Rare and Potentially Pathogenic Variants in Hydroxycarboxylic Acid Receptor Genes Identified in Breast Cancer Cases.

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Abstract

Background: Three genes, clustered together on chromosome 12, comprise a group of Hydroxycarboxylic Acid Receptors (HCARs), *HCAR1*, *HCAR2*, and *HCAR3*. These paralogous genes encode different G-protein coupled receptors that are responsible for detecting glycolytic metabolites and controlling fatty acid oxidation. Though better known for regulating lipid metabolism in adipocytes, more recently HCARs have been functionally associated with breast cancer proliferation/survival; *HCAR2* has been described as a tumor suppressor, and *HCAR1* and *HCAR3* as oncogenes. Thus, we sought to identify genetic variants in *HCAR1*, *HCAR2,* and HCAR3 that could potentially be associated with breast cancer.

Methods: Due to the extreme homology between *HCAR1*, *HCAR2,* and *HCAR3*, primers were carefully designed to amplify each gene separately through nested PCRs followed by Sanger sequencing. Forty-six unrelated breast cancer cases were screened for rare, non-synonymous coding variants.

Results: Upon screening, a total of four variants were identified in four different cases, each with estrogen receptor-positive (ER+) breast cancer. The variants were identified exclusively in *HCAR1* and *HCAR3*. In *HCAR1*, two highly conserved and potentially damaging missense variants were identified, c.58C>G;p.Pro20Ala and c.721C>T;p.Leu241Phe. Statistical analyses revealed that c.58C>G;p.Pro20Ala was in significantly more cases than controls. In *HCAR3*, in addition to the breast cancer-associated missense variant c.560G>A;p.Arg187Gln, a frameshift mutation, c.1117delC;p.Gln373Lysfs*82, was detected that greatly extends the C-terminus and changes the secondary and tertiary protein structure.

Conclusions: Due to the important role of HCARs in breast cancer, it is vital to understand how these genetic variants play a role in breast cancer risk and proliferation, as well as their consequences on treatment strategies. Due to the small sample size, additional and larger studies will be needed to validate these findings. Nevertheless, the identification of these potentially pathogenic variants supports the need to investigate their functional consequences.

Background

There are three known genes clustered together on chromosome 12 that comprise a group of Hydroxycarboxylic Acid Receptors (HCARs), *HCAR1*, *HCAR2*, and *HCAR3*. With extreme homology, these paralogous genes encode three different heterotrimeric G-protein coupled receptors (GPCRs), also commonly referred to as GPR81, GPR109A, and GPR109B, respectively, that are responsible for detecting glycolytic metabolites and controlling the rate of fatty acid oxidation (1, 2). GPCRs are characterized by their 7-transmembrane domain structure with an N-terminus that interacts with extracellular components and a C-terminus that is responsible for transmitting intracellular signals. Since GPCRs are embedded in the cellular membrane and initiate signal transduction, they eliminate the challenge of intracellular drug administration and are very effective molecular drug targets, targeted by 30–50% of existing pharmaceuticals (3).

Though better known for their regulation of lipid metabolism in adipocytes, more recently HCARs have been functionally associated with breast cancer (BC) (1, 2, 4–7). For instance, *HCAR2* is described as a tumor suppressor gene because it exhibits a 70% reduction of cell-surface expression in primary BC cells and its cellular expression is essential for the initiation of apoptosis by its endogenous ligands (4). In contrast, *HCAR1* and *HCAR3* are considered oncogenes that show notably increased mRNA expressions in BC cells compared to controls and result in BC cell death when knocked down (5, 6). In fact, knockdown of *HCAR3* has been demonstrated to result in BC cell death through uncontrolled up-regulation of fatty acid oxidation, which can be mitigated by the introduction of fatty acid oxidation inhibitors (5). Furthermore, upon activation by lactate or butyrate, *HCAR1* increases the expression of DNA repair proteins and subsequently increases Hela cells' resistance to doxorubicin (7). Since doxorubicin is a common BC chemotherapy drug and reduced doxorubicin sensitivity is observed during BC treatment, potentially mediated by extracellular matrix proteins (8), *HCAR1* could be contributing to the resistance.

The metabolic function, differential expression, and promising therapeutic potential of HCARs make their involvement in BC tumorigenesis an interesting research topic. With potential implications in cancer precision medicine, it is important to identify inherited genetic variants that could be associated with BC risk, molecular subtype, and drug metabolism. This manuscript details the genetic screening of *HCAR1*, *HCAR2*, and *HCAR*3 in 46 BC cases from the Alabama Hereditary Cancer Cohort (AHCC) (9) to identify rare variants with potentially damaging effects.

Methods

Study cohort

Forty-six unrelated BC cases from the AHCC, who self-described as being White/European American, enrolled in a hereditary cancer genetics research study through Auburn University Institutional Review Board-approved protocols, 14–232 or 15–111, which was previously described by Bishop *et al*. (9). Informed consent was obtained through writing for all study participants. In brief, the study criteria included BC-affected individuals with a family history of the disease or diagnosed with BC under the age of 45 years. Blood samples were obtained from all participants and genomic DNA was extracted for genetic analyses.

Genetic analyses: Due to the extreme homology between *HCAR1* (NM_032554.3), *HCAR2* (NM_177551.3), and *HCAR3* (NM_006018.2), primers were carefully designed using Primer3Plus to amplify each gene separately through nested PCRs (Supplementary information: Tables S1, S2, and S3 and Figures S1, S2, and S3). External primers were designed for each gene that initially amplified a large (> 3.1 Kb) PCR product from genomic DNA. The forward and reverse external primers were respectively located upstream and downstream of each transcript and uniquely hit once in the genome according to University of California, Santa Cruz (UCSC) BLAT (10, 11). Overall, this design increased specificity and avoided amplification of the other paralogous genes. Internal primers were designed to subsequently amplify all regions of each large amplicon. For this study, which screened for coding variants in each gene, only internal primers that targeted coding regions were used for mutation analysis (Supplementary information: Tables S1, S2, and S3 and Figures S1, S2, and S3). Touchdown PCRs were carried out using a final annealing temperature of 56 °C. Extension times varied according to amplicon size (1 minute per 1 Kb), and betaine was required for GC rich amplicons. Regarding the internal primer sets, the initial PCR product was the template. PCR products of internal primer sets were sent for purification and Sanger sequencing at Eurofins Genomics. Sequences were analyzed using Mutation Surveyor (Soft Genetics). Similar to the

screening/validation process previously described (12), a systematic approach was followed for variant detection and validation. This involved screening genomic DNA from “Tube A” for the initial PCR of each study participant and, upon variant detection, validating variants by performing another initial PCR but using “Tube B.” Rare and non-synonymous coding variants (with MAFs of < 1% in Exome Variant Server (EVS) (13) European Americans) were validated.

Some internal primer sets would have amplified multiple products if the template was genomic DNA. To demonstrate that nested PCRs increased specificity, we used UCSC In-silico PCR (hg38 assembly) to determine genomic DNA amplicons (11), BLASTn to align/compare those amplicons (14, 15), and then viewed electropherograms of our nested PCR products, particularly at positions that varied between the amplicons (i.e., Supplementary information: Figure S4, confirming *HCAR3* c.560G > A;p.Arg187Gln).

For each validated variant, Fisher’s exact tests were performed to compare allele frequencies in BC cases versus controls. Two control datasets were used for these calculations, EVS (13) (European Americans) and gnomAD (European (non-Finnish)) (16). P-values of < 0.05 were considered to be statistically significant and were not corrected for multiple testing. Pathogenicity of each missense variant was predicted using Sorting Intolerant From Tolerant (SIFT) (17, 18), PROVEAN (19), Polyphen2 (20), PANTHER (21), and MutationTaster (22), and amino acid conservation was assessed using WebLogo (23). Regarding *HCAR3* p.Gln373Lysfs*82, Mutalyzer (24), Phyre2 (25), I-TASSER (26, 27), and PSORT (28, 29) were used to determine differences between the wildtype and mutant protein.

Results

A total of four rare non-synonymous variants were identified in four different BC cases, two in *HCAR1* and two in *HCAR3* (Table 1 and Supplementary information: Table S4). In *HCAR1*, two highly conserved missense variants were identified, both with conflicting pathogenicity predictions (Table 1 and Fig. 1). *HCAR1* c.58C > G;p.Pro20Ala, located at the N-terminus of the HCAR1 protein in the first extracellular topological domain (Fig. 1), was primarily predicted to be pathogenic through various prediction software and determined to be associated with BC risk (Table 1). The variant was detected in a female with an infiltrating lobular carcinoma (estrogen receptor-positive (ER+) and human epidermal growth factor receptor 2-negative (HER2-)) diagnosed at 35 years of age and a family history of BC and other cancers in first-, second- and third-degree relatives. This individual did not have clinical genetic screening but screened negative for clinically significant mutations through our research-based multi-gene panel screening (12). Regarding *HCAR1* c.721C > T;p.Leu241Phe, a statistically significant difference was not identified between cases and controls, and two of the five prediction software suggested it was pathogenic (Table 1). It is located directly at the junction of the sixth transmembrane domain and the third extracellular loop, but specifically embedded within the cell membrane (Fig. 1). It was detected in a female diagnosed with grade I or II DCIS (ductal carcinoma in situ) with necrosis at 42 years. The tumor was ER + and progesterone receptor-positive (PR+). This individual did not have a family history of BC but had a first- and second-degree relative diagnosed with other cancer types. Lastly, in the absence of clinical genetic screening, previous research-based screening did not reveal any clinically relevant mutations (12).

Table 1
HCAR1 and *HCAR3* rare non-synonymous variants.

EA	Coding change	Protein change	GRCh38 Position	rs ID	Alleles	MAF (%)			Comparing AHCC BC ca		
									EVS (European Americans)		g (F
						AHCC BC cases	Population Controls (data repositories)		p-value	Odds ratio	p v
							EVS (European Americans)	gnomAD (European (non-Finnish))			
HCAR1	c.58C > G	p.Pro20Ala	chr12:122730282	rs148912167	G > C	1.087	0.000^	0.000	0.011	Inf CI_{95}[2.40-Inf]	0
	c.721C > T	p.Leu241Phe	chr12:122729619	rs140482291	G > A	1.087	0.151	0.183	0.139	7.25 CI_{95}[0.17–49.3]	0
HCAR3	c.560G > A	p.Arg187Gln	chr12:122716178	rs373069919	C > T	1.087	0.012	0.028	0.021	93.99 CI_{95}[1.19-6881.84]	0
	c.1117delC	p.Gln373Lysfs*82	chr12:122715621	N/A	delG	1.087	0.012	0.005	0.022	90.33 CI_{95}[1.14-6625.78]	0

^not detected in EVS; therefore, used "# of EA Samples Covered".

Two variants were identified in *HCAR3* that appear to be associated with BC (Table 1). Despite a statistically significant difference in minor allele frequencies (MAFs) between cases and controls, *HCAR3* c.560G > A;p.Arg187Gln, located in an extracellular loop (Fig. 1 and Supplementary information: Figure S4), was unanimously predicted to be benign (Table 1). It was detected in a female diagnosed with moderately differentiated, infiltrating ductal carcinoma (ER+, PR +

and HER2-) at the age of 38 years. Interestingly, this individual was previously determined to have a frameshift mutation in the clinically relevant BC susceptibility gene *NBN* (30) (through our research-based multi-gene panel screening (12)), but lacks a family history of the disease. Lastly, an extremely rare *HCAR3* frameshift mutation (Table 1) was identified in a female diagnosed with ER + PR + and HER2- infiltrating ductal carcinoma at 62 years of age. She had previous negative clinical genetic screening, which was confirmed through our research-based screening (12). The mutation, c.1117delC;p.Gln373Lysfs*82, greatly extends the C-terminus of HCAR3 and changes the secondary and tertiary protein structure (Fig. 2). Moreover, PSORT predicted a loss of a prenylation motif, as well as a gain of an ER Membrane Retention Signal and gain of a peroxisomal targeting signal in the mutant HCAR3 (Table 2). The individual with this variant had one first- and two second-degree relatives with BC, along with other cancers in additional first- and second-degree relatives. No additional family members participated in the study for segregation analyses.

Table 2
PSORT predictions of HCAR3 wildtype and mutant, p.Gln373Lysfs*82.

HCAR3 protein	ER Membrane Retention Signals:	NUCDISC: discrimination of nuclear localization signals	Tripeptide SKL-motif	Prenylation Motif	k-NN (k-nearest neighbors algorithm)			
Wildtype	KKXX-like motif in the C-terminus: NONE	content of basic residues: 9.8%	peroxisomal targeting signal in the C-terminus: NONE	CCIE	55.6%: endoplasmic reticulum	22.2% vacuolar	11.1% nuclear	11.1% Golgi
Mutant	KKXX-like motif in the C-terminus: KMGK	content of basic residues: 11.5%	peroxisomal targeting signal in the C-terminus: GKL	NONE	77.8%: endoplasmic reticulum	11.1% vacuolar	0.0% nuclear	11.1% Golgi

Discussion

Upon screening 46 European American BC cases from the AHCC for rare non-synonymous variants in *HCAR1*, *HCAR2*, and *HCAR3*, a total of four variants were identified in four different BC cases. These variants were exclusively identified in *HCAR1* and *HCAR3*, which is notable considering their suggested oncogenic role and requirement for BC proliferation and survival, compared to the demonstrated tumor suppressor properties of *HCAR2* (4–6).

The HCAR1 protein is known as the lactate receptor, and, upon HCAR1 binding, lactate inhibits lipolysis (1, 2, 31). Liu *et al.* identified ligand-binding pockets in HCAR1, specifically demonstrating that particular missense variants in transmembrane domains three (p.Arg99Ala), six (p.Tyr233Ala and p.Arg240Ala), and seven (p.Thr267Ala) diminished the response of HCAR1 to L-lactate (Fig. 1A) (31). It is important to note the proximity of p.Arg240Ala to one of the missense variants detected in this study, p.Leu241Phe, within the sixth transmembrane domain (Fig. 1A). The vital function of Arg240 in lactate binding, as well as the identification of other critical residues directly located at a transmembrane and extracellular (Arg71) (32) or cytoplasmic (Ala110) (33) loop junction (Fig. 1A), hints towards the importance of the highly conserved Leu241 and the potential damaging effects of an amino acid substitution at that location. Coincidentally, the other *HCAR1* missense variant detected in a BC affected-individual in this study, p.Pro20Ala, is also near a transmembrane/extracellular domain junction and specifically located in the extracellular N-terminal domain (Fig. 1A). Another key, extracellular residue with similar junction proximity, Cys88, abolishes receptor activity when substituted with an Ala or Ser. Furthermore, two other critical cysteine residues, Cys6 and Cys7, are located in the N-terminus (Fig. 1A) (32); thus, the highly conserved Pro20 may also be vital for protein function. The need to further study p.Pro20Ala is compounded by the fact that the N-terminus, despite being highly variable between different GPCRs and not extensively studied, is important for ligand binding, dimerization, signaling, and surface expression (32, 34–36); additionally, p.Pro20Ala was determined to be genetically associated with BC.

Considering that several of the prediction software suggested both *HCAR1* p.Pro20Ala and p.Leu241Phe were pathogenic, determining their functional involvement in BC is critical. To date, all functionally assessed *HCAR1* variants have been deemed loss-of-function (highlighted (in color) in Fig. 1A) (31–33). However, with HCAR1 typically being regarded as critical for BC proliferation and survival by controlling lipid/fatty acid metabolism(5, 6), a loss-of-function mutation would presumably result in BC cell death. Interestingly, knocking down *HCAR1* has different effects on different BC molecular subtypes (5, 6). For instance, knocking down *HCAR1* in a HER2-enriched BC cell line, HCC1954 (ER-, PR-, HER2+), and triple negative BC cell line, HCC38 (ER-, PR-, HER2-), resulted in a significant decrease of cell viability within 48 hours of transfection. However, there was no significant change in viability regarding the luminal B cell line, BT-474 (ER+, PR+, HER2+), similar to the non-tumorigenic epithelial breast cell line, MCF12A (5). Furthermore, when *HCAR1* was knocked down in the luminal A BC cell lines, MCF-7 and T47D, cell viability decreased (6). In our study, *HCAR1* p.Pro20Ala and p.Leu241Phe were detected in individuals diagnosed with luminal subtypes. Specifically, the individual with p.Pro20Ala had luminal A BC (ER + and HER2-), which according to Lee *et al.* requires *HCAR1* to proliferate (6). The individual with p.Leu241Phe was ER + and PR + but HER2 status was unknown; thus, the subtype could not be confirmed as luminal A or B. If it was luminal B, *HCAR1* expression would not be not required for survival (5), whereas it would be required for luminal A (6). In additional to knock down studies, Lee *et al.* investigated *HCAR1* expression levels in different BC molecular subtypes and noted that ER + BC cell lines expressed *HCAR1* at a higher level (6); this ER + BC-association has also been reported for another GPCR, GPR30 (37). Thus, it is important to determine if *HCAR1* p.Pro20Ala and p.Leu241Phe are specifically associated with ER + BC, as well as if they are loss- or gain-of-function mutations.

HCAR3, which is only found in higher primates, is the receptor for 3-hydroxylated β-oxidation intermediates, particularly 3-hydroxy-octanoate (1, 2). When activated, HCAR3 inhibits free fatty acid release from cells, providing a negative feedback mechanism to offset stimuli that promote lipolysis and fatty acid oxidation. Knocking down *HCAR3* in BC cell lines BT-474, HCC1954, and HCC38 induced cell death, suggesting that HCAR3 has oncogenic properties. Introducing fatty acid oxidation inhibitors mitigated the knock down effects, confirming that uncontrolled up-regulation of fatty acid oxidation promotes BC cell death; thus, HCAR3 plays an vital role in controlling fatty acid metabolism in BC cells (5). Accordingly, one can presume that the two BC-associated *HCAR3* variants identified in this study, p.Arg187Gln and p.Gln373Lysfs*82, have gain-of-function effects. However, it is important to note that *HCAR3* knock down effects have not been assessed in luminal A BC cell lines, which is the molecular subtype reported in the two BC-affected individuals with each *HCAR3* variant. Numerous *HCAR3* genetic variants have been reported in publically available databases (13, 16), as well as through polymerase chain reaction (PCR)-based

techniques (38), and while their pathogenic effects have not been functionally assessed, our study suggests that rare non-synonymous variants in *HCAR3* may enhance the receptor's ability to control fatty acid metabolism. Nonetheless, *HCAR3* p.Arg187Gln, located in the third extracellular topological domain (Fig. 1), was unanimously predicted to be benign. Even though prediction software have been shown to misclassify known pathogenic variants (39), it is important to note that HCAR2, which shares 95% sequence identity with HCAR3, has a glutamine at that overlapping position. Though we have confirmed *HCAR3* p.Arg187Gln through nested PCR (Supplementary information: Figure S4), it is unknown if this change would affect the function of HCAR3. That being said, with such slight differences between the two proteins, perhaps each alteration is key to protein function. On another note, this variant was detected in an early onset BC case also determined to harbor a clinically significant frameshift mutation in *NBN* (12, 30). The interaction of these two variants and their combined ability to promote BC is unknown, but, intriguingly, expression of both *HCAR3* and *NBN* have been reported to be dysregulated in oocytes of older women, when investigating why aneuploidy pregnancies occur in women of older ages. Overall, this observation suggests that these genes may play a role in proper chromosome segregation and maintaining genomic integrity, which is a phenomenon also disrupted in cancer (40, 41).

The *HCAR3* frameshift mutation, p.Gln373Lysfs*82, significantly extends the C-terminal, cytoplasmic tail of the mutant HCAR3 protein and changes the secondary and tertiary protein structure (Fig. 2). Again, based on the suggested oncogenic role of HCAR3 in BC, *HCAR3* p.Gln373Lysfs*82 may potentially result in a gain-of-function. Interestingly, distinct mutation profiles, corresponding to clusters of nonsense and frameshift mutations in the C-termini of GPCRs, GPR34, CCR6, and CCR4, have been reported in mucosa-associated lymphoid tissue (MALT) lymphoma and adult T cell leukemia/lymphoma (ATLL) as gain-of-function mutations (42–44). Even though the nonsense and frameshift mutations reported in *GPR34*, *CCR6*, and *CCR4* truncate the encoded proteins, PSORT predicted that *HCAR3* p.Gln373Lysfs*82 abolishes a prenylation motif in a manner similar to how the *GPR34* mutations eliminate a key phosphorylation motif and ultimately dysregulate the receptor's desensitization process (42, 43). Additionally, the mutant HCAR3 protein gains an ER Membrane Retention Signal, potentially affecting internalization patterns, which is also disrupted with *CCR4* gain-of-function mutations (42, 44). Contrarily, the addition of a predicted peroxisomal targeting signal to the mutant HCAR3 hints toward protein degradation, a loss-of-function mechanism, and, on a similar note, read-through mutations that result in mutant proteins with C-terminal extensions in *PNPO* and *HSD3B2* cause hereditary disorders through protein degradation (45). Nonetheless, *GATA3* frameshift mutations that extend the C-terminus are the most common somatic mutation identified in The Cancer Genome Atlas (TCGA) BC patients and display gain-of-function activity (46). Loss-of-function *GATA3* mutations were also identified, demonstrating that both loss- and gain-of-function mutations can be identified in the same gene and associated with BC. Similarly, *TP53*, a clinically valid BC susceptibility gene, has both tumor suppressor and oncogenic properties (47–49). Thus, the exact functional consequences of *HCAR3* p.Gln373Lysfs*82 may be complex but are important to elucidate, especially considering the extensive homology between HCAR3 and HCAR2, and that HCAR2 has domains in the cytoplasmic, C-terminus vital for receptor export, internalization, constitutive activity, and desensitization (50).

Conclusions

HCAR1, *HCAR2*, and *HCAR3* are three genes clustered on chromosome 12 that encode HCARs, known GPCRs that play a critical role in lipid metabolism, even in the context of BC proliferation and survival. Upon genetic analysis of a cohort of BC-affected individuals, potentially damaging, non-synonymous genetic variants in *HCAR1* and *HCAR3* were identified that could alter receptor function. Though the sample size is small, the identification of genetic associations bolsters the need to investigate the functional consequences of these variants, but certainly these associations need to be validated in additional and larger cohorts. Ultimately, it is vital to understand how these genetic variants play a role in BC risk and proliferation, as well as their consequences on treatment strategies, particularly regarding the use of doxorubicin, a commonly prescribed BC chemotherapy drug (7, 8).

List Of Abbreviations

AHCC: Alabama Hereditary Cancer Cohort

ATLL: adult T cell leukemia/lymphoma

BC: breast cancer

DCIS: ductal carcinoma in situ

ER: estrogen receptor

EVS: Exome Variant Server

GPCR: G-protein coupled receptor

HCAR: hydroxycarboxylic acid receptor

HER2: human epidermal growth factor receptor 2

MAF: minor allele frequency

MALT: mucosa-associated lymphoid tissue

PCR: polymerase chain reactions

PR: progesterone receptor

SIFT: sorting intolerant from tolerant

Declarations

Ethics approval and consent to participate: This study was approved by the Auburn University Institutional Review Board (protocols 14-232 and 15-111). Informed consent was obtained through writing for all study participants.

Consent for publication: Written informed consent for publication of identifying images or other personal or clinical details was obtained from all of the participants.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions:

Conceptualization of study: NDM

Genetic analysis: CMS, KS, MRB, NDM

Writing and editing of manuscript: CMS, JT, NDM

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Figures

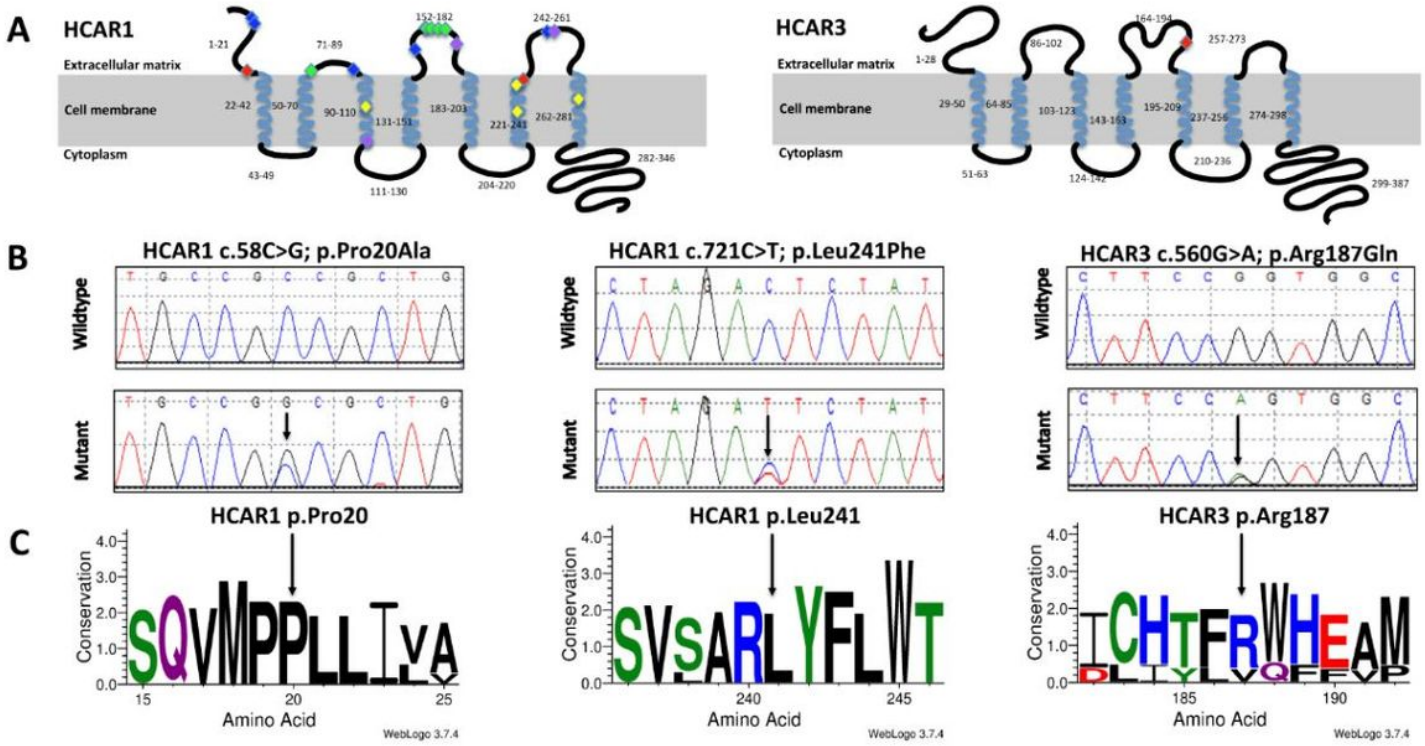

Figure 1

Rare missense variants in HCAR1 and HCAR3. A) Topological illustrations of HCAR1 (Q9BXC0) and HCAR3 (P49019) with highlighted missense variants (red: HCAR1 Pro20Ala and Leu241Phe, and HCAR3 Arg187Gln identified in BC cases in this study; yellow: HCAR1 transmembrane domain variants, Arg99Ala, Tyr233Ala, Arg240Ala, and Thr267Ala, reported by Liu et al. to diminish the response to L-lactate [31]; green: HCAR1 residue Arg71 and motif Cys165-Glu166-Ser167-Phe168 identified by Kuei et al. as being critical for protein function [32]; blue: HCAR1 extracellular Cys residues, Cys6, 7, 88, 157, 165 (green) and 252, reported by Kuei et al. to abolish receptor activity when substituted with Ala or Ser [32]; purple: HCAR1 Ala110Val, Ser172Leu, and Asp253His identified by Doyle et al. as loss-of-function variants [33]). B) Sanger sequencing comparisons of wildtype and mutant. C) WebLogo amino acid conservation. The y-axis shows relative sequence conservation and the height of symbols in the stack show relative frequency for that position (HCAR1 sequences were aligned with the following orthologues: Homo sapiens (Q9BXC0), Mus musculus (Q8C131), Canis lupus familiaris (B9UM26), Pan troglodytes (G2HJ56), Pongo pygmaeus (A0A4Y1JWL9), and Pan paniscus (A0A2R8ZEB9); HCAR3 sequences were aligned with: Homo sapiens (P49019), Xenopus laevis (A0A1L8I0Z3), Pan troglodytes (H2RAM9), Pongo abelii (H2NJ01), Pan paniscus (A0A4Y1JWN7), and Pongo pygmaeus (A0A4Y1JWR4)).

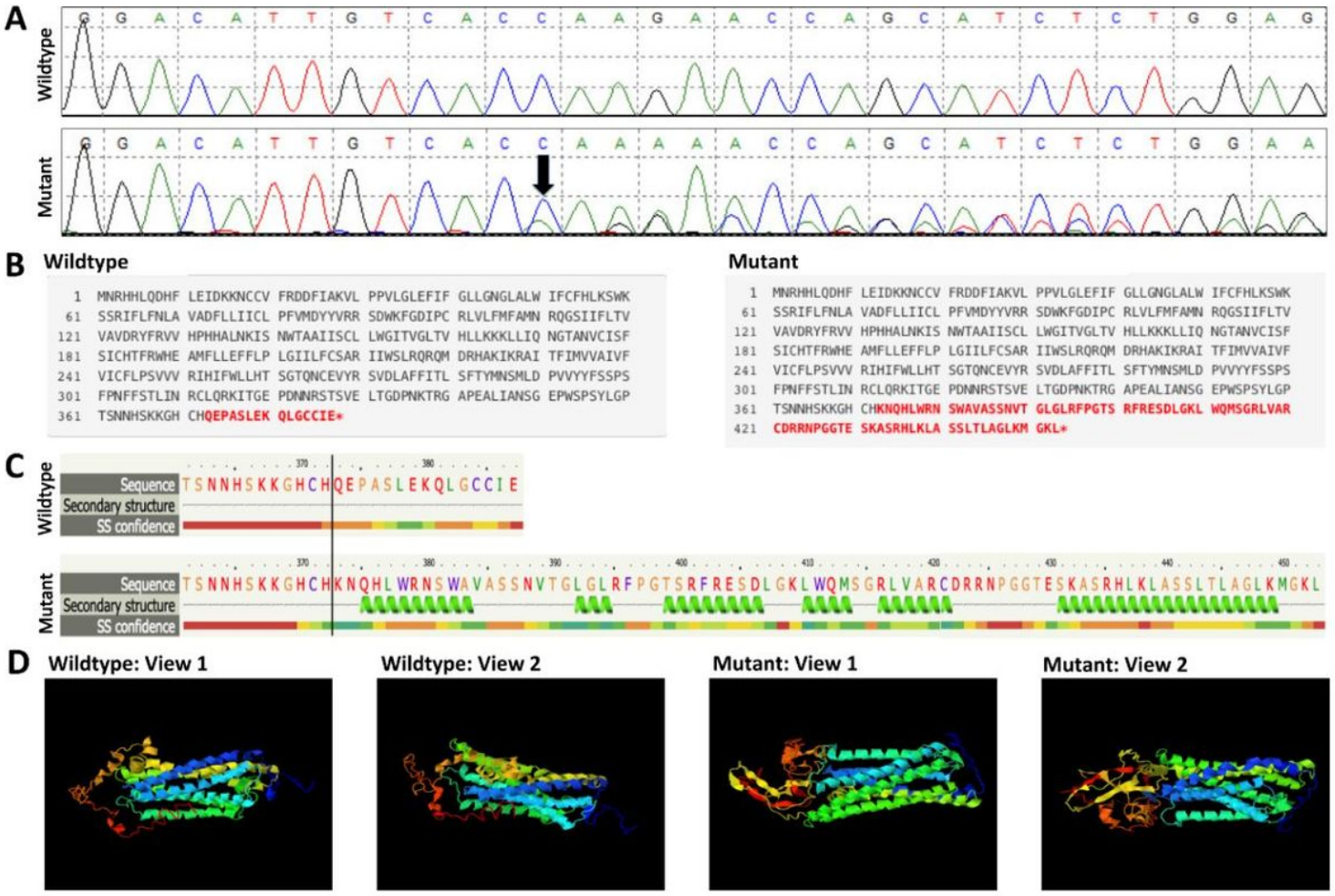

Figure 2

HCAR3 frameshift mutation, c.1117delC;p.Gln373Lysfs*82. A) Sanger sequencing comparisons of wildtype and mutant, illustrating the deletion of the cytosine. B) Mutalyzer comparison of wildtype and mutant, highlighting the different C-termini in red. C) Phyre2 protein prediction software displaying secondary structure differences between wildtype and mutant. D) I-TASSER protein prediction comparisons of the wildtype and mutant protein (each in two different views) to show the C-terminus extension and difference in tertiary structures; PyMOL was used to display the molecular graphics.

Supplementary Files

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- SupplementaryInformation.pdf