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**Diagnostics of hereditary connective tissue disorders by genetic next generation sequencing**

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## Abstract

***Aims:*** This quality study aimed to review the indications, reports, and clinical consequences of 438 diagnostic next generation sequencing (NGS) gene panel analyses for hereditary connective tissue disorders (HCTD).

***Methods:*** Molecular analyses were retrieved from the laboratory database and patient journal and compared to clinical information in the requisition and journal, classified according to the Human Phenotype Ontology.

***Results:*** In 123 of 438 NGS analyses, 156 sequence variants were reported in 33 of 54 genes analyzed. NGS analyses and, in some cases, post-analytic assessment resulted in pathogenic variants in 41 (9%) and variants of uncertain significance in 83 (19%) of analyses. While cardiovascular abnormalities were the most common phenotype noted in requisitions, no specific organ system could be identified in which symptoms represent a preferable indication for the analysis. Certain health issues recorded in the journal were found to be frequently left out of requisitions.

***Conclusions:*** The interpretation of genetic sequence variants continues to be a significant challenge in HCTD. Although not associated with the highest diagnostic yield, cardiovascular disease and family history may be suitable indications for NGS due to the clinical consequences of the identification of a causative sequence variant for a vascular HCTD in patients and relatives.

**Keywords: Next generation sequencing, diagnostic gene panel, hereditary connective tissue disorders, unclassified sequence variants**

## Introduction

The connective tissue constitutes a large portion of the human body and pathological changes may affect many organs. Hereditary connective tissue disorders (HCTD) comprise a wide specter of conditions with overlapping features caused by alterations in genes encoding components that regulate the structure and function of the connective tissue (Van Laer *et al.,* 2013). Many of the conditions show variable intrafamiliar expressivity (Colombi *et al.,* 2015; Murphy-Ryan *et al.,* 2010). Several traits that are often seen in these conditions, such as hypermobility of joints, and less frequently arterial aneurysms and dissections, constitute phenocopies in the general population (Grahame, 1999). Therefore, clinical diagnosis is challenging. Because vascular HCTD require specific surveillance and treatment, notably more frequent controls, different cutoff values for vascular surgery, indication for pharmacological treatment, and restriction measures in physical activity, it is of high value for clinical decision support to uncover a genetic cause in order to adjust treatment and follow up of the patient and enable predictive testing for their relatives (Verstraeten *et al.,* 2017).

The HCTD are associated with significant genetic heterogeneity and private mutations. The introduction of next generation sequencing (NGS) enabled the rapid retrieval of extensive amounts of genetic information (Rehm *el al.,* 2013, Hoyer *et al.,* 2015; diResta et al., 2018), at the same time generating new challenges in interpretation. In HCTD, the pathogenic variants are frequently missense mutations resembling benign genetic variation. In 2015 the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) published standards and guidelines to aid the geneticist in classification of genetic sequence variants, based on different types of defined evidence (Richards S et al., 2015).

*Background and aim of the study*

Until 2013, Sanger sequencing of a limited number of genes was the sole genetic sequencing method available at our hospital's laboratory. A diagnostic gene panel for HCTD was commenced as a clinical service in 2013, using an Illumina platform for exome sequencing with bioinformatic filtering for 34 HCTD genes in the first version, and increasing the number throughout the study period to 53 genes in the fourth version. A 54th gene, TNXB, was excluded from the last version due to quality issues. Classification of variants was accomplished in the laboratory. From spring 2015, the ACMG-AMP standards and guidelines were used. The criteria for inclusion of a gene in the HCTD gene panel was a documented

causal variant in >1 unrelated family with HCTD. The 53 genes in the latest panel are associated with at least 55 HCTD according to the database, Online Mendelian Inheritance in Man (Table 1).

The aim of this quality study was to evaluate the NGS clinical service for HCTD at a medical genetic department. The endpoints were phenotypic traits noted on the requisition and in the patient journal, reported genetic sequence variants, and recorded information on post-analytic assessment of reported sequence variants.

## Materials and Methods

The protocol was assessed and the study classified as a quality study by the regional ethics committee. The investigators' access to the laboratory information system and patient journal was approved by the data protection officer and Department of Clinical Service, respectively, at Oslo University Hospital.

*Retrieval of genotypes*

The analyses to be studied were identified through an in-house database, and the genotypes were retrieved from SWISSLAB Laboratory Information System (Roche Diagnostics IT Solutions GmbH, Berlin, Germany), which also enclosed the requisitions and reports. Requisitions for the analyses were available in the DIPS Electronic Patient Record (DIPS AS, Bodø, Norway). Variants uncovered by NGS analysis and the accompanying interpretation from the laboratory were registered. For the first 100 analyses it was noted in the patient journal and the current and previous laboratory information systems (SWISSLAB and SHIRE Genetics Data Management Systems (Genial Genetics, Chester, UK)) if a negative result from Sanger sequencing had preceded the NGS analysis, and if so, which genes had been sequenced.

The laboratory information system was also used to examine if the sequence variants had been reassessed and reclassified after submission of the original lab reports, and if so, on which grounds. Using this system, it was also examined whether subsequent mRNA analysis had been carried out in order to investigate a reported variant of unknown significance (VUS) with predicted effect on RNA splicing. For cases where the inheritance of the specific condition related to a reported heterozygous sequence variant was autosomal recessive, the information system was used to examine whether semi-quantitative copy number analysis (Multiplex ligation-dependent probe amplification, MLPA) had been performed.

*Registration of phenotype information*

Scanned requisitions were retrieved from the laboratory information system. Seven requisitions were missing. The hospital department of each requester was registered, as well as the patient's gender and year of birth. The phenotypes stated on the requisitions were classified according to the human phenotype ontology (HPO) (www.hpo.jax.org 12.12.2018) on the levels of organ abnormality and specific HPO features. HPO is a digital tool facilitating

standardized registering of symptoms and clinical findings (Kohler et al., 2017, Robinson et al., 2008) containing about 11 000 different digitalized traits (Groza et al., 2015) organized in a hierarchical system with a transitive dependency between features in different organ abnormality categories. Only HPO features for HCTD associated with a gene in the panel were used, however certain features were not registered due to relevance. For instance, fever and nausea were not considered to be beneficial information. The first 100 analyses only included patients with a medical journal available at the hospital where the study was performed. For these patients, clinical information both from the requisitions and in the patient journal, were classified and registered separately. For the following 338 analyses, phenotype information from the requisition only was registered.

**Results**

Of 452 performed HCTD NGS analyses, 14 were excluded from the study due to lack of a patient journal at the hospital where this study was performed. Of the 438 analyses, 329 (75%) were requested by physicians at this hospital, and 169 (51%) of these were from the Department of Medical Genetics. Of the included analyses, 234 (53%) were of men. The mean age of the patients undergoing analysis was 45 years. Sequence variants were reported in 123 (28%) of the NGS analyses. Of these, a pathogenic variant was reported in 36 (8%), and one or more VUS was reported in 92 (21%) analyses. More than one variant was reported in 24 (5%) of the analyses. Three variants were found in a gene that had previously been Sanger sequenced, one missense and two splicing variants. In total, 156 sequence variants (142 different variants, of which 80% were missense mutations) were reported in 33 of the 54 genes that were included in the diagnostic panel during the study period (Table 1). Based on molecular genetic analysis and preanalytic clinical information, 120 (77%) variants were classified as VUS and 36 (23%) as likely pathogenic or pathogenic variants. Pathogenic or likely pathogenic variants were reported in 11 (20%) of the genes in the panel.

Symptoms from the cardiovascular system were mentioned in 290 of the 438 requisitions (66%), constituting the most common indication for analysis (Figure 1). Of the more frequently mentioned cardiovascular features were dilatation of the ascending aorta or dissection of the descending aorta (Table 2*)*. Symptoms from the skeletal system were the second most reported organ abnormality on the requisition (in 165 requisitions; 38%), with joint hypermobility, scoliosis, and arachnodactyly as the most frequent traits. Symptoms from the integument were the third most reported organ abnormality, present on 103 requisitions (24%). The most commonly noted integument traits were hyperextensible skin, bruising susceptibility, atrophic scars and striae distensae. In 215 (49%) of requisitions it was stated that the patient had a positive family history for HCTD or similar, constituting the second most frequent indication for analysis.

For the most frequent indications, abnormality of the cardiovascular or skeletal system or family history, the likelihood of reporting a pathogenic or likely pathogenic variant was 8%. For abnormalities of the integument the likelihood was 6%. In the less frequent indication, abnormality of the eye, the likelihood of reporting a pathogenic or likely pathogenic variant

was 17%. Table 2 shows that the likelihood varied for different traits within organ categories. The possible effect on likelihood in combinations of symptoms was not assessed.

For the first 100 analyses, the information in the requisition was compared with information in the patient's electronic journal at the hospital (Figure 2), showing that 79% of the patients had symptoms from the cardiovascular system, 74% from the skeletal system, and 64% from the nervous system recorded in the journal. Sanger sequencing for selected genes had previously been performed in 51% of the first 100 analyses without any pathological findings.

Sequence variants from several analyses had undergone further investigation after the original report from the laboratory. In cases where additional evidence could be used to classify a variant, suggestions were noted in the original report. This included mRNA analysis for intronic variants predicted to alter mRNA splicing, testing of parents to determine if the variant was de novo, or segregation analysis. In other cases reassessment was prompted by the laboratory due to new publications in literature or databases. After post-analytic assessment including all these sources of evidence, sequence variants from 21 analyses had been reclassified. Of these, variants from four analyses had been reclassified from likely pathogenic to pathogenic. Further, VUS from five analyses had been classified as likely pathogenic and VUS from 12 analyses as likely benign, resulting in 41 analyses (9%) in 13 (22%) genes with a likely pathogenic or pathogenic variant (Figure 3) and 83 analyses (19%) that still had one or more VUS in the entire study sample. The reassessment included mRNA analysis in four analyses and MLPA in one analysis. No diagnosis resulted from the MLPA analysis. In cases where no known or likely pathogenic variant was identified, the patient and first degree relatives were referred to surveillance according to their clinical and family history. In cases with a strong suspicion of a HCTD, reanalysis with an updated gene panel was suggested with submission of a new requisition after 1-2 years, as more HCTD genes are expected to be identified. It was not investigated in the present study whether this was carried out later.

NGS analysis and post-analytic assessment resulted in confirmation of a clinical diagnosis in 41 patients. In another patient, two separate HCTD were uncovered (Riise et al., 2018). Patients with a likely or confirmed pathogenic variant were referred to a multidisciplinary team consisting of a contact nurse, thoracic and vascular surgeon, pediatric cardiologist, consultants in physical medicine and clinical genetics, respectively, and staff from the national resource center for rare connective tissue disorders. When indicated, an

ophthalmologist associated with the team would investigate patients in the eye clinic. Patients were given advice about precautionary measures, if possible referred for initiation of preventive measures, and encouraged to contact the resource center and patient organization. Genetic counseling including information on prenatal diagnosis for grave disorders was offered. The probands were encouraged to inform their relatives that predictive genetic testing could be offered after mandatory genetic counseling according to the Norwegian Biotechnology Act. Many relatives subsequently received genetic counseling and underwent predictive or diagnostic genetic testing. All individuals that tested positive were offered surveillance by the multidisciplinary team.

## Discussion

A diagnostic yield of 8% is within the reported range from other HCTD diagnostic gene panels (Campens et al., 2015; Hicks et al., 2018; Proost et al., 2015; Renner et al., 2019, Wooderchak-Donahue et al., 2015) where a pathogenic variant is uncovered in 4-35% of cases. As the diagnostic yield is dependent on the selection of the samples to be tested, low yield may reflect too liberal or off target indications for analysis. The abundance of phenocopies in HCTD is a well-known challenge, and may contribute to the low yield. However, this also makes NGS a suited method for HCTD diagnostics. Among the studies that report higher yield is the study by Hicks et al.. This study includes predictive genetic testing of relatives for a known family mutation, which increases the likelihood of positive findings. In the present study, NGS analysis was offered only to patients without a known family mutation. Other studies with higher reported diagnostic yield (Proost et al.,2015; Poninska et al., 2016) are performed for vascular HCTD, with smaller gene panels more relevant to these specific conditions. This decreases the clinical variability of patients included, and might explain the higher percentage of genetic findings compared to this study, which includes a more diverse patient population.

NGS analysis was preceded by negative Sanger sequencing analysis in 51% of the first 100 analyses performed in this study. Fewer of the last 338 NGS analyses were preceded by Sanger sequencing. Patients with well-known causes of HCTD, such as mutations in *FBN1*, *TGFBR1*, *TGFBR2*, *COL1A1*, *COL1A2*, *COL5A1*, *COL5A2*, *COL3A1*, *ACTA2* and *MYH11* were hence randomly excluded from the cohort prior to NGS analysis, influencing the diagnostic yield and the distribution of reported mutations (Figure 1). Accordingly, NGS uncovered a relatively high number of presumed pathogenic variants in genes associated with the newer types of Loeys Dietz syndrome, especially type 3 and 4, which were not available for Sanger sequencing. In contrast to Loeys-Dietz syndrome (MacCarrick et al., 2014), diagnosis of Ehlers-Danlos and Marfan syndromes depends on clinical criteria (Beighton et al., 1998; Loeys BL et al., 2010), making Sanger sequencing of specific genes the preferred first-tier method in patients fulfilling those criteria, followed by NGS only if the primary analysis does not uncover any pathological findings (Arslan-Kircher et al., 2016). In cases with ambiguous or non-syndromic clinical presentation, NGS is less costly than sequential Sanger-sequencing of three or more semi-large genes (Wooderchak-Donahue et al., 2015).

A considerable number of unclassifiable variants were reported from the 438 NGS analyses included in the study. From the first 67 analyses, two or three VUS were in some cases reported in addition to a pathogenic variant in the same sample. During the study period, the laboratory changed this practice. In the last 371 analyses no VUS were reported where a pathogenic or likely pathogenic variant was identified in another gene. This change to a more restrictive, context-dependent reporting practice implies that two patients with the same VUS may receive different reports from the laboratory.

Several issues hamper the interpretation of sequence variants in HCTD. Most of the diseases have autosomal dominant inheritance, where a heterozygous mutation is sufficient to set a diagnosis. However, most disease causing variants are private missense mutations, which can be difficult to distinguish from normal genetic variation. Furthermore, segregation analysis are hard to perform in these families due to phenocopies, reduced penetrance, and early death in vascular HCTD, the latter causing a lack of available DNA from relatives. A large number of genes contributed to the pathogenic variants and VUS that were identified from the diagnostic gene panel in this study. A VUS cannot be ruled out as the cause of disease, and therefore continuation of the broad selection of genes might be appropriate. On the other hand, if certain genes only generate VUS, one could argue that these are of no clinical benefit.

The clinical information on the requisition represents the indication for analysis as well as aiding in variant classification in the laboratory. One of the key strengths of this study is, in addition to the high number of analyses compared to other reports, the study of requisitions. The study has general limitations due to the lack of systematic physical examination of patients included in the study. Therefore, correlations between phenotype and genotype in HCTD could not be determined, but a summary of the information that was noted in the requisitions was made. The likelihood of finding a pathogenic sequence variant seemed similar for symptoms in different organ systems (Figure 2). Due to the insufficient number of findings, statistical analyses were not performed. Cardiovascular findings, which was the most frequent phenotype noted in requisitions, were no more often associated with a pathogenic or likely pathogenic finding than any of the other commonly noted indications. This may be explained by the phenocopy, multifactorial cardiovascular disease, in the general population. Nonetheless, due to the abundance of requisitions containing information on cardiovascular traits, the identification of a pathogenic variant in a vascular HCTD constituted a high percentage of findings and had significant consequences for the families. The likelihood of a pathogenic finding where there was a combination of indications, for instance

a combination of an organ abnormality with family history, could not be calculated due to low numbers. Also, we acknowledge that the lack of precise family history data represents a weakness of this study as criteria are not defined for this parameter.

Certain health issues were rarely mentioned in the requisitions, despite being recorded in the journal (Figure 3). A striking discrepancy was seen between information on symptoms from the nervous system in the medical journal versus requisitions. According to the journals, cerebral aneurysm, dural ectasia, headache and fatigue were frequent neural symptoms, but headache and fatigue were rarely mentioned in the requisition. It is possible that some physicians did not recognize that these complaints, being common in the general population, are even more frequent symptoms in HCTD (Bathen el al., 2014; Samantha, 2018).

*Conclusion*

In 438 analyses that included a number of samples which had been subject to Sanger-sequencing of well-known genes with a negative result, NGS and post-analytic assessment resulted in reports of a pathogenic sequence variant in 9% of analyses. While the present study could not identify any specific organ system in which symptoms represent a preferable indication for this analysis, the most frequent indications, cardiovascular disease and family history, may be suitable for NGS due to the clinical consequences of identification of causative genetic sequence variants for the patients and relatives. The interpretation of sequence variants is a significant challenge in NGS of HCTD and measures should be taken to improve and standardize variant classification, including implementing sharing of variants and their classification in international databases as standard practice. Over time, this improvement may reveal which clinical indications are most suitable for NGS panel analysis for HCTD.

## Author Disclosure Statement

No competing financial interests exist.

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Table 1. Number of reported sequence variants per gene included in the panel

| Gene | Pathogenic* variants | Variants of Unknown Significance(VUS) | Phenotype (OMIM) | Phenotype (OMIM) # |
|---|---|---|---|---|
| *ACTA2* | 5 | 1 | Moyamoya disease 5<br>Aortic aneurysm, familial thoracic | 614042<br>611788 |
| *ADAMTS10* | 0 | 5 | Weill-Marchesani syndrome 1, recessive | 277600 |
| *ADAMTS2* | 0 | 7 | Ehlers-Danlos syndrome, dermatosparaxis type | 225410 |
| *ADAMTSL4* | 0 | 0 | Ectopia lentis, isolated, autosomal recessive | 225100 |
| *ALDH18A1* | 0 | 1 | Cutis laxa, autosomal recessive, type IIIA | 219150 |
| *ATP6V0A2* | 0 | 1 | Cutis laxa, autosomal recessive, type IIA | 616603 |
| *B3GALT6* | 0 | 0 | Ehlers-Danlos syndrome, spondylodysplastic type, 2<br>Spondyloepimetaphyseal dysplasia with joint laxity, type 1, with or without fractures | 615349<br>271640 |
| *B4GALT7* | 0 | 2 | Ehlers-Danlos syndrome, spondylodysplastic type, 1 | 130070 |
| *BGN* | 0 | 0 | Meester-Loeys syndrom<br>Spondyloepimetaphyseal dysplasia, X-linked | 300989<br>300106 |
| *C1R* | 0 | 0 | Ehlers-Danlos syndrome, periodontal type, 1 | 130080 |
| *C1S* | 0 | 0 | Ehlers-Danlos syndrome, periodontal type, 2 | 617174 |

| | | | | |
|---|---|---|---|---|
| *CHST14* | 0 | 0 | Ehlers-Danlos syndrome, musculocontractural type 1 | 601776 |
| *COL11A1* | 0 | 2 | Stickler syndrome, type II<br>Marshall syndrome<br>Fibrochondrogenesis 1 | 604841<br>154780<br>228520 |
| *COL1A1* | 1 | 0 | Caffey disease Ehlers-Danlos syndrom, klassisk type<br>Ehlers-Danlos syndrome, arthrochalasia type, 1<br>Osteogenesis imperfecta, type I<br>Osteogenesis imperfecta, type II<br>Osteogenesis imperfecta, type III<br>Osteogenesis imperfecta, type IV | 114000<br>130060<br>166200<br>166210<br>259420<br>166220 |
| *COL1A2* | 1 | 4 | Ehlers-Danlos syndrome, arthrochalasia type, 2<br>Ehlers-Danlos syndrome, cardiac valvular type<br>Osteogenesis imperfecta, type II<br>Osteogenesis imperfecta, type III<br>Osteogenesis imperfecta, type IV | 617821<br>225320<br>166210<br>259420<br>166220 |
| *COL2A1* | 3 | 7 | Stickler syndrome, type I | 108300 |
| *COL3A1* | 0 | 5 | Ehlers-Danlos syndrome, vascular type | 130050 |
| *COL4A5* | 0 | 1 | Alport syndrome | 301050 |
| *COL5A1* | 3 | 5 | Ehlers-Danlos syndrome, classic type, 1 | 130000 |

| | | | | |
|---|---|---|---|---|
| *COL5A2* | 0 | 5 | Ehlers-Danlos syndrome, classic type, 2 | 130010 |
| *COL9A1* | 0 | 0 | Stickler syndrome, type IV | 614134 |
| *EFEMP2* | 0 | 1 | Cutis laxa, type IB | 614437 |
| *ELN* | 0 | 2 | Supravalvar aortic stenosis<br>Cutis laxa, autosomal dominant | 185500<br>123700 |
| *EMILIN1* | 0 | 0 | Hereditary connective tissue disease autosomal-dominant | |
| *FBLN5* | 0 | 2 | Cutis laxa, autosomal recessive, type IA<br>Cutis laxa, autosomal dominant 2 | 219100<br>614434 |
| *FBN1* | 12 | 6 | Marfan syndrome | 154700 |
| *FBN2* | 1 | 7 | Contractural arachnodactyly, congenital | 121050 |
| *FKBP14* | 0 | 0 | Ehlers-Danlos syndrome, kyphoscoliotic type, 2 | 614557 |
| *FLNA* | 0 | 0 | Cardiac valvular dysplasia, X-linked<br>Frontometaphyseal dysplasia 1<br>Melnick-Needles syndrome<br>Otopalatodigital syndrome, type I<br>Otopalatodigital syndrome, type II<br>Terminal osseous dysplasia | 314400<br>305620<br>309350<br>311300<br>304120<br>300244 |
| *FOXE3* | 0 | 1 | Aortic aneurysm, familial thoracic 11, susceptibility to | 617349 |
| *LOX* | 0 | 0 | Aortic aneurysm, familial thoracic 10 | 617168 |
| *LTBP2* | 0 | 1 | Weill-Marchesani syndrome 3, recessive | 614819 |

| | | | | |
|---|---|---|---|---|
| *LTBP4* | 0 | 2 | Cutis laxa, autosomal recessive, type IC | 613177 |
| *MED12* | 0 | 0 | Lujan-Fryns syndrome | 309520 |
| *MFAP5* | 0 | 0 | Aortic aneurysm, familial thoracic 9 | 616166 |
| *MYH11* | 0 | 9 | Aortic aneurysm, familial thoracic 4 | 132900 |
| *MYLK* | 0 | 5 | Aortic aneurysm, familial thoracic 7 | 613780 |
| *NOTCH1* | 0 | 13 | Aortic valve disease 1 | 109730 |
| *PLOD1* | 0 | 5 | Ehlers-Danlos syndrome, kyphoscoliotic type, 1 | 225400 |
| *PRKG1* | 0 | 0 | Aortic aneurysm, familial thoracic 8 | 615436 |
| *PYCR1* | 0 | 0 | Cutis laxa, autosomal recessive, type IIB<br>Cutis laxa, autosomal recessive, type IIIB | 612940<br>614438 |
| *RIN2* | 0 | 4 | Macrocephaly, alopecia, cutis laxa, and scoliosis | 613075 |
| *SKI* | 0 | 0 | Shprintzen-Goldberg syndrome | 182212 |
| *SLC2A10* | 1 | 1 | Arterial tortuosity syndrome | 208050 |
| *SLC39A13* | 0 | 0 | Ehlers-Danlos syndrome, spondylodysplastic type, 3 | 612350 |
| *SMAD2* | 0 | 0 | Aortic aneurysm, familial thoracic | |
| *SMAD3* | 3 | 6 | Loeys-Dietz syndrome, type 3 | 613795 |
| *SMAD4* | 0 | 0 | Juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome<br>Myhre syndrome Juvenile polyposis | 175050<br>139210 |

| | | | | |
|---|---|---|---|---|
| *TGFB1* | 0 | 2 | Camurati-Engelmann disease | 131300 |
| *TGFB2* | 5 | 0 | Loeys-Dietz syndrome 4 | 614816 |
| *TGFB3* | 0 | 1 | Loeys-Dietz syndrome 5 | 615582 |
| *TGFBR1* | 0 | 3 | Loeys-Dietz syndrome 1 | 609192 |
| *TGFBR2* | 1 | 1 | Loeys-Dietz syndrome 2 | 610168 |
| *TNXB* | 0 | 1 | Ehlers-Danlos syndrome, classic-like, 1 | 606408 |

*Pathogenic= Classified as pathogenic or likely pathogenic

Table 2. Phenotypic information on requisitions* and reported pathogenic sequence variants

| Phenotype information on requisition | Report of no pathogenic variant | Report of pathogenic variant |
|---|---|---|
| | n | n (%) |
| *Abnormality of the cardiovascular system* | | |
| HP 0004942 Aortic aneurysm | 132 | 13 (9) |
| HP 0002647 Aortic dissection | 78 | 6 (7) |
| HP 0005294 Arterial dissection | 23 | 1 (4) |
| HP 0002617 Dilatation | 22 | 0 |
| HP 0006702 Spontaneous coronary artery dissection | 15 | 0 |
| HP 0002636 Aneurysm of an abdominal artery | 14 | 0 |
| HP 0012158 Carotid artery dissection | 14 | 0 |
| HP 0001647 Bicuspid aortic valve | 11 | 1 (8) |
| HP 0004944 Cerebral aneurysm | 11 | 0 |
| HP 0005116 Arterial tortuosity | 7 | 1 (13) |
| HP 0001654 Abnormal heart valve morphology | 7 | 1 (13) |
| HP 0025019 Arterial rupture | 6 | 0 |
| HP 0002170 Intracranical hemorrhage | 5 | 0 |
| HP 0031653 Abnormal heart valve physiology | 4 | 1 (20) |
| HP 0002619 Varicose veins | 3 | 0 |
| HP 0100026 Arteriovenous malformation | 3 | 0 |
| | | |
| *Abnormality of the skeletal system* | | |
| HP 0001382 Joint hypermobility | 98 | 2 (2) |
| HP 0001166 Arachnodactyly | 30 | 0 |
| HP 0002829 Arthralgia | 26 | 1 (4) |
| HP 0002650 Scoliosis | 20 | 4 (17) |
| HP 0000767 Pectus excavatum | 14 | 0 |
| HP 0000768 Pectus carinatum | 7 | 2 (22) |
| HP 0001373 Joint dislocation | 9 | 0 |
| HP 0001385 Hip dysplasia | 5 | 0 |

| | | |
|---|---|---|
| HP 0010754 Abnormality of the temporomandibular joint | 4 | 0 |
| HP 0001384 Abnormality of the hip joint | 2 | 1 (33) |
| HP 0001555 Asymmetry of the thorax | 3 | 0 |
| HP 0002757 Recurrent fractures | 3 | 0 |
| HP 0003834 Shoulder dislocation | 3 | 0 |
| HP 0000939 Osteoporosis | 3 | 0 |
| HP 0001388 Joint laxity | 3 | 0 |
| | | |
| *Abnormality of the integument* | | |
| HP 0000974 Hyperextensible skin | 42 | 1 (2) |
| HP 0000978 Bruising susceptibility | 37 | 0 |
| HP 0001075 Atrophic scars | 36 | 0 |
| HP 0001065 Striae distensae | 19 | 1 (5) |
| HP 0001030 Fragile skin | 5 | 0 |
| HP 0000977 Soft skin | 5 | 0 |
| HP 0010648 Dermal translucency | 4 | 0 |
| HP 0001058 Poor wound healing | 4 | 0 |

*Only phenotypes registered more than twice are included in the table

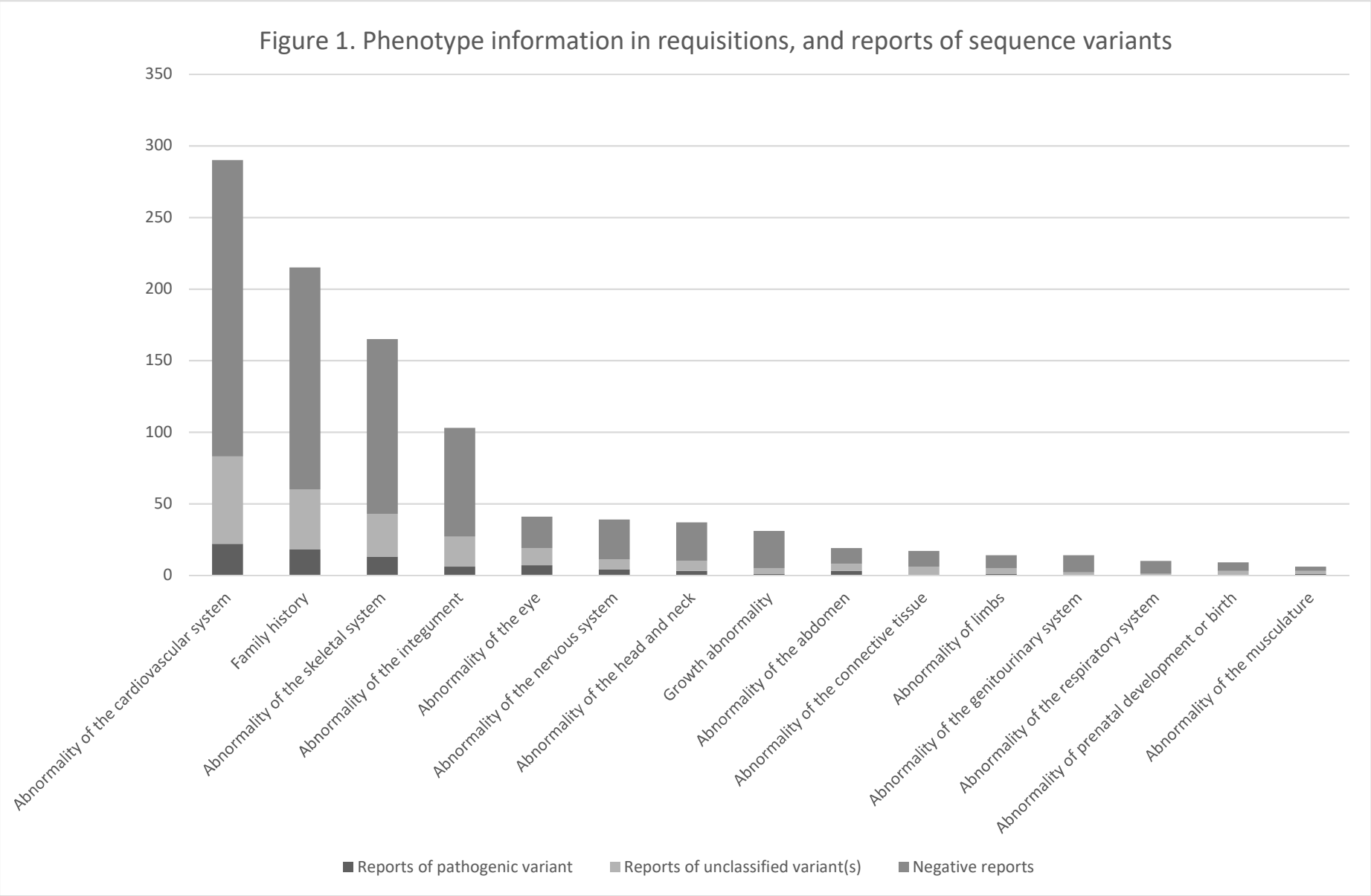


Figure 1. Phenotype information in requisitions, and reports of sequence variants
350
300
250
200
150
100
50
0
Abnormality of the cardiovascular system
Family history
Abnormality of the skeletal system
Abnormality of the integument
Abnormality of the eye
Abnormality of the nervous system
Abnormality of the head and neck
Growth abnormality
Abnormality of the abdomen
Abnormality of the connective tissue
Abnormality of limbs
Abnormality of the genitourinary system
Abnormality of the respiratory system
Abnormality of prenatal development or birth
Abnormality of the musculature
Reports of pathogenic variant
Reports of unclassified variant(s)
Negative reports

Figure 2. Phenotype information in requisitions and in patient journals (first 100 analyses)

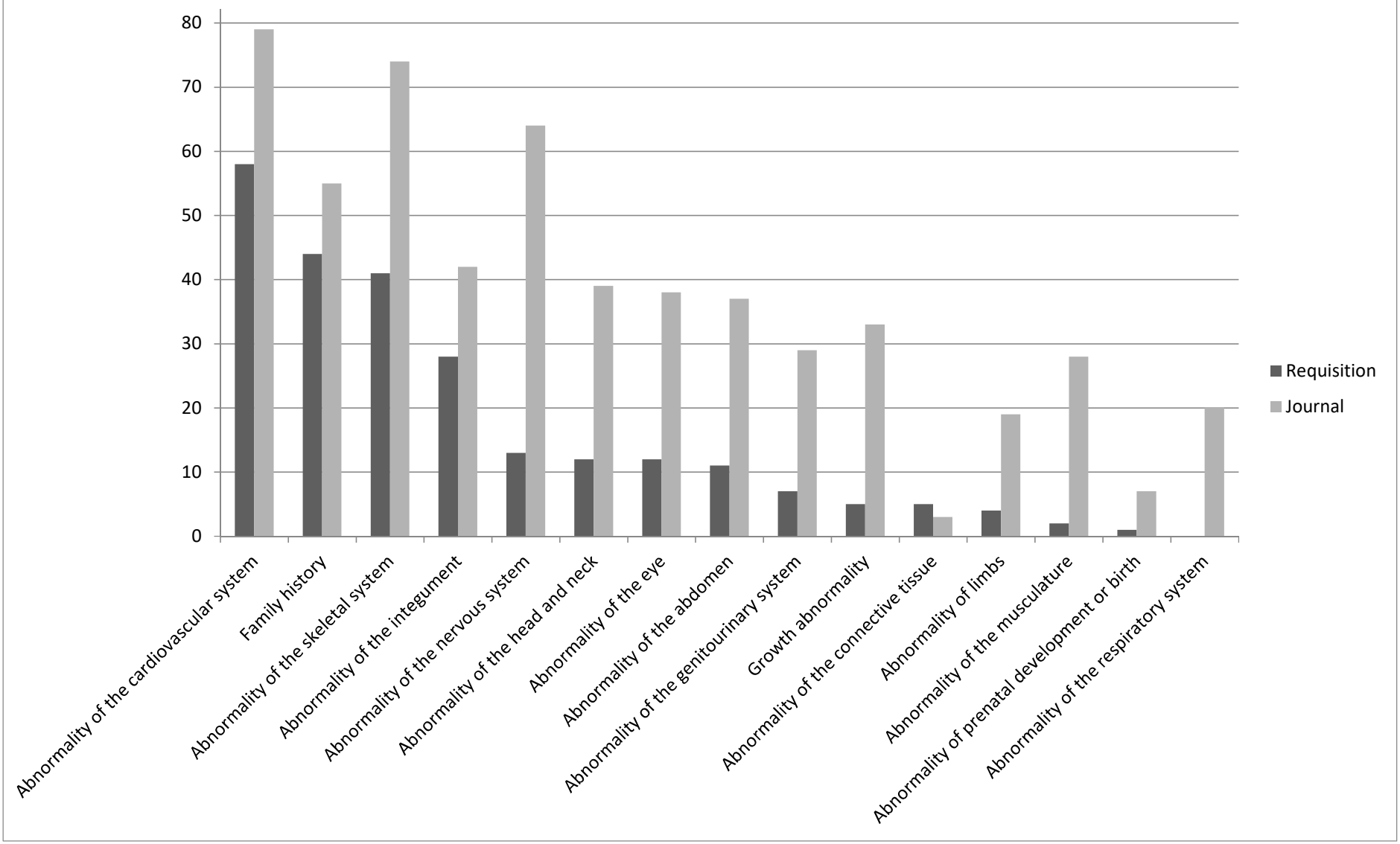
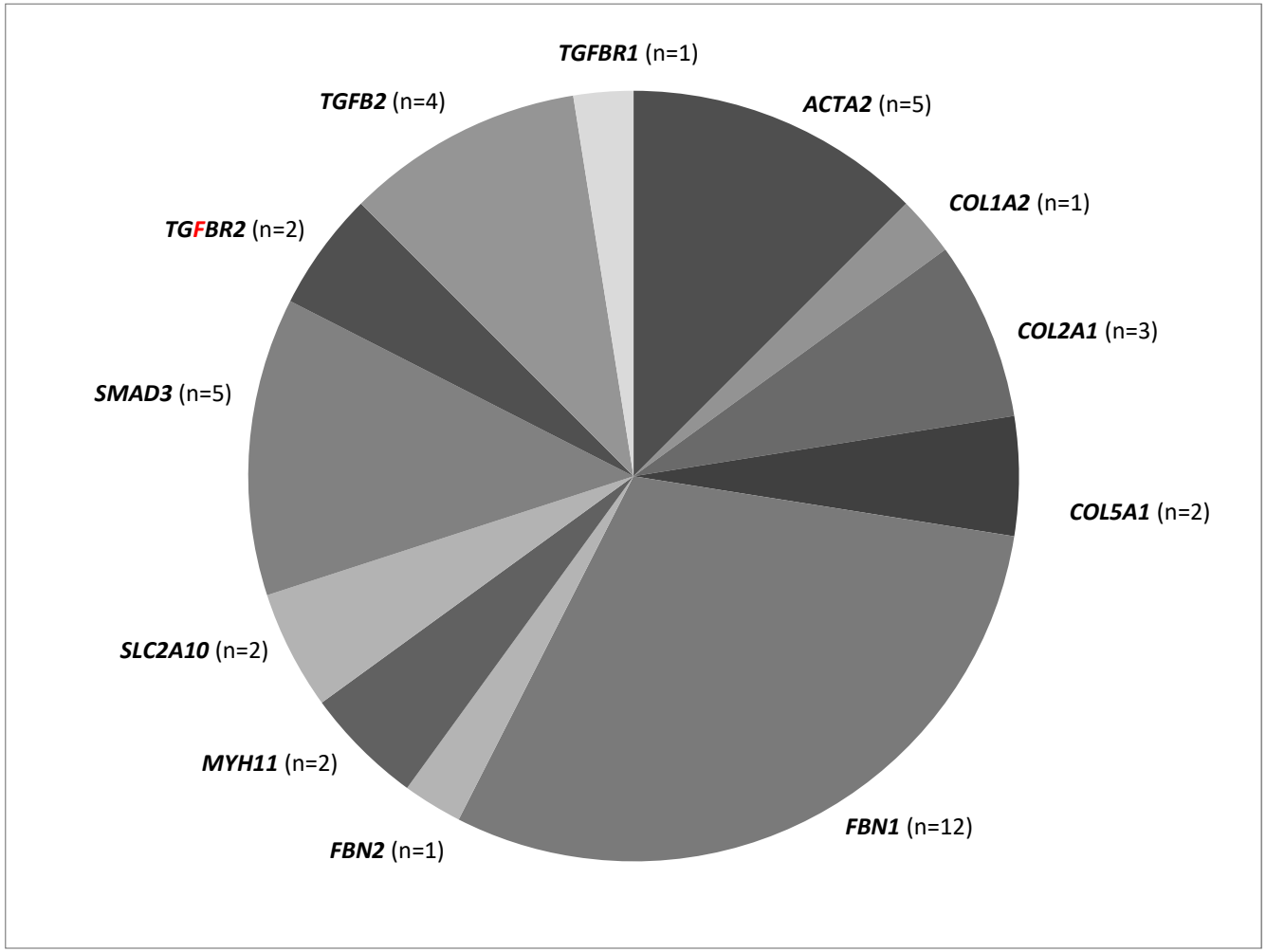


Figure 3. Pathogenic and likely pathogenic sequence variants (n) per gene after post-analytical assessment


TGFBR1 (n=1)
ACTA2 (n=5)
COL1A2 (n=1)
COL2A1 (n=3)
COL5A1 (n=2)
FBN1 (n=12)
FBN2 (n=1)
MYH11 (n=2)
SLC2A10 (n=2)
SMAD3 (n=5)
TGFBR2 (n=2)
TGFB2 (n=4)