early detection as the best tool for survival, especially recognising that risk-reducing measures were not available for men and limited to risk-reducing mastectomy for women given that LFS pose more of a "whole body cancer risk" in comparison with organ-specific inherited cancer predisposition syndromes (Werner-Lin *et al.*, 2022).

These studies all evaluated psychosocial outcomes in relation to WB-MRI. An earlier study on 31 participants from 2010 by Lammens *et al,* evaluated cancer worry and benefits/risk of participating in the surveillance program in the Netherlands including annual breast imaging, annual medical consultation and tailored screening. Their results reflect the same outcomes as the later ones with a perceived benefit of early cancer detection (90%) and sense of control (84%) and security (77%) (Lammens, Bleiker*, et al.*, 2010). In conclusion, there seems to be reports of an overall satisfaction with surveillance including WB-MRI and a sense of more control and less cancer worry. A number of the mentioned studies report, however, negative aspects such as many hospital visits, difficulties to travel to a specialised centre, and insurance problems. In addition, there is a

risk of selection bias in these studies, as those who completed questionnaires also participated in the surveillance program.

The six mentioned studies (summarised in Supplementary Table S1) are from Australia (Mcbride *et al.*, 2017), Great Britain (Bancroft *et al.*, 2020), Germany (Rippinger *et al.*, 2020), the Netherlands (Lammens, Bleiker*, et al.*, 2010) and the US (Ross *et al.*, 2017) (Werner-Lin *et al.*, 2022). All but the one by (Werner-Lin *et al.*, 2022) included 17 to 46 study participants. Werner-Lin and colleagues presented results from 66 interviews including 117 carriers. Two of these studies have reported that patients plead for a standardised surveillance (Ross *et al.*, 2017; Rippinger *et al.*, 2020). Another interesting aspect is the reflection mentioned in the publications connected to a possible insurance problem due to the diagnosis, and problems with coverage for the WB-MRIs. This reflects each country's health care system and private insurance policies.

1.11.3.3. Psychosocial reactions to being a carrier

In addition to the burden of surveillance, cancer treatment, loss of loved ones, and perceived lack of control over major life changes over all ages, another dimension is all this in relation to being an AYA, aged 15-39 years (Werner-Lin *et al.*, 2020). These formative years imply important psychosocial developmental stages and have been reported to affect development of both peer and intimate relationships, autonomy, and views of self-identity. Core developmental tasks such as financial independence or childbearing may be inflicted by being a carrier and/or having undergone cancer treatment. This could potentially lead to psychosocial crises, when society's expectations on particular life stages cannot be fulfilled (Forbes Shepherd *et al.*, 2020).

In addition, yet another dimension needing special consideration is in the case of *de novo* variants (occurring in 7-20% (Gonzalez *et al.*, 2009; Renaux-Petel *et al.*, 2018), where no family history is present to guide in important medical decisions.

1.12. PREVENTION

1.12.1. Lifestyle interventions

In addition to surveillance for early cancer detection, the aspect of cancer prevention is important. To decrease cancer risk by making lifestyle interventions such as healthier diet, smoking cessation and increased physical activity is a way to possibly lower the personal cancer risk. A descriptive German study of 70 carriers and their relatives identified women carriers as being less likely to smoke and had a healthier diet than male carriers and compared to their relatives (Nees *et al.*, 2022). It is known that these interventions decrease cancer risk in the general population. However, prospective studies in the setting of h*TP53*rc are needed even though one might argue that the personal risk reduction in the light of having a very high life-time risk might be small, this still needs to be explored. At least, this might be another risk-reducing strategy that individuals with h*TP53*rc can choose to obtain along with risk-reducing surgeries.

1.12.2. Chemoprevention

For a drug to be a putative chemopreventative agent, it has to be well tolerated by the patients. Even if the side effects are not severe, they should not impact on the individual's life to a degree of non-compliance and the adverse effects must always be weighed against the potential benefits of delaying or even preventing cancer development. As *TP53* is disrupted in many human cancers, it is an attractive target for treatment. Compounds tested in clinical trials act either by reactivating mutant TP53 forms back to a functional

form, or by inhibiting the interaction between wild-type TP53 and its inhibitor MDM2/MDM4 (Duffy *et al.*, 2022). One of these compounds is APR-246 (also known as PRIMA-1 or eprenetapopt). This is a small, first-in-class molecule that induces apoptosis in TP53 mutated cancer cells by restoring wild-type p53 functions (Lambert *et al.*, 2009). It also promotes tumour cell death by increasing oxidative stress (Peng *et al.*, 2013; Bykov *et al.*, 2016). The anti-cancer effect of APR-246 have been studied in several phase I/II trials on patients with cancer, but none of them have been performed on individuals with h*TP53*rc, and it would be highly interesting to evaluate whether there is a possibility to use the drug as a chemopreventative agent in healthy g*TP53* carriers.
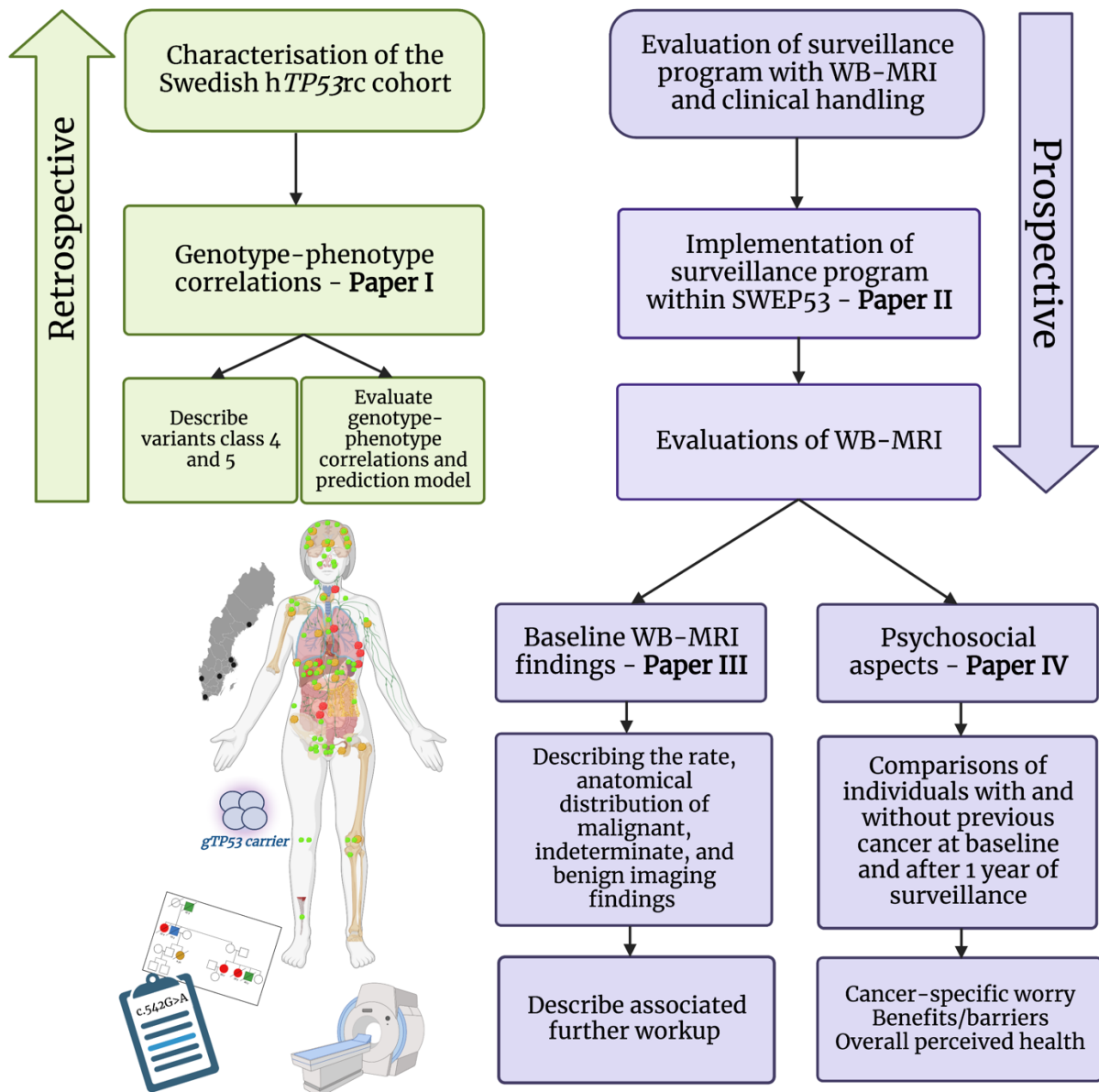
As a preventative agent has to be tolerable with few and acceptable side effects for long-term use, it is reasonable to use well-known drugs för this purpose. This approach of drug-repurposing common drugs like metformin and aspirin is of ongoing interest, and the first clinical trial is currently recruiting g*TP53* carriers for the evaluation of metformin with regards to cancer-free survival and overall survival. Metformin is thought to have a role in many of the classical "hallmarks of cancer", by inhibiting chronic inflammation, telomere dysfunction, angiogenesis, oxidative stress, immune dysregulation and metabolic reprogramming (Pantziarka and Blagden, 2022).

SUPPLEMENTARY TABLE S1. Psychosocial evaluation of participation in surveillance with WB-MRI

Country	Reference	No of participants	Study design	Methods
AUSTRALIA	McBride *et al* (2017) SMOC+	17 of whom 11 had previous ca but eligible at 50% risk also.	Prospective sub-study within WB-MRI surveillance.	Mixed interviews (2) analysed in accordance with grounded theory and questionnaires at baseline, 2 weeks post WB-MRI, 12, 26, 52 weeks post WB-MRI.
Summary *Australia*: A significant reduction in participants' mean anxiety from baseline to two weeks post WB-MRI (1.2, 95% CI 0.17 to 2.23 p = 0.025), indicative of some benefit. Qualitative analysis: participants are emotionally supported and contained by the screening program despite being informed about the current lack of evidence around efficacy of screening in terms of cancer morbidity or mortality. Perceived risks; feelings of abandonment by the research team is a risk when the study ends. Families with *TP53* variants need ongoing support due to the impact on the whole family system.				
GERMANY	Rippinger *et al* (2020).	46 of whom 43 had previous cancer.	13 centres, questionnaires on surveillance at each centre (not a specified program), post-hoc adherence vs non-adherence, otherwise descriptive.	CWS + LFS items on worry regarding variant, partner etc. and study specific questionnaire on risk factors.
Summary *Germany*: Efficient counselling is required as well as an accessible, well-organised, interdisciplinary, standardised surveillance program to increase adherence and psychological coping.				
THE NETHERLANDS	Lammens *et al* (2010).	45 of whom 18 were at risk but was recommended surveillance, in total 32 were recommended surveillance.	Descriptive and comparing adherence with non-adherence. Surveillance: organ-targeted surveillance based on family cancer history.	CWS, Questionnaire on perceived benefits and barriers.
Summary The Netherlands: Perceived benefits of finding cancer early (90%) sense of control (84%) and security (77%). Negative impact on health insurance (42%).				

Country	Reference	No of participants	Study design	Methods
GREAT BRITAIN	Bancroft *et al* (2019) SIGNIFY.	44 carriers, 44 controls.	Matched case-control.	HADS, CWS, HQ, IES, Spielberger, SF-36, PR, SSQ, acceptability of screening.
Summary *Great Britain*: Overall higher mean cancer-related worry in the carriers, but this was not negatively impacted by the WB-MRI screening. In carriers with additional work-up due to a finding on the scan, the levels of anxiety were not higher compared to carriers with a normal scan.				
USA	Ross *et al* (2017), LEAD Study (MD Anderson).	20 of whom 17 had a previous cancer.	Descriptive.	Grounded theory, semi-structured phone interviews of persons already taking part of the surveillance program with WB-MRI.
Summary *USA LEAD*: Benefits of screening include early detection, peace of mind, centralised screening, knowledge providing power, and screening making LFS seem more liveable. Perceived drawbacks included logistical issues, difficulty navigating the system, screening being draining, and significant negative emotional reactions such as anxiety, fear, and scepticism. Regardless of the emotions that were present, 100% of participants planned on continuing screening in the program.				
USA	Werner-Lin *et al* (2022), The National Cancer Institute's LFS study.	117 carriers from 45 families in 66 interviews.	Nested within the WB-MRI study, prospective over five years, descriptive.	Grounded theory, and interpretive description of interviews.
Summary *USA NCI*: The constant monitoring during surveillance leads to a perception of the embodied risks of being a carrier, and connects carriers within families with each other. Normalisation of aesthetic changes occurred. Carriers considered early detection as the best tool for survival, especially recognising that risk-reducing measures were not available for men and limited to risk-reducing mastectomy for women given that LFS poses more of a "whole body cancer risk" in comparison with organ-specific inherited cancer predisposition syndromes.				

Abbreviations for different questionnaires: CWS = Cancer Worry Score, HADS = Hospital Anxiety and Depression Scale, HQ = Health Questionnarie, IES = Impact of Events Scale, PR = perceived risk, SF-36 = the 36-Item Short Form Health Survey, Spielberger = Spielberger State Anxiety Inventory B, SSQ = Screening Satisfaction Questionnaire.

Abbreviations for studies: LEAD = Li-Fraumeni Education and Early Detection, SIGNIFY = magnetic reSonance ImaGing screeNing In Li-Fraumeni sYndrome: An exploratory whole-body MRI study, SMOC+ = Multi-Organ Cancer prone syndromes.

Heritable *TP53*-related cancer syndrome in Sweden: Characterisation of genotype–phenotype correlation and surveillance

Figure 17. Overview and specific research aims of this thesis "Heritable TP53-related cancer syndrome in Sweden: Characterisation of genotype-phenotype correlation and surveillance. Paper I is a retrospective study of all known gTP53 carriers in Sweden while Papers III-IV are prospective and part of the national SWEP53 study. WB-MRI = whole-body MRI.

2. AIMS OF THE THESIS

2.1. OVERALL AIM

The overall aim of this thesis is to characterise the Swedish constitutional *TP53* (g*TP53*) cohort from a molecular, genetic and phenotypic perspective and to evaluate the clinical handling within a surveillance program including whole-body MRI (WB-MRI) (Figure 17).

2.2. SPECIFIC AIMS

- To characterise the Swedish g*TP53* cohort, especially by exploring genotype-phenotype correlations (**Paper I**).

- To implement (**Paper II**) and evaluate a surveillance programme for individuals with h*TP53*rc in Sweden:
    - In relation to other programmes globally (**Paper II**).
    - Characterising the rate and anatomical distribution of imaging findings (**Paper III**).
    - Outlining the associated further work-up needed due to surveillance with WB-MRI (**Paper III**).
    - Evaluate the feasibility and psychosocial impact of participation in surveillance with WB-MRI (**Paper IV**).

3. MATERIALS AND METHODS

3.1. OVERVIEW OF STUDY I-IV

The cohort in **Paper I** included all individuals with a verified pathological/likely pathological germline *TP53* variant, with methods summarised in Table 4. For **Papers II-IV**, different study designs were performed with study participants within the prospective SWEP53 study. All individuals in SWEP53 were included in the retrospective **Paper I**, with the addition of data on all g*TP53* carriers outside of SWEP53. Data collection for all studies and the clinical handling required nation-wide collaborations (Figure 18).

Table 4. Overview of Papers I-IV

	Study design	Cohort	Data collection	Data analysis
Paper I	Retrospective	All individuals in Sweden with a verified *TP53* variant class 4/5, all ages, from January – March 2022. 188 carriers in 91 families	November 2020 – March 2022	• Unpaired two-tailed t test • Mann-Whitney U test • ANOVA • Kruskal-Wallis test
Paper II	Prospective, descriptive	The enrolment of the first participants within SWEP53; 41 adults, 11 children	April 2019 – December 2019	Descriptive of current inclusion status and comparisons with other study protocols
Paper III	Prospective, observational cross-sectional	Sixty-one adults within SWEP53 eligible for surveillance with WB-MRI with a WB-MRI at baseline	April 2016 – May 2021	Descriptive statistics at one time point
Paper IV	Prospective, observational cohort study	Sixty adults within SWEP53 undergoing WB-MRI and with 1 year in the study	April 2016 – October 2021	Descriptive statistics with between group comparisons, linear regression
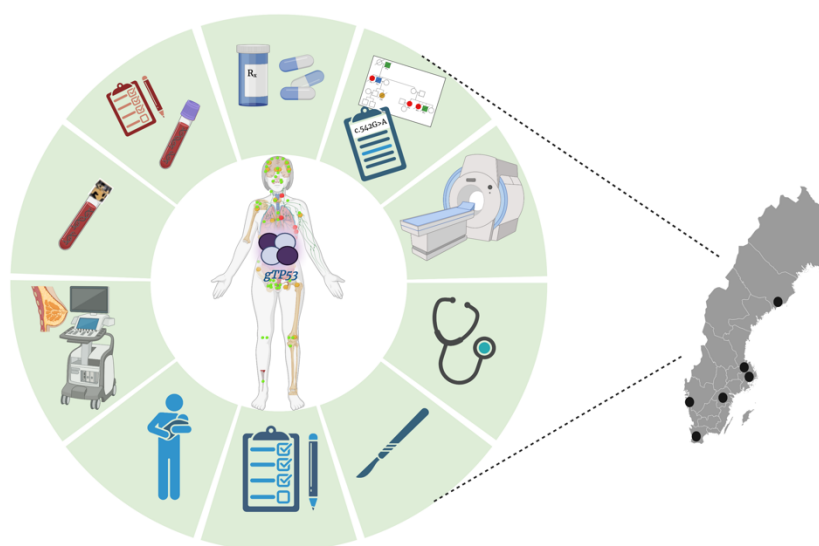
Figure 18. The clinical handling and the research on individuals with gTP53 variants in Sweden. h*TP53*rc is a rare cancer risk syndrome, and the general knowledge among health care professionals is therefore scarce. The clinical handling is challenging and is still under development. Individualisation and adaptation to the different families and their different needs requires tight collaborations between all study sites to ensure for the best possible handling including surveillance, biobanking, clinical check-ups, questionnaires, family planning, data collection to the registry, and treatment adaptations.

3.2. THE NATIONAL GERMLINE *TP53* COHORT AND SWEP53

3.2.1. The national g*TP53* cohort; genotype-phenotype associations

Paper I is a retrospective study where we gathered data on all known g*TP53* carriers with an ACMG class 4 or 5 germline variant in Sweden, regardless of age and ethnicity, since the testing started at each participating centre with the earliest data from January 2000 and up until March 2022. The information on the *TP53* variants and pedigrees were collected from the six departments of clinical genetics in Sweden (Umeå, Uppsala, Karolinska (Stockholm), Linköping, Sahlgrenska (Gothenburg) and Lund). Ethics approval number: 2020/02826 with the amendment 2021/06932-02. Data collection is ongoing to ensure an updated database on h*TP53*rc families in Sweden.

3.2.1.1. Data collection

Pedigrees that were used within the clinical investigation for genetic counselling at each study site were obtained for all individuals and served as a base for registering cancer diagnoses and family phenotypes including gender, tumour type, age of tumour onset, number of primary tumours and, when available, pathologic report of tumours. Data on variant testing included date of testing, variant type and original variant classification. All data were compiled in a registry within REDCap (version 11.1.15) at the coordinating site at Karolinska Institutet, Stockholm. All families were classified as either fulfilling the classical Li-Fraumeni syndrome (classical LFS) criteria (Li *et al.*, 1988) (Malkin *et al.*, 1990), the revised Chompret criteria (Bougeard *et al.*, 2015), HBC criteria according to Swedish national guidelines ((Rcc). 2022), or when genetic screening was performed outside any of the mentioned criteria, as "Other".

3.2.1.2. Variant classification and prediction

All variants classified before the *TP53* specific guidelines in 2021 (Fortuno *et al.*, 2021) were reclassified according to the new criteria by two researchers independently and compared to the previous classification in accordance with the ACMG/AMP criteria (Richards *et al.*, 2015). We also evaluated all missense variants regarding their phenotypic

impact on prediction for LFS rather than HBC using our previously published prediction model based on protein structural parameters previously (Liu *et al.*, 2021). By outlining the phenotypic pattern of tumours in the families with regards to the specific missense variant in this extended national cohort, the threshold for predisposition of LFS was recalibrated to 0.65 on a scale from 0 to 1. The threshold was originally reported as set to 0.5 in the previous publication by (Liu *et al.*, 2021).

3.2.2. The outline of the Swedish Constitutional *TP53* Study (SWEP53)

For Papers II-IV, the patients were recruited within the nation-wide prospective SWEP53 Study. All individuals with a clinically actionable germline *TP53* variant of ACMG class 4 or 5 were offered study participation, regardless of age. Individuals were identified as carriers of a disease-causing *TP53* variant by one of these three testing procedures: (1) through gene panel testing either within the clinical workup of participants with a suspected hereditary breast cancer, or through extended gene panel testing within a national research study aiming at identifying novel high risk genes for breast cancer, (2) targeted testing of *TP53* due to multiple primary cancers or family history fulfilling the classical LFS (Li *et al.*, 1988) or Chompret 2015 criteria (Bougeard *et al.*, 2015), or (3) through carrier testing of a healthy individual for a pathogenic *TP53* variant previously identified in the family.

Inclusion started with three pilot participants in 2016. Formal inclusion began at the main study site at the Karolinska University Hospital (Stockholm) in 2017. The other five sites, Umeå University Hospital, Uppsala University Hospital, Linköping University Hospital, Sahlgrenska University Hospital (Gothenburg), and at Skåne University Hospital (Lund), started inclusion at different later time points. The last site started inclusion by 2020. Recruitment was mainly performed through the departments of clinical genetics and the hereditary cancer units, while the clinical assessment was carried out at the oncological clinics for children and adults. Ethics approval number: 2015/1600-3 with amendments 2017/1527-32 and 2018/1690-32.

In Paper II, we describe the surveillance program within SWEP53. The publication compares the Swedish program with previously published ones from other countries. The SWEP53 comprises four different parts: (1) A study participant registry (mandatory for all participants, including questionnaire on general health and medications) and three optional parts; (2) A biobank including collection of genomic DNA from white blood cells, cell-free DNA (cfDNA) and tumour DNA where applicable, (3) A surveillance program, and (4) A psychosocial evaluation of the surveillance program. All known adult eligible carriers regardless of age are offered to take part of the surveillance program offering annual WB-MRI, MRI of the breasts and brain, as well as breast ultrasound, as previously described in Figure 15. A specifically tailored surveillance program is offered for individuals 0-18 years old with a 50% risk of being a g*TP53* carrier, or with a verified class 4 or 5 clinically actionable variant, including ultrasound of the abdomen and urine corticosteroid profiles. Individuals between 15 and 18 years who were confirmed carriers could choose to participate in the adult protocol. Clinically motivated further examinations are performed upon need and in accordance with clinical practice.

3.2.3. WB-MRI surveillance and associated work-up

The third study (**Paper III**) evaluates the baseline findings of the WB-MRIs for adult participants (≥18 years old). The individuals were enrolled at all six university hospitals In Sweden with a department of clinical genetics as previously mentioned, and information on their radiological reports was obtained. The rate, anatomical distribution of malignant,

indeterminate, and benign imaging findings, as well as the associated further workup generated by the baseline WB-MRI, were described. Those who had performed their baseline WB-MRI between April 1st 2016 and May 1st 2021 were included in this analysis.

3.2.4. Psychosocial impact of WB-MRI surveillance

Paper IV was a psychosocial evaluation of the SWEP53 surveillance program with WB-MRI among the adult cohort population. Questionnaires evaluating responses for the Cancer Worry Scale (CWS), benefit and barriers to participation, and the 36-Item Short Form Health Survey (SF-36) from the baseline visit and at the one-year follow up visit were analysed. Completed questionnaires were collected by local coordinators and sent to the national study coordinator (Meis Omran). Questionnaires at the one-year follow-up were completed prior to information of the results from the WB-MRI. The items at the first assessment corresponded to the participants expectations of the surveillance prior to their first WB-MRI, and their perception of participation after one year in the study. All questionnaires for baseline and the one-year visit, received from the different study sites between April 2016 to October 2021, were included for analysis in this paper. Comparisons were made between individuals *with* and *without* a previous cancer. We also obtained and presented sociodemographic details and personal history of cancer from a study specific questionnaire filled in at inclusion. Family history of cancer and death due to cancer amongst family members were collected from pedigrees. Personal history of cancer diagnoses was also confirmed in medical records. There were no restrictions regarding recency of previous cancer.

3.3. STATISTICAL ANALYSIS

In **Paper I**, continuous variables from two entities (two different groups) were evaluated by unpaired, two-tailed t test or Mann-Whitney U test, and continuous variables from three entities (*i.e.*, three types of families) were compared by using ANOVA analysis or Kruskal-Wallis test. Statistical significance was set at $p \leq 0.05$.

Paper II did not require statistical analysis as this was a description of the outline of surveillance within SWEP53, with comparisons of the Canadian 'Toronto protocol'.

In **Paper III**, all data was observational and presented as numbers and proportions.

In **Paper IV**, descriptive statistics were used to present sociodemographic and clinical data such as mean, range, counts and proportions. For the specific psychosocial measures, several different methods were used and processed in Stata version 17.

- For the questionnaires *Cancer Worry Scale*, *perceived benefits* and *barriers to participation*: differences in these ordinal items were tested by using the Mann-Whitney U test. These three questionnaires were presented as items.
- *SF-36*: Presented as eight aggregated subscales. For these subscales, linear regression was used to estimate and test group differences. Results are presented as mean differences together with 95% confidence intervals and Wald p values. Cronbach's coefficient alpha was calculated to determine internal consistency reliability for the different subscales in SF-36. The scale ranges from 0 to 1, and measures whether the responses are consistent between items. Generally, an $\alpha \geq 0.7$ is considered to be acceptable. Indirect standardisation using the age and the sex distribution in the study cohort together with normative data was used to calculate expected population mean scores for the subscales.

3.4. PSYCHOSOCIAL MEASURES

Within SWEP53, we have chosen to use the Cancer Worry Scale, Perceived benefits and barriers, and The 36-item Short Form Survey.

3.4.1.1. Cancer Worry Scale (CWS)

The Cancer Worry Scale (CWS) measures cancer-specific distress and is an eight-item questionnaire with a response format ranging from 1 to 4 or 5 ("Not at all" to "A lot" or to "Almost always"). The original six items assess own cancer worry, (Lammens, Bleiker, *et al.*, 2010) (Lerman *et al.*, 1991; Watson *et al.*, 1998) with the later addition of two items concerning worries about family members and worries regarding future cancer operations (Douma *et al.*, 2010). The summative score is between 8 and 35, a higher score indicates more cancer worry. A cut off score of ≥14 has been reported to correlate with clinical significant cancer worry (Custers *et al.*, 2014).

3.4.1.2. Perceived benefits and barriers

An important part of the perception of the surveillance with WB-MRI is to characterise the participant's view of perceived benefits and barriers to surveillance. For this purpose, we chose a questionnaire measuring perceived benefits (five statements) and barriers (six statements). This is an 11-item questionnaire based on previously performed work by other researchers.(Champion, 1984; Kash *et al.*, 1992; Madalinska *et al.*, 2007; Lammens, Bleiker, *et al.*, 2010) The response format consists of 5-point scales from "Does not agree at all" to "Agrees completely".

3.4.1.3. The 36-Item Short Form Health Survey (SF-36)

Health-related quality of life (HRQoL) was assessed by the 36-Item Short Form Health Survey (SF-36) version 1.0, (Ware and Sherbourne, 1992) a widely used questionnaire first validated in a Swedish cohort in 1995 (Sullivan *et al.*, 1995). SF-36 consists of eight subscales; physical functioning (PF, ten items), role limitations due to physical functioning (role physical, RP, four items), bodily pain (BP, two items), general health (GH, five items), vitality (VT, four items), social functioning (SF, two items), role limitations due to emotional functioning (role emotional, RE, three items) and mental health (MH, five items). RP, BP, VT, SF, RE, and MH all focus on the perceived health status during the last four weeks, ranging from no limitations at all in daily life to severe limitations. PF and GH translates into overall physical functioning (from "limited a lot in performing physical activities including bathing or dressing to "performs all types of physical activities including the most vigorous without limitations due to health") and general health perception (from "believes personal health is poor and likely to get worse" to "believes personal health is excellent"). Higher levels on each scale indicate higher HRQoL.

3.5. ETHICAL CONSIDERATIONS

3.5.1. Considerations in the retrospective analysis

Paper I included the retrospective collection of sensitive genetic information, but the data is presented anonymously, and at a group level, without any presentations of the actual pedigrees. By taking these measures, we have minimised the risk of identification despite h*TP53*rc being a rare syndrome. In the case of reclassification of a variant with altered surveillance or treatment implications, this would be reported back to the participating site as would have been done in the clinical routine.

3.5.2. Aspects of the SWEP53 Study

The studies within SWEP53 (**Paper II-IV**) include not only individual persons with h*TP53*rc, but in many cases their relatives whom are also carriers. This impose some special considerations with regards to the study designs, such as the limitations to being able to conduct a randomised clinical trial (RCT). An RCT typically presents the golden standard for the aggregation of relevant and reliable outcomes from clinical trials. However, in the case of rare heritable syndromes, it would be unethical to offer some family members inclusion in WB-MRI surveillance, while others would have to be in a control arm without surveillance despite of previously published data on the possible survival benefits of surveillance (Villani *et al.*, 2011; Villani *et al.*, 2016; Ballinger, Best*, et al.*, 2017).

In the case for SWEP53, the ethical permission from the Swedish Ethical Review Authority was granted in November 2015, and the study started inclusion on a large scale during 2017. At the time of outlining the study design for SWEP53, there was only one published study on surveillance with WB-MRI (Villani *et al.*, 2011), where survival comparisons were made with historical controls. Since then, both an 11-year follow-up of that cohort (Villani *et al.*, 2016) and a meta-analysis on baseline WB-MRI (Ballinger, Best*, et al.*, 2017) have been published.

4. RESULTS AND DISCUSSION

4.1. PAPER I - CHARACTERISATION OF HERITABLE *TP53*-RELATED CANCER SYNDROME IN SWEDEN – A RETROSPECTIVE NATIONWIDE STUDY OF GENOTYPE-PHENOTYPE CORRELATIONS IN 91 FAMILIES

4.1.1. Main findings

We identified and collected data on 91 families with h*TP53*rc since the testing for *TP53* variants started in Sweden, with the earliest data from January 2000 up until March 2022. After reclassification in accordance with the *TP53*-specific criteria (Fortuno *et al.*, 2021), five variants in eight families were down-graded to either class 2 or 3. The remaining 83 families harboured 42 different disease-causing *gTP53* variants (class 4 or 5). Within these families, 176 carriers were found of which 113 had a previous history of cancer (164 tumours) (Figure 19). Mean ages for tumour onset among missense variants compared to other variants were 36 vs 31.5 years, but this was not statistically significant (p=0.17).
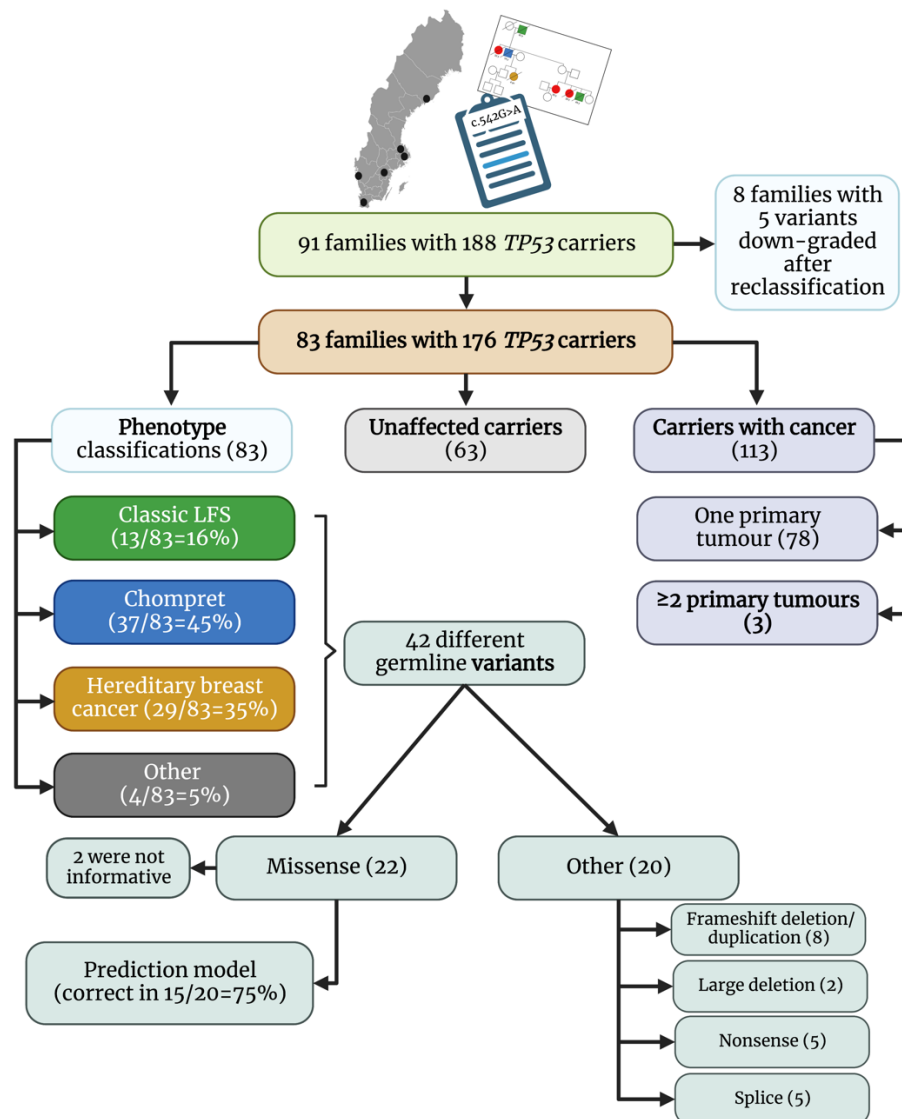

Figure 19. Results from the Swedish retrospective gTP53 characterisation (genotype-phenotype correlations) study. *The prediction model was used only on missense variants to predict the phenotype to be either LFS/Chompret or hereditary breast cancer.*

The most common malignancies were breast cancer (81 tumours in 71 patients), followed by CNS tumours (16 tumours in 16 patients), and soft tissue sarcomas (14 tumours in 13 patients). The age of first tumour onset differed between the three family phenotypes, with a lower age at first tumour onset for classic LFS of 28 years, 33 years for Chompret and 42 for HBC (Figure 20). Around one-third of patients (35/113) developed more than one primary tumour. The mean vs. median time to the second primary tumour onset was 9 and 6 years, respectively, in both the classic LFS (range 2-32 years) and Chompret families (range 0-25 years), while it was 10 and 9 years respectively among patients in the HBC families (range 0-25 years). Fifteen of the twenty (75%) informative missense variants were phenotypically predicted in agreement with our previously published prediction model (Liu *et al.*, 2021). Thus, when adequate information on the family history of cancers were available in pedigrees with missense variants (in 20/22 families), our prediction model correctly identified the familial phenotype (HBC or LFS/Chompret) in 75%.

4.1.1.1.   c.542G>A - a potential Swedish founder

The most frequently observed variant was the c.542G>A/p.R181H, identified in 22% of all families (18/83) families and in 62% (18/29) of families with HBC making it a potential Swedish founder variant, primarily associated with an HBC phenotype.
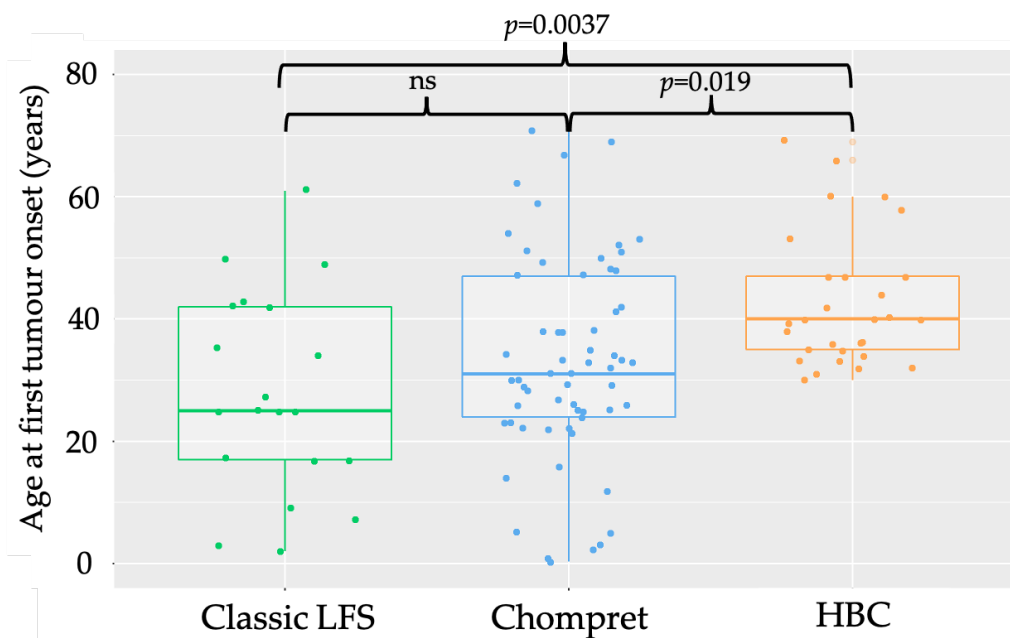
Figure 20. Comparison of the affected germline TP53 variant carriers from classic LFS, Chompret and HBC families in the age of first tumour onset. *The age of first tumour onset was significantly different among patients from these three family phenotypes (Kruskal-Wallis test, p=0.0037). The Mann-Whitney U test was used to evaluate the statistical difference between the two types of families. p≤0.05 is considered significant.*

4.1.2.   Discussion

The main aim of this study was to perform the first nation-wide characterisation of Swedish g*TP53* carriers, in order to outline the genotype-phenotype variation in families, thereby improving the genetic counselling and clinical handling of these individuals. Among the 113 affected carriers, 35 developed more than one primary tumour. The median time to the second tumour onset was six years, indicating that these patients need continuing follow-up. As this study was based on information from pedigrees and the report from genetic testing, it was not possible to asses if the second primary tumours occurred within previous irradiation fields, but this is a subject for upcoming studies to explore further. Of note, among the originally 47 different *TP53* variants that were

identified in the 91 families, 17 variants were reclassified using the *TP53*-specific criteria; five were upgraded (from class 4 to 5) and 12 were downgraded; seven variants from class 5 to 4 and the remaining five variants to non-actionable (*i.e.* to class 3 or 2). This is in agreement with several publications indicating that it is more common to downgrade variants than to upgrade them (Esterling *et al.*, 2020), indicating that is of importance to continuously repeat classification of variants and to the adjust clinical handling of the families.

The 18 families harbouring the c.542G>A variant represent the largest published phenotype outcome, and we therefore suggest that this might be a Swedish founder variant, possibly with a predominantly hereditary breast cancer phenotype. Importantly, this has to be explored further with complementary haplotype analysis. Within this study, we have created a national registry with data on Swedish g*TP53* carriers. As every study site provided the pedigrees based on their local files/registries, we might have missed some families or carriers depending on the accessibility at each clinic. In the future, as it becomes more and more common with whole genome sequencing, it is reasonable to think that we will gain more knowledge on different phenotypes as more genotypes emerge from the main stream testing. As h*TP53*rc is a rare syndrome, it would be especially important to extend collaborations internationally to have as much data as possible to evaluate a wider phenotypic presentation. Therefore, it would be of great value to repeat a similar but international study within the coming years, with the aim to being able to stratify clinical surveillance.

4.2. PAPER II - WHOLE-BODY MRI WITHIN A SURVEILLANCE PROGRAM FOR CARRIERS WITH CLINICALLY ACTIONABLE GERMLINE *TP53* VARIANTS - THE SWEDISH CONSTITUTIONAL *TP53* STUDY SWEP53

4.2.1. Main findings

In this paper, we were aiming to describe the Swedish surveillance program and to compare it with the pivotal "Toronto protocol" published in 2011 (Villani *et al.*, 2011). At the time (April 2016- December 2019), 41 adults and eleven children had been enrolled in SWEP53. All eligible adults informed about the study had accepted participation, and everyone but one participant chose to accept all four parts of SWEP53. Regarding the children, we did not require *TP53* carrier testing for enrolment. Five of the eleven children were confirmed carriers and they all participated in surveillance. The remaining six children had a parent being a verified g*TP53* carrier, and 3/6 underwent surveillance.

Comparisons with the pioneer 'Toronto protocol' (Villani *et al.*, 2011) were made (Table 5 and 6). In addition, the data collected for this paper constitute the base for a Swedish registry over g*TP53* carriers, the first of its kind in Sweden.

Table 5. Comparisons between the Toronto protocol and the SWEP53 protocol. From Paper II.

	Breast cancer	Brain tumor	Soft tissue and bone sarcoma	Leukaemia or lymphoma	Colon cancer	Melanoma
The Toronto protocol (2011) Adults	Monthly breast self-examination starting at age 18 years Clinical breast examination twice a year, starting at age 20–25 years, or 5–10 years before the earliest known breast cancer in the family Annual mammography and breast MRI screening starting at age 20–25 years, or at earliest age of onset in the family Consider risk-reducing bilateral mastectomy	Annual brain MRI	Annual rapid total body MRI Ultrasound of abdomen and pelvis every 6 months	Blood test every 4 months: complete blood count, erythrocyte sedimentation rate, lactate dehydrogenase	Colonoscopy every 2 years, beginning at age 40 years, or 10 years before the earliest known colon cancer in the family	Annual dermatological examination
The SWEP53 Protocol Adults	Monthly breast self-examination starting at age 18 years Clinical breast examination once a year, starting at age 18 Annual breast MRI screening starting at age 18 and breast ultrasounds 6 month after the MRI Consider risk-reducing bilateral mastectomy	Annual brain MRI	Whole-body MRI	Only if there are known cases in the family	Only if there are known cases in the family. 10 years before the earliest known colon cancer in the family	Annual dermatological examination

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Table 6. Study protocols for children within the Toronto protocol and the SWEP53 Study. From Paper II.

|	Adrenocortical carcinoma	Brain tumor	Soft tissue and bone sarcoma	Leukaemia or lymphoma
The Toronto protocol (2011) children	Ultrasound of abdomen and pelvis every 3–4 months Complete urinalysis every 3–4 months Blood tests every 4 months: β-human chorionic gonadotropin, alpha-fetoprotein, 17-OH-progesterone, testosterone, dehydroepiandrosterone sulfate, and rostenedione	Annual brain MRI	Annual rapid total body MRI	Blood test every 4 months: complete blood count, erythrocyte sedimentation rate, lactate dehydrogenase
The SWEP53 Protocol children	Ultrasound of abdomen and pelvis every 3–4 months Complete urinalysis every 3–4 months	None unless suspicion raised at the clinical check-up performed every 3 months	None unless suspicion raised at the clinical check-up performed every 3 months	None unless suspicion raised at the clinical check-up performed every 3 months

4.2.2. Discussion

The most striking difference between the Swedish protocol and the published international ones at the time, was the children's protocol. The children's protocol within SWEP53 was developed in communication with the Swedish Childhood Solid Tumour Working Group, with two major differences in relation to imaging protocols from other studies; 1) the children do not have to be verified g*TP53* carriers, the requirement is instead having a 50% risk, and 2) the Swedish protocol offers WB-MRI only to adolescents aged 15-18 years old while those below 15 years are instead offered surveillance with abdominal ultrasound and analysis of urine corticosteroids in addition to clinical check-up (Figure 15). The children's protocol requires contact with the health care every six weeks, as the imaging and clinical visits are performed every three months, and the urine collection in between those visits. For the adults, the SWEP53 is less comprehensive than the Toronto protocol as screening for leukaemia, lymphoma and colon cancer is only performed upon known cases in the family. The annual mammography within the Toronto protocol have been switched to breast ultrasound in SWEP53, to minimise the irradiation. Even so, it is difficult to know how to manage families with PV with reduced penetrance such as only adult-onset cancers, or mainly breast cancer predisposition. Female carriers in these families are usually offered annual breast surveillance with breast-MRI and ultrasound (avoiding ionising radiation from annual mammograms) and/or risk-reducing mastectomy. It is not known if they have an increased risk of other tumour types. Patients with h*TP53*rc are heterogenous and considerations should be done in the future with regards to their own individual cancer risk, depending on their genotype and also the family history. As an example, if there is a family history of colon cancer, regular colonoscopy should be considered. Earlier detection might not change the prognosis, but the rationale for clinical surveillance in persons with h*TP53*rc is supported by the need for early detection because of the high risk of developing secondary tumours due to irradiation and DNA-damaging chemotherapy. If we manage to find tumours at the earliest possible state, we need to find them when they are asymptomatic and small enough to only require surgery for complete treatment. This strategy minimises the need of DNA-damaging interventions (such as radiotherapy/ chemotherapy), thereby reducing the risk of treatment-induced secondary tumours.

The SWEP53 study is the first surveillance program in the northern countries, and with a nation-wide approach offering inclusion to all g*TP53* carriers regardless of phenotypic differences in the families. It remains for future studies to conclude if the Swedish approach with a more limited screening is beneficial, or if there would be a need to extend or adjust the protocol.

4.3. PAPER III - WHOLE-BODY MRI SURVEILLANCE-BASELINE FINDINGS IN THE SWEDISH MULTICENTRE HEREDITARY *TP53* -RELATED CANCER SYNDROME STUDY (SWEP53)

4.3.1. Main findings

In 61 participants undergoing a baseline WB-MRI, a total of 88 lesions were found. Out of these, 58 were benign and did not require additional work-up. Nineteen carriers, 31% (19/61), had 30 lesions requiring work-up, most commonly additional imaging and/or fine needle aspiration or biopsy. Out of these, 16 carriers (16/19=84%) had 21 lesions that were eventually diagnosed as benign. The remaining 3/19 (16%) had 9 malignant lesions (Figure 21). One individual had a new primary cancer, another had disseminated disease, and the third had a recurrent cancer. Notably, all three of them were asymptomatic at the time of inclusion. The most common additional work-up was radiological (24 new imaging procedures were performed after the baseline WB-MRI), followed by 15 other types of interventions such as fine needle aspirations (8), referrals (5) or surgical procedures/gastroscopy (2).
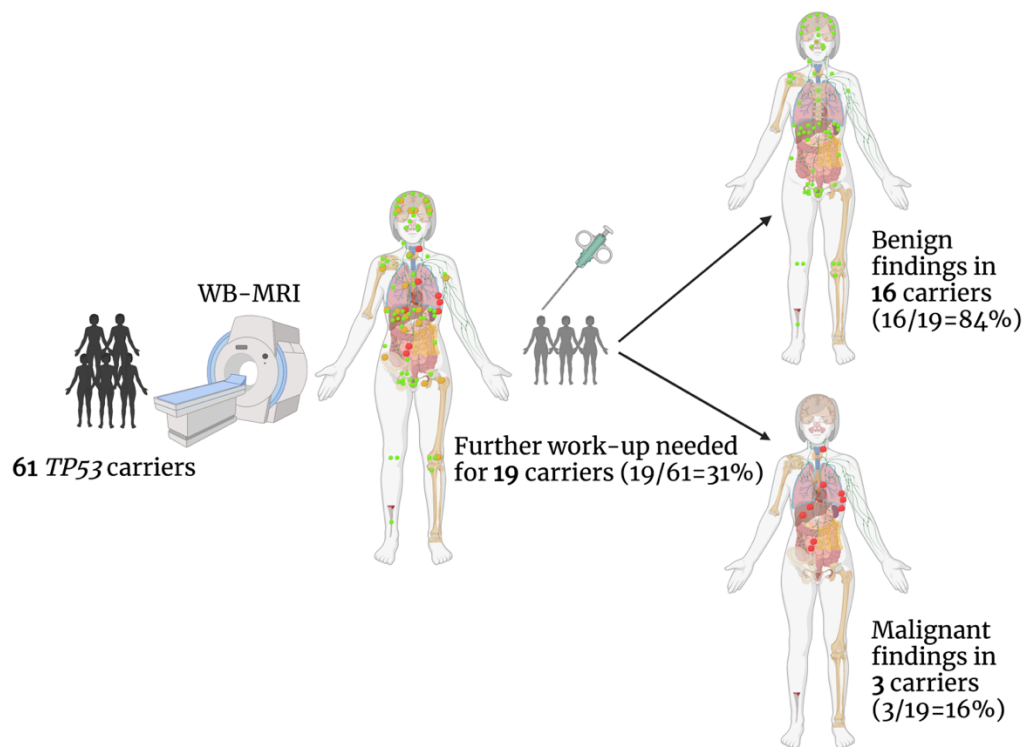
Figure 21. Results of the baseline WB-MRI within the SWEP53 Study (Paper III). *The location of the lesions detected are illustrated with coloured dots. Green lesions indicate benign findings, yellow corresponds to intermediate and red to malignant findings.*

4.3.2. Discussion

With this study, we evaluated WB-MRI as a surveillance strategy for healthy and asymptomatic g*TP53* carriers. After baseline WB-MRI imaging, additional work-up was required in one third of all study participants. This rate is in line with a previously published meta-analysis on 578 g*TP53* carriers reporting "false positives" on imaging to be 42.5%. This number however, included recurrences of pre-existing cancers and newly diagnosed metastatic cancers in addition to the benign findings, potentially explaining the higher frequency. An important lesson is the fundamental importance of having a continuous dialogue among the different health care providers in order to offer the best possible care to these carriers that have a high life-time cancer risk.

4.4. PAPER IV - WHOLE-BODY MRI SURVEILLANCE IN *TP53* CARRIERS IS PERCEIVED AS BENEFICIAL WITH NO INCREASE IN CANCER WORRY REGARDLESS OF PREVIOUS CANCER – DATA FROM THE SWEP53 STUDY

4.4.1. Main findings
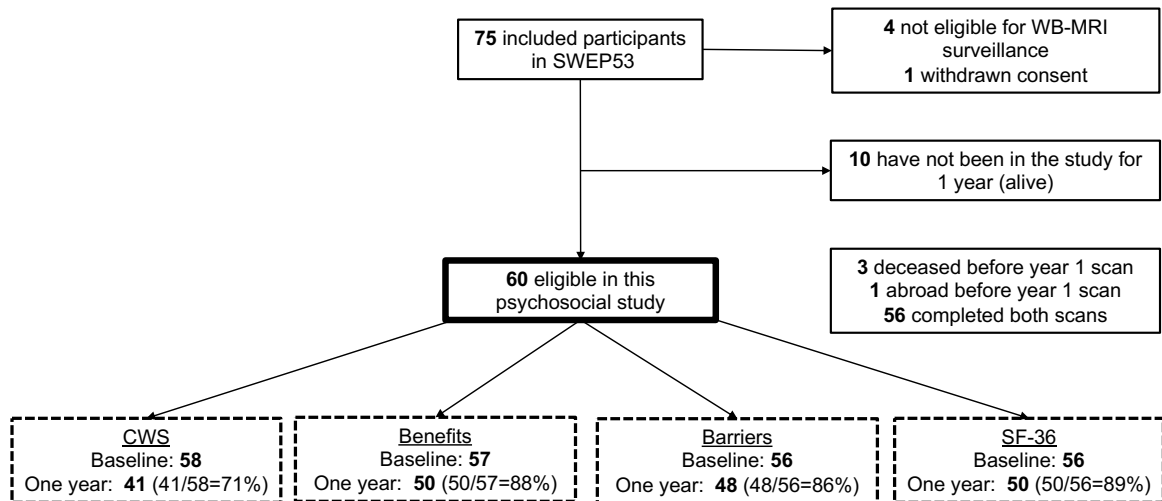
Figure 22. Flow-chart of the 60 eligible gTP53 carriers in the psychosocial study within SWEP53 (Paper IV). *All participants were evaluated with four different questionnaires at inclusion and after one year. The response rates for each questionnaire at baseline and at the one-year analysis. CWS = Cancer Worry Scale.*

As presented in Figure 22, a total of 60 eligible participants underwent the first year of surveillance within SWEP53 between April 2016 and October 2021. The response rate varied between 71-89% among the four different questionnaires. The lowest response rate of 71% was for CWS, but no systematic differences (sex, age, and previous cancer) were found between responders and non-responders at the two time points. At baseline, there were group differences within all items except for the question "How often do you worry about the chance of family members developing cancer?". After one year in surveillance, no statistically differences were found with regards of changes in response categories among those *with* previous cancer compared to the ones *without*, with the exception of performing daily tasks. In individuals *without* previous cancer, 18/19 (95%) reported no changes with regards to if the risk of getting cancer had affected their ability to perform daily tasks. 38% (8/21) in the group *with* previous cancer reported lower levels of cancer worry at one year. For SF-36, Cronbach's alpha indicated a good internal consistency among the eight subscales (α=0.74-0.93, median 0.87). When compared to a normative age- and gender-adjusted population, study participants scored clinically significantly lower on "vitality" and "emotional role functioning". In comparisons of the study participants with regards to previous cancer or not at baseline, the group with previous cancer scored significantly lower on three items (physical functioning, role limitations due to emotional functioning and general health). These between-group differences did not remain at the time of the one-year evaluation, with the exception of "social functioning" that emerged (Figure 23).
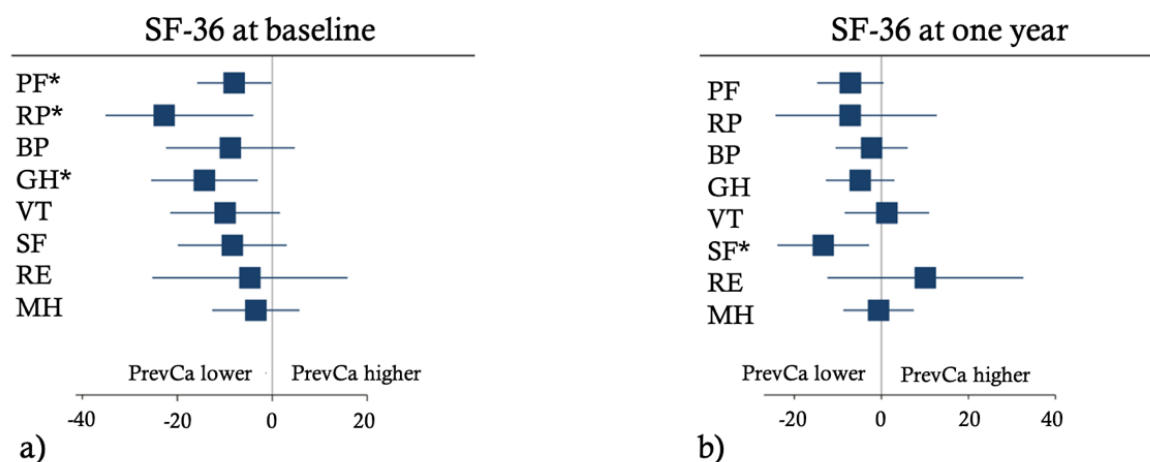
Figure 23. The 36-Item Short-Form Health Survey (SF-36), comparison of those with previous cancer (PrevCa) with individuals without previous cancer. Responses at a) baseline and b) at year 1, adjusted for baseline. Higher levels on each scale indicate higher HRQoL. Standard regression used. BP = bodily pain, GH = general health, MH = mental health, PF = physical functioning, RE = role limitations due to emotional functioning, RP = role limitations due to physical functioning, SF = social functioning, VT = vitality. At baseline, participants with previous cancer scored statistically significantly (*) lower on three subscales; PF, RP, and GH compared to individuals without previous cancer. At the one-year follow-up, between group differences emerged for SF with lower scores in the previous cancer group. No other differences were found for the other variables at that assessment point. Adapted from Figure 3 in Paper IV.

In summary, the study was perceived as beneficial and did not lead to increased cancer worry regardless of previous cancer history or not.

4.4.2. Discussion

One of the limitations with our study is the limited sample size of only 60 participants, reflecting on the rarity of this syndrome. However, to the best of our knowledge, this is the largest published quantitative psychosocial cohort to date. Most participants reported positive attitudes towards the surveillance, and few reported barriers to participation. Previous studies (Mcbride *et al.*, 2017; Ross *et al.*, 2017; Bancroft *et al.*, 2020; Rippinger *et al.*, 2020) have pointed towards mainly a benefit of participation, which could reflect a selection bias in that participants wanting to undergo surveillance choose inclusion, thereby reflecting a positive attitude from the beginning. It would be possible to present data as means, but we regarded it to be more informative to present the changes over time, as we wanted to evaluate the participation in the surveillance program. However, a strength of our study is the use of standardised questionnaires and the longitudinal study design, allowing for the comparisons of the participants expectations of surveillance at the study entry, with the perceived benefits, barriers, and cancer worry after one year of surveillance. For the future, evaluations of the psychological burden of repeated screening and the impact of interventions such as more imaging and/or invasive procedures need to be explored. Until then, professional teams with special expertise on h*TP53*rc are needed to provide for psychological support.

5. CONCLUSIONS

In **Paper I**, we report the first national genotype-phenotype characterisation of all individuals with h*TP53*rc in Sweden since testing for g*TP53* started. The g*TP53* missense variant c.542G>A/p.R181H was the most commonly occurring and could possibly be a new Swedish founder variant, mainly associated with a hereditary breast cancer predisposition. We also evaluated our previously published prediction model for missense g*TP53* variants, with correct phenotype prediction in 75% of the families.

In **Paper II**, we presented the first structured surveillance program for individuals with h*TP53*rc in a Scandinavian setting. The Swedish program differs from the pivotal "Villani protocol" by being less comprehensive and offering WB-MRI only for those ≥15/18 years old.

In **Paper III**, we conclude that WB-MRI can detect asymptomatic cancers in asymptomatic g*TP53* carriers, and that benign and indeterminate imaging findings could lead to the need of further (unnecessary) follow-up procedures such as complementary imaging and invasive biopsies. However, 16% of g*TP53* carriers that proceeded to further work-up were diagnosed with an asymptomatic malignant disease, which is considered to be a high proportion, and could therefore motivate the need of additional work-up. To ensure for an adequate secondary work-up, a multidisciplinary team and a clinical infrastructure is needed in each hospital for adequate management.

In **Paper IV**, we present a quantitative psychosocial evaluation of surveillance with WB-MRI among g*TP53* carriers, based on the largest published cohort to date. WB-MRI surveillance was viewed as beneficial by the participants regardless of if they had previously been diagnosed with cancer, and participation did not increase cancer worry in any of the two groups after one year of surveillance.

6. POINTS OF PERSPECTIVE

The work of this thesis explores different aspects from genotype-phenotype correlations to psychosocial issues and clinical handling of individuals and families with heritable *TP53*-related cancer (h*TP53*rc) syndrome, commonly referred to as the Li-Fraumeni syndrome.

One challenge in a cancer risk syndrome with such a wide phenotypic variation as the h*TP53*rc is the individualised stratification of surveillance modalities. With increased understanding of the underlying genotypic mechanisms behind different phenotypic presentations, a more family tailored surveillance could be designed and is in line with the personalised medicine dogma. Certain g*TP53* variants appear to be associated with specific risk profiles while others may be more dependent of additional genetic background profiles (*i.e.,* SNPs), and could vary between different ethnical groups. The introduction of a polygenic risk score based approach would thus improve the individualisation of surveillance strategies. To exemplify; carriers of the potential Swedish founder g*TP53* variant c.542G>A/p.R181H presented in **Paper I**, appear to have an association with mainly a breast cancer risk (at least in carries of Swedish ethnicity). They could potentially only be offered breast surveillance as they may not benefit from WB-MRI, thus possibly reducing the need of unnecessary follow-up interventions of unclear incidental findings as discussed in **Paper III**.

In a best-case scenario, tailored surveillance in accordance with known family phenotype and polygenic risk scores could be combined with the use of for example liquid biopsies, such as cfDNA, for improved early tumour detection. Image surveillance could then be more organ specific guided by cfDNA findings, instead of the whole-body protocol presented in **Paper II**. In **Paper IV**, we explored psychosocial aspects of being a g*TP53* carrier. It is important to offer psychosocial support by professional teams with special expertise on h*TP53*rc to meet the patients' and families' needs throughout life, especially at critical developmental events such as during adolescence and young adulthood. Thus, the future lays within personalised cancer prevention instead of "one size fits all".

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