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Familial hypercholesterolemia: enhancing actionability through the recall-by-genotype experience

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Familial hypercholesterolemia:

enhancing actionability through the recall-by-genotype experience

Abstract:

Recall-by-genotype studies in population-based biobanks enable to develop interventions to improve life quality and prevent health complications for individuals at a high risk of a genetic disorder, identifying them first through genomic data. Additionally, electronic health records can be used to further refine the phenotype and recall those most at risk. In this thesis, electronic health records, pharmacogenomics data, and a participant survey were used to assess the long-term impact of the familial hypercholesterolemia recall-by-genotype study conducted in the Estonian Biobank during 2016-2018. The follow-up consisted of 34 formerly recalled participants and 291 non-recalled biobank participants with the same genetic profile, acting as controls.

Keywords:

Familial hypercholesterolemia, recall-by-genotype, lipid-lowering treatment adherence

CERCS:

B110 Bioinformatics, medical informatics, biomathematics, biometrics; B790 Clinical genetics

Perekondlik hüperkolesteroleemia: sekkumisvõime arendamine läbi genotüübil põhineva tagasiside uuringukogemuse

Lühikokkuvõte:

Genotüübil põhineva tagasiside uuringud populatsioonipõhistes biopankades võimaldavad välja töötada sekkumisi, mis tõstavad elukvaliteeti ja aitavad ära hoida tüsistusi inimestel, kellel on kõrge risk päriliku haiguse avaldumiseks, selekteerides nad kõigepealt geeniandmete kaudu. Lisaks on võimalik kasutada elektroonilisi terviseandmeid fenotüübi täpsemaks käsitluseks, et kutsuda tagasisideks vastuvõtule kõrgeima koondriski tasemega isikud. Antud töös kasutati Eesti geenivaramusse koondatud elektroonilisi terviseandmeid, farmakogenoomika andmeid ja geenidoonoritel läbiviidud küsitlust, et hinnata varem teostatud perekondliku hüperkolesteroleemia alase genotüübil põhineva tagasiside uuringu (2016-2018) pikaajalist mõju. Jätku-uuring hõlmas 34 varem tagasisidet saanut ja võrdlusgrupina 291 samasuguse geneetilise profiiliga tagasikutsumata geenidoonorit.

Võtmesõnad:

Perekondlik hüperkolesteroleemia, genotüübil põhineva tagasiside uuring, lipiide langetava ravi järgimus

CERCS:

B110 Bioinformaatika, meditsiiniinformaatika, biomatemaatika, biomeetrika; B790 Kliiniline geneetika

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TERMS, ABBREVIATIONS AND NOTATIONS

ACMG	American College of Medical Genetics
APOB	Apolipoprotein B
ApoB100	Apolipoprotein B-100, predominant isoform of ApoB
ASCVD	Atherosclerotic cardiovascular disease
ATC	Anatomical Therapeutic Chemical Classification System
CAC	Coronary artery calcium (score)
CAD	Coronary artery disease
CPIC	The Clinical Pharmacogenetics Implementation Consortium
DDD	Defined Daily Dose
DLCN	Dutch Lipid Clinic Network
EAS	European Atherosclerosis Society
ECG	Electrocardiogram
EstBB	Estonian Biobank
EHR	Electronic health record
FH	Familial hypercholesterolemia
FHCL1	Familial hypercholesterolemia, type 1
FHCL2	Familial hypercholesterolemia, type 2
FHCL3	Familial hypercholesterolemia, type 3
FHCL4	Familial hypercholesterolemia, type 4
FHSC	Familial Hypercholesterolemia Studies Collaboration
GOF	Gain-of-function (variant)
HMG-CoA	3-hydroxy-3methylglutaryl-coenzyme A
ICD-10	International Classification of Diseases, 10th revision
IDL	Intermediate-density lipoprotein
IMT	*Intima media* thickness
KEGG	Kyoto Encyclopedia of Genes and Genomes

LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
LDLR	Low-density lipoprotein receptor
LDLRAP1	Low-density lipoprotein receptor adaptor protein 1
LLT	Lipid-lowering treatment, group of drugs that treat FH by lowering the cholesterol level
OMIM	Online Mendelian Inheritance in Man database
PCSK9	Proprotein convertase subtilisin/kexin type 9
RbG	Recall-by-Genotype, studies where subjects are first identified by genomic data and then recalled for more detailed phenotyping
SLCO1B1	Solute carrier organic anion transporter family member 1B1
VLDL	Very-low-density lipoprotein

INTRODUCTION

The major advances made in sequencing technology in the last few decades have generated an unprecedented amount of genomic data. Although these methods are still constantly being perfected, one of the principal challenges now is to put this data into clinical practice. Additionally, the Estonian health care system has implemented the use of electronic health data registries, providing basis to create an overview of the patient's clinical phenotype. Both the genomic data and clinical phenotype data intersect at the Estonian Biobank, granted by the consent of the biobank's participants. The recall-by-genotype study takes advantage of precisely this combination – potentially pathogenic variant carriers are first identified by genomic data and then a selection for recall (and return of results) is refined using the clinical phenotype data available.

Familial hypercholesterolemia is one of the most common Mendelian disorders known; individuals heterozygous for FH are estimated to be about 1:500 to 1:200 in Northern European populations (Nordestgaard *et al.*, 2013). Generally caused by a defect in *APOB*, *LDLR* or *PCSK9* genes, FH as a mainly cardiovascular disorder presents itself as premature atherosclerotic disease with significantly elevated cholesterol levels, particularly LDL-C levels (Kumar *et al*., 2015). A recall-by-genotype pilot study for FH was conducted at the Estonian Biobank in 2016-2018 (Alver *et al.*, 2018); this thesis aims to conduct a follow-up study in order to assess the impact of the recalls on the participants' health nearly half a decade later, and implement the potential of linked electronic health records in doing so.

A manuscript (first author M. Nurm) describing the course of the study and the results presented in this thesis was submitted to the journal *Frontiers in Genetics* on 04.05.2022 and is currently under review.

The protocol (and further amendments) for this study was approved by the Ethics Review Committee on Human Research of the University of Tartu and Estonian Committee on Bioethics and Human Research (approval no 1.1-12/3015).

1 LITERATURE REVIEW

1.1 Recall-by-Genotype (RbG) studies

Despite their novelty, Recall-by-Genotype (RbG) studies have already proven their value in population-based biobank-related studies. The RbG study design relies on a 'genotype-first' approach, where individuals harboring genetic variants of interest are first identified by genomic data. Next, electronic health records and clinical phenotype data collected during recall are used to determine the individuals most at risk for developing the disease in question (Corbin *et al.*, 2018).

The necessity for RbG studies stems from the swift advancements in DNA sequencing technology and particularly in the cost-effectiveness of these methods that, among other things, tend to lead to the discovery of *de novo* variants in disease-associated genes (Stessman *et al*., 2014). The technological advancements have also raised the question of incidental findings as the line between diagnosis and screening has become increasingly blurred. Incidental findings (or secondary findings) are defined as genetic results of potential medical importance that have been discovered during genetic testing where these findings were not the primary objective of the particular test (Saelaert *et al.*, 2018). However, in the context of biobanks, where the participants have signed up to receive feedback on their actionable genetic variants, genetic results that may ordinarily be considered incidental findings can turn out to be the actionable results expected by the participants.

Pathogenicity assessment remains a significant challenge for many previously described genetic variants, and the establishment of novel genotype-phenotype connections plays a crucial role in elucidating the clinical impact of a variant (Kelly *et al.*, 2018). RbG studies also provide a more solid basis for drawing causal inferences regarding population-based genomic studies (Corbin *et al.*, 2018). The RbG study design has demonstrated its effectiveness at the Estonian Biobank (EstBB) (Leitsalu *et al.*, 2016, 2020; Alver *et al.*, 2018) as well as elsewhere (Haukkala *et al.*, 2013; Ormondroyd *et al.*, 2020).

Along with the opportunities arising from genome and exome sequencing, there are also concerns regarding the possible influence of the knowledge of personal genetic risk variant carrier status on the often ostensibly healthy individuals participating in such a return of results (Leitsalu *et al.*, 2020). For instance, studies have found that confirmation of a genetic finding in the diagnosis of familial hypercholesterolemia (FH) may influence families to comply more efficiently with their recommended treatment plan even in the absence of

symptoms' manifestation (Defesche *et al.*, 2017). Therefore, researchers should take care in disclosing the potential impact of a genetic variant so as not to cause undue stress but still provide sufficient information, enabling the recipients to make informed choices in improving their health and general life quality (Ravitsky and Wilfond, 2007; Haukkala *et al.*, 2013).

1.2 The EstBB RbG pilot study 2016-2018

This work is a follow-up to the FH RbG pilot study conducted at the EstBB from 2016 to 2018, the results of which were published in (Alver *et al.*, 2018). In the pilot study, participants were recalled based on FH-related genetic findings in their exome sequencing (ES) or genome sequencing (GS) data (n=4776). 27 EstBB participants (of whom 21 responded positively) with FH-associated gene variants were identified and invited for a physical examination to detect early FH manifestations and to have their blood drawn for biochemical analyses. Additionally, family members were recruited through cascade screening, resulting in a final cohort of 41 confirmed FH variant carriers with an *APOB, LDLR,* or *PCSK9* variant. *APOB, LDLR,* and *PCSK9* have also been included in the American College of Medical Genetics (ACMG) list of genes where secondary findings should be reported back to patients because the intervention would be significantly beneficial in improving life quality (Kalia *et al.*, 2017). Cascade screening of family members for detection of FH is a tried and proven method, as seen in (Lee *et al.*, 2019). All participants received feedback at the end of their visit to explain their genetic findings and final diagnosis, along with recommendations for their treatment plan.

1.3 Estonian Biobank

The Estonian Biobank (EstBB), with its more than 205 000 participants (i.e., about 20% coverage of the country's adult population), has become a recognized source of international scientific research and offers a valuable basis for quick developments toward innovative personalized approaches in healthcare locally. Next to multilevel molecular datasets (DNA, RNA, proteomics, microbiome), the enrichment of phenotype data is provided through regular linkage to nationwide electronic health records (EHRs) and specialized health registries. Both the initial cohort health questionnaires and the EHRs have been recorded by medical specialists, thereby ensuring the reliability of the data. On top of the previous, the availability of clinical-level results for lab measurements and medical investigations supports creating applications for lifetime health surveillance and effective disease prevention.

1.4 Familial hypercholesterolemia (FH)

Familial hypercholesterolemia (FH) is a systemic disorder with mainly cardiovascular consequences. It is caused by a defect in the gene encoding the low-density lipoprotein (LDL) receptor, LDLR, or in a gene influencing a biochemical pathway related to the LDL receptor. An interruption of the normal LDLR pathway leads to abnormally elevated low-density lipoprotein cholesterol (LDL-C) levels in the body that induces premature atherosclerosis and myocardial infarction (Kumar *et al*., 2015). According to the Online Mendelian Inheritance in Man (OMIM) database (accessed on 07.05.2022), FH can be divided into four types: type 1 or FHCL1, caused by a defect in the *LDLR* gene; type 2 or FHCL2, caused by a defect in the *APOB* gene; type 3 or FHCL3, caused by a defect in the *PCSK9* gene, and type 4 or FHCL4, caused by a defect in the *LDLRAP1* gene. However, in most cases, FH is believed to stem in an autosomal dominant form from a pathogenic variant in *LDLR*, *APOB*, or *PCSK9* (Berberich and Hegele, 2018).

FH is one of the most common Mendelian disorders known. Heterozygotes with only one relevant mutant gene represent around 1 in 500 individuals globally (Kumar *et al*., 2015), but FH has been found to be more prevalent specifically in Northern European populations, with a frequency of about 1 in 200 individuals (Nordestgaard *et al.*, 2013; Benn *et al.*, 2016). Individuals homozygous for FH are very rare – between 1 in 160 000 and 1 in 1 000 000 (Cuchel *et al.*, 2014). FH heterozygotes have been shown to have a two- to three-fold increase in plasma LDL-C levels from birth, whereas, for homozygotes, there may be a five- to six-fold elevation in LDL-C levels compared to normal that would also lead to a much more severe phenotype (Kumar *et al*., 2015). The usual values of total cholesterol and LDL-C for FH heterozygotes have been found to range between 9-14 mmol/l and 5-10 mmol/l, respectively (Hovingh *et al.*, 2013).

1.4.1 LDL receptor and FH pathogenesis

While elevated cholesterol levels and premature heart attacks were initially set on a genetic backdrop by Carl Müller in 1938, it was the Nobel prize-winning work of Goldstein and Brown in 1985 that defined the association between LDL receptor gene mutations and coronary atherosclerosis in FH patients (Goldstein and Brown, 2015). The cholesterol circulating in the body in plasma does so primarily in the form of LDL-C (Kumar *et al*., 2015); the LDL receptor is the major receptor responsible for plasma LDL-C uptake in hepatic cells (Vos *et al*., 2018).

In healthy individuals, the LDL particle cycles through the body as shown in Figure 1. The liver cell secretes a very-low-density lipoprotein (VLDL) particle carrying triglycerides and cholesteryl esters into the bloodstream, then the particle is cleaved by lipoprotein lipase in capillaries of adipose tissue or muscle. The resulting particle, intermediate-density lipoprotein (IDL), contains fewer triglycerides and more cholesteryl esters than VLDL but retains apoproteins B-100 and E that were also present in VLDL. LDLR then takes up about 50% of the newly released IDL in the hepatic cells to be recycled into VLDL particles once more. The rest of the IDL particles undergo metabolic processing where most of the triglycerides and apolipoprotein E are removed, resulting in cholesterol-rich LDL particles eventually removed from plasma by one of two mechanisms. One of these mechanisms is hepatic clearance through binding by the LDLR, which accounts for about 70% of all plasma LDL and the second mechanism works through the mediation of a scavenger receptor. As such, LDLR is responsible for both binding the IDL to liver cell membranes for recycling as well as for the hepatic clearance of LDL from the bloodstream (Kumar *et al*., 2015).

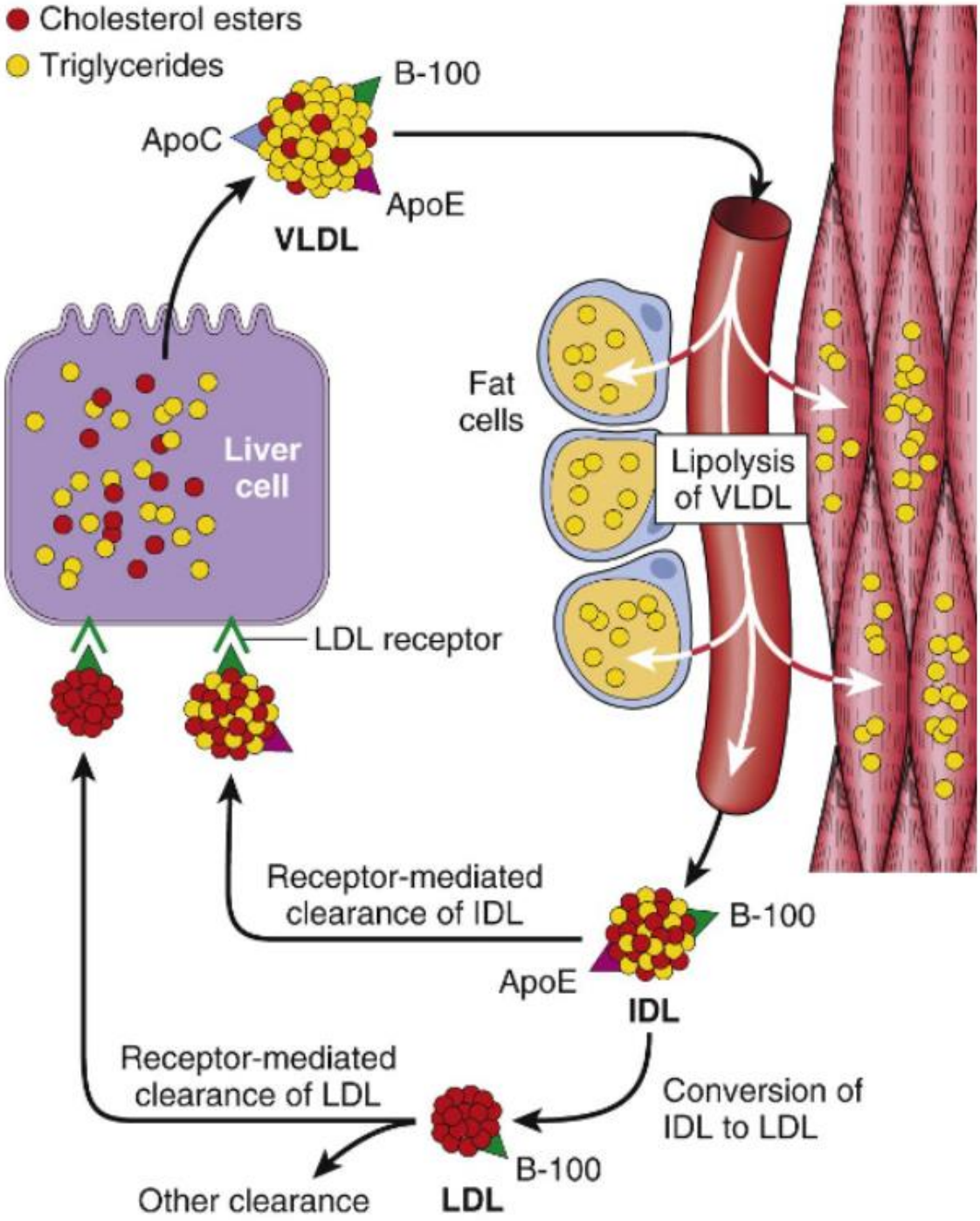

Figure 1. The cycle of LDL particles in the body (figure from Kumar *et al.*, 2015). ApoC – apolipoprotein C, ApoE – apolipoprotein E, B-100 – apolipoprotein B-100, IDL – intermediate-density lipoprotein, VLDL – very-low-density-lipoprotein, LDL – low-density lipoprotein.

The LDLRs, clustered in clathrin-coated pits on the plasma membrane, bind LDL particles to the cell surface, after which the LDLR-LDL particle complex is internalized inside the cell and transported to endosomes. From endosomes, the whole complex can either be sent

to lysosomes for degradation, or the receptor can be recycled and transported back to the cell membrane to resume its former purpose (Vos *et al*., 2018). The free cholesterol, released from LDL degradation, suppresses further cholesterol synthesis and LDLRs in the cell to avoid an overaccumulation of cholesterol. However, in individuals heterozygous for FH, the number of normal LDLRs is reduced by half, and in homozygotes, normal LDLRs are essentially nonexistent. This leads to both defective clearance of LDL as well as excessive LDL synthesis. Additionally, a more significant proportion of IDL particles is diverted for plasma LDL particle generation as the IDL cannot enter liver cells for recycling as it normally would (Kumar *et al*., 2015). The intracellular processing of the LDLR-LDL complex can be found in Figure 2.

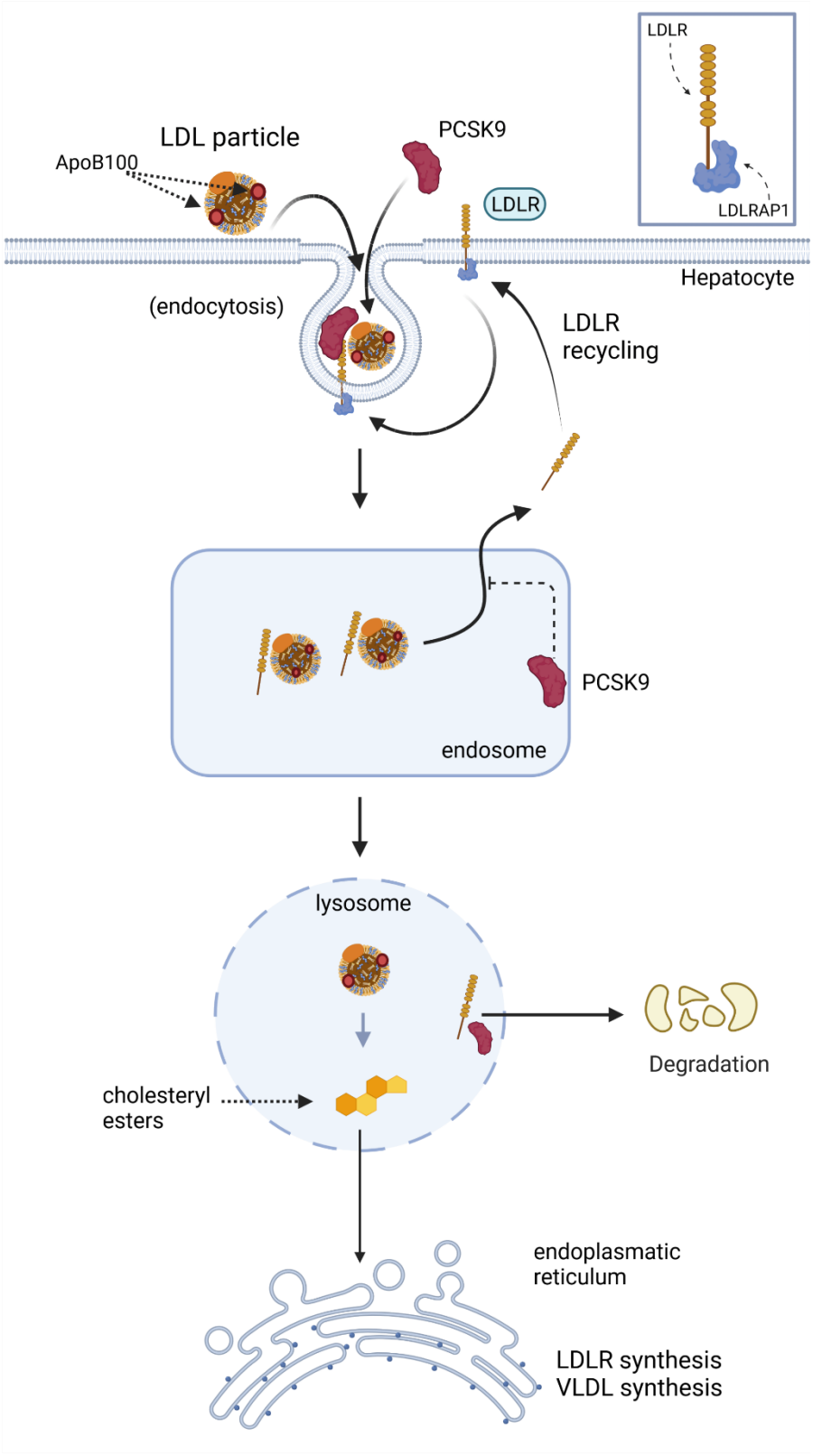

Figure 2. The uptake and subsequent processing of the LDL-LDLR complex in the hepatocyte. The LDL particle forms a complex with the LDLR at the mediation of the ligand ApoB100 and PCSK9. The complex is then endocytosed and incorporated into the endosome, where LDLR can be either directed to be recycled back to the cell surface or sent to degradation in the lysosome by PCSK9. In the lysosome, LDLR and PCSK9 proteins are

degraded, and the LDL particle is broken down into cholesteryl esters that are then transported to the endoplasmatic reticulum and used for new LDLR or VLDL synthesis. LDL – low-density lipoprotein, LDLR – low-density lipoprotein receptor, ApoB100 – Apolipoprotein B-100, LDLRAP1 – low-density lipoprotein receptor adaptor protein 1, PCSK9 - Proprotein convertase subtilisin/kexin type 9, VLDL – very-low-density lipoprotein. Created with web application BioRender, using the cholesterol metabolism pathway in KEGG and Gabcova-Balaziova *et al*. (2015) as references.

The circulating excess LDL-C and VLDL remnants eventually accumulate in the subendothelial compartment of the arteries and oxidize there (Schwenke and Carew, 1989). This process is augmented in arterial curves and branches where the blood flow exerts higher stress on the blood vessels (Davies, 1995). Oxidized particles begin to attract pro-inflammatory immune cells and induce their proliferation in the subendothelial compartment, eventually leading to migration of vascular smooth muscle cells that activates the production of a fibrous cap. These reactions lead to progression of atherosclerosis through the creation of hardened plaques in the vessels (Moore and Freeman, 2006), potentially inducing premature ischemic disease due to lumen occlusion.

1.4.2 FH-associated genes included in the ACMG list

1.4.2.1 LDLR gene

It has been estimated that pathogenic *LDLR* gene variants are responsible for 80-85% of all FH cases, which would mean that the vast majority of all FH patients are carriers of a defective LDLR gene (Nordestgaard *et al.*, 2013). *LDLR* lies on the short arm of chromosome 19 (19p13.2) and contains 18 exons, as described in the ClinVar database (read on 12.05.2022). A very rare form of FH that is also autosomal recessive is caused by a mutation in the low-density lipoprotein receptor adaptor protein 1 (LDLRAP1) gene. Impaired *LDLRAP1* alleles cause a dysfunctional LDLR complex formation and inhibit LDL uptake (Garcia *et al.*, 2001).

As mentioned previously, the LDLRs are vital to the LDL cycle in the body through the role they play in the hepatic clearance of LDL particles. LDLR gene defects have been classified into five groups: null alleles (total failure of receptor protein synthesis), folding defects impairing transport to Golgi complex, defects affecting the receptor's ability to bind LDL particles, defects resulting in a failure to localize in the cell membrane's coated pits and defects interrupting the receptor recycling process in endosomes (Kumar *et al.*, 2015).

1.4.2.2 APOB gene

The *APOB* gene lies on the short arm of chromosome 2 (2p24.1) and contains 29 exons. The protein produced from *APOB*, apolipoprotein B (ApoB), is the principal apolipoprotein in chylomicrons and LDL particles and also serves as a ligand for the LDL receptor (ClinVar database, 12.05.2022). ApoB100, the full-length form of ApoB, is the predominant isoform and is found in VLDL, LDL, and VLDL remnants (Contois *et al.*, 2009). *APOB* gene variants represent ca. 2-5% of FH cases in Northern Europe but appear to be less frequent in other populations; *APOB* variants have also been described as having variable penetrance (Singh and Bittner, 2015).

1.4.2.3 PCSK9 gene

The Proprotein convertase subtilisin/kexin type 9 (PCSK9) gene lies on the short arm of chromosome 1 (1p32.3) and contains 14 exons (ClinVar database, 12.05.2022). PCSK9 variants have been found to account for around 1% of all FH cases (Austin *et al.*, 2004). The PCSK9 protein is a proprotein convertase that binds to the LDLR on the cell surface and is crucial for the proper degradation of LDL receptors in endosomes (Lagace *et al.*, 2006). Therefore, gain-of-function (GOF) *PCSK9* variants inhibit LDLR function (Nordestgaard *et al.*, 2013) by inducing conformational changes post-transcription in the LDLR so that it becomes impossible to divert the receptor to recycling endosomes and thus reduce the number of functional LDL receptors in the cell (Peterson *et al.*, 2008).

1.4.3 Clinical symptoms and diagnosis of FH

While FH may vary considerably phenotypically, the extent of LDL-C elevation has been found to be the hallmark characteristic of phenotypic severity (Chemello *et al.*, 2021). Clinically, FH is characterized by the triad of hypercholesterolemia (elevation of LDL-C), premature coronary artery disease (CAD) preceded by atherosclerosis, and tendon or cutaneous xanthoma (Teramoto *et al.*, 2014). Achilles tendon xanthoma is a common feature of FH as the deposition of cholesterol crystals within the tendon is often followed by glycosaminoglycan accumulation, leading to xanthoma formation. X-ray radiography can be used to detect xanthomas, and Doppler ultrasonography can aid in tracking changes in xanthoma development (Tanaka *et al.*, 2021).

Arcus corneae also figures among the characteristic FH features, but only in about 30% of cases (Teramoto *et al.*, 2014). Arcus corneae emerges because of cholesterol deposits located in the corneal margins; when the deposits are concentrated in the skin of the eyelids, then

this manifestation is referred to as a xanthelasma. Arcus corneae and xanthelasmas can be observed in the course of physical examination (Pannu and Sharma, 2017). Skin xanthomas and vascular atherosclerosis of all types of blood vessels may develop in FH-affected individuals at an early age (Kumar *et al*., 2015).

The course and grade of atherosclerosis can be tracked in the body by performing an exercise electrocardiogram (ECG) to assess cardiac health, computer tomography for calculating coronary artery calcium (CAC) score, and carotid ultrasound to assess intima-media thickness (IMT); as was done in the RbG pilot study to find evidence of subclinical atherosclerosis (Alver *et al.*, 2018). The latter two methods are used to evaluate the extent of artery wall fibrosis and potential vessel occlusion.

Various scoring systems have been developed to assess the likelihood of FH diagnosis in a patient. One of these is the Dutch Lipid Clinic Network (DLCN) criteria that were also used by Alver *et al.* (2018) and can be applied in a clinical setting as described by Nordestgaard *et al.* (2013). The different categories used for classification assign a certain number of points when the statements are found to be true; family history, clinical history, the results of physical examination and biochemical analyses, and a confirmed genetic result in an FH-associated gene are taken into account. The diagnosis of FH is confirmed when the score is more than 8 points, FH is considered probable with 6-8 points, possible with 3-5 points, and unlikely below 3 points. A confirmed pathogenic variant in an FH-associated gene alone gives 8 points.

When FH is left untreated, the main health complications that may arise are stroke, myocardial infarction, and peripheral vascular disease (Defesche *et al*., 2017), which all have atherosclerosis as the underlying cause (Mozaffarian *et al.*, 2015). It has been found that nearly 3-6% of myocardial infarction survivors qualify to be diagnosed with FH (Kumar *et al*., 2015), and a study conducted using the European Atherosclerosis Society (EAS)'s global Familial Hypercholesterolemia Studies Collaboration (FHSC) registry found that the prevalence of coronary disease amongst adults with a probable or definite FH diagnosis (including both clinical and genetic diagnoses) was 17.4% and rising with concentrations of untreated LDL-C (Vallejo-Vaz *et al.*, 2021). According to the Rare Diseases database, untreated FH heterozygotes have a 10- to 20-fold increased risk of developing CAD compared to normal (Rare Diseases database, accessed on 20.05.2022).

1.4.4 State of FH worldwide and in Estonia

It has been acknowledged that there is a lack of systematic overview of FH prevalence in most countries. The country with the most comprehensive national FH research and patient handling in the world is considered to be the Netherlands (Singh and Bittner, 2015). Studies have suggested that there are about 30-35 million persons with FH worldwide, of whom only 3 million have been diagnosed (Gidding *et al.*, 2015). Considering the lack of a systemic approach to handling FH and its high expected prevalence worldwide, FH is likely to be underdiagnosed and undertreated in many regions (Nordestgaard *et al.*, 2013; Sturm *et al.*, 2018; Wilemon *et al.*, 2020). The former underappreciation of FH prevalence and subsequent insufficient prioritization of the disease may have led to a lack of awareness regarding FH among healthcare workers and health policy makers, despite the high burden untreated FH places on healthcare systems (Vallejo-Vaz *et al.*, 2015).

There is no recent confident data on the prevalence of FH in Estonia yet, however, clinical attention towards individuals with high LDL-C and awareness of the genetic basis of the disease have improved rapidly. This is reflected in the establishment of Estonian FH registry in two main hospitals in Estonia – North Estonia Medical Centre and Tartu University Hospital, and the latest developments in the related field of clinical practices are guided by The Estonian Society of Cardiology (according to the European Atherosclerosis Society website, accessed 23.05.2022).

1.5 Lipid-lowering treatment (LLT)

The most important approach to preventing or mitigating FH-related health complications is lowering LDL-C levels in blood to a certain threshold. The main class of drugs used for this purpose is statins (Baigent *et al.*, 2010). Statins assert their effect through a reversible inhibition of the 3-hydroxy-3methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in the cholesterol synthesis pathway in hepatic cells. HMG-CoA reductases convert hydroxy-methyl-coenzyme A into mevalonic acid that acts as a precursor to sterols. As such, statins interrupt cholesterol synthesis, which leads to an increase in LDLRs through a feedback loop (Zilmer *et al.*, 2015). Other lipid-lowering treatments (LLT) include fenofibrate, ezetimibe, and PCSK9 inhibitors that can be used alone or in combination with statins (Lloyd-Jones *et al.*, 2017).

According to current Estonian treatment guidelines, the level of LDL-C in FH patients should fall by at least 50% to be sustainable. A 50% decrease is only guaranteed by high-intensity statin treatment with 20-40 mg rosuvastatin or (40)-80mg atorvastatin, as reported by Hedman (2022). The guidelines published by the European Society of Cardiologists in 2021 suggest that the LDL-C target in FH patients without additional major risk factors should be lowered from the former 2.5 mmol/l to 1.8 mmol/l and in patients with atherosclerotic cardiovascular disease (ASCVD) risk from 1.8 mmol/l to 1.4 mmol/l (Visseren *et al.*, 2021). The EAS study analyzing the FHSC registry found that the use of combination therapy (statins with another LLT) would more likely result in LDL-C levels lower than 1.8 mmol/l than one type of LLT alone (Vallejo-Vaz *et al.*, 2021). However, LLT does not need to be instated immediately after a diagnosis of FH has been made. For instance, Adham *et al.* (2018) suggest that LLT should be prescribed only after three months of a well-observed dietary regimen have been attempted but have not yielded the necessary results (Adham *et al.*, 2018). The EAS study also reported that the median LDL-C level in untreated patients was 5.43 mmol/l, whereas the same value was 4.23 mmol/l for patients on LLT (Vallejo-Vaz *et al.*, 2021), which illustrates the utility of LLT in lowering LDL-C levels but also highlights that there may be a number of patients on LLT that do not adhere properly to their treatment plans or should receive more intensive treatment in order to reach target LDL-C levels. The key to halting ASCVD progress in FH may rather be continuous LLT adherence over several years, even with LDL-C levels higher than the value recommended in guidelines (Kastelein *et al.*, 2008).

Despite the proven efficacy of LLT in lowering LDL-C levels and preventing FH complications, LLT adherence is generally considered poor (Casula *et al.*, 2016; Langslet *et al.*, 2021). One possible explanation for this is the occurrence of statin-related side effects, the most predominant of these being myopathy (Mach *et al.*, 2018). Another explanation may be a lack of sufficiently thorough education on the importance of LLT in managing FH, owing once again to the neglect of a systemic approach.

2 THE AIMS OF THE THESIS

The aims of this thesis are as follows:

1. To collect long-term clinical and psychosocial evidence on the impact of return of high-risk actionable genetic findings to the research participants:
   - analyze the participants' evaluations to, and the long-term effects of the FH RbG pilot study;
   - Analyze the diagnostic and therapeutic performance of individuals with similar genetic profile in the healthcare system;
   - Explore the capacity of integrating the selected phenotype-/disease-related parameters into a profile that would potentially enable a more personalized therapeutic approach.

2. Make use of the datasets available at the biobank for automatic analysis to explore their potential for long-term patient surveillance.

3 EXPERIMENTAL PART

The experimental part of the thesis was performed in the form of a follow-up study to the original RbG pilot study (2016-2018) and was conducted at the EstBB 4-6 years after the initial recalls. EHRs were used to collect the participants' general information, ICD-10 codes, drug prescription data, and LDL-C values and to create individual disease timelines with pharmacogenomic profiles as an exemplary visual aid for better long-term surveillance of FH. A participant survey was conducted to gauge their personal opinions and reactions to the impact that the recalls had had on their life and health over a more extended period of time.

3.1 MATERIALS AND METHODS

3.1.1 Materials

3.1.1.1 Study subjects

The study involved 325 EstBB participants – 34 formerly recalled during the RbG pilot study and 291 as controls who are yet to be recalled. Only RbG pilot study participants who had signed up as gene donors by fall 2021 were eligible to take part in the follow-up study. The 291 controls were selected on the basis of the gene variant list published in Alver *et al.* (2018) Supplementary Table 3 and also included RbG pilot study participants who had been invited for a return of results but were not able to attend the visit. A more detailed overview of the participants' repartition between the follow-up study, RbG pilot study, and Alver *et al*. (2018) can be seen in Figure 3.

As such, the first subset of controls consisted of biobank participants whose FH-associated gene variants had been confirmed by Sanger sequencing (n=55) prior to the follow-up study. The second subset was made up of biobank participants that had confirmed FH-associated genetic variants in both the genotyping array dataset as well as the imputed dataset (n=225). A third, small subset was retrieved of individuals with either an FH-associated genetic finding in the genotyping array dataset or the imputing dataset and who were selected for quality control (n=11). The second and third subsets' genetic findings were also confirmed by Sanger sequencing. Whereas the participants in Alver *et al.* (2018) were selected based on findings from exome sequencing or genome sequencing data (n=4776), the follow-up search expanded this search to all biobank participants whose genotyping array (Global Screening

Array (GSA), Illumina Inc., San Diego, USA) data or imputed data (based on the Estonian population-specific reference panel) was available at the time of the study (n=201146).

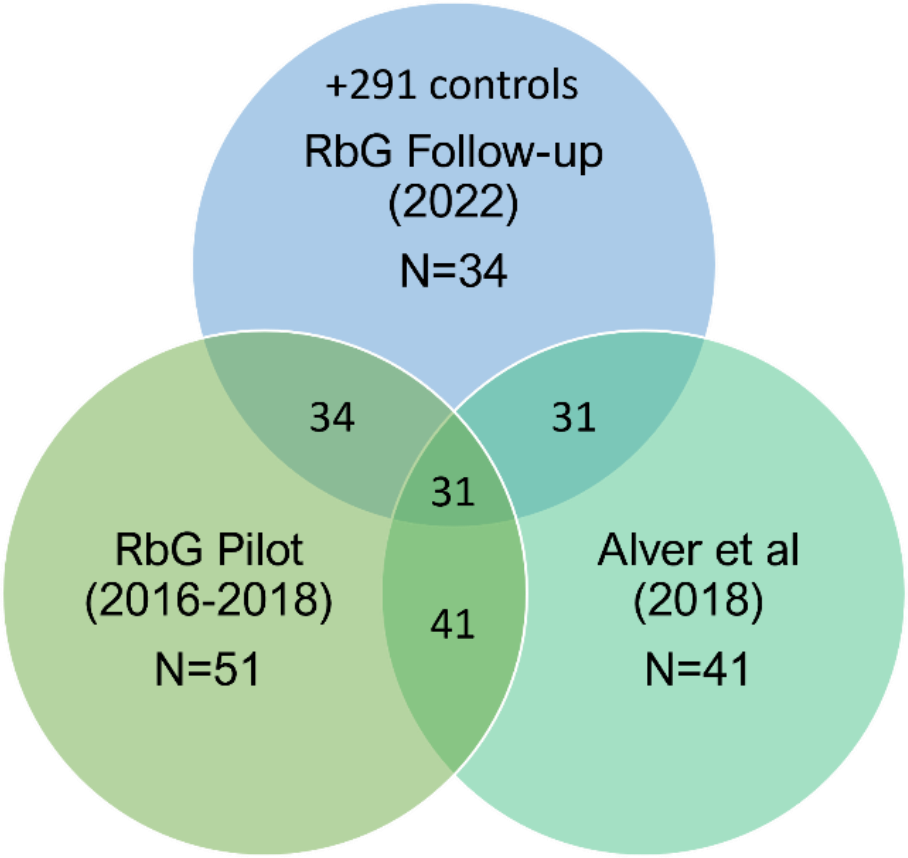

Figure 3. Repartition of recalled participants across the RbG pilot study, follow-up study and published results from Alver *et al.* (2018). Only the recalled participants from the RbG pilot study who had signed up as biobank participants by fall 2021 were eligible to participate in the follow-up study.

3.1.1.2 EHRs linked to the EstBB

The EHRs spanning over a time window of up to 18 years (2004 - 2022) were retrieved for all study participants as described in a prior EstBB publication (Leitsalu *et al.*, 2015). All biobank participants had given their consent to make their EHRs available for scientific research. The participants' general information (age, sex, height, weight, smoking status), International Classification of Disease, 10th revision (ICD-10) diagnosis codes, and data of prescribed medicines (Anatomic Therapeutical Chemical Classification System/Defined Daily Dose (ATC/DDD) codes, dosage, and purchase data) were obtained from EHRs and used for analysis.

3.1.1.3 Pharmacogenomics dataset

EstBB participants' genotype data was classified into predictive pharmacogenomic phenotypes for 11 clinically important pharmacogenes using a previously developed pipeline (Reisberg *et al.,* 2019). In the current study, the output for *SLCO1B1* gene genotypes and the corresponding pharmacogenomic risk predictions were created by the Estonian Genome Center's pharmacogenomics group according to The Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines (The Clinical Pharmacogenetics Implementation Consortium, accessed 20.12.2021) in force up to the end of 2021 (Wilke *et al.*, 2012; Ramsey *et al.*, 2014). These risk assessments were used to assign study participants into three risk categories: normal risk of developing statin-induced myopathy, higher than normal, and much higher than normal, and the warnings were applied only to simvastatin, atorvastatin, and pravastatin.

3.1.1.4 Recalled participants' survey

The survey questionnaire was created in Estonian by the medical genetics workgroup's genetic counselor, Liis Leitsalu, using previously published survey instruments (Marteau and Bekker, 1992) and relevant studies (Brehaut *et al.*, 2003). The questionnaire consisted of 40 questions regarding the perceived usefulness of the genetic results that participants had received (including how they understood the information presented to them), the impact that knowing about their genetic findings had had on their lives, the participants' evaluation of the healthcare system's efficiency in integrating this information, and whether they had experienced any possible FH complications after their recall visits. For questions with five possible answers given in the range of 'disagree' to 'agree', the responses were converted to a numeric scale of 1-5, with values <3 demarcating a negative attitude towards the statement and those >3 a positive one.

Of the 34 recalled participants of the RbG pilot study, 32 were recontacted by mail and asked to fill out the questionnaire (Appendix I) four to six years after their initial recall. The participants were asked to return the filled form in 14 days. Two participants of the initial cohort had to be excluded from the survey as one participant could not be contacted due to changed contact information, and one had passed away in 2018. In the event of no response in 14 days, the participants were contacted by telephone. Further no response was taken to imply that participants had declined to take part in the follow-up study.

3.1.2 Analysis methods

A combination of Python (v3.8.5) scripts generated by the author with pandas (v1.1.3), seaborn (v0.11.0), numpy (v1.19.2), matplotlib (v3.3.2), plotly (v.5.6.0) libraries and the Python Standard Library (statistics, re, and datetime modules) and manual data processing in Excel was used in the analysis of the linked EHRs datasets. P-values presented in Table 3 were calculated with Langsrud's online 2-tailed Fisher's exact test calculator (accessed 20.03.2022), which uses Agresti (1992) as a reference (Agresti, 1992).

The scripts used have been made available at a public GitHub repository: https://github.com/mnurm/MSc-FH-follow-up

3.1.2.1 Data integration for precise phenotyping: ICD-10 codes, general information, and clinical lab measurements

ICD-10 codes were extracted from the linked registries in a consolidated manner – each row in the table represented one diagnosis code per individual. The table with all 325 individuals consisted of 21 111 rows and included variables such as earliest date of diagnosis, ICD-10 diagnosis code, the latest date of diagnosis, how many times the diagnosis had been mentioned in the individual's EHRs and where. Aside from searches for particular diagnosis codes, ICD-10 codes were reduced to 3-character category codes with each code counted only once for each individual.

General information such as participant's age, sex, BMI, and smoking status had been gathered when the participant signed up for the biobank. Tables were created to summarize the most important EHR findings. Clinical lab results (total cholesterol and LDL-C values) were collected from linked EHRs, principally from the lab analyses registry. Where an individual had several timepoints for the analyte's measurement, the average of all measurements was taken into account when calculating overall average and median values for each study group.

3.1.2.2 Drug prescription data analysis

Drug prescription data was preprocessed akin to the ICD-10 diagnosis codes' table, where one row represented one drug prescription per person. Only prescriptions containing ATC/DDD codes beginning with C10A or C10B were extracted from the registry. The table containing data on the whole cohort consisted of 7 743 rows and included variables such as prescription numbers, ATC/DDD codes, prescription treatment indication (as an ICD-10 code), the commercial name of the drug, the active substance, drug prescription date, drug purchase date, number of packages purchased, number of pills in package, dosage and form

of medication (e.g. tablet with thin polymer coating). Before further analysis the dataset had to be cleaned of rows with NaN (blank) values and filtered only for purchased prescriptions.

To visualize LLT adherence, a Gantt chart was created in Python using the plotly library showing the periods of LLT use from 2004 to March 2022 among recalled participants with LLT prescriptions (n=27). The periods covered by LLT use were defined from the date of drug purchase, as recorded in the drug prescription registry, to the end date of prescription consumption, as the number of packages multiplied by the number of pills and added to the purchase date as the number of days (thus assuming that participants consumed one tablet per day). The formula for these calculations was adjusted as needed on a case-by-case basis; for example, when a participant purchased several prescriptions on the same day or when the start dates for subsequent packages had to be adjusted based on the consumption end dates for previous packages. In this study, consistent LLT use was defined as use without a gap >6 months. More detailed data on LLT use are depicted in the Medication use graphs section in in the Appendix.

3.1.2.3 Pharmacogenomics dataset

Pharmacogenomic profiles were created for the 27 recalled participants using LLT and included the following information: age, sex, the FH-associated genetic variant, LLT adherence, previous FH-related medical history (ICD-10 codes), statin-adjusted total cholesterol and LDL-C values (original values divided by 0.8 and 0.7, respectively) measured during feedback visits of the RbG pilot study, statin-induced myopathy risk assessment, and an overview of the participant's survey responses regarding treatment plan adherence and health (Appendix III and IV). LLT adherence was presented in these profiles as medication use graphs (Appendix V), created in Python with matplotlib library.

3.2 RESULTS AND DISCUSSION

3.2.1 Overview of recalled and non-recalled participants

A summary of the identified FH-associated variants in both recalled and non-recalled participants is provided in Table 1. Of 325 participants, 60.9% (n=198) were carriers of an *APOB* variant, 42.3% (n=123) of an *LDLR* variant, and 1.7% (n=5) of a *PCSK9* variant. The *APOB* variants are depicted in Figure 4, *PCSK9* in Figure 5 and *LDLR* in Figure 6. Similarly to the findings reported by Alver *et al.* (2018), the most frequent FH-associated variants identified in the follow-up cohort were p.Arg3527Gln (rs5742904) in the *APOB* gene and p.Cys329Tyr (rs761954844) in the *LDLR* gene. As the non-recalled group of the follow-up

study was retrieved using the genotyped and imputed data from the full EstBB cohort, these two FH-linked variants were estimated to be the most prevalent in the Estonian population. The study cohort also included two individuals with a novel variant, originally discovered in the RbG pilot study but not covered by Alver *et al*. (2018).

We found that the recalled cohort and the non-recalled control group were overall comparable in terms of general phenotypic characteristics (i.e., height, weight, body mass index, age, sex) (Table 2). Along with the similar genetic composition in FH-associated variants, this indicates that the major general confounding factors had little impact on the study results and that the most significant effect on outcomes is likely to stem from knowledge of their carrier status.

Table 1. FH-associated genetic variants detected in the follow-up cohort. Participants with *LDLR* p.Val436Ala (rs779732323) and *PCSK9* p.Ala103Ser (novel) were assigned to the control group because they declined feedback visits offered by Alver *et al.* (2018) in 2016-2018. *One individual had both *APOB* p.Arg3527Gln (rs5742904) and *LDLR* p.His250Arg (rs1256668310) variants. SNV, single nucleotide variant; rs, dbSNP reference number; RbG, recall by genotype; GS, genome sequencing; ES, exome sequencing.

Gene RefSeq protein ID	**SNP**	**RbG partici-pants**	**Controls**	**Sum**	**Alver *et al* 2018 (GS/ES)**
APOB* NP_000375		**22**	**176**	**198**	
	p.Arg3527Gln (rs5742904)	20	176	196	11
	p.Gly861Glu (rs1663664782)	1	0	1	
	p.Cys4217Alafs3* (novel)	1	0	1	
LDLR* NP_000518		**11**	**112**	**123**	
	p.His250Arg (rs1256668310)	1	22	23	2
	p.Leu401His (rs121908038)	1	2	3	2
	p.Ala431Ser (rs28942079)	1	13	14	1
	p.Arg633His (rs754536745)	1	0	1	1
	p.Cys329Tyr (rs761954844)	3	72	75	5
	p.Arg215Cys (rs764042910)	2	1	3	1
	p.Gly396Ala (rs766474188)	1	0	1	1
	p.Arg115Cys (rs774723292)	1	0	1	1
	p.Val436Ala (rs779732323)	0	2	2	1
PCSK9 NP_777596		**1**	**4**	**5**	
	p.Arg357Cys (rs148562777)	1	3	4	1
	p.Ala103Ser (novel)	0	1	1	

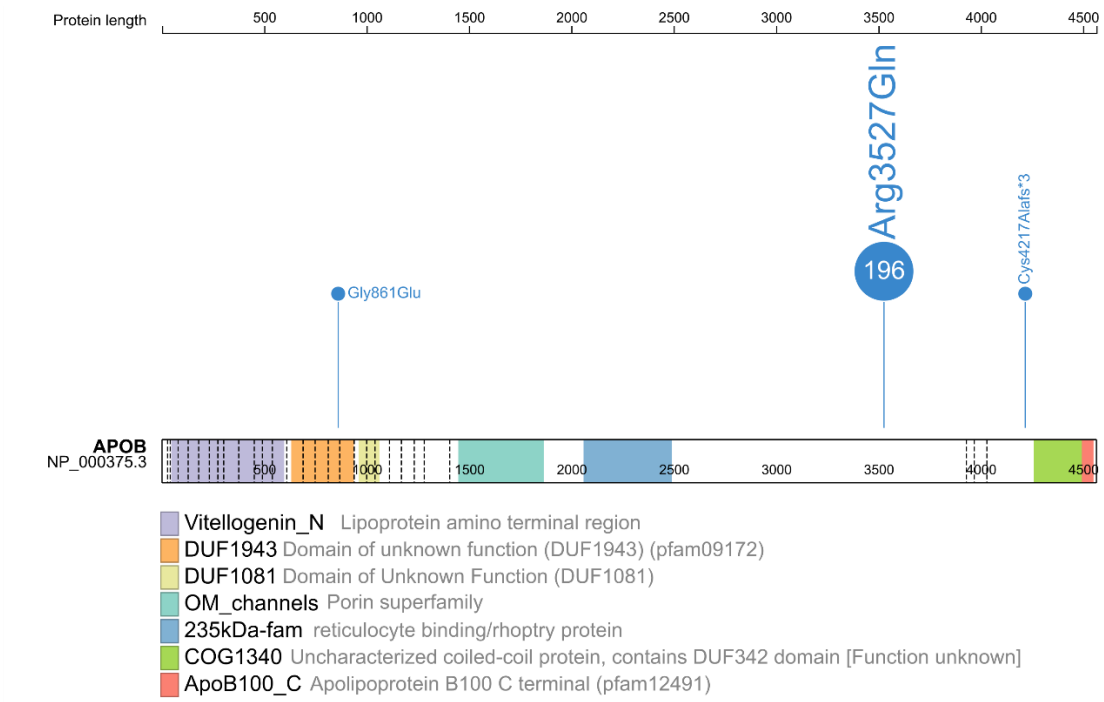

Figure 4. *APOB* variants (as amino acid changes) identified in the study placed on the APOB protein. The numbers right next to variant names show the number of study participants carrying the specific variant. If no number is specified, then the variant had only one carrier. Colored regions on the protein denote domains of (potential) functional significance; dotted lines signify exon limits. The Cys4217Alafs*3 is a stop-gained variant, cutting off the C-terminal part of ApoB100. The image was created using web application Proteinpaint.

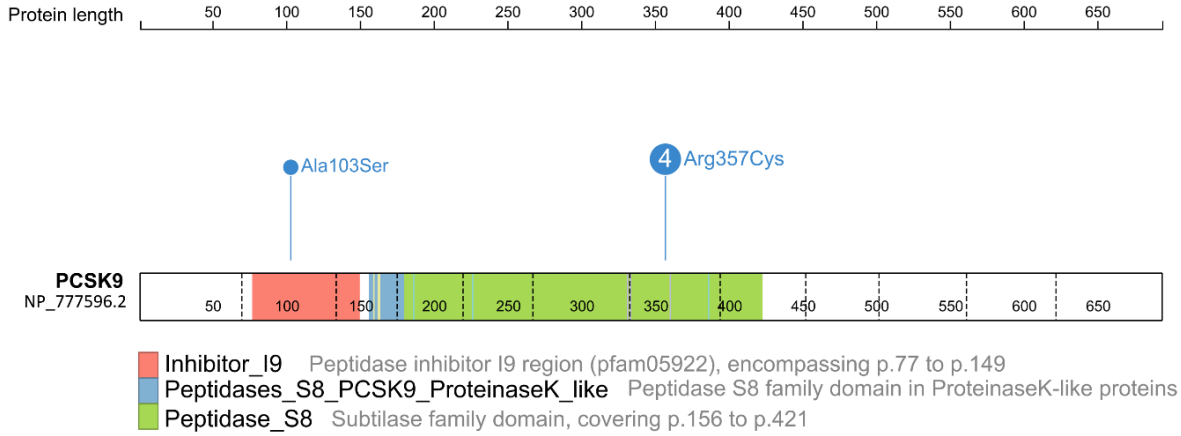

Figure 5. *PCSK9* variants (as amino acid changes) identified in the study placed on the PCSK9 protein. The numbers right next to variant names show the number of study participants carrying the specific variant. If no number is specified, then the variant had only one carrier. Colored regions on the protein denote domains of (potential) functional significance; dotted lines signify exon limits. The 'p.' in the color code legend indicates the position of the changed amino acid in the protein. The image was created using web application Proteinpaint.

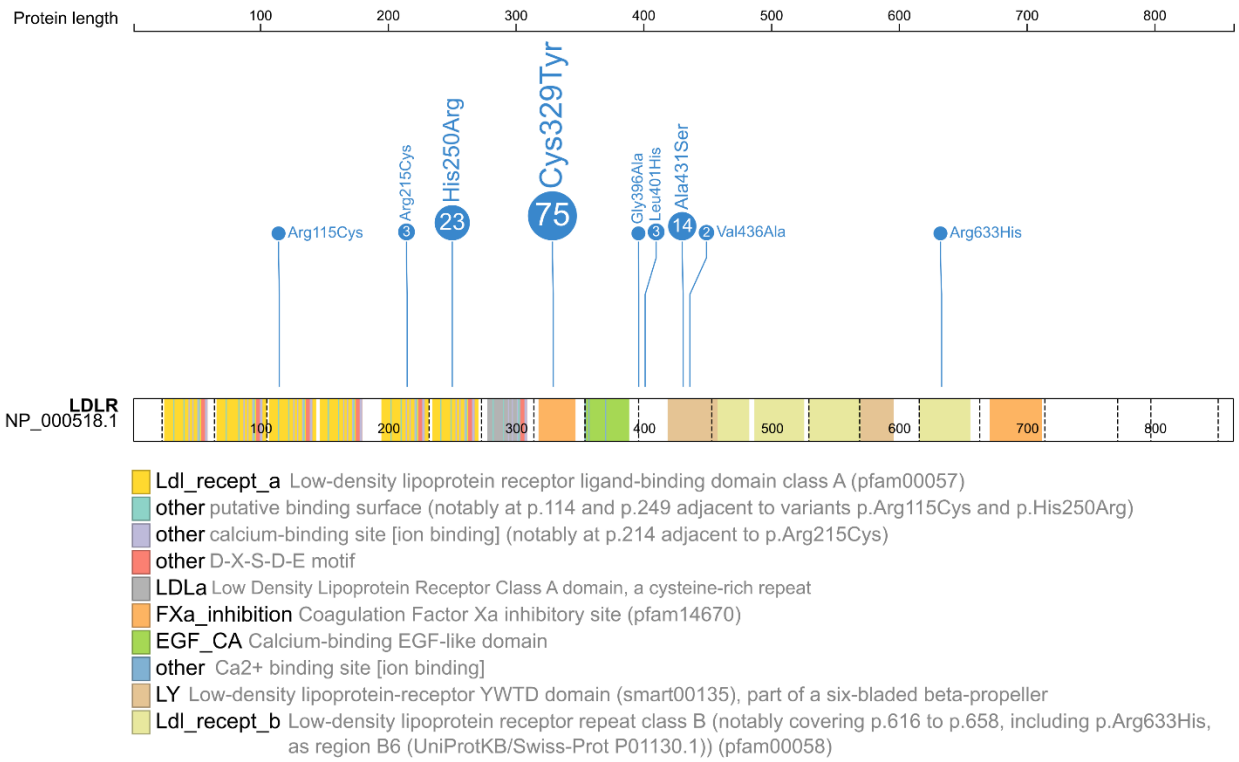

Figure 6. *LDLR* variants (as amino acid changes) identified in the study placed on the LDLR protein. The numbers right next to variant names show the number of study participants carrying the specific variant. If no number is specified, then the variant had only one carrier. Colored regions on the protein denote domains of (potential) functional significance; dotted lines signify exon limits. The 'p.' in the color code legend indicates the position of the changed amino acid in the protein. Region B1 (of LDLR repeat class B domain) covering p.397 to p.438 is an additional functional domain not pictured here. The image was created using web application Proteinpaint.

Table 2. Cohort characteristics**.** RbG - recall-by-genotype; BMI - body mass index.

	RbG participants (n=34)	**Controls (n=291)**
Gender – male/female (%)	16/18 (47.1/52.9%)	105/186 (36.1/63.9%)
Age, range (yrs)	Median 49.5 (29-84)	Median 49 (21-100)
BMI	Mean 25.5 (18.2-44.3)	Mean 26.3 (17.2-50.8)
Weight (kg)	Mean 76.4 SD 19.0	Mean 76.2 SD 15.7
Height (cm)	Mean 172.3 SD 11.0	Mean 170.1 SD 9.1
Smoking (%)		
Never	19 (55.9%)	138 (47.4%)
Former	7 (20.6%)	70 (24.1%)
Current	8 (23.5%)	67 (23.0%)
Unknown	0	16 (5.5%)

3.2.2 Survey results

The survey was sent out to 32 recalled participants nearly half a decade after their primary feedback visits and was returned by 24 (75%). Two participants contacted the EstBB to decline to participate in the survey, one because of bad personal experience with statin-related side effects and another for unknown reasons.

The respondents' assessment of the feedback received was overwhelmingly positive (Figure 7). As the answers were scaled to numeric values 1-5 where possible, mean values >3 signify agreement with a particular statement unless specified otherwise. On average, the participants agreed that "it was the right decision" to attend the feedback visit (4.96/5), and that they "wished to have been informed earlier about the genetic finding and the potential health risks" (4.29/5). The respondents asserted that they would "make the same choice if they had to do it over again" (5/5) and largely agreed that the knowledge of their genetic finding did not cause them distress ("I am able to cope with having this genetic finding in my family," 4.92/5). Average scores for the negative statements "I regret my choice" and "the choice did me a lot of harm" were 1.17/5 and 1.04/5, respectively. The respondents did not definitively agree or disagree with statements about access to healthcare and the improvement of their treatment and/or condition.

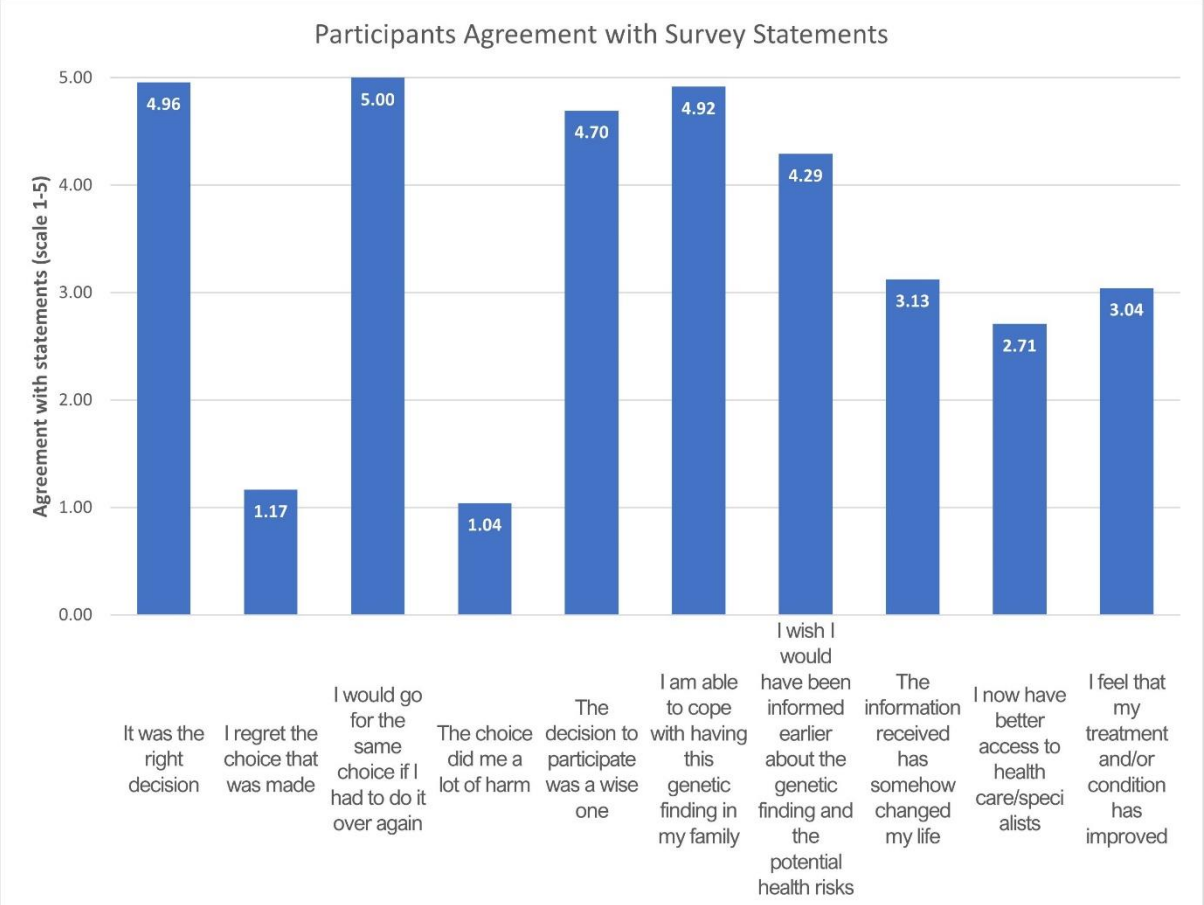

Figure 7. Mean values for answers given to the first portion of the RbG feedback survey where the participants were asked to rate their agreement with the following statements (n=24). The answers were converted to numeric values according to the scale: 'agree' - 5, 'slightly agree' - 4, 'unsure' - 3, 'slightly disagree' - 2. 'disagree' - 1. Mean values above three signify agreement with the specific statement.

The follow-up study's high survey response rate reflects EstBB participants' continuing willingness to cooperate with biobank inquiries, even several years after their feedback visits. This willingness is likely to stem partially from participants' interest in their general physical health and awareness of their carrier status, as well as positive attitudes toward and gratitude for the disclosure of their genetic findings; several participants in the study indicated that they would have liked to have had knowledge of their genetic findings earlier and most reported that they had made the right choice to receive genetic feedback.

Regarding aspects of the healthcare system with respect to their genetic findings, the respondents subjectively assessed the consistency of follow-up the lowest (3.71/5), followed by access to healthcare (3.96/5) (Figure 8). Access to medication was regarded the highest (4.38/5), followed by the clarity of recommendations (4.13/5). The participants' satisfaction with access to medication suggests that the cost or poor supply of drugs is not a substantial barrier for patients adhering to their treatment plans in Estonia.

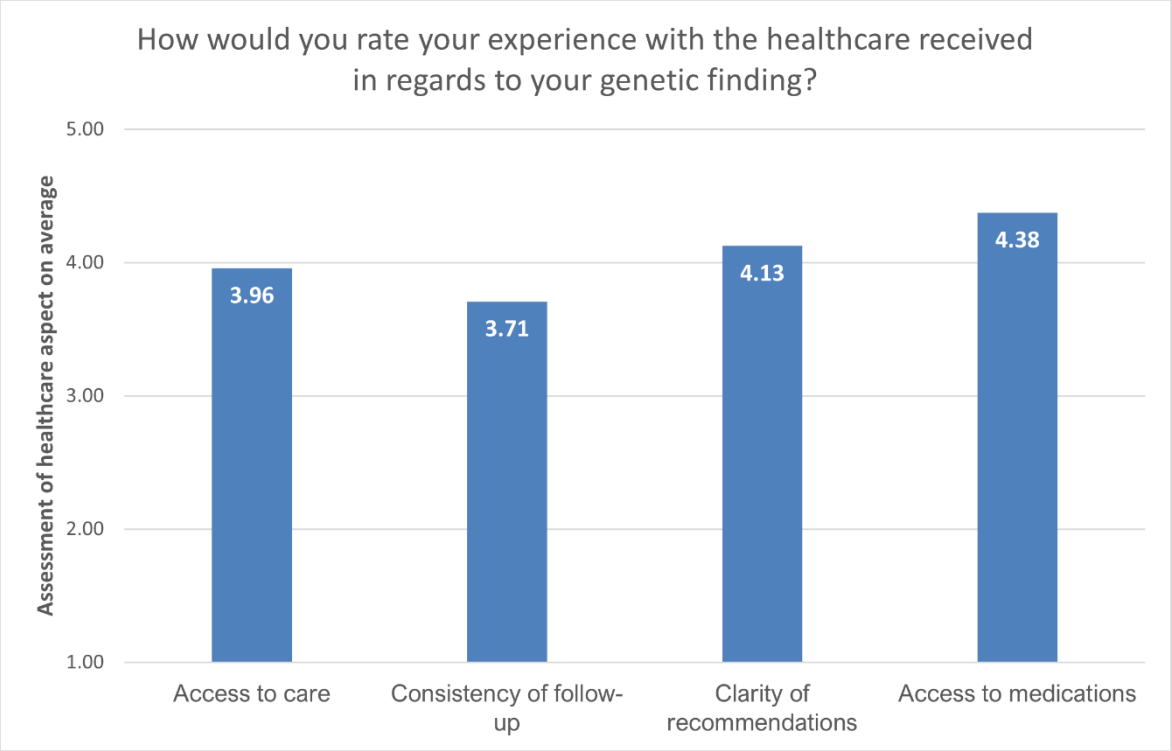

Figure 8. Overview of the assessment of the current state of the healthcare system by recalled participants (n=24). The answers are presented as mean values of provided answers converted to numeric values: 'very good' - 5, 'good' - 4, 'unsure'-3, 'satisfactory'- 2, 'unsatisfactory' -1. Mean values above three signify satisfaction with the specific healthcare aspect.

When asked about the potential complications related to FH, most respondents replied 'yes' only when asked about the detection of a high cholesterol level after their feedback visit (Figure 9). Regarding all other potential complications (e.g., myocardial infarction, stroke, arrhythmia, chest pain after strenuous exercise, vertigo, balance problems, or being diagnosed with cardiovascular disease), the vast majority of respondents affirmed that these health problems had not occurred.

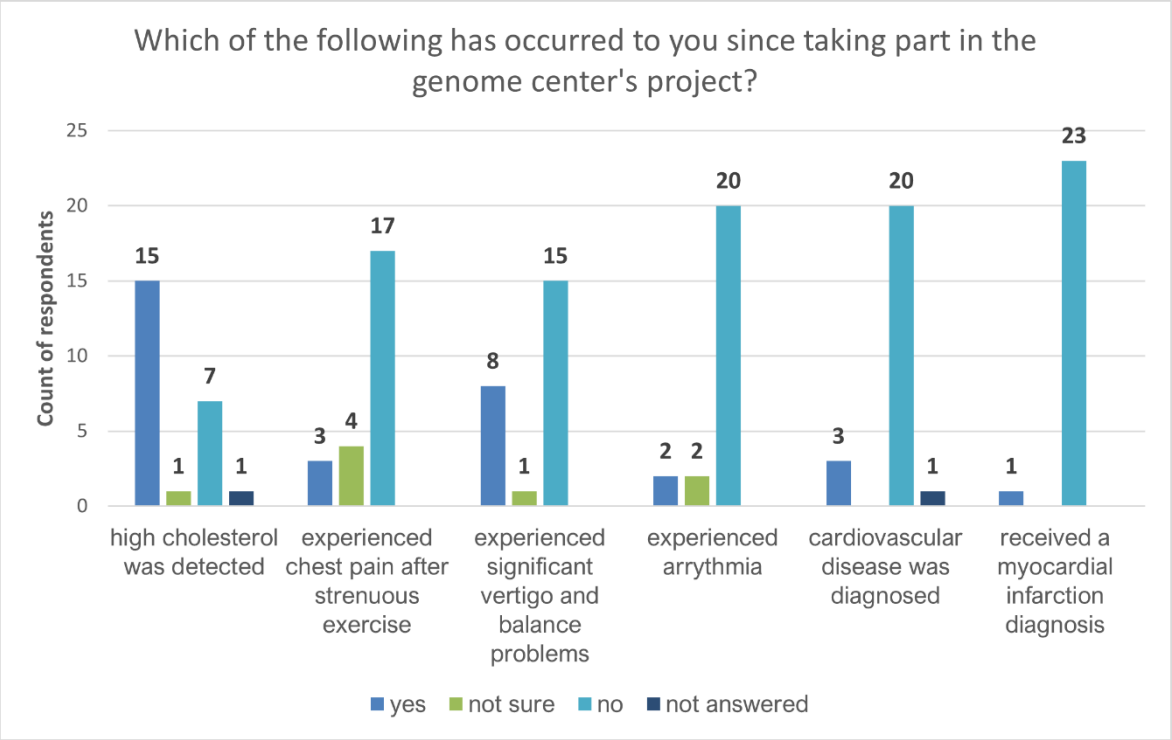

Figure 9. Feedback on health issues potentially related to hypercholesterolemia as reported by recalled participants (n=24).

Most (92%) of the participants responded "yes" or "yes, partly" to the question about whether they were following the treatment plans by their doctors; 4% responded "yes, but not in the last six months", and another 4% reported that they were not following their treatment plans (Figure 10). Nearly two-thirds (63%) of respondents affirmed having made at least some changes in their diet after receiving genetic feedback ("likely agree", 50%; "agree", 13%). The responses "disagree", "likely disagree" and "unsure" made up 21%, 4%, and 8% of the total, respectively. This question was left unanswered by 4% of the respondents (Figure 11).

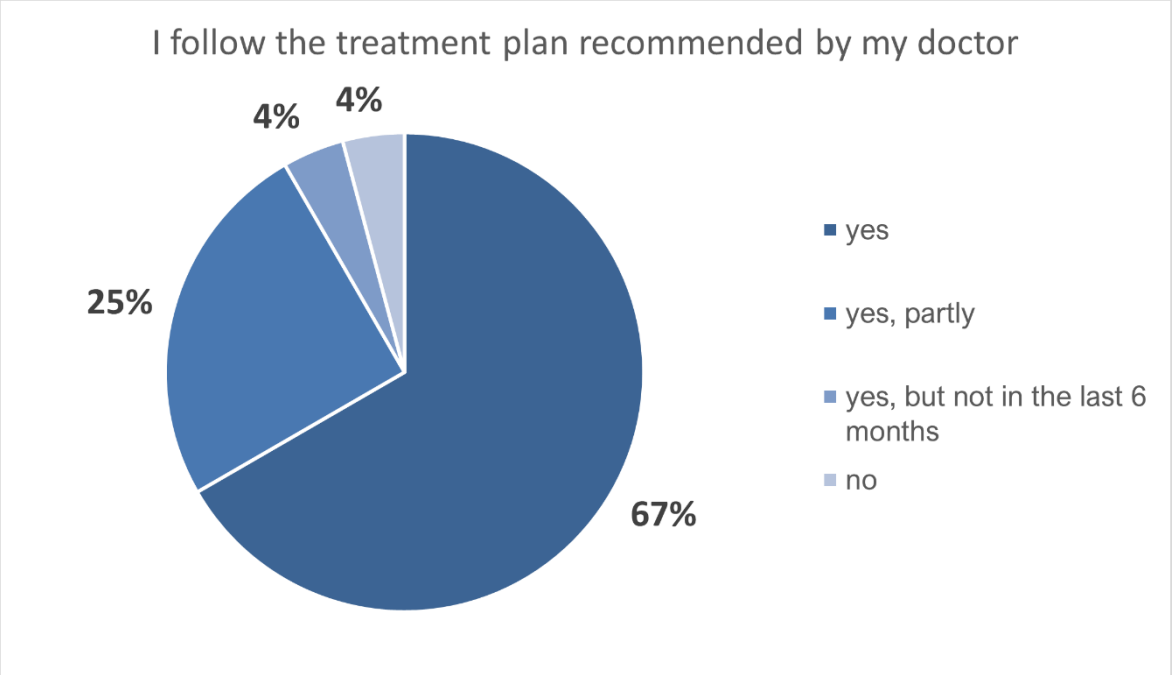

Figure 10. Recalled participants' self-reported assessment of their adherence to the treatment plan proposed by their doctor (n=24).

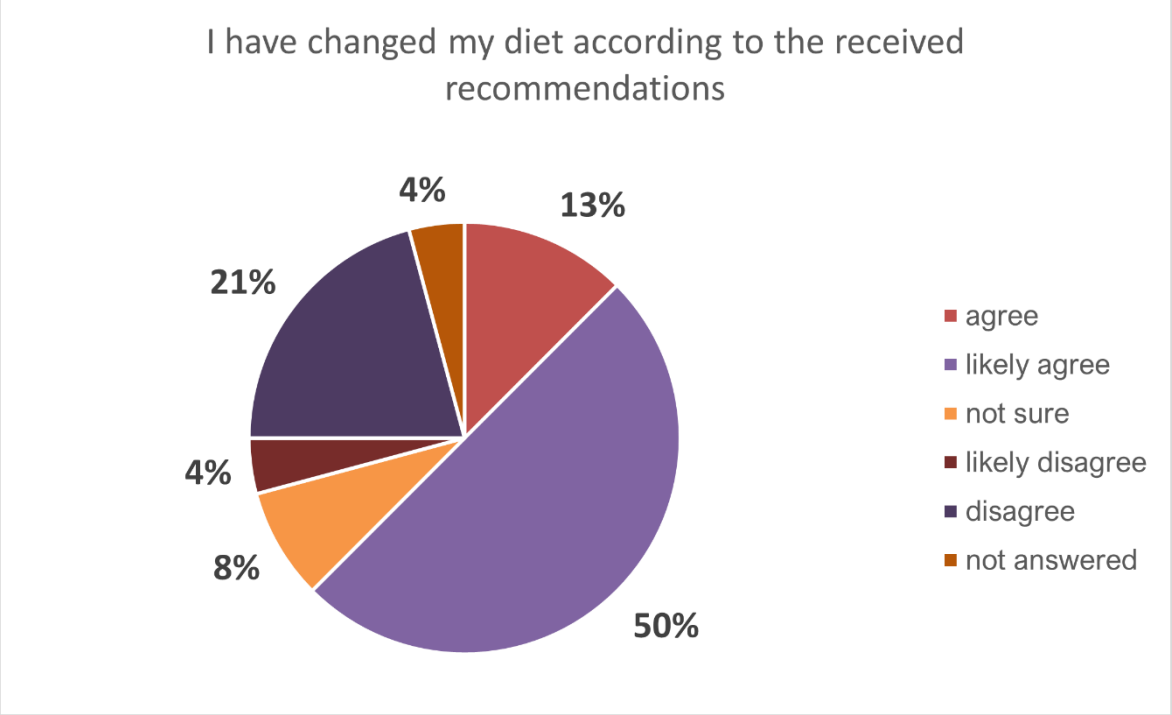

Figure 11. Overview of the participants' feedback to the question 'How have you changed your habits after receiving information on the genetic variant you carry?' in regard to changes in diet (n=24).

Although the importance of LLT in the management of FH is not negligible (Cuchel *et al.*, 2014), treatment plans for this small cohort did not necessarily involve LLT alone or at all,

as dietary changes and various supplements could be tried for some time before or together with LLT prescription if patients' medical histories and clinical characteristics allowed for it. These results, along with responses indicating a poor understanding of the importance of LLT in general, may explain several participants' indications that they did follow their treatment plans while having poor LLT adherence.

Additionally, participants' answers to questions about how they changed their smoking habits, physical activity level, and with whom they shared the information about their carrier status are outlined in Appendix II.

3.2.3 ICD-10 diagnosis codes, drug prescriptions, and pharmacogenomics

The most relevant FH-associated findings were derived from a synthesis of the ICD-10 diagnosis codes, drug prescription registry, and pharmacogenomic data of the study participants and have been summarised in Table 3. The recalled cohort had significantly more diagnoses of lipoprotein metabolism disorders (ICD-10 E78*) and pure hypercholesterolemia (ICD-10 E78.0; the same code used for FH diagnosis in Estonian clinical practice) than the non-recalled group (94.1% vs. 67.0%, $p<0.001$; and 82.4% vs. 46.0%, $p<0.001$, respectively). Furthermore, the recalled group contained significantly more LLT users than the non-recalled group (79.4% vs. 53.3%, $p<0.005$). Statin-induced myopathy risks were similar in the recalled and non-recalled participant groups (higher than normal, 29.4% and 28.5%, respectively; much higher than normal, 2.9% and 2.4%, respectively).

Significantly larger proportions of the recalled cohort than the control group were diagnosed with pure hypercholesterolemia (ICD-10 code E78.0) and E78* in general. The same applied to LLT prescription. Consistent with the conclusions of Alver *et al.* (2018), these findings indicate that FH remains underdiagnosed and undertreated in Estonia. Despite the recalled participants' relatively low survey assessment of follow-up consistency and access to healthcare, these findings also indicate that the return of genetic results and the associated feedback visits improved the participants' visibility in the Estonian healthcare system and access to FH treatment compared with the control group, whose disease severity should match that of the recalled cohort.

Participants in the recalled cohort also reported very few potential FH complications, such as myocardial infarction and stroke, which may be attributable to their early receipt of hypercholesterolemia-related diagnoses and statin prescription when disease progression could still be stalled. However, further investigations would be necessary to assert this hypothesis.

Table 3. Principal FH-related findings. The statin risk warning is applicable only for simvastatin, atorvastatin, and rosuvastatin. *ICD-10 codes were reduced to three characters, with each code counted only once for each individual.

	RbG participants n=34	**Controls n=291**	**p-value (<0.005)**
All diagnoses* (excluding Z codes)	34 people with 1413 diagnosis codes – on average 41.6 per person	291 people with 11857 diagnosis codes – on average 40.7 per person	
Participants with E78 diagnosis code (including all subsets)	32 (94.1%)	195 (67.0%)	<0.001
Participants with E78.0 Pure hypercholesterolemia diagnosis code	28 (82.4%)	134 (46.0%)	<0.001
Users of any lipid-lowering medication	27 (79.4%)	155 (53.3%)	<0.005
Statin side effect (myopathy) risk assessment according to genotype			
- Normal risk	23 (67.6%)	201 (69.1%)	
- Higher risk	10 (29.4%)	83 (28.5%)	
- Much higher risk	1 (2.9%)	7 (2.4%)	

About 30% of all participants in this study were at a greater than normal risk of developing myopathy as a side effect of statin use; about 3% of participants had genotypes corresponding to a risk much higher than normal. As myopathy is the most predominant statin-related adverse effect and a major reason for poor LLT adherence (Mach *et al.*, 2018), the results presented here suggest the role of pharmacogenomic predisposition in LLT adherence and highlight the value of pharmacogenomic profiling for adherence improvement.

However, the CPIC guidelines are being updated alongside continuous refinements in scientific knowledge (Cooper-DeHoff *et al.*, 2022), and more comprehensive genotypes, including variants from CYP2C9 and ABCG2 genes and additional recommendations for all statins, will replace the corresponding part of the EstBB pharmacogenomics predictions pipeline in the nearest future. Using the same approach as a matter of principle and the scripts developed here to visualize the individual timelines of disease-related landmarks, pharmacogenomic profiles, and treatment adherence, a solid basis has been created to develop a practical and dynamic application for everyday medicine.

LLT adherence was explored to evaluate the consistency of LLT purchase and to expose any potential gaps in treatment based on how long the prescribed tablets should have lasted in case of continuous administration (Figure 12). It is noticeable that most of the participants continued treatment with the medication that was first described to them (59.3%). Of all participants on LLT, atorvastatin was prescribed at least once to 70.4%, rosuvastatin to 63.0%, and the combination LLT therapy (rosuvastatin + fenofibrate or rosuvastatin + ezetimibe) to 14.8%.

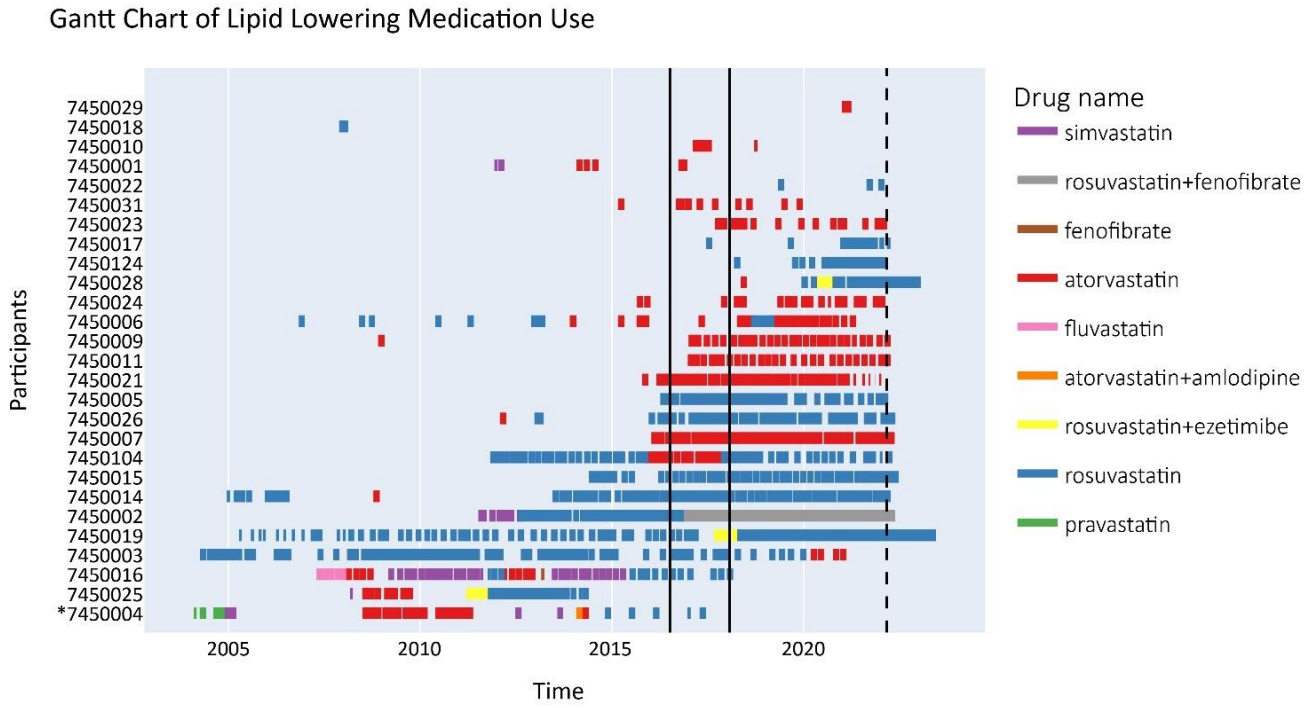

Figure 12. Gantt chart depicting lipid-lowering treatment for recalled participants from 2004 until 2022 (n=27). The time period between the two solid black lines signifies the period of RbG pilot visits (2016-2018) in the Alver *et al.* (2018) study. The dashed line signifies the end of the drug prescription registry follow-up. The lines crossing the end of follow-up indicate purchase of a significant stock of medication in advance. Drug name on this graph indicates the active substance of the prescribed LLT, not the brand name. Participant #7450004 passed away in 2018 (denoted by *).

The widespread use of atorvastatin within the study cohort is not surprising, as it was the best-selling drug in the early 2000s (Kogawa *et al*., 2019) and has remained one of the most popular lipid-lowering medications in more recent years (Pijlman *et al.*, 2010; Casula *et al.*, 2016). Additionally, rosuvastatin was among the most popular LLT medications in the study carried out by Casula *et al*. (2016).

The LLT users were categorized in three groups characterized by:

i) poor overall adherence (participants #7450029 - #7450024 in the order listed on Figure 1; n=11);

ii) consistent LLT use at the feedback visit or started shortly after the feedback (participants #7450006 - #7450019; n=13);

iii) consistent LLT use for ≥2 years before the feedback visits but had, for various reasons, stopped doing so (participants #7450003 - #7450004; n=4).

Treatment adherence varied among the small cohort of recalled participants on LLT; the largest proportion of these participants began using lipid-lowering medications consistently during or shortly after their feedback visits, but a second group of roughly the same size had poor overall LLT adherence. The small sample of LLT users may have resulted in greater variance than would likely be present in the general population. However, given the generally poor LLT adherence reported in other studies (Phan *et al.*, 2014; Casula *et al.*, 2016) and several of our participants' clear improvement in LLT adherence (e.g., #7450009) or consistent LLT use right after the feedback visit (e.g., #7450011), it appears reasonable to speculate that the EstBB intervention had a positive impact on LLT adherence. The full scale of this effect would need to be explored with larger cohorts.

The third group of LLT users had periods of consistent (≥2 years) adherence prior to the feedback visits but stopped LLT entirely before 2022. This group contains participants who changed LLT medication at least once; all except one had tried at least three different forms of LLT. Treatment discontinuation was recorded for one participant who died in 2018. Other known reasons for LLT discontinuation were subsequent pregnancies and breastfeeding for a young female participant (although she was found to have remarkably high total cholesterol and LDL-C levels in 2016), and another participant reported myopathy-related side effects that persisted despite several changes in her LLT treatment plan over the years. It is noteworthy that this participant's pharmacogenomic profile did also indicate a higher risk of myopathy as a statin use side effect.

3.2.4 Lipid measurement results

Although LDL-C values are major indicators of LLT use success and overall progress of the disease, the results regarding LDL-C and total cholesterol values remain inconclusive in this study. The linked EHRs (not including results measured during the RbG pilot study) provided measured total cholesterol values for 153 EstBB participants (121 controls and 32 recalled participants) and LDL-C values for 122 study participants (89 controls and 33 recalled participants). Otherwise put, 41.6% and 30.6% of controls had had their total cholesterol and LDL-C values measured, respectively; the same proportions are 94.1% and 97.1%, respectively, for former RbG participants. While the significant difference in favor of RbG

participants could be taken to mean a better follow-up and visibility post feedback visits, it is noteworthy that there were 155 controls on LLT and only 89 had LDL-C measurements available from automatically retrievable linking data from EHRs. As only two controls had been prescribed LLT on other indications than an E78* ICD-10 diagnosis code, it stands to reason that almost all of the controls on LLT should have had at least one LDL-C value measured prior to 2022. Thus, it is likely that not all lab analyses have been made available through EHRs and that the lipid measurement results presented here are inconclusive. It can be hypothesized that this may be due to a discord between results being submitted through the official lab analyses registry versus results only being recorded as plain text in epicrises.

When also including the LDL-C values measured during the RbG visits, the average and median LDL-C values for both recalled participants and controls are nearly the same – on average, the control group had an LDL-C value of 4.91 mmol/L (median 4.80 mmol/L) and the recalled group 4.94 mmol/L (median 4.87 mmol/L). Taking into account the small sample sizes and potentially missing data, it is unlikely that these values accurately reflect the impact of the RbG visits but do highlight the likelihood that neither of the study groups has reached the suggested target LDL-C levels.

SUMMARY

In the course of this thesis, a follow-up study was conducted to the FH RbG pilot study first carried out at the EstBB in 2016-2018, with the main goal to gauge the impact of the recalls on the health and handling of the participants in the Estonian healthcare system 4-6 years post-recall. The follow-up was performed for two groups: 34 formerly recalled pilot study participants as cases and 291 biobank participants with the same genetic profile as controls. The consequences of the FH RbG approach were dissected through the following aspects:

- A survey was conducted for recalled participants. 75% of all available RbG participants responded and the overall reaction was clearly positive. On average, the respondents gave a rating of 4.96/5 when asked whether it had been the right decision to attend the recalls, 4.92/5 when asked if they were able to cope with the genetic results, and all agreed that they would make the choice to attend the recalls again. Most respondents affirmed not having had hypercholesterolemia-related health complications post-recall.
- ICD-10 diagnosis codes showed a significantly higher percentage of RbG participants with documented E78.0 diagnosis than controls (82.4% vs. 46.0%).
- Drug prescription registry data showed a significantly higher proportion of RbG participants receiving LLT than controls (79.4% vs. 53.3%).
- A Gantt chart illustrating the drug prescription registry data was created to describe LLT adherence amongst RbG participants and found variable adherence within the small cohort.
- The pharmacogenomics dataset showed that nearly a third of the study participants had higher risk than normal of developing statin-induced myopathy as a side effect, suggesting that more personalized profiles could help improve LLT adherence.
- The LDL-C values from the lab analyses registry proved inconclusive and registry linkage outputs likely need additional data retrieval approaches to fill the gaps.

The recalls were received positively by the biobank participants and have appeared to have raised the participants' visibility in the Estonian medical system, but a larger scale study would be needed to solidify the results presented here. Personalized pharmacogenomic profiles could potentially help to improve LLT adherence. In conclusion, it is likely that FH currently remains underdiagnosed and undertreated in Estonia, but both the related scientific developments and substantial systematic changes in medical practices are ready here to turn a new page.

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APOB apolipoprotein B [Homo sapiens (human)]. https://www.ncbi.nlm.nih.gov/gene/338 (12.05.2022)

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St Jude Children's Research Hospital. ProteinPaint. https://proteinpaint.stjude.org/ (15.05.2022)

Appendix

I. Survey questionnaire

Dear study participant!

In 2016-2018, you took part in an earlier scientific study conducted by the Estonian Genome Center of the University of Tartu, „Defining the familial hypercholesterolemia phenotype and subsequent processing in a clinical setting when a genetic finding has been detected in a genome-wide study". In the course of the project you attended a cardiologist's consultation and at the end of the study we shared with you the results of our findings.

This questionnaire is a continuation of the aforementioned study. By answering this questionnaire, you would provide us with valuable information regarding how you assess now the information you received during the project and whether there have been any notable changes in your health behaviours and habits due to this knowledge. Please answer the following questions by marking the answer you find most suitable. Some questions may already be familiar to you, but do not let this disturb you. We would ask you to mark the answers according to how you are feeling right now.

Please recall your earlier decision to receive personalized genetic feedback. Please mark below to what degree you agree with the following statements, by ticking the answer you find most suitable. Please put down an answer for all the statements.

It was the right decision	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
I regret the choice that was made	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
I would go for the same choice if I had to do it over again	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
The choice did me a lot of harm	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
The decision was a wise one	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
I am able to cope with having this genetic finding in my family	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
I wish I would have been informed earlier about the genetic finding and the potential health risks	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐

The information received has somehow changed my life	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
I now have better access to health care / specialists	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐
I feel that my treatment and/or condition has improved	Agree ☐ Slightly agree ☐ Slightly disagree ☐ Disagree ☐ Unsure ☐

Have you shared information on your genetic finding with anybody?

Spouse/partner	Yes ☐	Not yet, but planning to ☐	No ☐	Not possible ☐
Children	Yes ☐	Not yet, but planning to ☐	No ☐	Not possible ☐
Siblings	Yes ☐	Not yet, but planning to ☐	No ☐	Not possible ☐
Parents	Yes ☐	Not yet, but planning to ☐	No ☐	Not possible ☐
Physician	Yes ☐	Not yet, but planning to ☐	No ☐	
Other _____	Yes ☐	Not yet, but planning to ☐	No ☐	

If you shared information regarding your genetic finding, what was the response?

_____

If you are not planning to share information regarding the genetic finding with anyone, what is the main reason for doing so?

_____

Which of the following has occurred to you since taking part of the genome center's project?

high cholesterol was detected	Yes ☐	No ☐	Unsure ☐
cardiovascular disease was diagnosed	Yes ☐	No ☐	Unsure ☐
experienced significant vertigo and balance problems	Yes ☐	No ☐	Unsure ☐
experienced arrythmia	Yes ☐	No ☐	Unsure ☐
experienced chest pain after strenuous exercise	Yes ☐	No ☐	Unsure ☐
received a myocardial infarction diagnosis	Yes ☐	No ☐	Unsure ☐
received a stroke diagnosis	Yes ☐	No ☐	Unsure ☐

Which of the following activities have you done since participating in genome center's familial hypercholesterolemia project? Please respond to each of the statements by marking the most appropriate answer.

I have recommended my family members to get a doctor consultation

Yes ☐ Not yet, but planning to ☐ No ☐

I am being monitored by my family physician or a specialist doctor regarding familial hypercholesterolemia.

Yes, I have seen a doctor regarding this matter within the past year ☐

Yes, but it has been more than a year yet less than two years since the last visit ☐

No, I have not seen a doctor regarding this matter within the past two years ☐

No, I have not seen a doctor regarding this matter since the participating in the project ☐

No, I have not seen any doctors since the project ☐

I follow the treatment-plan recommended by my doctor

Yes ☐

Yes, partially ☐

Yes, but not within the past 6 months ☐

No, I stopped more than 6 months ago ☐

No ☐

How would you rate your experience with the healthcare received in regard to your genetic finding?

Access to care	Very good ☐	Good ☐	Satisfactory ☐	Unsatisfactory ☐	Unsure☐
Consistency of follow-up	Very good ☐	Good ☐	Satisfactory ☐	Unsatisfactory ☐	Unsure☐
Clarity of recommendations	Very good ☐	Good ☐	Satisfactory ☐	Unsatisfactory ☐	Unsure☐
Access to medications	Very good ☐	Good ☐	Satisfactory ☐	Unsatisfactory ☐	Unsure☐

What did you appreciate the most?

_____

_____

What has been the greatest shortcoming?
_____
_____

How have you changed your habits after receiving information on the genetic variant you carry?

Smoking	Increased	☐
	Decreased	☐
	Same	☐
	Unsure	☐
	Never smoked	☐
	Quit smoking	☐
Physical activity	Increased	☐
	Decreased	☐
	Same	☐
	Unsure	☐

I have changed my diet according to the received recommendations

Agree	☐
Slightly agree	☐
Slightly disagree	☐
Disagree	☐
Unsure	☐

Other
_____

Where have you sought additional information from? Please mark all that apply.

Asked my physician ☐ From the Internet ☐ Other _____

I have not sought additional information ☐

Do you have any recommendations on what the genome center could have done differently during the familial hypercholesterolemia project?

_____

_____

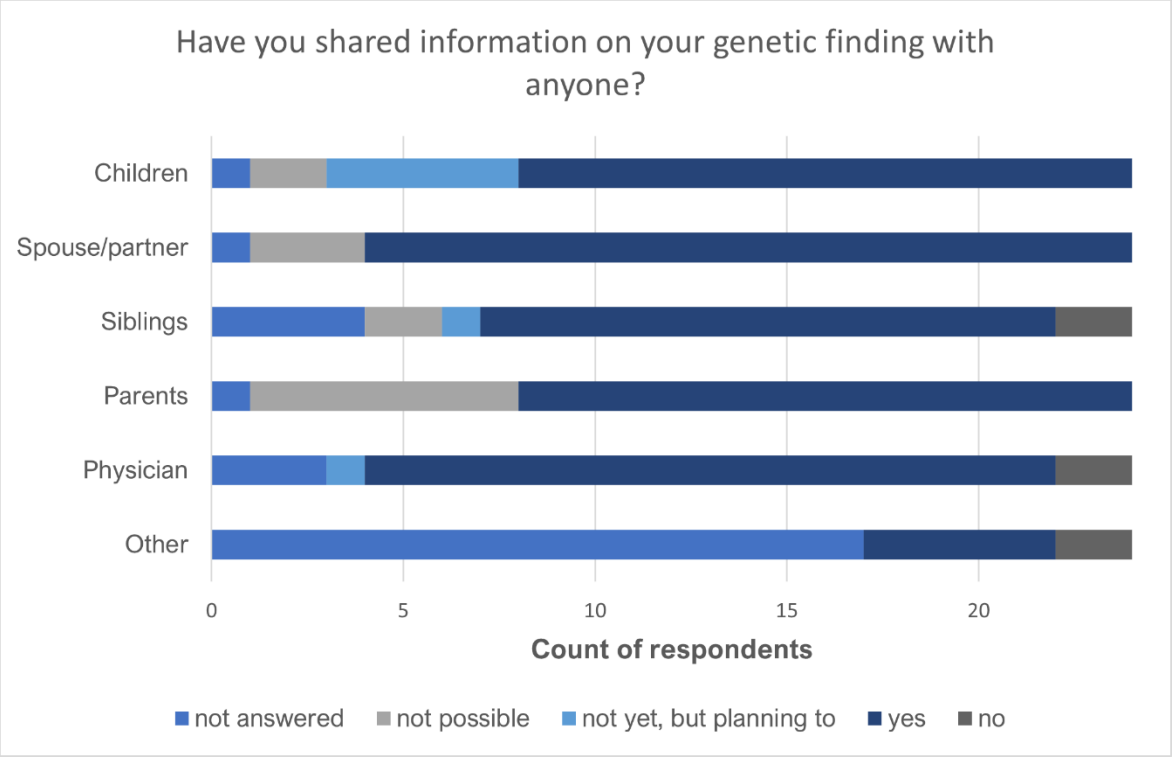
Do you have any recommendations or suggestions on what could be done differently in the current health care system when a person with a familial hypercholesterolemia genetic variant turns to a doctor?

_____

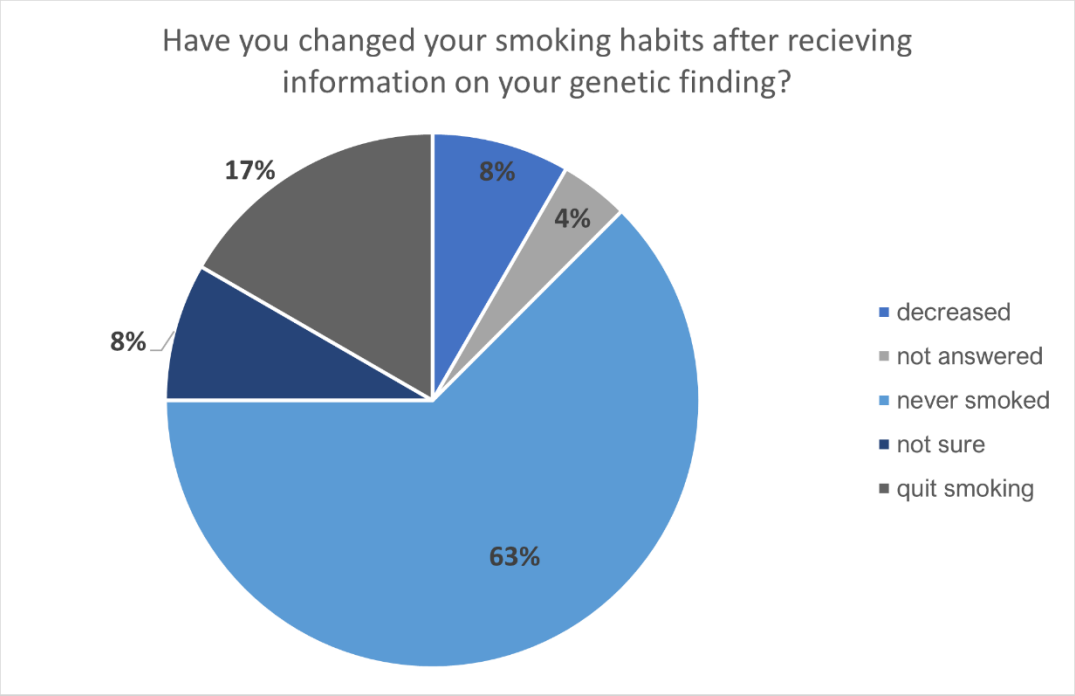
_____

Thank You!

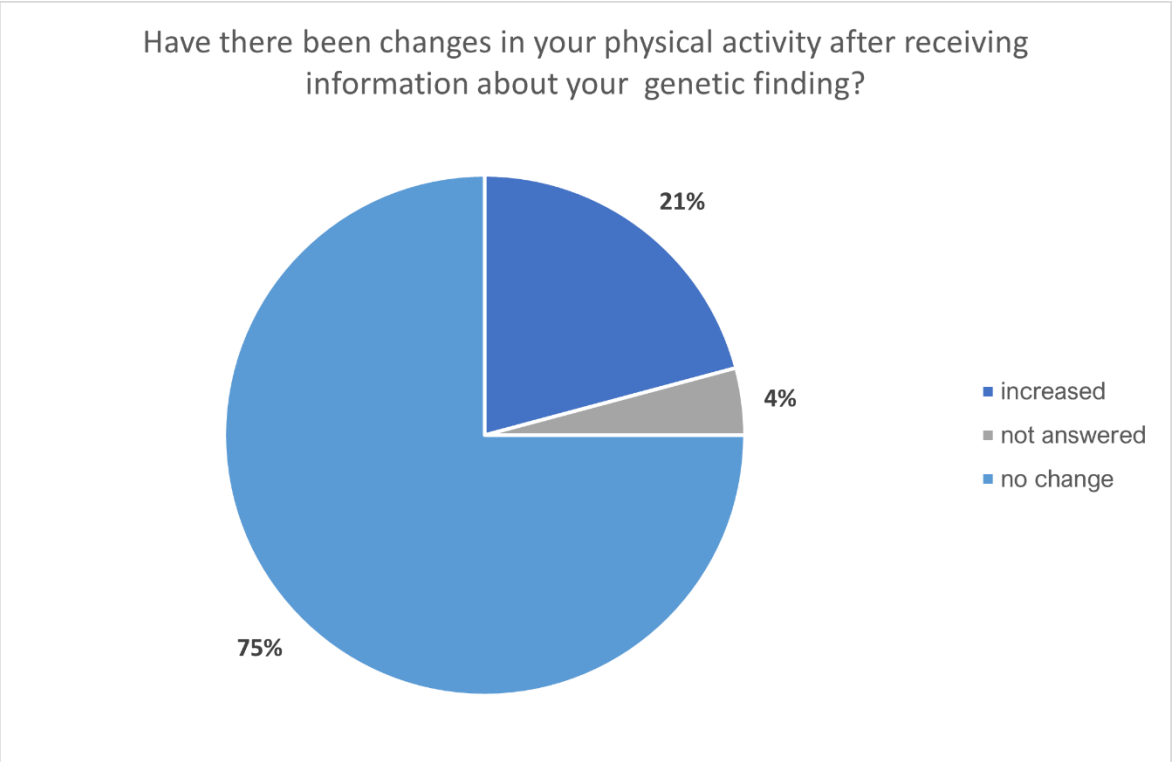
II. Supplementary Figures



Supplementary Figure S1. Overview of participants' feedback about whether they have shared knowledge about their genetic finding and with whom (n=24).



Supplementary Figure S2. Overview of participants' feedback on how their smoking habits have changed after receiving genetic feedback (n=24).



Supplementary Figure S3. Overview of participants' assessment on how their physical activity level has changed after receiving genetic feedback (n=24).

III. Pharmacogenomic profiles of RbG participants on LLT

The profiles were created to give an overview of the participants LLT adherence and related factors (general patient information, genetic finding, relevant health information, clinical analyses, statin side effect risk). TC – total cholesterol, LDL – low-density lipoprotein. [1]original total cholesterol and LDL values from Alver et al (2018)'s study were recalculated for statin users in the follow-up study (divided by 0.8 for total cholesterol and by 0.7 for LDL); [2]methodology described in Reisberg et al (2019).

Participant	Sex (M/F) and Age (in 2022)	dbSNP reference number (rs)	Gene	TC (mmol/l) (2016)[1]	LDL (mmol/l) (2016)[1]	Treatment adherence profile	Statin side effect risk assessment[2]	Health information (FH-related ICD-10 codes)	Feedback date
7450001	M39	rs5742904	APOB	7.4	5.9	Inconsistent treatment adherence	Normal risk of myopathy	E78.0 and E78.9 in 2011, R00 in 2016	09-2016
7450002	M61	rs5742904	APOB	6.6	5.4	Good treatment adherence - pre- and post-study	Higher risk than normal of myopathy	I10 in 2006, E78.0, I11 and I11.0 in 2011, E78 in 2015, I25.1, I11.9, I20.8, E78.2 in 2016, E78.9 in 2019	07-2016
7450003	F55	rs761954844	LDLR	6.8	4.5	Good treatment adherence - pre- and post-study	Normal risk of myopathy	E78.0 in 2001, E78.4 in 2004, Z13.6 and K80.8 in 2005, E78.2 in 2006, I20.8 in 2008, E78.5 in 2014	09-2016
7450004	M82 (deceased in 2018)	rs5742904	APOB	8.2	6.0	Good treatment adherence for at least a 2 year period, adherence irregular after 2012	Normal risk of myopathy	I50 in 1956, I22 in 1981, I25.1 in 2003, E78, I11.0, I25.2, I11.9, I50.1 in 2004, K80.5 in 2006, E78.2, I50.0 in 2007, E78.1 in 2012, I70.0 in 2013, I48 in 2014, I25 in 2018	07-2016
7450005	F47	rs5742904	APOB	7.0	5.0	Good treatment adherence - pre- and post-study	Normal risk of myopathy	I47.9, E78 in 2010, E78.0 in 2016	11-2016
7450006	M34	rs761954844	LDLR	10.9	9.2	Adherence from poor to good in the past few years	Normal risk of myopathy	E78.0 in 2004, E78.2 in 2013, I25.2 in 2018	09-2016

7450007	M43	rs28942079	LDLR	7.5	6.3	Good treatment adherence - pre- and post-study	Normal risk of myopathy	E78.0 in 2012, E78 in 2014, E78.2, Z03.5 in 2016,	09-2016
7450009	M60	rs5742904	APOB	8.9	6.3	Good treatment adherence post study	Normal risk of myopathy	E78.0 in 2008, I25.1 in 2016	12-2016
7450010	F51	rs5742904	APOB	6.6	4.5	Poor treatment adherence pre- and post-study	Higher risk than normal of myopathy	E78.2 in 2008, E78.0 in 2016,	01-2017
7450011	M41	rs5742904	APOB	6.4	5.0	Good treatment adherence post-study	Normal risk of myopathy	I10 in 2003, E78.2 in 2016	12-2016
7450014	M80	rs754536745	LDLR	5.3	3.9	Good treatment adherence - pre- and post-study	Normal risk of myopathy	E74 in 2004, I11, I11.9, E78.0, I11.0, I50.1, E78.8 in 2008, I50.9, I20.8, I35.8, I35.0, I25, I20 in 2009, I25.1 in 2016	06-2017
7450015	F53	rs5742904	APOB	5.9	4.0	Good treatment adherence for periods of time with large gaps in-between	Normal risk of myopathy	E78.5 in 2013, I10 2014, E78.0 in 2016	06-2017
7450016	F85	rs5742904	APOB	10.0	9.1	Good treatment adherence - pre-occurrence of side effects (myopathy). Declined treatment after as per her own words.	Higher risk than normal of myopathy	E78.0, E78 in 2007, I11.9 in 2009, I10 in 2012	06-2017
7450017	M55	rs5742904	APOB	7.0	5.7	Poor adherence	Normal risk of myopathy	I49, K80 in 2003, I10, I11.9 in 2009, E78.0 in 2016, E78 in 2019	05-2017

7450018	F61	rs5742904	APOB	11.0	9.1	Poor adherence	Higher risk than normal of myopathy	E78.0 in 2005, E78 in 2007, E78.0 in 2016, I78.8 in 2021	05-2017
7450019	F60	rs761954844	LDLR	9.9	5.8	Good treatment adherence - pre- and post-study	Higher risk than normal of myopathy	E78 in 2004, I11.9 in 2005, E78.0 in 2007, E78.4 in 2018,	09-2017
7450021	F73	rs5742904	APOB	5.8	4.2	Good treatment adherence - pre- and post-study, although with no-table gaps	Higher risk than normal of myopathy	K80 in 2003, I10 in 2004, I11, I11.0, I49, I50 in 2005, I20 in 2006, I11.9, I48 in 2007, I49.1, I49.3 in 2009, E98 in 2010, I20.3 in 2013, E78.2 in 2015, E78.0, I25.1 in 2016, I25.8 in 2019	06-2017
7450022	F38	rs148562777	PCSK9	7.5	5.1	Poor treatment adherence	Normal risk of myopathy	I49, I49.8 in 2011, E78.0 in 2016	05-2017
7450023	F47	rs5742904	APOB	7.2	5.2	Poor treatment adherence	Normal risk of myopathy	E78 in 2009, E78.2 in 2016, E78.0 in 2017	09-2017
7450024	F50	rs5742904	APOB	7.1	5.8	Inconsistent treatment adher-ence, but with visible improve-ment in later years	Normal risk of myopathy	E78.0 in 2009, E78.2 in 2015, I10 in 2017, E78.4 in 2019,	10-2017
7450025	38	rs5742904	APOB	12.7	10.4	Variable treat-ment adherence, stopped taking treatment alto-gether after hav-ing several subse-quent pregnan-cies and breast-feeding	Higher risk than normal of myopathy	E78, E78.2, E78.0 in 2008, E78.4 in 2021, LDL values over 10!	06-2017

7450026	M70	rs764042910	LDLR	8.0	5.5	Good treatment adherence overall, but with notable gaps	Normal risk of myopathy	E78.0 in 2007, I10, I11.9, E78.2 in 2009, I70.2 in 2016	09-2017
7450028	M53	rs5742904	APOB	7.6	5.5	Irregular treatment adherence post-study	Higher risk than normal of myopathy	E78 in 2009, E78.0 in 2015	09-2017
7450029	M37	rs121908038	LDLR	7.9	6.5	Poor treatment adherence	Normal risk of myopathy	E78.2 in 2017	10-2017
7450031	F62	novel	APOB	6.3	4.5	Inconsistent treatment adherence pre- and post-study	Normal risk of myopathy	I10 in 2004, I11.9 in 2006, E78.2 in 2013, E78.0 in 2017	09-2017
7450104	F57	rs5742904	APOB	6.5	4.8	Good treatment adherence, but with notable gaps	Normal risk of myopathy	I10 in 2010, I11.0, E78.0 in 2011, E78, I11, I11.9 in 2016	02-2017
7450124	F37	rs5742904	APOB	6.2	4.4	Poor adherence	Normal risk of myopathy	I10 in 2004, E78.0 in 2017, E78.2 in 2021	01-2018

The profiles are accompanied by the survey results overview in Appendix IV and medication use graphs in Appendix V.

IV. Selected survey results related to pharmacogenomic profiles

Participant #7450001

Would have preferred to know about their genetic results earlier. Has not recommended family members to consult a doctor. Unsure whether his treatment or health has improved. Follows the treatment plan partially. Considers medical help as good (accessibility, follow-up, recommendations, and medication access).

Participant #7450002

Would have preferred to know about their genetic results earlier. Has not recommended family members to consult a doctor. Unsure whether his treatment or health has improved. Follows the treatment plan partially. Considers medical help as good (accessibility, follow-up, recommendations, and medication access).

Participant #7450003

Would have preferred to know about their genetic results earlier. Unsure whether his treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help satisfactory (accessibility, follow-up, recommendations) and medication access good.

Participant #7450004

NA (deceased in 2018)

Participant #7450005

Would have preferred to know about their genetic results earlier. Tends to disagree that her treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (accessibility, recommendations and medication access) and follow-up good. greatest shortcoming is lack of awareness of FH.

Participant #7450006

Would have preferred to know about their genetic results earlier. Tends to agree that his treatment or health has improved. Plans to recommend family members to consult a doctor. Partially follows the treatment plan. Considers medical help as very good (accessibility, recommendations and medication access) and follow-up good. Considers his own motivation as the greatest challenge.

Participant #7450007

Would have preferred to know about their genetic results earlier. Tends to agree that her treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (accessibility, recommendations, medication access and follow-up).

Participant #7450009

Would have preferred to know about their genetic results earlier. Agrees that his treatment or health has improved. Has not recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (recommendations, medication access and follow-up) and accessibility as good.

Participant #7450010

Would have preferred to know about their genetic results earlier. Slightly disagrees that her treatment or health has improved. Has recommended family members to consult a doctor. Partially follows the treatment plan. Considers medical help good (accessibility, recommendations, medication access) and follow-up satisfactory.

Participant #7450011

Would have preferred to know about their genetic results earlier. Disagrees that his treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (accessibility, recommendations, medication access and follow-up).

Participant #7450014

Would have preferred to know about their genetic results earlier. Tends to agree that his treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as good (accessibility, and follow-up) of very good (recommendations, medication access).

Participant #7450015

Would have preferred to know about their genetic results earlier. Tends to agree that her treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as good (recommendations, and follow-up) of very good (accessibility, medication access).

Participant #7450016

Declined to fill out the survey

Participant #7450017

Did not fill out the survey

Participant #7450018

Did not fill out the survey

Participant #7450019

Would have preferred to know about their genetic results earlier. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as good (accessibility) or very good (recommendations, follow-up, and medication access).

Participant #7450021

Uncertain if would have preferred to know about their genetic results earlier. Disagrees that her treatment or health has improved. Has not recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as satisfactory (accessibility, follow-up, recommendations) or good (medication access).

Participant #7450022

Would have preferred to know about their genetic results earlier. Tends to agree that her treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (accessibility) or good recommendations, medication access, and follow-up).

Participant #7450023

Would have preferred to know about their genetic results earlier. Uncertain if her treatment or health has improved. Has recommended family members to consult a doctor. Partially follows the treatment plan. Considers medical help as very good (accessibility and medication access) or good (recommendations and follow-up).

Participant #7450024

Changed contact information

Participant #7450025

Would have preferred to know about their genetic results earlier. Uncertain if her treatment or health has improved. Has recommended family members to consult a doctor. Does not follow the treatment plan. Considers medical help as good (accessibility) or is unsure (recommendations, follow-up, medication access).

Participant #7450026

Unsure if would have preferred to know about their genetic results earlier. Unsure if his treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (accessibility, follow-up, recommendations, and medication access).

Participant #7450028

Would have preferred to know about their genetic results earlier. Tends to agree that his treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as good (accessibility, follow-up, recommendations, and medication access).

Participant #7450029

Would have not preferred to know about their genetic results earlier. Disagrees that his treatment or health has improved. Has recommended family members to consult a doctor. Followed the treatment plan at first. Considers medical help as very good (accessibility, follow-up, recommendations, and medication access).

Participant #7450031

Would have preferred to know about their genetic results earlier. Disagrees that her treatment or health has improved. Has not yet recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (medication access), satisfactory (follow-up, recommendations) or is unsure (access).

Participant #7450104

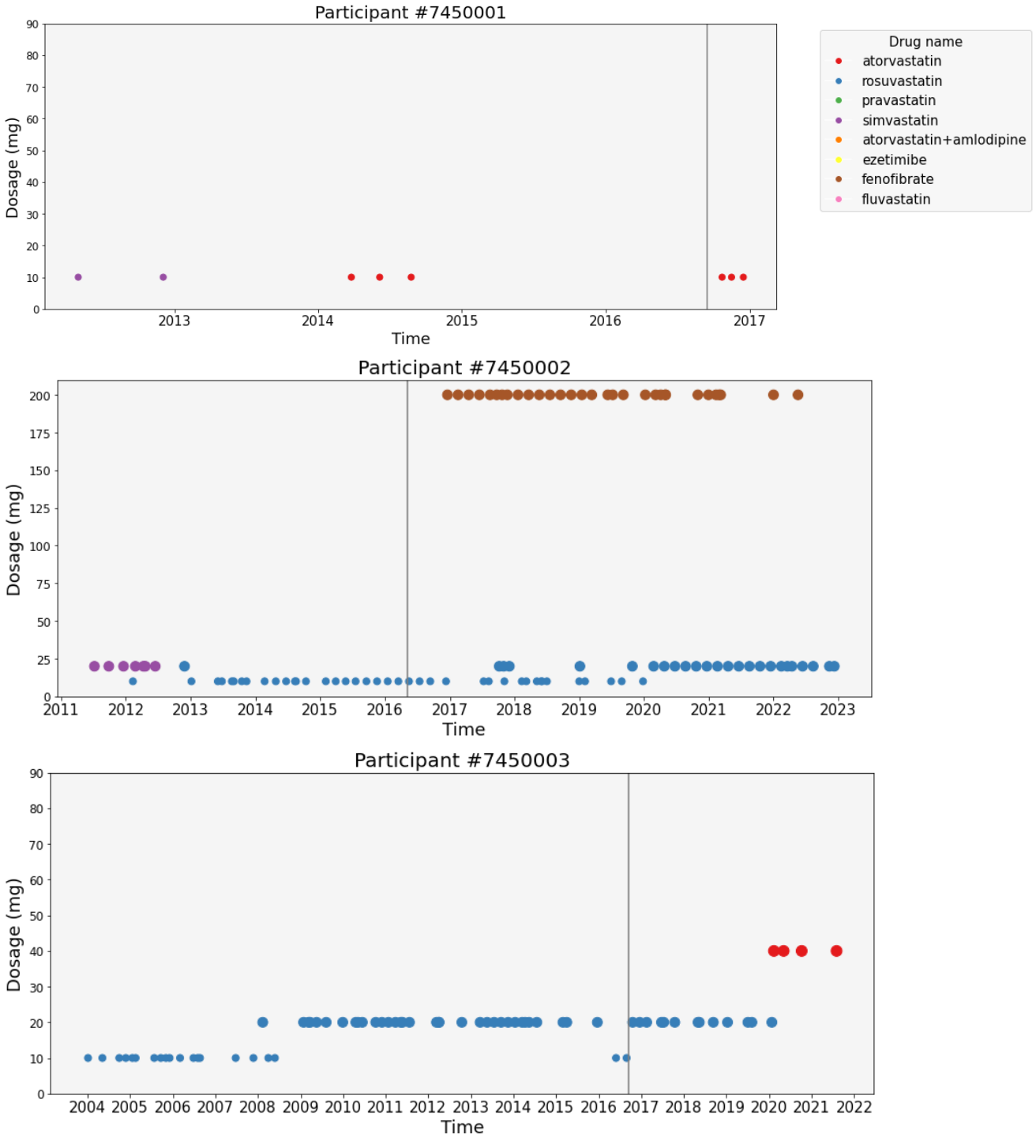
Would not have preferred to know about their genetic results earlier. Disagrees that her treatment or health has improved. Has recommended family members to consult a doctor. Follows the treatment plan. Considers medical help as very good (recommendations and medication access) or is unsure (accessibility, follow-up).

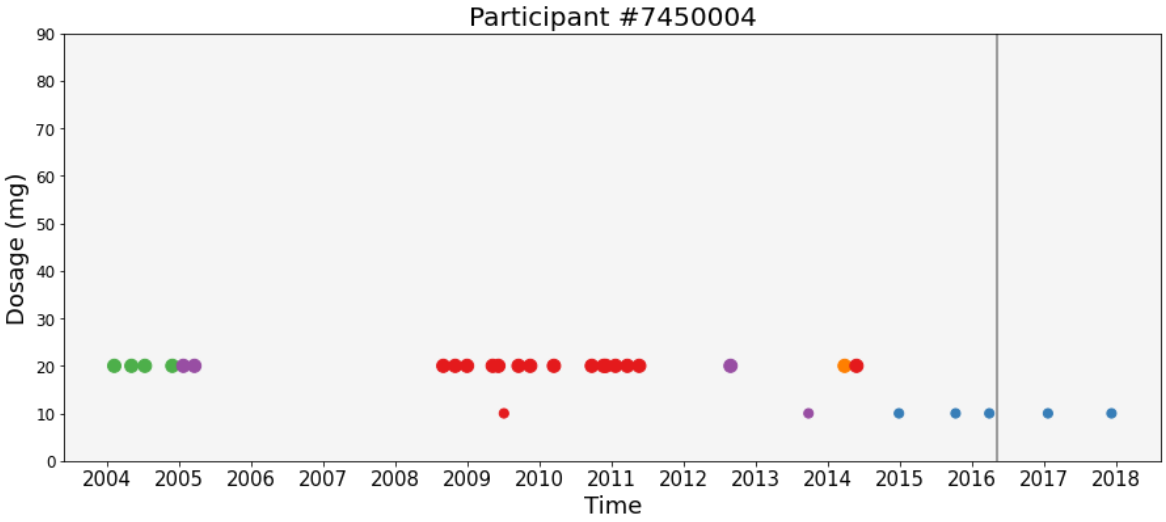
Participant #7450124

Did not fill out the survey

V. Medication Use Graphs

Medication use graphs were created in Python, using matplotlib. Legend is visible on the graph of participant #7450001. Grey vertical line denotes the date of the visit of genetic finding disclosure.

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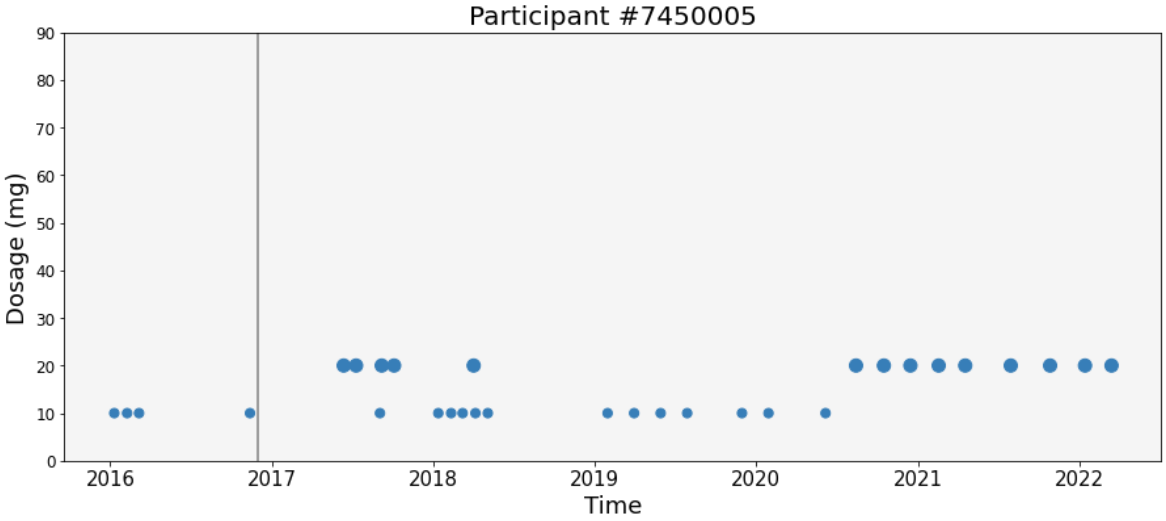
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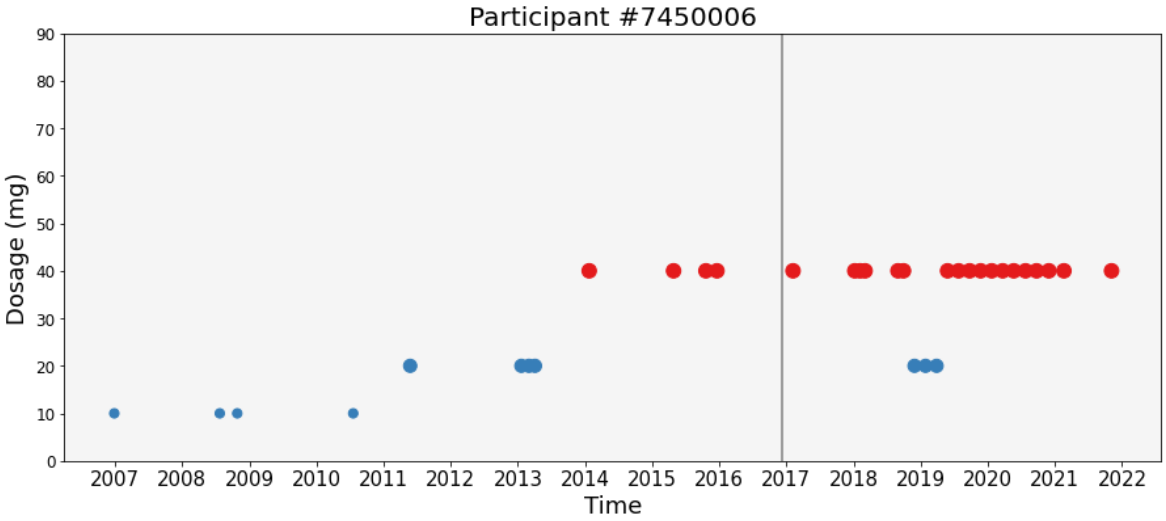
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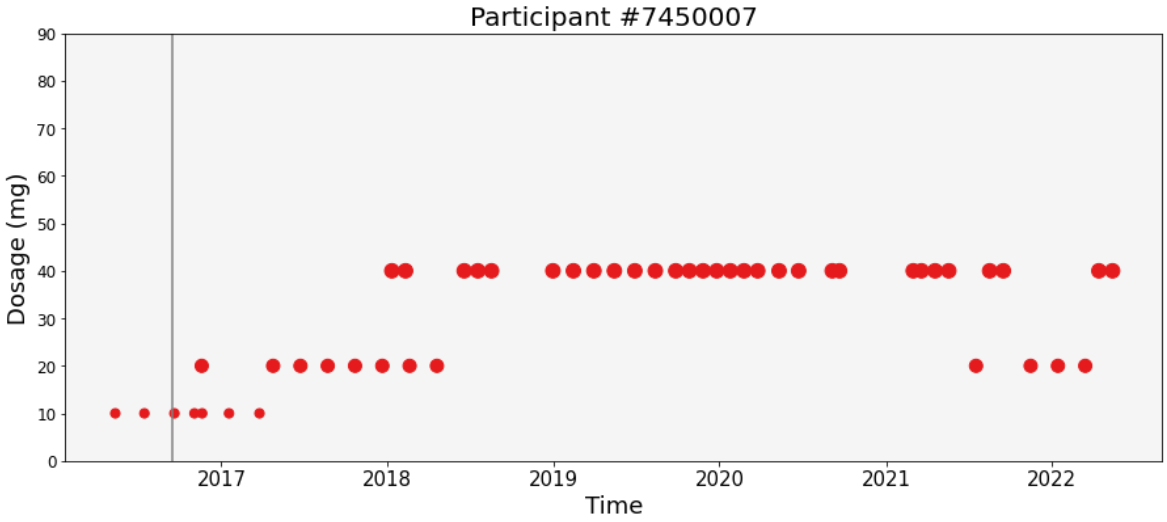
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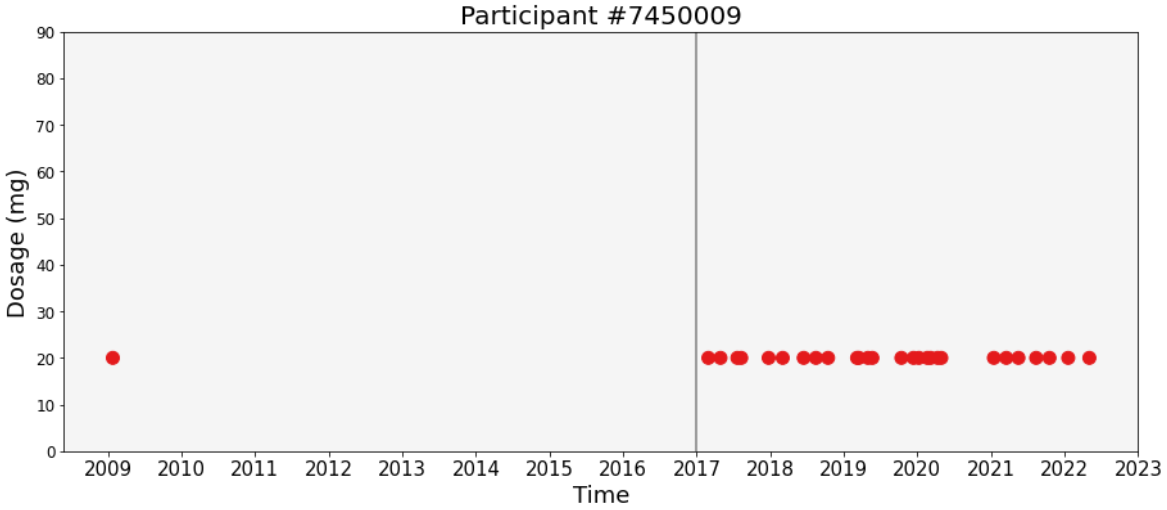
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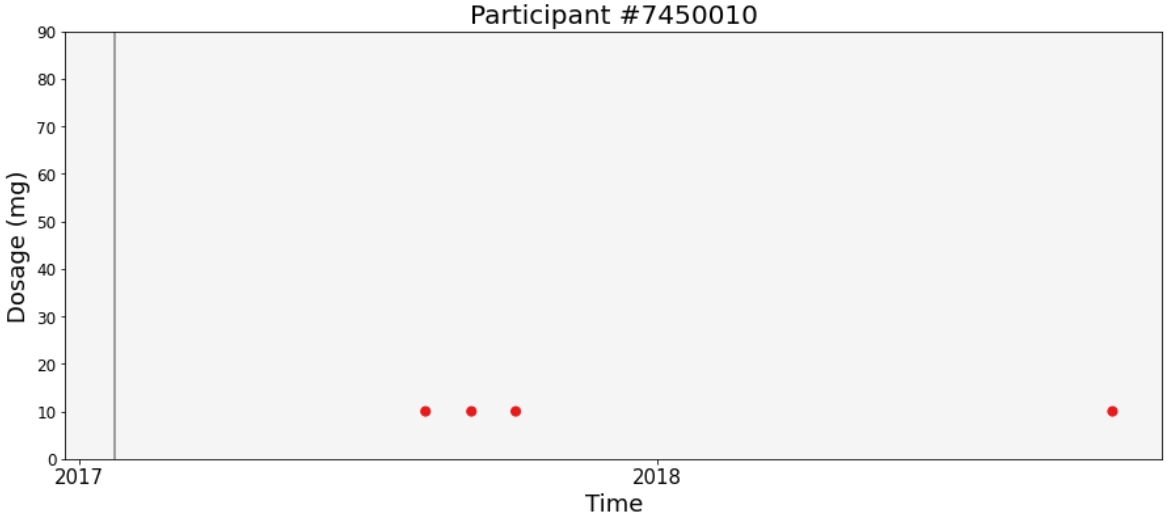
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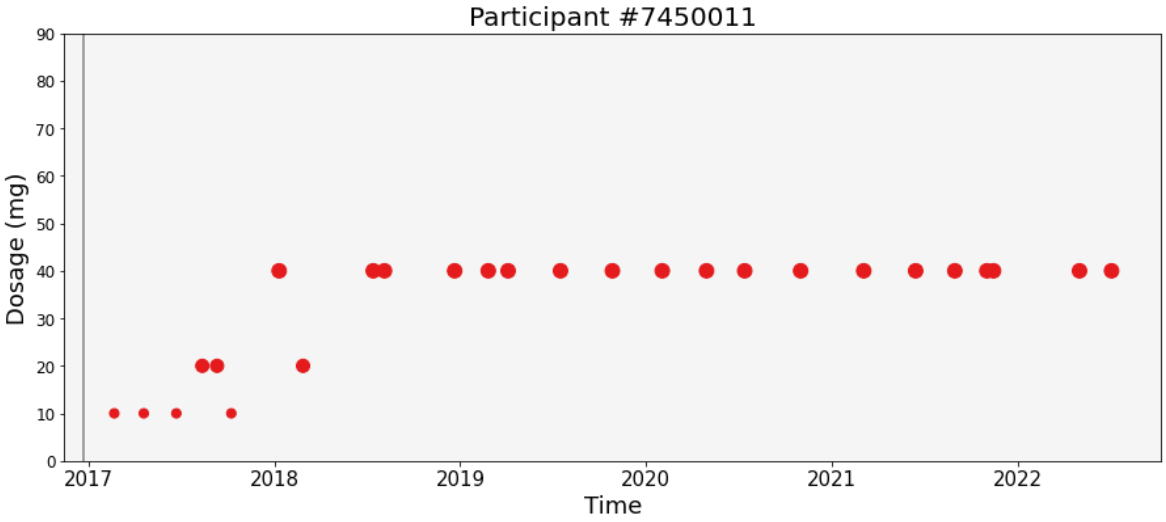
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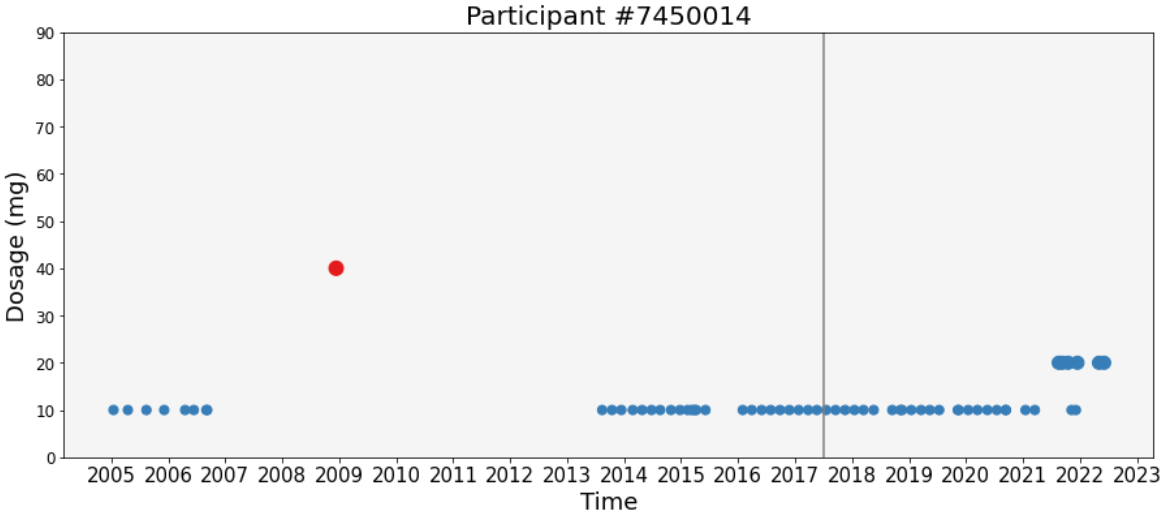
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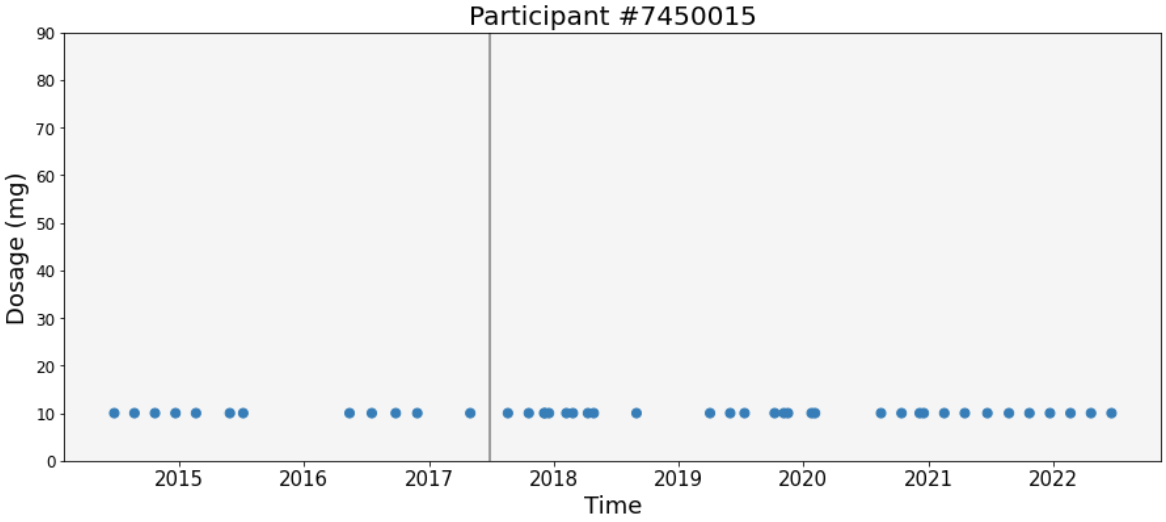
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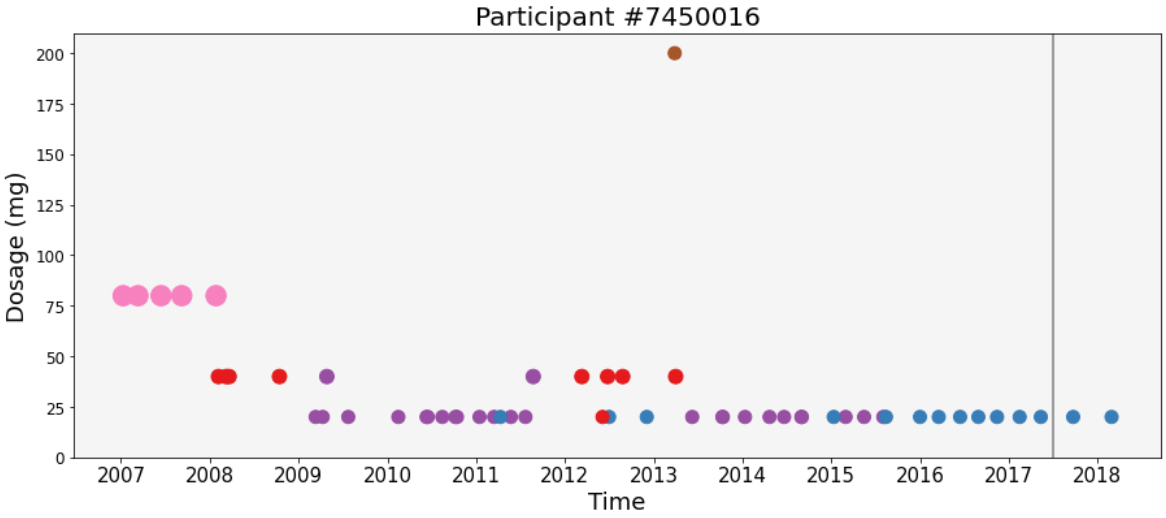
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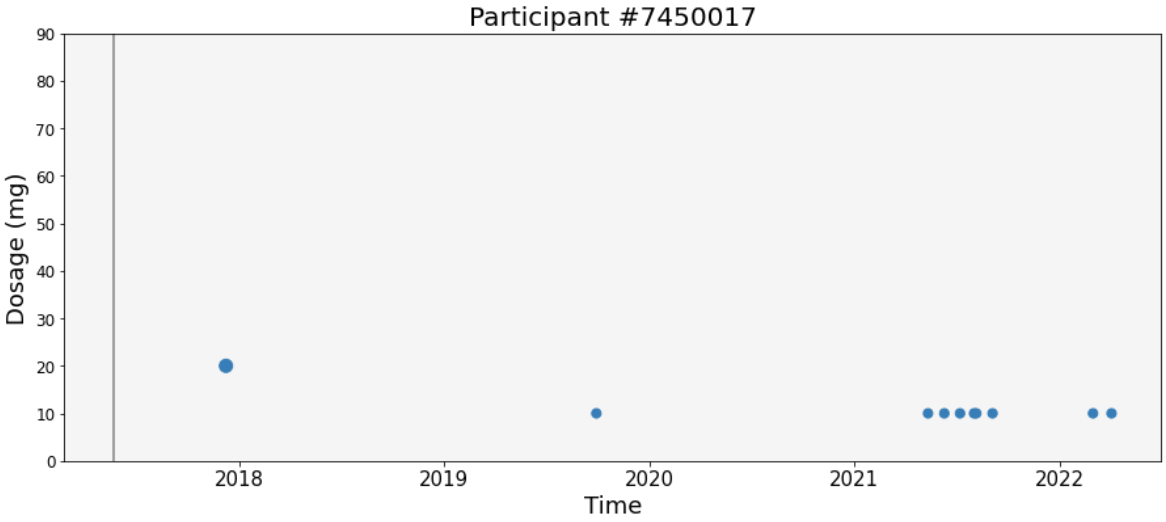
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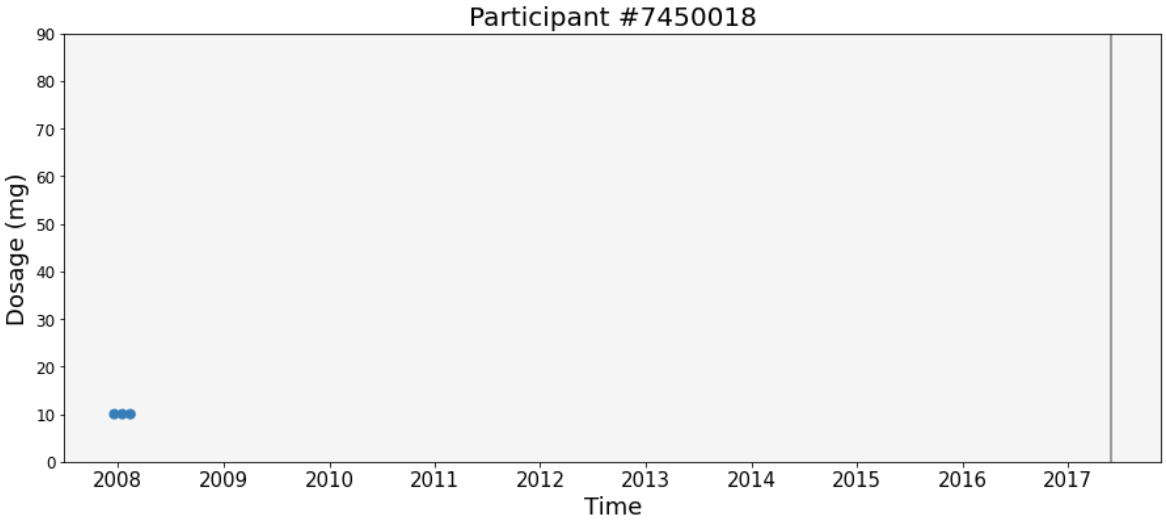
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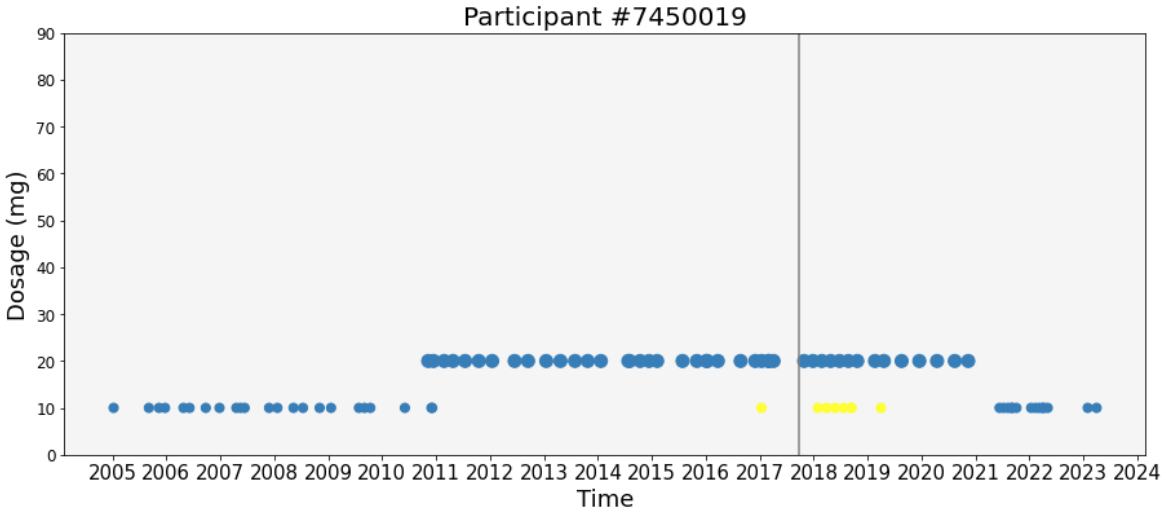
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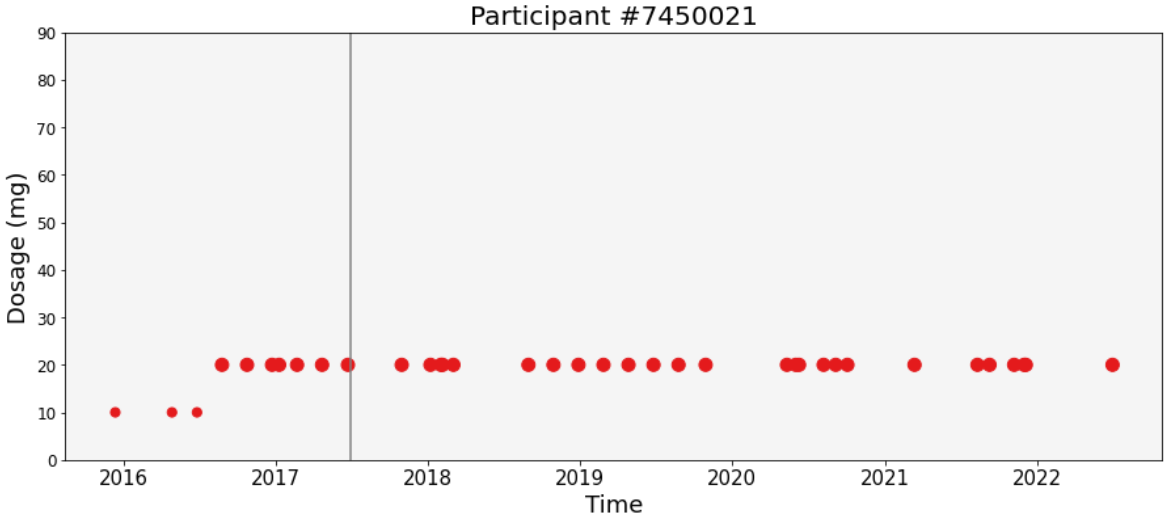
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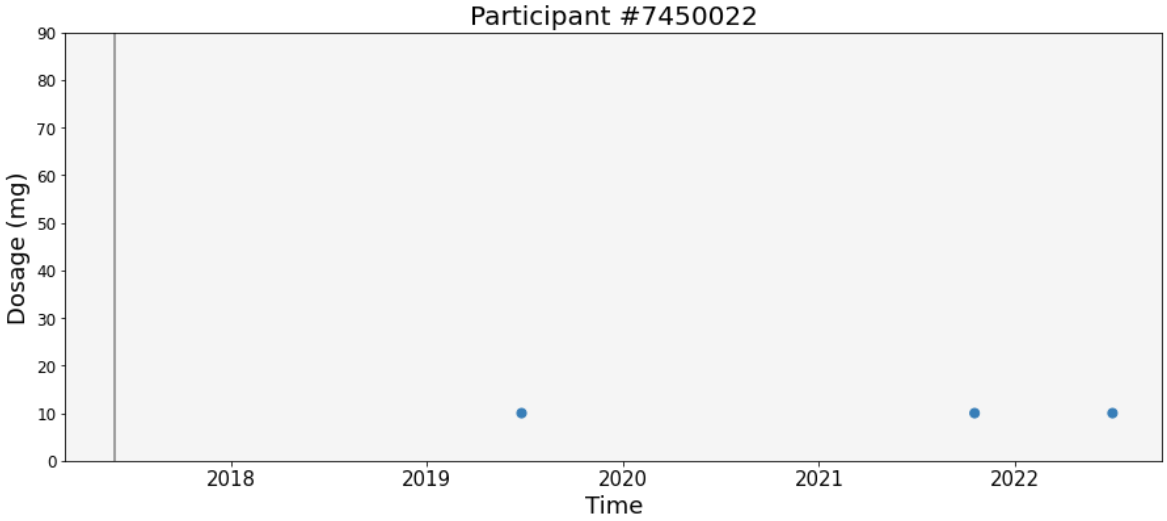
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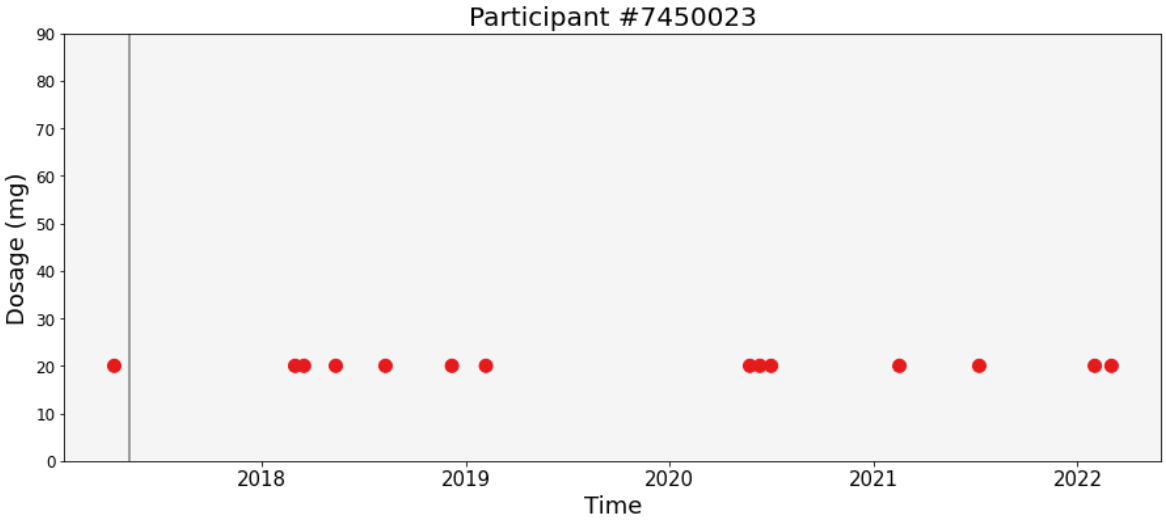
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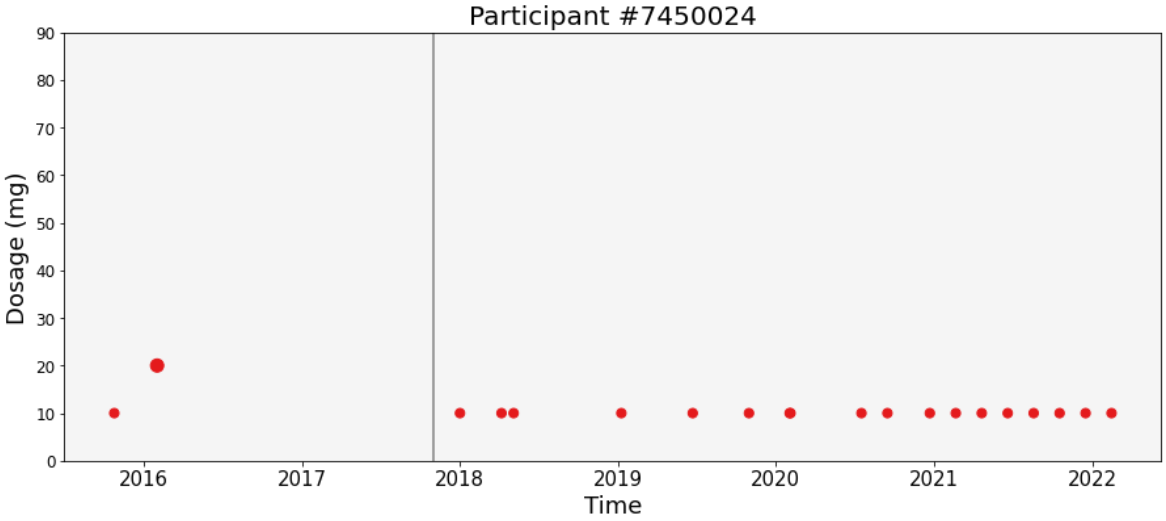
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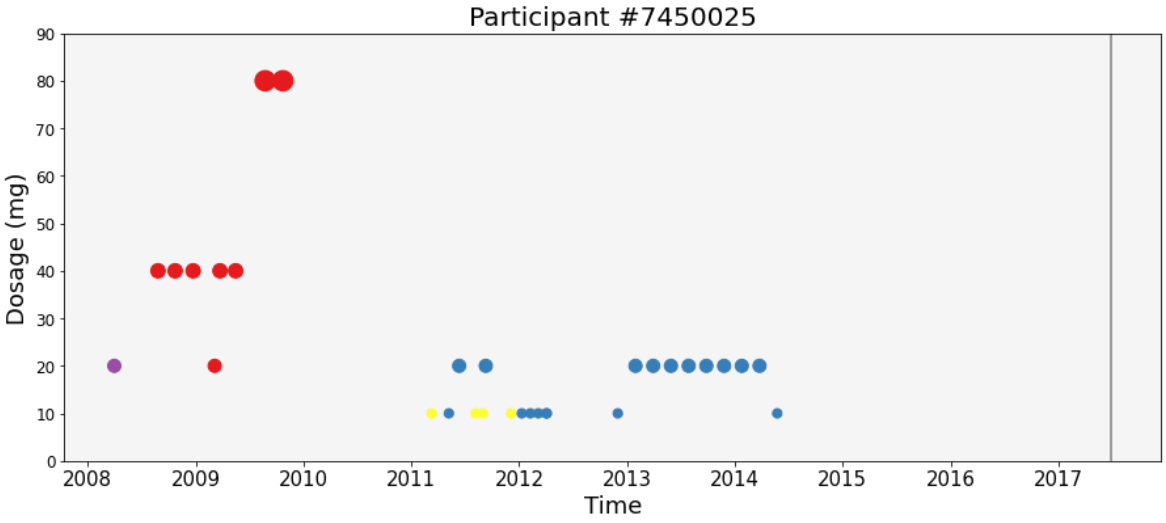
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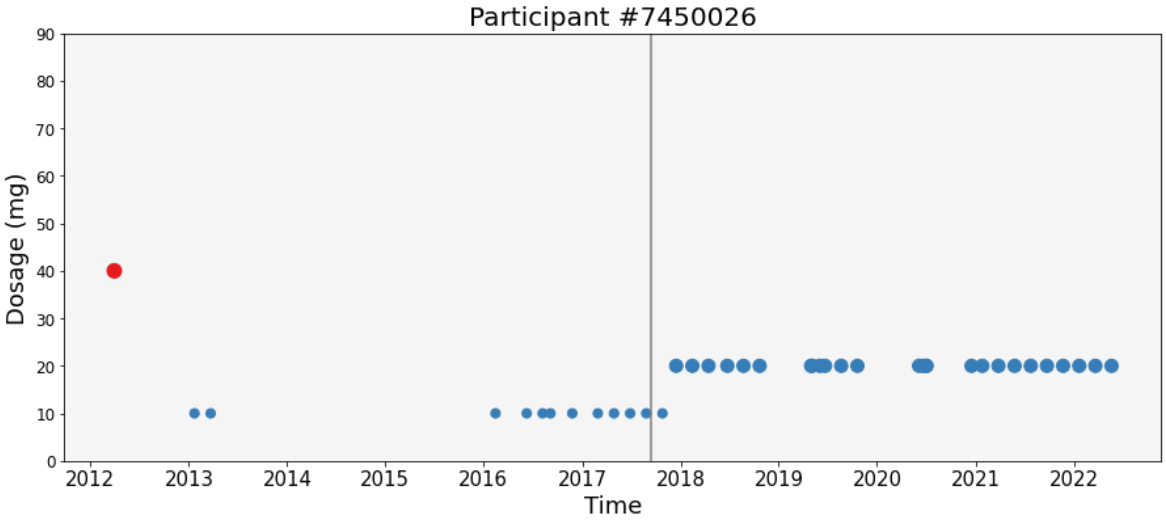
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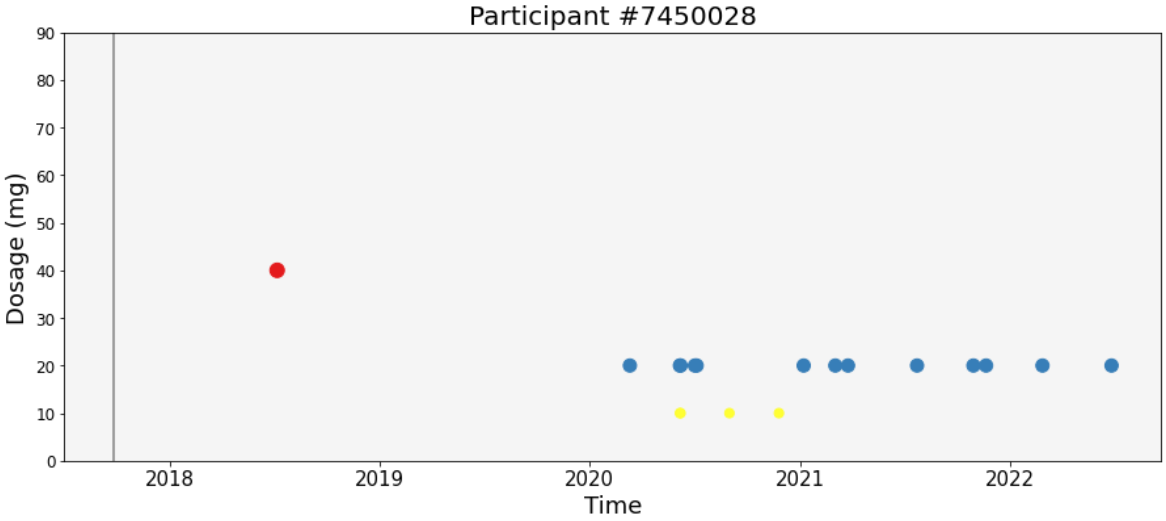
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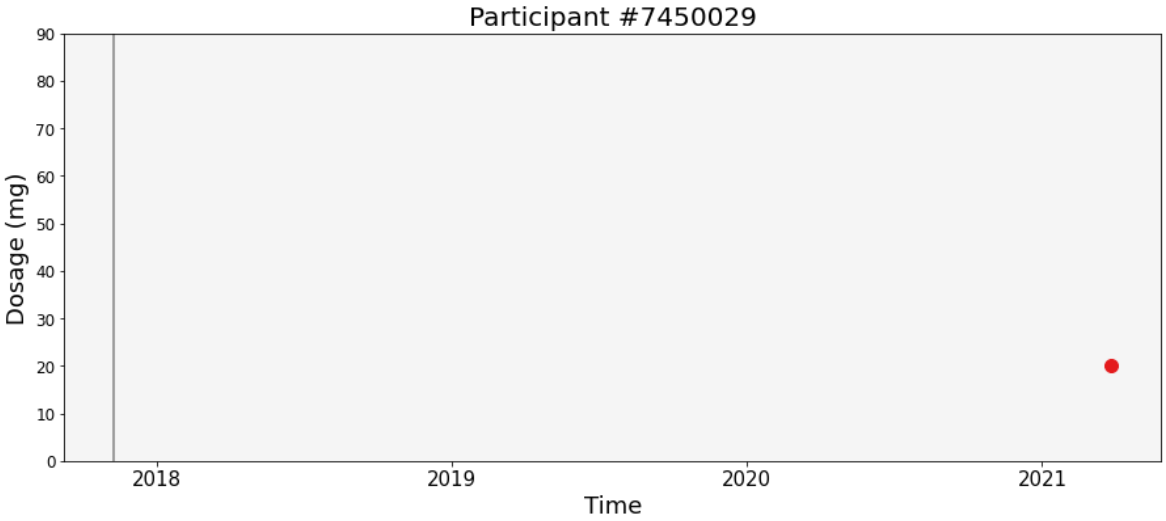
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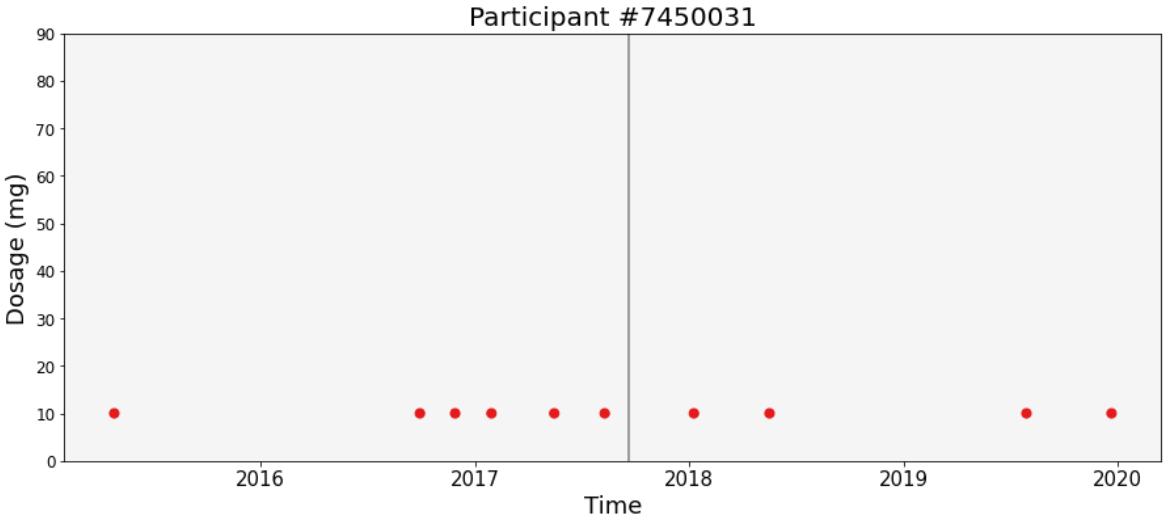
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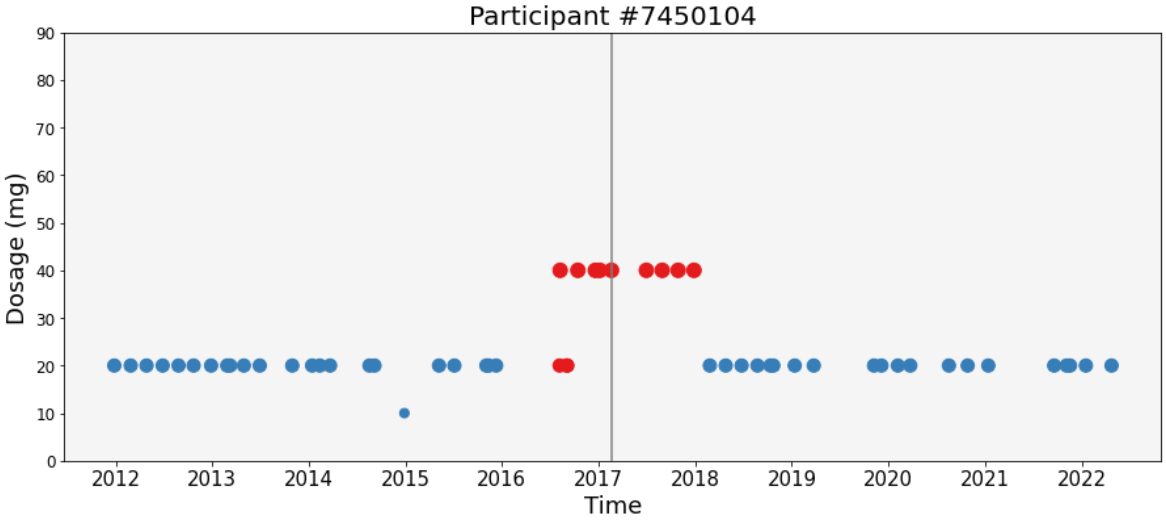
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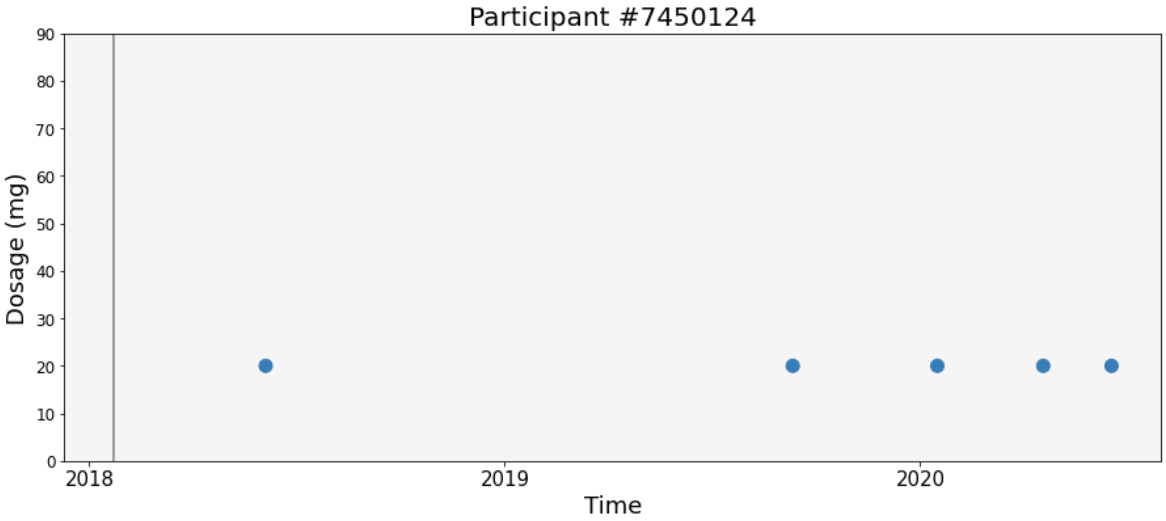
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26/05/2022