The HudsonAlpha CLINICAL SERVICES LAB

601 Genome Way, Room 3001, Huntsville, AL 35806

Phone: (256) 327-9670 Fax: (256) 327-9760

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Patient Name	Jane Doe	**Ordering Physician**	Dr. Gene	**Specimen Type**	Blood
Date of Birth	01/01/1970	**Date of Report**	04/01/2021	**Test Type**	WGS
Race/Ethnicity	Caucasian	**Sample Collection Date**	01/01/2021	**Accession #**	ABCDEFG
Gender	Female	**Sample Received Date**	01/01/2021	**Provider Accession ID**	8675309
		Test Request Date	01/01/2021		

Clinical Indications

The patient is a 51-year-old female with a history of breast cancer, coronary artery disease, hypercholesterolemia, and mild cognitive impairment. We were requested to perform elective whole genome sequencing on a sample from the patient (SID123456).

Results Summary of Primary Findings

The following variant(s) were identified in gene(s) related to the indication for testing.

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification[1]
CYLD, NM_015247.2	5' Coding Exon	chr16:50749868A>G (GRCh38) c.170A>G p.His57Arg	Heterozygous	Frontotemporal dementia and/or amytrophic lateral sclerosis 8 (OMIM 619132)	Autosomal Dominant	Variant of Uncertain Significance

[1] Based on ACMG guidelines (Richards, 2015)

Interpretation and Recommendation Summary for Primary Findings

The patient is heterozygous for a potentially clinically significant variant in CYLD. Pathogenic variants in this gene are associated with frontotemporal dementia and/or amytrophic lateral sclerosis 8. Frontotemporal dementia and/or amytrophic lateral sclerosis 8 have some overlap with this patient's reported presentation, suggesting that this individual may have this disease.

These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Genetic counseling is recommended to discuss the implications of this report. Please note that variant classification and/or interpretation, as well as our knowledge of the genome, will change over time as more information becomes available. Therefore, reanalysis of the genome at regular intervals should be considered.

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Other Variants of Medical Significance (Secondary Findings)

The following variant(s) were identified in gene(s) not related to the indication for testing.

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification[1]
CTH, NM_001902.5	Interior Coding Exon	chr1:70415987C>T (GRCh38) c.200C>T p.Thr67Ile	Heterozygous	Cystathioninuria (OMIM 219500)	Autosomal Recessive	Pathogenic
GALT, NM_000155.3	Interior Coding Exon	chr9:34649032G>T (GRCh38) c.855G>T p.Lys285Asn	Heterozygous	Galactosemia (OMIM 230400)	Autosomal Recessive	Pathogenic
HFE, NM_000410.3	Interior Coding Exon	chr6:26092913G>A (GRCh38) c.845G>A p.Cys282Tyr	Heterozygous	Hemochromatosis (OMIM 235200)	Autosomal Recessive	Pathogenic

[1] Based on ACMG guidelines (Richards, 2015)

Interpretation and Recommendation Summary for Secondary Findings

The patient is heterozygous for a potentially clinically significant variant in CTH. Pathogenic variants in this gene are associated with cystathioninuria. The clinical presentation of cystathioninuria is significantly divergent from this patient's described phenotype. This result suggests that this individual is a carrier for this disease.

The patient is heterozygous for a potentially clinically significant variant in GALT. Pathogenic variants in this gene are associated with galactosemia. The clinical presentation of galactosemia is significantly divergent from this patient's described phenotype. This result suggests that this individual is a carrier for this disease.

The patient is heterozygous for a potentially clinically significant variant in HFE. Pathogenic variants in this gene are associated with hemochromatosis. The clinical presentation of hemochromatosis is significantly divergent from this patient's described phenotype. This result suggests that this individual is a carrier for this disease.

These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Genetic counseling is recommended to discuss the implications of this report. Please note that variant classification and/or interpretation, as well as our knowledge of the genome, will change over time as more information becomes available. Therefore, reanalysis of the genome at regular intervals should be considered.

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Detailed Description of Primary Findings

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
CYLD, NM_015247.2	5' Coding Exon	chr16:50749868A>G (GRCh38) c.170A>G p.His57Arg	Heterozygous	Frontotemporal dementia and/or amytrophic lateral sclerosis 8 (OMIM 619132)	Autosomal Dominant	Variant of Uncertain Significance

Genomic Position	Variant Frequency
chr16:50749868A>G (GRCh38)	1/143320 of chromosomes in gnomAD Genome 3/249346 of chromosomes in gnomAD Exome

Variant Interpretation and Disease Information

The patient is heterozygous for a potentially clinically significant variant in CYLD. Pathogenic variants in this gene are associated with frontotemporal dementia and/or amytrophic lateral sclerosis 8. Frontotemporal dementia and/or amytrophic lateral sclerosis 8 have some overlap with this patient's reported presentation, suggesting that this individual may have this disease.

The c.170A>G variant in the CYLD gene has not been published, to our knowledge. The Genome Aggregation Database (gnomAD) reports that the c.170A>G variant was observed in 1/143320 and 3/249346 alleles for genome and exome data, respectively; consistent with the expected frequency of a pathogenic variant in frontotemporal dementia and/or amytrophic lateral sclerosis 8. Multiple lines of computational evidence support a deleterious effect of the p.His57Arg variant on the gene or gene product. In summary, this collective evidence supports c.170A>G as a variant of uncertain significance for frontotemporal dementia and/or amytrophic lateral sclerosis 8.

Frontotemporal dementia and amytrophic lateral sclerosis 8 arise from the presence of a single pathogenic variant in CYLD.

Detailed Description of Secondary Findings

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
CTH, NM_001902.5	Interior Coding Exon	chr1:70415987C>T (GRCh38) c.200C>T p.Thr67Ile	Heterozygous	Cystathioninuria (OMIM 219500)	Autosomal Recessive	Pathogenic

Genomic Position	Variant Frequency
chr1:70415987C>T (GRCh38)	975/143254 of chromosomes in gnomAD Genome 1628/251408 of chromosomes in gnomAD Exome

Variant Interpretation and Disease Information

The patient is heterozygous for a potentially clinically significant variant in CTH. Pathogenic variants in this gene are associated with cystathioninuria. The clinical presentation of cystathioninuria is significantly divergent from this patient's described phenotype. This result suggests that this individual is a carrier for this disease.

The c.200C>T variant in the CTH gene has been previously reported in patients with cystathioninuria (ClinVar Variation ID 2939; Espinós, 2010; Kraus, 2009; Wang, 2003; Zhu, 2008). The c.200C>T variant has been shown to damage the gene or gene-product based on a well-established functional assay, catalytic activity (Kraus, 2009; Zhu, 2008). The prevalence of the c.200C>T variant in affected individuals with cystathioninuria is significantly increased compared to the prevalence in controls (Espinós, 2010; Kraus, 2009; Wang, 2003). Multiple lines of computational evidence support a deleterious effect of the p.Thr67Ile variant on the gene or gene product. The c.200C>T variant has been reported as pathogenic by a reputable source (ClinVar Variation ID 2939). In summary, this collective evidence supports c.200C>T as a pathogenic variant for cystathioninuria.

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Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
GALT, NM_000155.3	Interior Coding Exon	chr9:34649032G>T (GRCh38) c.855G>T p.Lys285Asn	Heterozygous	Galactosemia (OMIM 230400)	Autosomal Recessive	Pathogenic

Genomic Position	Variant Frequency
chr9:34649032G>T (GRCh38)	24/143282 of chromosomes in gnomAD Genome 35/251490 of chromosomes in gnomAD Exome

Variant Interpretation and Disease Information

The patient is heterozygous for a potentially clinically significant variant in GALT. Pathogenic variants in this gene are associated with galactosemia. The clinical presentation of galactosemia is significantly divergent from this patient's described phenotype. This result suggests that this individual is a carrier for this disease.

The c.855G>T variant in the GALT gene has been previously reported in patients with galactosemia (Berry, 2021; Chhay, 2008; ClinVar Variation ID 3621; Coelho, 2014; Viggiano, 2015). The c.855G>T variant has been shown to damage the gene or gene-product based on a well-established functional assay, GALT enzyme activity (Chhay, 2008; Coelho, 2014; Viggiano, 2015). The prevalence of the c.855G>T variant in affected individuals with galactosemia is significantly increased compared to the prevalence in controls (Berry, 2021). The c.855G>T variant occurs in a known mutational hot-spot (https://franklin.genoox.com/clinical-db/variant/snp/chr9-34649029-G-T). The Genome Aggregation Database (gnomAD) reports that the c.855G>T variant was observed in 24/143282 and 35/251490 alleles for genome and exome data, respectively; consistent with the expected frequency of a pathogenic variant in galactosemia. The c.855G>T variant has been observed to be in trans with a pathogenic variant based on segregation analysis in individuals with galactosemia (Chhay, 2008; Viggiano, 2015). A different amino acid change, p.Lys285Arg, at the same position has been previously reported as pathogenic (ClinVar Variation ID 25277). The p.Lys285Asn missense variant occurs in a gene GALT that is associated with galactosemia where missense is a common mechanism of disease (https://franklin.genoox.com/clinical-db/variant/snp/chr9-34649029-G-T). Multiple lines of computational evidence support a deleterious effect of the p.Lys285Asn variant on the gene or gene product. The c.855G>T variant has been reported as pathogenic by a reputable source (ClinVar Variation ID 3621). In summary, this collective evidence supports c.855G>T as a pathogenic variant for galactosemia.

Gene & Transcript	Location	Variant	Zygosity	Disease or Phenotype	Inheritance Pattern of Disease	Variant Classification
HFE, NM_000410.3	Interior Coding Exon	chr6:26092913G>A (GRCh38) c.845G>A p.Cys282Tyr	Heterozygous	Hemochromatosis (OMIM 235200)	Autosomal Recessive	Pathogenic

Genomic Position	Variant Frequency
chr6:26092913G>A (GRCh38)	5448/143266 of chromosomes in gnomAD Genome 8344/251236 of chromosomes in gnomAD Exome

Variant Interpretation and Disease Information

The patient is heterozygous for a potentially clinically significant variant in HFE. Pathogenic variants in this gene are associated with hemochromatosis. The clinical presentation of hemochromatosis is significantly divergent from this patient's described phenotype. This result suggests that this individual is a carrier for this disease.

The c.845G>A variant in the HFE gene has been previously reported in patients with hemochromatosis (ClinVar Variation ID 9; Feder, 1996; Feder, 1997). The c.845G>A variant has been shown to damage the gene or gene-product based on a well-established functional assay, cell trafficking and degradation (Feder, 1997). The prevalence of the c.845G>A variant in affected individuals with hemochromatosis is significantly increased compared to the prevalence in controls (Feder, 1996). Multiple lines of computational evidence support a deleterious effect of the p.Cys282Tyr variant on the gene or gene product. The c.845G>A variant has been reported as pathogenic by a reputable source (ClinVar Variation ID 9). In summary, this collective evidence supports c.845G>A as a pathogenic variant for hemochromatosis.

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Pharmacogenomic Testing

Drug	Dosing Guidelines	Gene	Variant	Zygosity
efavirenz	PA166182603	CYP2B6	chr19:g.41006936G>T (GRCh38) c.516G>T (NM_000767.5) CYP2B6*9 cytochrome P450 family 2 subfamily B member 6	Heterozygous
amitriptyline citalopram escitalopram clopidogrel voriconazole	PA166105006 PA166127638 PA166127638 PA166104948 PA166161537	CYP2C19	chr10:g.94761900C>T (GRCh38) c.-806C>T (NM_000769.4) CYP2C19*17 cytochrome p450 2C19 enzyme	Heterozygous
codeine amitriptyline tamoxifen	PA166104996 PA166105006 PA166176068	CYP2D6	chr22:g.42127803C>T (GRCh38) c.985+39G>A (NM_000106.6) CYP2D6*41 cytochrome p450 2D6 enzyme	Heterozygous
tacrolimus	PA166124619	CYP3A5	chr7:g.99672916T>C (GRCh38) c.219-237A>G (NM_000777.5) CYP3A5*3 cytochrome p450 3A5 enzyme	Homozygous
warfarin	PA166104949	CYP4F2	chr19:g.15879621C>T (GRCh38) c.1297G>A (NM_001082.5) CYP4F2*3 cytochrome P450 family 4 subfamily F member 2	Homozygous

CYP2B6 encodes cytochrome P450 family 2 subfamily B member 6 involved in the efficacy or metabolism of a number of drugs, including efavirenz. The table above details the variant(s) found in CYP2B6 for this patient. For dosing guidelines please use the links below.

CYP2B6 Dosing Guidelines
efavirenz: https://www.pharmgkb.org//guidelineAnnotation/PA166182603

CYP2C19 encodes cytochrome p450 2C19 enzyme involved in the efficacy or metabolism of a number of drugs, including amitriptyline, citalopram, escitalopram, clopidogrel, and voriconazole. The table above details the variant(s) found in CYP2C19 for this patient. For dosing guidelines please use the links below.

CYP2C19 Dosing Guidelines
amitriptyline: https://www.pharmgkb.org/guidelineAnnotation/PA166105006
citalopram: https://www.pharmgkb.org/guidelineAnnotation/PA166127638
escitalopram: https://www.pharmgkb.org/guidelineAnnotation/PA166127638
clopidogrel: https://www.pharmgkb.org/guidelineAnnotation/PA166104948
voriconazole: https://www.pharmgkb.org/guidelineAnnotation/PA166161537

CYP2D6 encodes cytochrome p450 2D6 enzyme involved in the efficacy or metabolism of a number of drugs, including codeine, amitriptyline, and tamoxifen. The table above details the variant(s) found in CYP2D6 for this

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patient. For dosing guidelines please use the links below.

CYP2D6 Dosing Guidelines
codeine: https://www.pharmgkb.org/guidelineAnnotation/PA166104996
amitriptyline: https://www.pharmgkb.org/guidelineAnnotation/PA166105006
tamoxifen: https://www.pharmgkb.org/guidelineAnnotation/PA166176068

CYP3A5 encodes cytochrome p450 3A5 enzyme involved in the efficacy or metabolism of a number of drugs, including tacrolimus. The table above details the variant(s) found in CYP3A5 for this patient. For dosing guidelines please use the links below.

CYP3A5 Dosing Guidelines
tacrolimus: https://www.pharmgkb.org/guidelineAnnotation/PA166124619

CYP4F2 encodes cytochrome P450 family 4 subfamily F member 2 involved in the efficacy or metabolism of a number of drugs, including warfarin. The table above details the variant(s) found in CYP4F2 for this patient. For dosing guidelines please use the links below.

CYP4F2 Dosing Guidelines
warfarin: https://www.pharmgkb.org/guidelineAnnotation/PA166104949

The Pharmacogenomics report is restricted to genetic variants that have been curated by PharmGKB as meeting the highest standard for evidence (category designated 1A or 1B) supporting a variant-drug association. All PharmGKB variants at the 1A or 1B level of evidence that have dosing guidelines are included in this pharmacogenomics report except the CYP2D6*5 variant and variants in the genes HLA-A, HLA-B, and CFTR. When two or more variants are reported within the same gene, all of the variants may come from one parent on the same chromosome (in cis). Alternatively each parent may contribute at least one of the variants (in trans). We are not able to determine whether the variants are in cis or trans for this individual. Whether the reported variants are in cis or trans may affect the individual's pharmacogenomics phenotype. Clinical interpretation of these results, especially in regard to specifc dosing pharmacogenomics recommendations, must be made in the context of other factors such as gender, age, weight, ethnicity, disease state, diet, organ function, concomitant therapy, and drug-drug interactions. The laboratory may include additional pharmacogenomic variants based on the clinical information provided.

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https://www.ncbi.nlm.nih.gov/clinvar/variation/2939

https://www.ncbi.nlm.nih.gov/clinvar/variation/3621

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https://www.ncbi.nlm.nih.gov/clinvar/variation/9

Sequencing and Analysis Methodology and Limitations

Whole Genome Sequencing was performed by HudsonAlpha Clinical Services Lab, LLC, an Alabama limited liability company ("CSL"), on the provided sample(s) using the Illumina NovaSeq 6000 sequencing platform. DNA was checked for concentration and quality. The DNA was then sonicated to a specific fragment size and prepared as a paired-end library with ligation of Illumina flowcell-specific adapter sequences and a unique barcode. The prepared library was then quality checked for adequate yield through fluorescence methods and quantitative polymerase chain reaction (PCR), as well as for appropriate library size and profile using bioanalysis. Libraries were clustered onto Illumina NovaSeq 6000 flowcells and sequenced using standard Illumina reagents and protocols.

After sequencing, reads were generated using Illumina's bcl2fastq and data were aligned to the human reference GRCh38. Quality metrics were evaluated at each stage of sample preparation and analysis to ensure quality data. Fragments mapping to multiple regions of the reference genome were removed from analysis, as were fragments having a low quality score. Duplicate fragments were removed from analysis. Variant calling then proceeded using unique, quality mappings.

Sequence variants were loaded into a custom software analysis application called Codicem for interpretation. Within Codicem all sequence variants were annotated with relevant reference information from established data sources to provide support for the interpretations set forth in this test report. Population allele frequencies were derived from data collected by the gnomAD consortium (Karczewski, 2020). A listing of data sources is available upon request. All primary, actionable secondary, and complex indel variants were confirmed by an orthogonal technology (Sanger (dideoxy) sequencing). Other reported variants were assessed by a validated algorithm and orthogonally confirmed when metrics indicated a possible false positive. Patient-specific orthogonal confirmation details are available upon request.

The ability of this test to identify abnormal variants (the analytical sensitivity) is dependent on the presence of the variants in the sequencing data provided to Codicem for evaluation. Statistical analysis shows that a minimum coverage of seven reads at a locus is required to reliably call heterozygous variants with a power equal to or greater than 99%. This depth of coverage is expected to yield an error rate comparable to the error rate that occurs in Sanger dideoxy terminator sequencing.

Regions of the human genome exist that are unable to be resolved by current technology, or are duplications of other regions which make accurate alignment difficult. Regions of the genome not fully known or where alignment is not yet possible are not reported by this test. Using this technology, it is only possible to sequence 90% to 95% of the human reference genome. The process uses an over-sequencing approach to achieve an average depth of coverage to support accurate variant calling over most regions of the genome. Analysis of the sequencing depth of all known coding regions of the genome is available upon request. A gene with insufficient coverage may harbor variants that are not detected by this test. A gene may also appear to have inadequate coverage when there is a deletion or insertion in the individual's gene sequence compared to the reference sequence.

This genome sequence test is designed to evaluate single nucleotide variants and small insertions or deletions within the human genome. This test is limited in its ability to detect mosaicism and chimerism. This technology is limited in its ability to accurately identify variants occurring in regions with high sequence identity to other regions of the genome (e.g. paralogous genes and pseudogenes). Certain types of sequence variation are difficult to identify using this test and have not been validated to be reliably detected for current clinical use. These include insertions, deletions, copy number variations, long repeat sequences, triplet repeat expansions, structural chromosomal

rearrangements, polyploidy, aneuploidy, repetitive regions such as mono-, di- and trinucleotide repeats, GC rich regions, intronic variants outside of canonical splice-sites, and epigenetic effects.

The clinical sensitivity of this test is affected by the genetic disorder sought in the patient. As WGS is used in the diagnostic evaluation of many different disorders, it is not possible to assign a single clinical sensitivity.

Primary/Secondary Bioinformatics Pipeline Version: Sentieon-201808.07/Strelka-2.9.10

Data Store Version: 0.60.0

Codicem Version (Annotation): 5.3.4

Codicem Version (Report): 5.5.2

Interpretation Limitations

The interpretations set forth in this report assume that all information provided to the CSL, including any family relationships and all information stated on the sample submission or test requisition form, are accurate and fully answered. Because of the limitation of today's sequencing technology and scientific knowledge, a genetic abnormality may still exist even if a variant is not included in this report. If specific clinical disorders are suspected, specific evaluation of known genes by alternate test methods should be considered. False positive, negative or misleading results may occur for reasons that include genetic variants, blood transfusions, bone marrow transplantation, erroneous representation of family relationship, or contamination of a specimen. However, the CSL has standard and effective procedures in place to protect against such technical and operational problems.

The interpretations set forth in this report are based only upon current scientific knowledge and technology. Each gene sequence is interpreted independently of all other gene sequences. This test attempts to use current scientific knowledge to identify possible genetic variants; however, current scientific knowledge about the function of variants, genes, and other portions of the genome, and the ways in which genetic disorders are inherited, is incomplete. For example, variants in different genes may sometimes interact to cause disease, and variants may modify the phenotype of a monogenic disease. It cannot be excluded that pathogenic variants were missed due to limitations inherent in the sequence analysis method used in this test.

These results should be interpreted in the context of an individual's medical evaluation, family history, and racial/ethnic background. The data, interpretations and results of this test are not intended to recommend or discourage any specific treatment plan, product or course of action in an individual's medical care.

The clinical implications of some variants may be uncertain at the time of analysis. As clinical testing and research continue, some variants which are presently identified as "variants of uncertain significance" in this report may later be identified as pathogenic or non-pathogenic variations based on advances in medical knowledge and new discoveries. Variants of uncertain significance have uncertain effects on gene function, have not been previously reported, or have been reported with inadequate or conflicting evidence regarding pathogenicity or clinical relevance. As such, please note that variant classification and/or interpretation may change over time if and when more information becomes available. Therefore, it is possible that re-interpretation of these test results after any scientific advancement related to a variant of uncertain significance could lead to new information about a medical condition or clinical disorder an individual may be experiencing. Such re-interpretation must be requested by an individual's health care provider, and will involve additional costs.

The identification of some variants, including those associated with disease, is limited by the current state of

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Patient Name	Jane Doe	**Ordering Physician**	Dr. Gene	**Specimen Type**	Blood
Date of Birth	01/01/1970	**Date of Report**	04/01/2021	**Test Type**	WGS
Race/Ethnicity	Caucasian	**Sample Collection Date**	01/01/2021	**Accession #**	ABCDEFG
Gender	Female	**Sample Received Date**	01/01/2021	**Provider Accession ID**	8675309
		Test Request Date	01/01/2021		

knowledge in the genomics field and the annotations of variants in currently available public and private databases.

Whole genome sequencing is often not able to identify the cause of an individual's medical issues, provide information about prognosis, disease severity or help guide medical screening or treatment. If these test results did not identify a genetic cause for a medical condition, it is still possible a genetically-determined, medical condition could exist. Therefore, these results should be supplemented with genetic counseling regarding the possible genetic findings and potential implications of the genetic information contained herein.

Variants found in the individual that are benign or likely benign, as identified in the medical literature based on ACMG criteria, are not generally reported but may be available upon request.

This test was developed, and the associated orthologous methods and performance characteristics were determined by, the CSL. It has not been cleared or approved by the U.S. Food and Drug Administration. To date, the U.S. Food and Drug Administration has determined that such clearance or approval is not necessary. These tests are used for clinical purposes, and therefore validation was done as required under the requirements of the Clinical Laboratory Improvement Act of 1988. These test results should not be regarded as investigational or for research. The chance of a false negative or a false positive result due to laboratory error cannot be completely excluded.

CLIA #01D2086581

CAP #8051488

Signatures

David Bick, MD, FACMG, Associate Director, Clinical Services Laboratory

Electronically signed by David Bick on 04/01/2021