No Evidence for the Pathogenicity of the *BRCA2* c.6937 + 594T>G Deep Intronic Variant: A Case–Control Analysis

Julie Dutil,¹ Lenin Godoy,¹ Rafael Rivera-Lugo,² Nelly Arroyo,¹ Elinette Albino,¹ Luis Negrón,³ Alvaro N. Monteiro,⁴ Jaime L. Matta,¹ and Miguel Echenique⁵

Background: The role of deep intronic variants in hereditary cancer susceptibility has been largely understudied. Previously, the *BRCA2* c.6937 + 594T>G variant has been shown to preferentially promote the inclusion of a 95 nucleotide cryptic exon and to introduce a premature termination codon. Our objective was to further assess the pathogenicity of the *BRCA2* c.6937 + 594T>G deep intronic variant.

Patients and Methods: We examined the association between *BRCA2* c.6937 + 594T>G and breast cancer (BC) risk in 464 BC cases and 497 noncancer controls from Puerto Rico.

Results: The overall frequency of the G allele was 2.1% in this population. There was no association between the TG/GG genotypes and BC risk in the uncorrected model and after correcting for confounders. There was only one carrier of the GG genotype. This individual did not have personal or family history of cancer and did not meet the National Comprehensive Cancer Network criteria for hereditary cancer genetic testing.

Conclusions: Although previous work has demonstrated that the *BRCA2* c.6937 + 594T>G variant affects splicing, this association study does not support a pathogenic role for the *BRCA2* c.6937 + 594T>G intronic variant in breast and ovarian cancer syndrome susceptibility. Furthermore, it emphasizes the need to take into account multiple diverse populations in association studies for the assessment of variant pathogenicity.

Keywords: hereditary breast and ovarian cancer syndrome, *BRCA2*, intronic variant, variant classification

Introduction

THE TUMOR SUPPRESSOR GENES *BRCA1* (OMIM No. 113705) and *BRCA2* (OMIM No. 600185) are necessary for the maintenance of genomic stability (Gudmundsdottir and Ashworth, 2006). Inherited pathogenic mutations in *BRCA1* and/or *BRCA2* have been found to increase the risk of developing breast and ovarian cancer in women, prostate and breast cancer (BC) in men, and other types of cancer in both men and women (Chen and Parmigiani, 2007). The identification of a *BRCA* pathogenic variant leads to clinical decisions regarding cancer prevention strategies such as increased surveillance and prophylactic surgeries, which are associated with improved outcomes (Scheuer *et al.*, 2002; Pruthi *et al.*, 2010).

It has been shown that for a subset of high-risk families, *BRCA1* and *BRCA2* testing for hereditary breast and ovarian cancer syndrome (HBOC) does not identify a causative variant (Caminsky *et al.*, 2016). Traditionally, diagnostic

screening for cancer risk assessment was performed on genomic DNA and limited to coding exons and intron/exon junctions (Frank *et al.*, 2002). As a result, ~79% of the *BRCA* variants reported in the Breast Cancer Information Core database are frameshifts, resulting in the insertion of a premature termination codon, missense or nonsense changes, leaving changes that may alter splicing largely unexplored (National Institutes of Health, 2017). Previous studies have proposed that splicing variants may account for a subset of HBOC cases (Sanz *et al.* 2010; Gambino *et al.*, 2015).

Recently, transcript analysis for individuals with strong familial cancer history that does not present pathogenic variants in coding sequences or at exon/intron junctions led to the identification of a deep intronic mutation in *BRCA2* (c.6937 + 594T>G; rs191253965), promoting the inclusion of a 95 nucleotide cryptic exon and introducing a premature termination codon (Anczuków *et al.*, 2012). Based on molecular analyses showing a preference for the cryptic splice site

¹Cancer Biology Division, Ponce Research Institute, Ponce Health Sciences University, Ponce, Puerto Rico.

²Department of Biology, University of Puerto Rico in Ponce, Ponce, Puerto Rico.

³Hematology-Oncology Program, VA Caribbean Healthcare System, San Juan, Puerto Rico.

⁴Cancer Epidemiology Program, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida.

⁵Cancer Center, Auxilio Mutuo Hospital, San Juan, Puerto Rico.

TABLE 1. ASSOCIATION BETWEEN *BRCA2* INTRON 12 c. 6937+594T>G AND BREAST CANCER RISK

Genotype	Controls, % (n)	Cases, % (n)	OR (95% CI), p		
			Crude	Adjusted ^a	Adjusted ^b
TT	96.2 (478)	95.5 (443)	Ref.	Ref.	Ref.
TG+GG	3.8 (19)	4.5 (21)	1.19 (0.63–2.27), 0.6	1.17 (0.58–2.38), 0.5	0.98 (0.34–2.76), 0.3

^aAdjusted for age, education level, and civil status (*n* missing = 185).

^bAdjusted for age, education level, civil status, age at menopause, use of estrogen replacement therapy, number of children, and breast feeding duration (*n* missing = 598).

CI, confidence interval, OR, odds ratio; Ref., referent.

compared with the canonical site, the authors proposed that this variant should be considered pathogenic (Anczuków *et al.*, 2012).

Due to an observed frequency of 3.8% for the rare allele in the Puerto Rico 1000 Genomes project sample, this population represents a unique opportunity to explore the role of this deep intronic mutation in conferring high risk for HBOC. The present study assesses the association of the *BRCA2* c.6937+594T>G variant with BC in Puerto Rican women, using an incident case–control study design. We present evidence against a role of this variant in conferring high risk to BC.

Patients and Methods

Study population

This study utilized an incident case–control design. The study was approved by the Ponce Health Sciences University–Ponce Research Institute Institutional Review Board. Participants (464 BC cases and 497 noncancer controls) were selected from our larger BC study, which has been previously described (Matta *et al.*, 2012; Morales *et al.*, 2013). Women 21 years or older, whose three out of four grandparents were born in Puerto Rico, are included in the study. Inclusion criteria for cases were patients who (1) were recently diagnosed histopathologically with primary BC and (2) were treatment-naïve (had not received chemotherapy, blood transfusions, or radiotherapy). Cases were not selected for family history or age of onset. All individuals recruited as controls had a negative mammography and breast examination within the last 6 months. Participants completed a standard questionnaire regarding their demographic information, hormonal and pregnancy history, and family history of cancer. In addition, for BC patients, a tumor pathology report was obtained. Personal and family history information was used to estimate pretest likelihood of mutation using the BRCAPRO (Parmigiani *et al.*, 1998) and Myriad II (Frank *et al.*, 2002) models. The carrier risk was calculated independently for maternal and paternal sides of each family.

Genotyping

Genomic DNA from lymphocytes of 464 BC patients and 497 controls was extracted using QIAamp DNA Mini Kit (Qiagen, Valencia, CA), following the manufacturer's protocol. Genotyping of *BRCA2* (c.6937+594T>G) (rs191253965) was conducted using a TaqMan Custom Allelic Discrimination Assay (Applied Biosystems, Foster City, CA). This assay consists of two primers for amplifying the sequence of interest and two TaqMan minor groove binding probes for detecting allele 1(T) and allele 2(G), using the fluorophores

FAM and VIC, respectively. PCR was performed in 5 μ L reaction volumes under the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. Genotypes were confirmed by Sanger sequencing in a subset of 29 individuals. Primers for PCR amplification and sequencing were as follows: 5'-TGATGCCATTACCGATCAGA-3' and 5'-GGGAATCCAAGCTGTCAAAA-3' (Anczuków *et al.*, 2012).

Statistical analyses

All statistical analyses were conducted with R version 3.2.1 implemented in R Studio (RStudio Team, 2015). Differences in frequency distributions were calculated by Pearson's chi-square test. The association between *BRCA2* intron 12 c.6937+594T>G variant and BC risk was analyzed by multiple logistic regression analysis using confounding factors as covariates. The impact of genotype on BC risk was quantified by an odds ratio (OR) and by calculating the 95% confidence interval (CI).

Results

The demographic, pregnancy, and hormonal history of the study population are summarized in Supplementary Table S1 (Supplementary Data are available online at www.liebertpub.com/gtmb). Overall, controls were younger ($p < 0.0001$), more likely to be married and less likely to be widows ($p < 0.0001$) than BC cases. When comparing reproductive risk factors, a significant difference was observed in the number of children ($p < 0.001$). For cases, menopause occurred later in life ($p = 0.004$), and they were less likely to have used estrogen replacement therapy ($p = 0.008$). Both groups were similar for all other variables measured.

Out of a total of 961 BC cases and controls, 18 nonaffected controls and 21 BC cases were heterozygous for the *BRCA2* c.6937+594T>G variant. In addition, one control was found to be homozygous for the G allele. The genotype frequencies for *BRCA2* c.6937+594T>G did not significantly deviate from the Hardy–Weinberg equilibrium in cases ($\chi^2 = 0.3021$, $p = 0.8$) or in noncancer controls ($\chi^2 = 0.587$, $p = 0.7$). There was no significant association when comparing the frequency of carriers (TG+GG) between BC cases and controls in the unadjusted model (OR = 1.19, 95% CI = 0.63–2.27, $p = 0.6$), after adjusting for age, education level, and civil status (OR = 1.17, 95% CI = 0.58–2.38, $p = 0.5$) or after adjusting for all confounders (OR = 0.98, 95% CI = 0.34–2.76, $p = 0.3$) (Table 1).

To further assess the potential clinical relevance of the *BRCA2* c.6937+594T>G variant, the prior carrier risks of homozygous and heterozygous carriers were estimated using

FIG. 1. Distribution of the prior carrier probability in carriers of the c.6937 + 594T>G alternate allele grouped by diseases status. BRCA carrier probability as estimated by the myriad tables (**a**) and BRCA2 carrier probability as estimated by the BRCAPRO model (**b**).

the Myriad tables and BRCAPRO tool (Fig. 1). The distribution and ranges of the pretest likelihood of carrying a BRCA2 mutation, as predicted by BRCAPRO and the Myriad tables, were not different in carriers of the c.6937 + 594 [G] allele versus controls. Overall, only a minority of the carriers (13%) met the National Comprehensive Cancer Network (NCCN) criteria (version 2.2016) for HBOC genetic testing (NCCN, 2016). It is also noteworthy that a 51-year-old participant was found to be homozygous for this variant. She had no personal history of cancer, did not meet the NCCN criteria for genetic testing, and her estimated prior risk of carrying a *BRCA* pathogenic variant was estimated at 1.5% and 0.1% with the Myriad tables and BRCAPRO model, respectively. This participant reported family history of endometrial cancer, but no family history of breast or ovarian cancer at any age.

Discussion

In the current study, we have assessed the potential pathogenicity of a *BRCA2* deep intronic variant, c.6937 + 594T>G, using genetic association and family history studies. Previous molecular studies have indicated that this variant preferentially activate a cryptic splice site within intron 12, leading to the inclusion of a 95 nucleotide cryptic exon in the *BRCA2* transcript (Anczuków *et al.*, 2012). We provide evidence that this variant is unlikely to represent a pathogenic variant in the context of high risk of BC. In the current study, we did not observe an association between the genotype at *BRCA2* c.6937 + 594T>G and BC risk.

Several hundreds of pathogenic variants have been identified in the *BRCA* genes (National Institutes of Health, 2017). Combined with the low prevalence of hereditary

cancers, each of these variants is expected to be rare in an unselected population. Due to a well-characterized founder effect, the Ashkenazi Jewish population presents higher prevalence of pathogenic variants in the *BRCA* genes, explained mostly by three founder mutations: *BRCA1* 185delAG, 5382insC, and *BRCA2* 6174delT (Neuhausen *et al.*, 1996; Tonin *et al.*, 1996). It is noteworthy that the combined frequency for these three variants in the Ashkenazi Jewish population adds up to 2.6% (Roa *et al.*, 1996; Frank *et al.*, 2002; Phelan *et al.*, 2002). In Hispanic populations, the *BRCA1* 185delAG variant is also the most frequent, reported in seven countries, including U.S. Hispanics (Dutil *et al.*, 2015). Yet, the reported frequency for this variant in the Exome Aggregation Consortium (ExAC) Database is <0.03% (ExAC, 2016). In the 1000 Genomes Project Phase 3 dataset, the [G] allele at *BRCA2* c.6937+594T>G has been observed in 10 individuals, which corresponds to an overall frequency of 0.2% (1000 Genomes Consortium, 2016). Since most of the carriers were observed in Puerto Ricans from Puerto Rico (PUR), the frequency of the rare allele was estimated to be 3.8% in that population (1000 Genomes Consortium, 2016). Although variant prevalence can vary significantly across race and ethnicity, it would be unlikely for a single pathogenic *BRCA* variant to reach such a high population frequency.

The classification of *BRCA* variants using epidemiological studies is usually complicated by the limited power associated with low population frequencies of the rare allele. Given that the observed allelic frequency for the [G] allele at *BRCA2* c.6937+594T>G is of 2.1% in this study and an estimated hereditary cancer prevalence of 0.2% (Janavičius, 2010), power calculations indicate that the current sample would be adequate for detecting a risk variant that is associated with an OR of 2.15-fold or higher with a power of 80.7%. Although *BRCA* pathogenic variants have been associated with ORs in the order of 3.2- to 6.8-fold (Ford *et al.*, 1998; Pruthi *et al.*, 2010), we cannot rule out that this variant have a more modest effect on risk as described for the polymorphic stop codon K3326X (Meeks *et al.*, 2015). However, for the purposes of clinical risk assessment, these variants should not be considered pathogenic (Spurdle *et al.*, 2016).

In *BRCA1* and *BRCA2*, functional *in vitro* assays revealed that 44–49% of the splicing variants predicted functional by bioinformatics approaches result in aberrant splicing (Sanz *et al.*, 2010; Acedo *et al.*, 2012). In a minigene assay, the *BRCA2* c.6937+549G>T variant was shown to systematically promote splicing from the cryptic splice site (Anczuków *et al.*, 2012). However, splicing of patients RNA is not always concordant with minigene assay results, which are single allele and lack the genomic context of the patient (Sanz *et al.*, 2010). In the neurofibromatosis type 1 gene (*NF1*), a splicing variant in exon 37 reproduces the exon skipping patterns observed in patients RNA only if the minigene construct spans exon 31–38, emphasizing the importance of the genomic context of neighboring exons in studying splicing mutations (Buratti *et al.*, 2006). The role of the genomic context and the splicing regulatory factors influencing the splicing outcomes in carriers of the *BRCA2* c.6937+549G>T variant remain to be determined. Nevertheless, although *in vitro* functional assays can provide valuable functional data for the clinical classification of splicing alterations, this information should be evaluated in the context

of additional evidence, such as family and epidemiological data (Thomassen *et al.*, 2012).

One of the limitations of this study is the younger age of controls when compared with cases. This is especially a concern since *BRCA2* mutations carriers tend to develop BC at later ages when compared with *BRCA1* carriers (Antoniou *et al.*, 2003). After correcting for age, there was no association between *BRCA2* c.6937+594T>G and BC risk. This observation is further supported by the family history characteristics, as carriers were not more likely to present with family history of the diseases. In fact, one individual (a control) was identified as a homozygous carrier of the [G] allele at this locus. Biallelic *BRCA2* carriers present with autosomal recessive Fanconi anemia, a genomic instability syndrome associated with developmental abnormalities, bone marrow failure, and a high predisposition to cancer (OMIM No. 605724). The nonpathogenicity of *BRCA* variants can therefore be assessed by the co-occurrence of a known pathogenic mutation in *trans* and the absence of the Fanconi anemia or other developmental delay phenotypes, to guard against the co-occurrence of hypomorphic variants (Domchek *et al.*, 2013; Eggington *et al.*, 2014). This individual did not present any indication of Fanconi anemia-related phenotypes or cancer.

Several groups have reported that splicing mutations may cause hereditary diseases (López-Bigas *et al.*, 2005), including breast and ovarian cancer syndrome (Sanz *et al.*, 2010; Thomassen *et al.*, 2012; Wappenschmidt *et al.*, 2012; Ahlborn *et al.*, 2015). As more comprehensive genetic analysis assays are incorporated in routine clinical testing, more splicing and variants are likely to be identified. Assessing the pathogenicity of these variants will be important to prevent the complications associated with clinical management of individuals found to carry a variant of unknown significance (Murray *et al.*, 2011). Our results also emphasize the utility of including diverse population in family history and epidemiologic studies aimed at classifying variants underlying genetic diseases.

Acknowledgments

The authors are grateful to all the women who participated in this study. We also want to thank the clinic teams at Auxilio Mutuo and Wanda Vargas at Ponce Research Institute. This work was supported by grant of the National Institute of Health (NIH), National Cancer Institute (NCI) (U54 CA163071, CA163068, and U01 CA116167) with the technical support of the Ponce Research Institute Molecular, and Genomics Core (M.A.G.I.C) (NIH-National Institute on Minority Health and Health Disparities (NIMHD) MD007579). Recruitment of participants was supported by grants from the NIH-National Institute of General Medical Sciences (NIGMS) (S06-GM008239 and 9SC1CA182846). R Rivera-Lugo received a fellowship from NIH-NIGMS (R25GM096955).

Author Disclosure Statement

No competing financial interests exist.

References

- 1000 Genomes Consortium (2016) 1000 Genome browser. Available at: <http://browser.1000genomes.org> (accessed on April 20, 2017).

- Acedo A, Sanz DJ, Durán M, *et al.* (2012) Comprehensive splicing functional analysis of DNA variants of the *BRCA2* gene by hybrid minigenes. *Breast Cancer Res* 14:R87.
- Ahlborn LB, Dandanell M, Steffensen AY, *et al.* (2015) Splicing analysis of 14 *BRCA1* missense variants classifies nine variants as pathogenic. *Breast Cancer Res Treat* 150:289–298.
- Anczuków O, Buisson M, Léoné M, *et al.* (2012) *BRCA2* deep intronic mutation causing activation of a cryptic exon: opening toward a new preventive therapeutic strategy. *Clin Cancer Res* 18:4903–4909.
- Antoniou A, Pharoah PDP, Narod S, *et al.* (2003) Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 72:1117–1130.
- Buratti E, Baralle M, Baralle FE (2006) Defective splicing, disease and therapy: searching for master checkpoints in exon definition. *Nucleic Acids Res* 34:3494–3510.
- Caminsky NG, Mucaki EJ, Perri AM, *et al.* (2016) Prioritizing variants in complete hereditary breast and ovarian cancer genes in patients lacking known *BRCA* mutations. *Hum Mutat* 37:640–652.
- Chen S, Parmigiani G (2007) Meta-analysis of *BRCA1* and *BRCA2* penetrance. *J Clin Oncol* 25:1329–1333.
- Domchek SM, Tang J, Stopfer J, *et al.* (2013) Biallelic deleterious *BRCA1* mutations in a woman with early-onset ovarian cancer. *Cancer Discov* 3:399–405.
- Dutil J, Golubeva VA, Pacheco-Torres AL, *et al.* (2015) The spectrum of *BRCA1* and *BRCA2* alleles in Latin America and the Caribbean: a clinical perspective. *Breast Cancer Res Treat* 154:441–453.
- Egginton JM, Bowles KR, Moyes K, *et al.* (2014) A comprehensive laboratory-based program for classification of variants of uncertain significance in hereditary cancer genes. *Clin Genet* 86:229–237.
- Exome Aggregation Consortium (ExAC) (2016) ExAC Browser. Available at: <http://exac.broadinstitute.org> (accessed on May 15, 2017).
- Ford D, Easton DF, Stratton M, *et al.* (1998) Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. *Am J Hum Genet* 62:676–689.
- Frank TS, Deffenbaugh AM, Reid JE (2002) Clinical Characteristics of individuals with germline mutations in *BRCA1* and *BRCA2*: analysis of 10,000 individuals. *J Clin Oncol* 20:1480–1490.
- Gambino G, Tancredi M, Falaschi E, *et al.* (2015) Characterization of three alternative transcripts of the *BRCA1* gene in patients with breast cancer and a family history of breast and/or ovarian cancer who tested negative for pathogenic mutations. *Int J Mol Med* 35:950–956.
- Gudmundsdottir K, Ashworth A (2006) The roles of *BRCA1* and *BRCA2* and associated proteins in the maintenance of genomic stability. *Oncogene* 25:5864–5874.
- Janavičius R (2010) Founder *BRCA1/2* mutations in the Europe: implications for hereditary breast-ovarian cancer prevention and control. *EPMA J* 1:397–412.
- López-Bigas N, Audit B, Ouzounis C, *et al.* (2005) Are splicing mutations the most frequent cause of hereditary disease? *FEBS Lett* 579:1900–1903.
- Matta J, Echenique M, Negron E, *et al.* (2012) The association of DNA Repair with breast cancer risk in women. A comparative observational study. *BMC Cancer* 12:490.
- Meeks HD, Song H, Michailidou K, *et al.* (2015) *BRCA2* polymorphic stop codon K3326X and the risk of breast, prostate, and ovarian cancers. *J Natl Cancer Inst* 108:dvj315.
- Morales L, Alvarez-Garriga C, Matta J, *et al.* (2013) Factors associated with breast cancer in Puerto Rican women. *J Epidemiol Glob Health* 3:205–215.
- Murray ML, Cerrato F, Bennett RL, *et al.* (2011) Follow-up of carriers of *BRCA1* and *BRCA2* variants of unknown significance: variant reclassification and surgical decisions. *Genet Med* 13:998–1005.
- National Comprehensive Cancer Network (2016) NCCN clinical practice guidelines in oncology: genetic/familial high risk assessment: breast and ovarian. Available at: www.nccn.org/professionals/physician_gls/f_guidelines.asp#detection (accessed on April 20, 2016).
- National Institutes of Health, National Human Genome Research Institute (NIH-NHGRI) (2017) Breast Cancer Information Core (BIC). Available at: <https://research.nhgri.nih.gov/projects/bic/index.shtml> (accessed on July 20, 2017).
- Neuhausen S, Gilewski T, Norton L, *et al.* (1996) Recurrent *BRCA2* 6174delT mutations in Ashkenazi Jewish women affected by breast cancer. *Nat Genet* 13:126–128.
- Parmigiani G, Berry DA, Aguilar O (1998) Determining carrier probabilities for breast cancer-susceptibility genes *BRCA1* and *BRCA2*. *Am J Hum Genet* 62:145–158.
- Phelan CM, Kwan E, Jack E, *et al.* (2002) A low frequency of non-founder *BRCA1* mutations in Ashkenazi Jewish breast-ovarian cancer families. *Hum Mutat* 20:352–357.
- Pruthi S, Gostout BS, Lindor NM (2010) Identification and management of women with *BRCA* mutations or hereditary predisposition for breast and ovarian cancer. *Mayo Clin Proc* 85:1111–1120.
- Roa BB, Boyd AA, Volcik K Sr, *et al.* (1996) Ashkenazi Jewish population frequencies for common mutations in *BRCA1* and *BRCA2*. *Nat Genet* 14:185–187.
- RStudio Team (2015) RStudio: Integrated Development for R. RStudio. Available at: www.rstudio.com (accessed on December 15, 2015).
- Sanz DJ, Acedo A, Infante M, *et al.* (2010) A high proportion of DNA variants of *BRCA1* and *BRCA2* is associated with aberrant splicing in breast/ovarian cancer patients. *Clin Cancer Res* 16:1957–1967.
- Scheuer L, Kauff N, Robson M, *et al.* (2002) Outcome of preventive surgery and screening for breast and ovarian cancer in *BRCA* mutation carriers. *J Clin Oncol* 20:1260–1268.
- Spurdle AB, Domchek S, Robson M, *et al.* (2016) Response. *J Natl Cancer Inst* 108:dvj315.
- Thomassen M, Blanco A, Montagna M, *et al.* (2012) Characterization of *BRCA1* and *BRCA2* splicing variants: a collaborative report by ENIGMA consortium members. *Breast Cancer Res Treat* 132:1009–1023.
- Tonin P, Weber B, Offit K, *et al.* (1996) Frequency of recurrent *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer families. *Nat Med* 2:1179–1183.
- Wappenschmidt B, Becker AA, Hauke J, *et al.* (2012) Analysis of 30 putative *BRCA1* splicing mutations in hereditary breast and ovarian cancer families identifies exonic splice site mutations that escape *in silico* prediction. *PLoS One* 7:e50800.

Address correspondence to:

Julie Dutil

Ponce Research Institute

Cancer Biology Division

Ponce Health Sciences University

395, Dr. Luis F. Sala Street

Puerto Rico

Puerto Rico

E-mail: jdutil@psm.edu