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Solving the genetic aetiology of hereditary gastrointestinal tumour syndromes– a collaborative multicentre endeavour within the project Solve-RD

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ABSTRACT

Background: Patients and families with suspected, but genetically unexplained (unsolved) genetic tumour risk syndromes lack appropriate treatment and prevention, leading to preventable morbidity and mortality. To tackle this problem, patients from the European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS) are analysed in the European Commission's research project "Solving the unsolved rare diseases" (Solve-RD). The aim is to uncover known and novel cancer predisposing genes by reanalysing available whole-exome sequencing (WES) data of large cohorts in a combined manner, and applying a multidimensional omics approach.
Approach: Around 500 genetically unsolved cases with suspected hereditary gastrointestinal tumour syndromes (polyposis, early-onset/familial colorectal cancer and gastric cancer) from multiple European centres are aimed to be included. Currently, clinical and germline WES data from 294 cases have been analysed. In addition, an extensive molecular profiling of gastrointestinal tumours from these patients is planned and deep learning techniques will be applied. The ambitious, multidisciplinary project is accompanied by a number of methodical, technical, and logistic challenges, which require the development and implementation of new analysis tools, the standardisation of bioinformatics pipelines, and strategies to exchange data and knowledge.
Results: and Outlook. The first re-analysis of 229 known and proposed cancer predisposition genes allowed solving 2–3% of previously unsolved GENTURIS cases. The integration of expert knowledge and new technologies will help to identify the genetic basis of additional unsolved cases within the ongoing project. The ERN GENTURIS approach might serve as a model for other genomic initiatives.

1. Introduction

Approximately 5–8% of solid malignancies are caused by Mendelian ("monogenic") inherited predispositions (Huang et al., 2018; Lu et al., 2015). These Mendelian hereditary tumour syndromes or genetic tumour risk syndromes (TRS) are attributable to more than 100 cancer

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predisposing genes (CPGs) involved in cell cycle regulation, cellular proliferation, and DNA repair (Rahman, 2014, Cancer Gene Census). Pathogenic germline variants in these genes represent the underlying cause of clinically distinct high-penetrant TRS, including various hereditary gastrointestinal tumour syndromes. The spectrum of pathogenic alterations encompasses single nucleotide variants, small insertions or deletions (indels), and large deletions or duplications. It is estimated that 1–2% of the general population are carriers of a predisposing variant for a Mendelian TRS (Grzymski et al., 2020; Samadder et al., 2021), pointing to around 5–10 million high-risk individuals across the European Union.

The precise identification and delineation of a TRS is an important task of medical geneticists and other health care professionals since pathogenic variant carriers have a considerably increased lifetime risk for a syndrome-specific spectrum of early-onset malignancies and benign tumours. TRS follow a certain inheritance pattern (mostly autosomal dominant inheritance, some with autosomal recessive inheritance) and thus, are accompanied by an increased tumour risk in relatives. When a TRS is diagnosed in an affected patient, the prognosis of asymptomatic carriers in the family can be decisively improved by early detection of premalignant and malignant lesions, which can be achieved through several established risk-adapted surveillance programs (Grzymski et al., 2020; Samadder et al., 2021). Furthermore, prophylactic surgery and tailored drug therapies are applied (Boku et al., 2019; Goyal et al., 2016). These approaches represent very successful examples of personalised medicine and preventive oncology. However, a large fraction of families with TRS is not identified as such or with a considerable delay of many years, leading to inappropriate surveillance and treatment that may result in preventable morbidity and mortality.

The implementation of high-throughput sequencing/next generation sequencing (NGS) facilitates accurate and prompt diagnosis through the simultaneous variant screening of a set of relevant genes (gene panels or multi-gene analysis). Nonetheless and depending on the phenotype and the specificity of symptoms, a presumed underlying genetic germline alteration cannot be found in a considerable portion of patients meeting established clinical criteria for a TRS. This might be due to i) specific variants in the screened genes that cannot be found by methods used in routine diagnostics (e. g. somatic mosaicism, variants in the promoter or intronic regions of the gene), ii) variants in genes unknown to be associated with a hereditary tumour syndrome, iii) a more complex genetic background, or iv) non-genetic risk factors. In these unsolved families, a clear diagnosis, specific surveillance, and treatment recommendations, as well as predictive testing of at-risk relatives cannot be applied.

Unsolved cases of presumed TRS are the subject of various local research projects across Europe, which use high-throughput sequencing to further elucidate genetic risk factors. Indeed, whole exome and genome sequencing (WES/WGS) data analysis has led to the discovery of new CPGs in the past years, and hereby increasing the diagnostic yield (Adam et al., 2016; Palles et al., 2013; Weren et al., 2018). However, so far only few novel Mendelian subtypes could be identified in a small fraction of probands indicating that uncovering a potential genetic aetiology in unexplained rare conditions remains a complex and challenging endeavour.

To address this medical problem, the research project "*Solving the unsolved rare diseases*" (Solve-RD; no. 779257), operating under the European Commission's Horizon 2020 framework, aims to uncover genetic predisposing factors in patients with yet unexplained but suspected hereditary conditions. To achieve this goal, the reanalysis of already existing WES data and the application of innovative "omics" approaches (genomics, transcriptomics, and epigenomics) are being performed in large combined patient cohorts by a multidisciplinary team of experts in the field. Within this ambitious initiative, four different European Reference Networks (ERNs) are collaborating on different rare or complex diseases. One of those is the ERN on *Genetic Tumour Risk Syndromes* (ERN GENTURIS) which provides a large patient cohort with suspected gastrointestinal TRS (www.genturis.eu) (Vos et al., 2019).

The development and establishment of this multidisciplinary network offers enormous opportunities for collaborations with synergistic effects, technical improvements, and fruitful scientific exchange resulting in successful and exciting research results. This article describes the collaborative actions of the ERN GENTURIS hereditary gastrointestinal tumour syndromes team within the Solve-RD project and preliminary results.

2. Hereditary gastrointestinal cancer

2.1. Hereditary colorectal cancer/polyposis syndromes

Around 5–10% of colorectal cancer (CRC) can be linked to a Mendelian cause (hereditary CRC) (Jasperson et al., 2010; Yurgelun et al., 2017). However, up to 30% of all CRCs present with an unexplained familial clustering (familial CRC) or very early onset. In fact, it has been estimated that constitutional genetic factors contribute to 15–40% of the aetiology of CRC (Graff et al., 2017; Lichtenstein et al., 2009). Hereditary CRC represents a group of different syndromes that can be broadly divided into Lynch syndrome, previously called hereditary non-polyposis colorectal cancer (HNPCC), and the gastrointestinal polyposis syndromes (Table S1), a detailed description and diagnostic criteria can be found elsewhere (Valle, 2017; Valle et al., 2019). However, the clinical presentation can be highly variable and overlapping. Only up to one-quarter of familial and early-onset CRCs can be attributed to known Mendelian syndromes, suggesting that a substantial portion of its heritability remains unexplained (Chubb et al., 2015). The inclusion criteria of probands with unexplained disease are summarised in Table S2.

The Mendelian syndromes are characterised by a high lifetime risk to develop gastrointestinal tumours, in particular CRC, and a varying risk for extraintestinal tumours and other symptoms (Table S1). Depending on the histologic type of polyps, several polyposis forms can be delineated, which differ in terms of age at onset, polyp number, and distribution (Valle, 2017). Lynch syndrome is caused by pathogenic germline variants in mismatch repair (MMR) genes, whereas polyposis syndromes are caused by variants in tumour suppressor genes and DNA repair genes, the latter are true especially to the novel forms (Table S1).

2.2. Hereditary gastric cancer

Likewise, less than 3% of gastric cancer (GC) can be attributed to a clear hereditary form, while familial clustering is observed in approximately 10% of the cases (van der Post et al., 2019). Two Mendelian GC-associated syndromes with a clear genetic cause have been established: the Hereditary Diffuse Gastric Cancer (HDGC) syndrome, caused by pathogenic germline variants in the *CDH1* or *CTNNA1* genes (Blair et al., 2020; Guilford et al., 1998); and the very rare Gastric Adenocarcinoma and Proximal Polyposis of the Stomach (GAPPS) syndrome, caused by specific pathogenic variants at the promoter 1B of the *APC* gene (Worthley et al., 2012) (Table S1). In addition, GC is part of the tumour spectrum of other Mendelian gastrointestinal TRS, in particular Lynch syndrome and juvenile polyposis. Familial intestinal gastric cancer (FIGC) is also perceived as a genetically determined GC-associated disease; however, a formal proof of genetic predisposition is yet to be identified (Carvalho et al., 2021).

2.3. Shared features

The likelihood to detect a pathogenic, disease-causing germline variant in one of the established genes is closely related to the phenotype and family history of the patient. Depending on the clinical criteria applied, in up to 50–70% of families with a suspected gastrointestinal tumour syndrome, no underlying genetic cause can be identified, although early-onset disease, familial clustering, or the syn- or

metachronous occurrence of dozens to hundreds of polyps strongly argues for an underlying genetic basis (Table S2). In particular, serrated polyposis seems to be a very heterogeneous condition, and only a small number of cases could be attributed to a Mendelian cause so far. In the past years, several candidate genes for hereditary CRC and unsolved, but clinically and pathologically confirmed HDGC families have been proposed by WES and candidate gene studies. However, most of these candidates explain only a small subset of cases or are not validated as high-penetrant causative genes, so their real contribution remains formally unproven (Chubb et al., 2015; Garcia-Pelaez et al., 2021; te Paske et al., 2021; Terradas et al., 2019; Valle et al., 2019).

3. ERN GENTURIS

The ERN GENTURIS is dedicated to all patients and their families with a suspected or confirmed genetic tumour risk syndrome (Vos et al., 2019). Due to the underlying hereditary basis, these individuals and their affected relatives need a specific, specialised, and multidisciplinary type of medical care. In particular, proper identification of relatives at risk as well as the prevention and early detection of tumours can prevent morbidity and mortality. ERN GENTURIS aims to improve health care standards for TRS across Europe and is heavily involved in research projects in this area. "Lynch syndrome and polyposis" represents one out of four ERN GENTURIS thematic groups, in which an increasing number of different gastrointestinal TRS are included. HDGC is integrated in the thematic group "Other rare – predominantly malignant TRS" given its rarity.

Altogether, 31 European expert centres (health care providers, HCP) from 19 different countries integrate the ERN GENTURIS (Fig. 1). Every year, around 35,000 patients with suspected TRS are seen and followed at ERN HCPs, including more than 7000 patients with familial/hereditary CRC/polyposis and a few thousands with familial/hereditary GC. Although many hundreds of patients with suspected TRS, which could not be explained by routine diagnostics of the most likely CPGs, have been exome sequenced, no highpenetrant genetic predisposition has been found so far in the vast majority (Schubert et al., 2020). These patients are willing to participate in research projects that aim at finding the genetic cause of their disease, and preventing cancer and death among yet unaffected relatives.

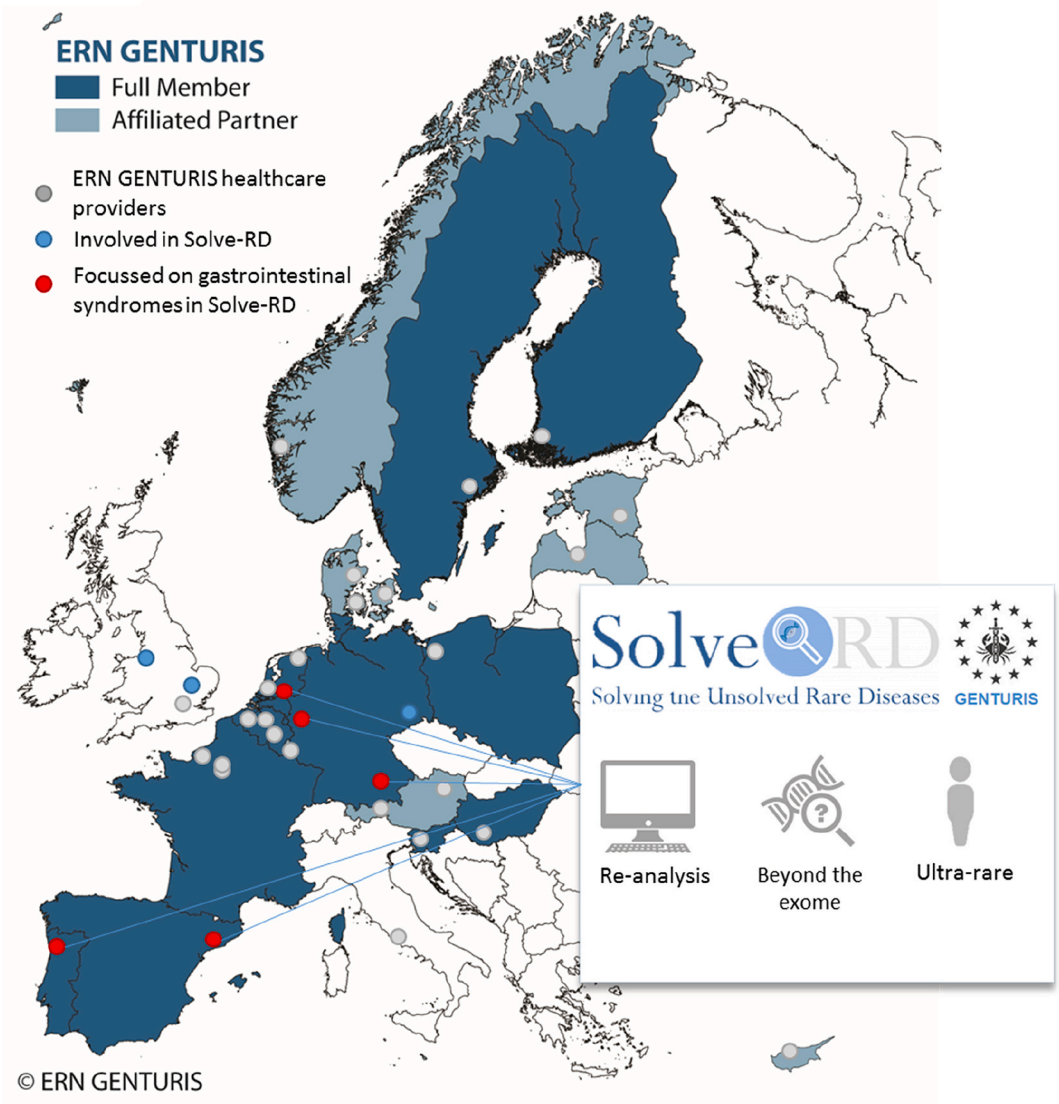

Fig. 1. Involved countries and health care providers (HCP) of ERN GENTURIS. Modified map (personal communication ERN GENTURIS coordinating team). Full member countries are shaded in dark blue, affiliated partners in light blue. Expert centres for gastrointestinal tumour syndromes involved in Solve-RD are marked in red, further contributing centres are marked in blue. Additional GENTURIS HCP are grey-coloured. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Solve-RD

The population prevalence of rare diseases is estimated to be 3.5–6% (Nguengang Wakap et al., 2020). The corner stone of the Solve-RD project (*www.solve-rd.eu*) are patients with a genetically undiagnosed rare disease. Although individually scarce, together these conditions represent a relevant group of patients across Europe, both from a medical point of view and concerning national health care systems. Solve-RD aims to identify a substantial number of novel predisposing genes/mechanisms for unsolved rare diseases and thereby, improve biological knowledge on these diseases, as well as associated diagnostics and medical care.

The basic first step approach of Solve-RD is the WES reanalysis of more than 19,000 patients with rare diseases. This core project represents the synergy between four different ERNs: ERN-RND, focussing on rare movement and cognitive disorders; EURO-NMD on neuromuscular disorders; ITHACA on rare congenital malformation syndromes and intellectual disability; and ERN GENTURIS on genetic tumour risk syndromes. The inter- and intra-ERN active collaborations and the access to state-of-the-art technologies have facilitated the creation of a consortium formed of leading clinicians, geneticists, biologists, bioinformaticians, and translational researchers, who share their knowledge about phenotypes, omics technologies, and data analyses (Zurek et al., 2021).

Solve-RD includes five work packages (WP) (Suppl. Fig. S1): WP1 - Universal accessibility for all researchers of the phenotypic and genetic data of each patient through a central database; WP2 - Improved data analysis algorithms and use of improved molecular and omics approaches to help unravelling the unsolved cases; and WP3 - translation of each successful discovery into clinical practice. WPs 1–3 are supported by WP4 connecting bioinformatics and knowledge management. The results of WPs 1–4 are coordinated by WP5 for the communication and integration of new findings to other (rare-disease) projects and associated ERNs.

Each collaborating ERN research group individually has limited resources and numbers of patients. Within Solve-RD, many small cohorts that have been studied separately in the past, become a unique large cohort. This pooled data approach, the application of standardised analyses and interpretation pipelines increases the analytical power and is expected to result in significant progress. Recent high-impact publications emphasise the major advantage of international collaborations for the diagnosis of rare disease patients (Ehrlich, 2019; Grolleman et al., 2019). For neighbouring or geographically close sites (like Nijmegen and Bonn, which are only 175 km apart but are separated by a border), merging of cohorts might be of particular interest, as patients may have close common ancestors resulting in shared genetic causes.

For each shared case, data are submitted to the RD-Connect *Genome-Phenome Analysis Platform* (GPAP), an integrative platform to connect phenotypic information, genomic data, and researchers (Lochmüller et al., 2018). Phenotypic details on index cases and family members are documented in GPAP-Phenostore. Genome-phenome analysis is facilitated by the RD-Connect GPAP as described elsewhere (Matalonga et al., 2021).

Each analysis in Solve-RD is planned and performed by a Data Analysis Task Force (DATF) and an ERN specific Data Interpretation Task Force (DITF) (Zurek et al., 2021) (Fig. 2). Therefore, experienced members from the DATF and DITF form working groups to deliberate on the best strategy and tools for the respective data analysis, e.g. concerning single nucleotide variants (SNVs) and copy number variants (CNVs). The final strategy is documented in project templates and submitted to the Solve-RD advisory board to monitor and coordinate the individual analyses of the working groups. The close collaboration and bidirectional interaction of DATF and DITF improves the implementation of novel innovative techniques and multi-disciplinary data

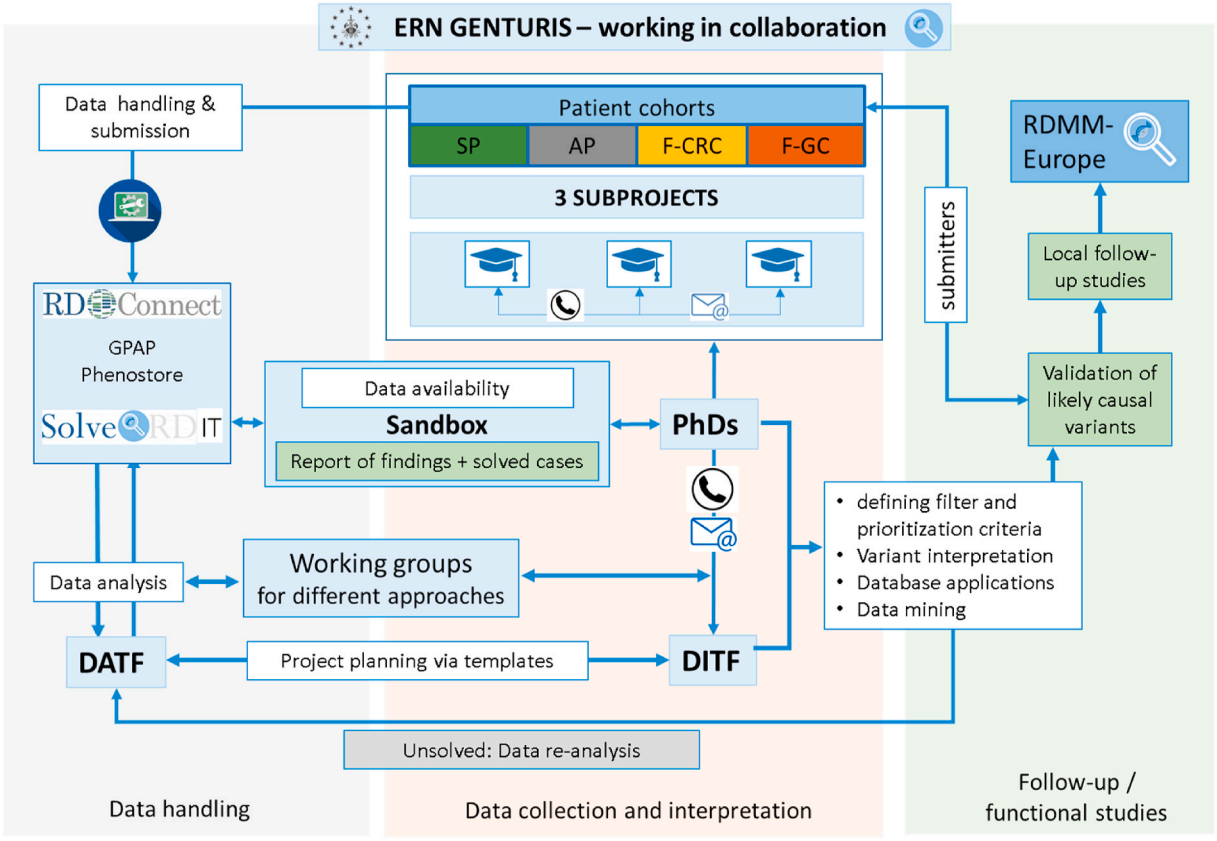

Fig. 2. The ERN GENTURIS collaborative workflow within Solve-RD. DATF: data analysis task force; DITF: data interpretation task force.

interpretation by clinical and molecular experts of the disease field.

5. The ERN GENTURIS approach

Within the Solve-RD project, three leading and three contributing expert centres of the ERN GENTURIS provide a cohort of around 500 patients with different types of unexplained, but suspected gastrointestinal TRS, where routine genetic germline diagnostics and a local cohort analysis of WES data could not identify a convincing cause of the disease. Specifically, the phenotypic spectrum encompasses adenomatous polyposis (AP), serrated polyposis (SP), early-onset/familial CRC (F-CRC), and early-onset/familial gastric cancer (F-GC) (Fig. 3); the inclusion criteria are summarised in Tab S2.

All patients that were included in this project provided informed consent for the use of their anonymised data and material for research on the genetic cause of their gastrointestinal TRS. Based on national laws and regulations, the specific informed consent varies among centres.

Three different areas using partly overlapping patient cohorts are defined within ERN GENTURIS to uncover predisposing genetic causes: (1) The "Unsolved cases" represent the whole group of unexplained ERN GENTURIS patients, where the reanalysis of the existing and combined clinical and WES data in a structured manner is expected to solve the underlying genetic cause or to identify promising candidate genes. (2) The ERN GENTURIS-specific approaches aim to perform an extensive molecular profiling of gastrointestinal tumours (polyps, cancers) by applying various omics and deep learning techniques to improve characterisation and subclassification of lesions, thereby reducing the heterogeneity within histological subgroups. Molecular subtypes characterised this way might in turn point to specific germline predispositions. (3) Within the area "Ultra-rare conditions", the most exceptional phenotypes within the ERN GENTURIS network are gathered for an extensive omics application including germline WGS (Fig. 4).

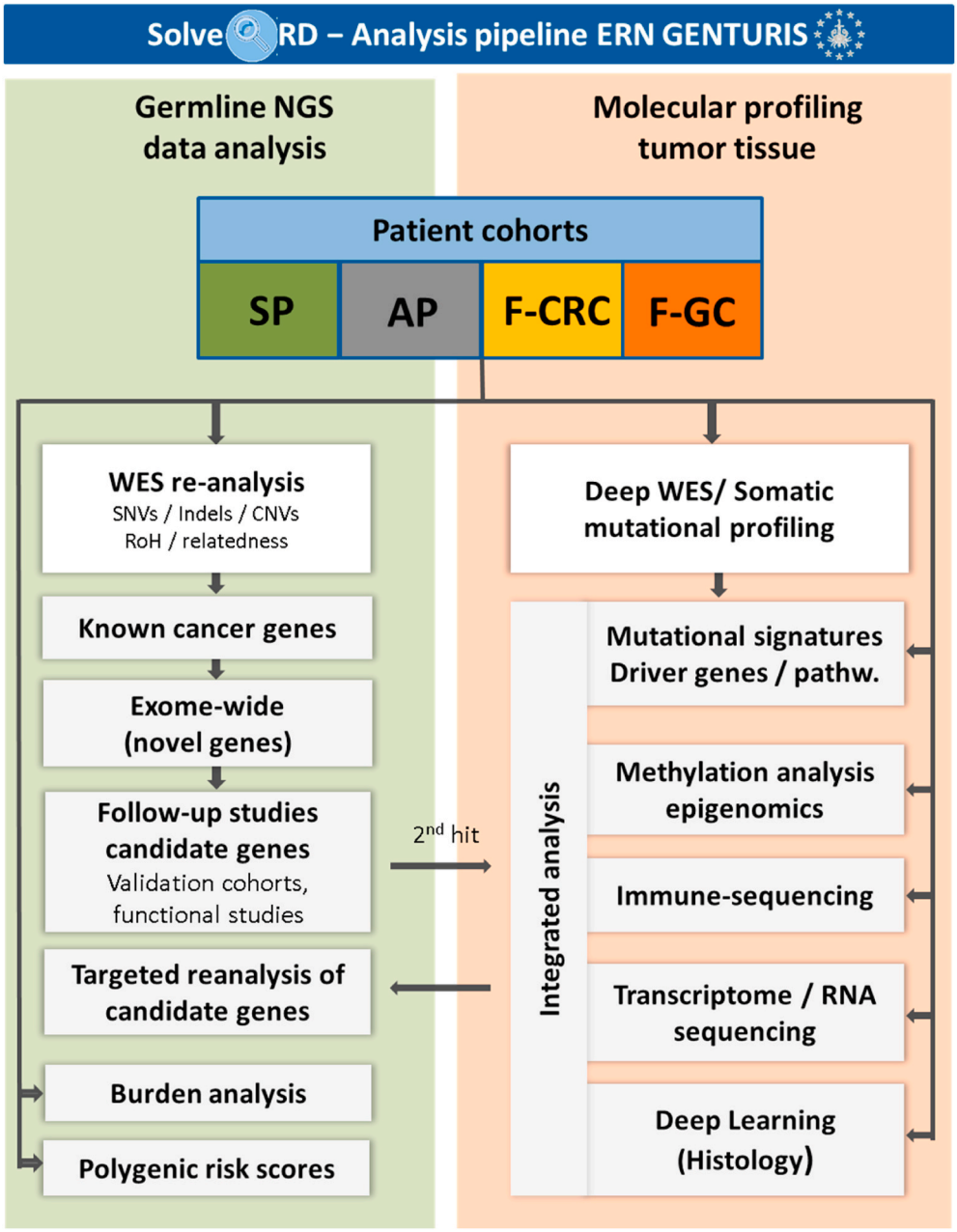

Fig. 3. Main contributors of ERN GENTURIS in Solve-RD and the related patient cohorts. The centres provide patients with different phenotypes. Two centres each share one PhD student in charge of a specific phenotypic group. CRC = colorectal cancer.

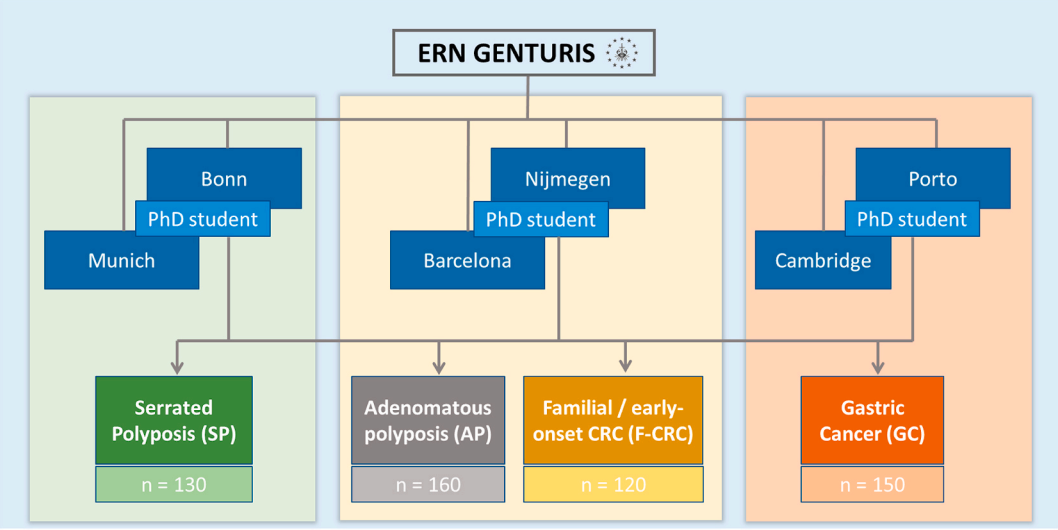

Fig. 4. The ERN GENTURIS analysis pipelines for germline and tumour analyses in Solve-RD. AP: adenomatous polyposis, SP: serrated polyposis, F-CRC: early-onset/familial colorectal cancer, F-GC: early-onset/familial gastric cancer. WES = whole exome sequencing; SNV = single nucleotide variant; CNV = copy number variant; Indels = insertion/deletion variants; RoH = runs of homozygosity.

Each area includes various subprojects with specific tasks and analysis strategies.

6. Collaboration works

This ambitious and multidisciplinary project is accompanied by a number of methodological, technical, and logistic challenges, which require the development and implementation of new analysis tools, the standardisation of bioinformatics pipelines (Matalonga et al., 2020), and strategies to exchange data and knowledge. Examples of those tools are the remapping analysis, but also the large-scale CNV analyses on WES/WGS data, as this is not routinely performed in most diagnostic and research labs.

The first approach is the reanalysis of WES data from 294 ERN GENTURIS patients aiming at the identification of pathogenic germline variants in a comprehensive panel of 229 established and proposed CPGs (te Paske et al., 2021). According to the intermediate results of this targeted approach, potential causative, phenotype-explaining variants were identified in six out of 294 cases of our initial cohort (2%) (Table 1, Suppl. Fig. S2), (Matalonga et al., 2021; te Paske et al., 2021). In another 7% of cases, potentially causative variants are under revision. Reasons for missing these variants during routine diagnostics are deviant phenotypic descriptions or insufficient clinical information, previous incomplete genetic diagnostics in the pre-panel era, or somatic mosaic cases escaping usual detection thresholds (te Paske et al., 2021). Furthermore, for some TRS the phenotypic spectrum seems to be broader than previously thought.

The preliminary findings already demonstrate that the reanalysis of existing data alone improves the diagnostic yield. Nonetheless, for some of the variants, a further laborious work-up including segregation analyses, transcript analyses, data mining, and functional studies is necessary to prove their causal relationship with the phenotype. Even by a comprehensive work-up, an appreciable number of rare variants cannot be classified as benign or pathogenic. Those Variants of Uncertain clinical Significance (VUS) are well known in the routine diagnostic setting. In this research project, the return of VUS to the attending physicians in charge is not intended routinely, but happens on an individual basis in certain cases with informed consent for this procedure where the affected gene is the very likely reason for the disease of the patient.

Currently, the analyses are being expanded genome-wide aiming at identifying novel candidate genes. To this end, the centres involved in the analysis are developing joint variant filtering approaches and refining current local pipelines. The Solve-RD cohorts from the other

Table 1
Solved cases in the Solve-RD GENTURIS cohort after variant analysis in a predefined set of 229 tumour-associated genes. AP, adenomatous polyposis; DGC, diffuse gastric cancer; FAP, familial adenomatous polyposis; GC, gastric cancer; hCRC, suspected hereditary colorectal cancer; JPS, juvenile polyposis syndrome; LS, Lynch syndrome; MAP, MUTYH-associated adenomatous polyposis.

Gene	Variant	Type of causative germline variant	Genotype	Phenotype of patient	Family history	Genetic diagnosis	References
APC/ SRP19	5:112175002–112200466del (GRCh37)[a]	Large deletion	Heterozygous	Polyposis	Affected brother	FAP	
MSH2	c.2314del; p.(Thr772Glnfs*40)	Frameshift	Heterozygous	hCRC	Unknown	LS	
MUTYH	c.1147delC; p.Ala385Profs*23	Frameshift	Homozygous	Polyposis	Unknown	MAP	
MUTYH	c.1437_1439delGGA; p.(Glu480del)	In-frame deletion	Homozygous	Polyposis	High occurrence of different cancer types in family	MAP	Matalonga et al. (2021)
PIK3CA	c.3140A > G; p.(His1047Arg)	Missense	Mosaic	GC	No family history	DGC	Matalonga et al. (2021), te Paske et al. (2021)
SMAD4	c.1231_1232delAG; p.(Ser411Leufs*17)	Frameshift	Heterozygous	Polyposis	Unsuspicious	JPS	Matalonga et al., (2021)

[a] This variant was identified in a copy number variant (CNV) analysis using whole exome sequencing data. The deletion could be confirmed by Multiplex Ligation Dependent Probe Amplification (MLPA) of the *APC* gene (*APC*:c.(3183_3928)_(8435_?)del).

ERNs with non-tumour phenotypes, which serves as a large control group concerning genetic artefacts and benign variants, as well as the multidisciplinary expertise, are major achievements to improve the identification of novel susceptibility genes. This step is considered as a discovery phase and currently, efforts are being made to recruit additional cohorts to validate future findings. However, the obvious genetic and clinical heterogeneity and the overlapping phenotypes of the entities are drawbacks to define the most efficient strategy: while large groups may increase the likelihood to identify recurrently mutated novel genes on the one hand, the mixture of varying phenotypes can result in dilution effects that may prevent the uncovering of promising genes on the other hand. For very rare Mendelian conditions, even the present cohorts might be too small to identify causative genes.

The ERN specific project, which has just begun, deals with the extensive analysis of tumour specimens derived from unexplained cases. It includes histologic reclassification, a comprehensive molecular profiling using WES and genome-wide methylation data, and deep learning techniques. This will add additional data layers, further increasing the complexity of data analyses and integration, and offer a unique opportunity to find novel biological relevant mechanisms and causes of TRS. As a prerequisite, the time-consuming collection of stored tumour tissue – usually Formalin-Fixed Paraffin-Embedded (FFPE) samples – and decisions on service providers for tumour WES are almost finished. While being a very promising approach, however, the complexity of this endeavour already led to some time delay of the respective subprojects compared to the intended work schedule.

7. Discussion and outlook

Through its integrated research program, Solve-RD aims at significantly increasing the diagnostic yield by reanalysing large cohorts, an improved variant calling workflow, and conceptionally developing strategies using novel molecular approaches. Reanalysis of WES/WGS data using state-of-the-art approaches is expected to solve 3–5% of all unexplained RD cases (Lelieveld et al., 2016). Similar results are obtained when broad gene panels are applied to cohorts of patients with suspected TRS in whom the genes most likely involved had been analysed beforehand during routine diagnostics (Henn et al., 2019). The preliminary frequency of solved cases in our cohort is in line with these findings, and is expected to increase, after the WES data have been screened for the whole spectrum of variants including large deletions and duplications (CNV analysis). Further, the reanalysis of additional TRS cases recently submitted to Solve-RD, is expected to improve the pick-up rate of novel TRS predisposing factors by the identification of more recurrently affected genes.

The evaluation of true and meaningful causative variants by functional analysis always remains a critical step to reduce false positives and increasing the clinical utility of the findings, thus, allowing rapid translation into clinical practice. To address this aspect, the *Rare Diseases Models & Mechanisms Europe* (RDMM-Europe) was established to evaluate the causal relevance of interesting candidate genes by functional analyses. This brokerage network shall stimulate collaborations between model organism investigators and clinical researchers for rare disease patients to enable more extensive follow-up studies. The RDMM brokerage platform was implemented to deal with this critical bottleneck step, creating the opportunity to increase cross-border collaborations between groups involved in variant interpretation and classification.

Depending on the phenotype and the analysis strategy, projects looking beyond germline WES data are expected to further solve a certain fraction of unexplained cases. In patient cohorts with early-onset severe disease such as intellectual disability or mitochondrial/muscular disorders, where a trio approach is often applied, the diagnostic yield highly increases by the implementation of novel diagnostic methods; 10% by moving from WES to WGS (Gilissen et al., 2014); 10–20% by adding transcriptomics (Cummings et al., 2017; Kremer et al., 2017) and at least 10% by other omics technologies and long read WGS (Ebert et al., 2021; Mantere et al., 2019).

Many of the unexplained TRS of the gastrointestinal tract are characterised by a rather late-onset manifestation, an invisible phenotype from the outside, which often is not recognised in relatives without endoscopic investigation, and a strong clinical variability so that a trio approach cannot be used. However, in contrast to the other ERNs, ERN GENTURIS deals with two kinds of phenotypic and genetic data: those from the patients (clinical data, germline genetics) and their tumours (molecular phenotypes, somatic variants). The integrated analysis of these data layers represents a unique possibility to improve biological insights and to uncover an underlying genetic predisposition. The characterisation of molecular and histological subtypes by multi-omics tumour analyses offers the possibility to reduce the assumed underlying constitutional genetic heterogeneity and thus, increases the likelihood to identify causative genes in more homogeneous subgroups of patients.

Notwithstanding, even by applying various omics approaches, the majority of patients will remain unexplained, mainly due to reasons mentioned above. Rare high-penetrant variants in non-coding functional regions of the genome and a multifactorial or polygenic aetiology based on moderate and low-penetrant variants can only be captured in larger patient cohorts and by using further methods, which may include WGS, association studies, enrichment/burden analyses, and polygenic scores. This long-lasting research will likely be conducted in various follow-up projects, but Solve-RD constitutes the personal, technical, and logistic infrastructure to further increase the cohorts, and to develop those large scale follow-up studies addressing complex questions which cannot be solved at the present moment.

Uncovering the aetiology of rare, presumed hereditary conditions can only be successful with the involvement of international, multidisciplinary teams. Moreover, the integration of complex data needs to be supported by an appropriate IT and research infrastructure (Fig. 2). In the past years, many collaborative structures have emerged, illustrating the great influence and power of joint work. For rare TRS, the Solve-RD collaboration is expected to represent a significant contribution although the project is faced with several challenges. The ERN GENTURIS approach might serve as a model for other genomic initiatives. The integration of expert knowledge and new technologies is expected to considerably improve the clarification of cases with yet genetically unsolved diseases.

Databases/URLs

Cancer Gene Census: https://cancer.sanger.ac.uk/census.

Genome-Phenome Analysis Platform (GPAP): https://platform.rd-connect.eu.

Human Phenotype Ontology (HPO): https://hpo.jax.org/app/

Orphanet Rare Disease Ontology (ORDO): https://www.ebi.ac.uk/ols/ontologies/ordo.

Online Mendelian Inheritance in Man (OMIM): https://www.omim.org/

Rare Diseases Models & Mechanisms Europe (RDMM-Europe): http://solve-rd.eu/rdmm-europe/

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Author statement

Conceptualization [AKS, ItP, JG-P, RMdV, NH, SA]; Resources [EH-F, VS-L, LV, IS, DH, CO, RMdV, NH, SA]; Investigation & Validation [AKS, ItP, JG-P]; Writing - Original Draft & Visualization [AKS, SA]; Writing - Review & Editing [ItP, JG-P, VS-L, EH-F, AL, SP, LV, IS, CO, RMdV, NH]; Supervision [CO, RMdV, NH, SA]; Data Curation & Project administration [Solve-RD-GENTURIS group]; Funding acquisition [NH].

Declaration of competing interest

The authors have no conflicts of interest to declare.

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Appendix A. Supplementary data

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