Breast Cancer Intrinsic Subtypes: A Critical Conception in Bioinformatics

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M.Sc. in Genetics

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

The University of Newcastle
Faculty of Science and Information Technology
School of Environmental and Life Sciences

Callaghan, NSW
Australia

September, 2016
Statement of Originality

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September, 2016

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Heloisa Helena Zaccaron Milioli

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Prof. Pablo Moscato

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Statement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

September, 2016

__________________________
Heloisa Helena Zaccaron Milioli

__________________________
Prof. Pablo Moscato
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To the best grandmother,

Helena Mafioleti Zaccaron
(Wherever you are)
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# Abbreviations

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<tr>
<td>ACR</td>
<td>Australasian Association of Cancer Registries</td>
</tr>
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<td>ACS</td>
<td>American Cancer Society</td>
</tr>
<tr>
<td>AIHW</td>
<td>Australian Institute of Health and Welfare</td>
</tr>
<tr>
<td>AJCC</td>
<td>American Joint Committee on Cancer</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen receptor</td>
</tr>
<tr>
<td>ARI</td>
<td>Adjusted Rand Index</td>
</tr>
<tr>
<td>BL1</td>
<td>Basal-like 1</td>
</tr>
<tr>
<td>BL2</td>
<td>Basal-like 2</td>
</tr>
<tr>
<td>BLBC</td>
<td>Basal-like breast cancer</td>
</tr>
<tr>
<td>BLIA</td>
<td>Basal-like immune-activated</td>
</tr>
<tr>
<td>BLIS</td>
<td>Basal-like immune-suppressed</td>
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<tr>
<td>ChIP-chip</td>
<td>Chromatin immunoprecipitation on chip</td>
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<tr>
<td>CIBEX</td>
<td>Center for information biology gene expression database</td>
</tr>
<tr>
<td>CIBM</td>
<td>Centre for Bioinformatics, Biomarker Discovery and Information-Based Medicine</td>
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<tr>
<td>CGH</td>
<td>Comparative genomic hybridization</td>
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<tr>
<td>CNA</td>
<td>Copy number aberration</td>
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<tr>
<td>CNV</td>
<td>Copy number variation</td>
</tr>
<tr>
<td>CTD</td>
<td>Comparative Toxicogenomic Database</td>
</tr>
<tr>
<td>DamID</td>
<td>DNA adenine methyltransferase identification</td>
</tr>
<tr>
<td>DAVID</td>
<td>Database for Annotation, Visualization and Integrated Discovery</td>
</tr>
<tr>
<td>DDBJ</td>
<td>DNA Data Bank of Japan</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EBI</td>
<td>European Bioinformatics Institute</td>
</tr>
<tr>
<td>EGA</td>
<td>European Genome-Phenome Archive</td>
</tr>
<tr>
<td>EpCAM</td>
<td>Epithelial cell adhesion molecule</td>
</tr>
<tr>
<td>ER</td>
<td>Oestrogen receptor</td>
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<tr>
<td>FGED</td>
<td>Functional Genomics Data Society</td>
</tr>
<tr>
<td>FOIPPA</td>
<td>Freedom of Information and Protection of Privacy Act</td>
</tr>
<tr>
<td>FS</td>
<td>Feature Selection</td>
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<tr>
<td>GEO</td>
<td>Gene Expression Omnibus</td>
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<tr>
<td>HER2</td>
<td>Human epidermal growth factor receptor 2</td>
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<tr>
<td>HREC</td>
<td>Human Research Ethics Committee</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>HTC</td>
<td>High content screening</td>
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<tr>
<td>HTS</td>
<td>High-throughput screening</td>
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<tr>
<td>ICGC</td>
<td>International Cancer Genomics Consortium</td>
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<tr>
<td>IDC</td>
<td>Invasive ductal carcinoma</td>
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<tr>
<td>IHC</td>
<td>Immunohistochemical</td>
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<tr>
<td>IHGSC</td>
<td>International Human Genome Sequencing Consortium</td>
</tr>
<tr>
<td>ILC</td>
<td>Invasive lobular carcinoma</td>
</tr>
<tr>
<td>IM</td>
<td>Immunomodulatory</td>
</tr>
<tr>
<td>JS</td>
<td>Jensen Shannon</td>
</tr>
<tr>
<td>Ki-67</td>
<td>Antigen identified by monoclonal antibody Ki-67</td>
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<tr>
<td>kNN</td>
<td>$k$ nearest neighbours</td>
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<tr>
<td>LAR</td>
<td>Luminal androgen receptor</td>
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<tr>
<td>lincRNA</td>
<td>long intergenic non-coding RNA</td>
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<td>MA</td>
<td>Memetic algorithm</td>
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<tr>
<td>MCC</td>
<td>Matthews’ Correlation Coefficient</td>
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<td>MDL</td>
<td>Minimum Description Length Principle</td>
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<td>METABRIC</td>
<td>Molecular Taxonomy of Breast Cancer International Consortium</td>
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<td>MIAME</td>
<td>Minimum Information About a Microarray Experiment</td>
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<td>microRNA</td>
<td>miRNA</td>
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<td>MGED</td>
<td>Microarray Gene Expression Data Society</td>
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<td>MS</td>
<td>Menopausal status</td>
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<tr>
<td>MST</td>
<td>Minimum Spanning Tree</td>
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<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
</tr>
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<td>NOS</td>
<td>Not otherwise specified</td>
</tr>
<tr>
<td>NPI</td>
<td>Nottingham prognostic score</td>
</tr>
<tr>
<td>NSC</td>
<td>Nearest Shrunken Centroids</td>
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<tr>
<td>NST</td>
<td>No special type</td>
</tr>
<tr>
<td>ORF</td>
<td>Open reading frame</td>
</tr>
<tr>
<td>PIPA</td>
<td>Personal Information Protection Act</td>
</tr>
<tr>
<td>PIPEDA</td>
<td>Personal Information Protection and Electronic Documents Act</td>
</tr>
<tr>
<td>PR</td>
<td>Progesterone receptor</td>
</tr>
<tr>
<td>PRC</td>
<td>Priority Research Centres</td>
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<tr>
<td>RHD</td>
<td>Research Higher Degree</td>
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<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>ROCK</td>
<td>Research Online Cancer Knowledgebase</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse-transcriptase Polymerase chain reaction</td>
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<td>Acronym</td>
<td>Description</td>
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<tr>
<td>SAM</td>
<td>Sentrix® Array Matrix</td>
</tr>
<tr>
<td>SCM</td>
<td>Subtype Classification Model</td>
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<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SSP</td>
<td>Single Sample Predictor</td>
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<tr>
<td>TCGA</td>
<td>The Cancer Genome Atlas</td>
</tr>
<tr>
<td>TEND</td>
<td>Trends in the Exploration of Novel Drug targets</td>
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<tr>
<td>TNBC</td>
<td>Triple-negative breast cancer</td>
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<tr>
<td>TNM</td>
<td>Tumour size, nodes, metastasis</td>
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<tr>
<td>TTD</td>
<td>Therapeutic Target Database</td>
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<tr>
<td>UCSC</td>
<td>University of California Santa Cruz</td>
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<tr>
<td>WEKA</td>
<td>Waikato Environment for Knowledge Analysis</td>
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Achievements

During my PhD, I applied for grants; submitted manuscripts for publication; and attended workshops, conferences and seminars. The relevant achievements are listed as follows:

Grants Awarded

- Hunter Medical Research Institute, 2014.

**JENNIE THOMAS MEDICAL RESEARCH TRAVEL GRANT (AUD $10,000)**


**HCRA TRAVEL GRANT (AUD $1,000)**


**HCRA PhD Research Award 2016 (AUD $5,000).**

- EMBL Australia PhD, 2016.

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- XII ELAG Course Fellowship (USD $700)

**Instituto Genética Para Todos – Brazil** (unable to attend)

Papers Published in Journals


**Abstracts Published**


Oral Presentations


Poster Sessions


**Other Presentations**

**Confirmation Year Presentation**

Faculty of Science and IT. The University of Newcastle, 2013.

*RHD candidates are required to submit the ‘Confirmation Year Report’ and present the research overview. In August 2013, I presented the preliminary results in the Faculty of Science and IT as an open seminar.*

**HCRA, ECR and PhD Student (HEAPS) Seminar Series**

Hunter Medical Research Institute. The University of Newcastle, 2014 and 2015.

*The HEAPS seminar series are organised by the Hunter Cancer Research Alliance (HCRA) for RHD students and supervisors. It is an opportunity for researchers to practice presenting (and critiquing) work in a local and highly supportive environment. In 2014 and 2015, I presented and discussed the results of my research as well as supported other researchers’ work.*

**HUBS3302 Bioinformatics Mini-Conference**

Faculty of Health and Medicine. The University of Newcastle, 2014 and 2015.

*The purpose of this event is to inspire students in the field and, specially, in their final project for the discipline. In the 2014 and 2015 Bioinformatics Mini-Conference, organised by Belinda Goldie, I presented my research on breast cancer.*
Science and Engineering Challenge

Faculty of Engineering and Built Environment. The University of Newcastle, 2014, 2015 and 2016.

The ‘Science and Engineering Challenge’ organise a number of events aimed at challenging students of all different ages in Science and Engineering. As part of the team, I coordinated activities in Tamworth (2014), Muswellbrook (2014), Dubbo (2015), Newcastle (2015), Central Coast (2016) and Narrabri (2016), and presented my research to the Rotary International (Australian Rotary Districts) in Tamworth and Dubbo.

Faculty Progress Seminar

Faculty of Science and IT. The University of Newcastle, 2015.

Students in the Faculty of Science and IT are required to present a Progress Seminar after completing 2 to 3 years of a PhD. In June 2015, I discussed the overall aims and results of my research and outlined my thesis to fellow RHD candidates and academics in the school.

Google Computer Science for High Schools

Faculty of Engineering and Built Environment. The University of Newcastle, 2015 and 2016.

The University of Newcastle's Computer Science 4 High Schools (CS4HS) is an introductory workshop for in-service and pre-service teachers (both at primary and secondary level), and career advisors focused on developing competencies included in the recently approved Digital Technologies curriculum and is accredited by BOSTES. In three events, I had the opportunity to explain the relevance of computer science to analyse biological/medical data.

Relevant Activities

Course: Winter School in Mathematical and Computational Biology

University of Queensland (UQ), Brisbane, 2013.
The winter school introduced mathematical and computational biology and bioinformatics to advanced undergraduate and postgraduate students, postdoctoral researchers and others working in the field. Important topics, such as mathematics, statistics, computer science, information technology, biology, chemistry and medical sciences and engineering, were selected for each day. Lectures and interactive discussions were ministered by national and international authorities.

Course: **European Molecular Biology Laboratory (EMBL) Australia PhD Course**
Australian National University (ANU), Canberra, 2014.

EMBL Australia offered to sixty students a unique introduction to research with the annual EMBL Australia PhD Course. The two-week program shows students how their research fits into the bigger picture of science, and introduces a range of fields including: bioinformatics, developmental biology, genomics, systems biology and regenerative medicine.

Course: **European Molecular Biology Laboratory (EMBL) Australia PhD Course**
Welcome Genome Campus, Hinxton, UK, 2016.

This course introduced a wide range of post-genome techniques including practical experience in performing (1) high-throughput RNAi screening, (2) microarray gene expression analysis and interpretation, using a range of commercial and academic software tools, (3) next-generation sequencing and alignment; (4) protein-protein interaction networks and integration with other data sources, and (5) pathway analysis. Laboratory work was based on the training of state-of-the-art methods and complementary approaches to address biological and medical questions.

Training: **Collaborative Research Training in Human Genetics and Bioinformatics**
Centre for Bioinformatics, Biomarker Discovery and Information-Based Medicine (CIBM). The University of Newcastle, 2014.

The CIBM established a research-training program in 2014 that contributed to improve the capacity of young investigators to conduct human genetics and bioinformatics research. The training promoted scientific collaborations between the University of Newcastle and international (undergraduate) students. The proposed program provided opportunities to generate expertise that could contribute to the
long-term goal of harnessing genetic knowledge and bioinformatics skills to diagnose, prevent, or treat diseases. Training activities were coordinated, facilitated and monitored by Prof. Pablo Moscato, A/Prof Regina Berretta and PhD student Heloisa Helena Milioli.

Short-term Exchange Program: **Cheminformatics and Chemogenomics Research Group (CCRG)**

Indiana University (IU), Bloomington USA, 2015.

*Further investigation on cheminformatics and toxicogenomics has been developed in collaboration with A/Prof. David J. Wild (May/June 2015), at the School of Informatics and Computing in Bloomington (USA). These approaches were used to delineate drug-targets for basal-like breast cancer, one of the most aggressive subtypes with limited therapy response. Further research, however, is required to design and perform in vitro tests.*

Organising Committee: **Australian Society for Medical Research (ASMR) Satellite Scientific Meeting**

Hunter Medical Research Institute (HMRI), Newcastle, 2015.

*This event showcases the recent research achievements of Hunter scientists, encourages postgraduate and student interactions and fosters collaboration between researchers within the Faculty of Health and Medicine, HMRI and the international community. In the 2015 edition, I was member of the committee.*
Abstract

Breast cancers have been uncovered by high-throughput technologies that allow the investigation at the genomic, transcriptomic and proteomic levels. In the early 2000s, the gene expression profiling has led to the classification of five intrinsic subtypes: luminal A, luminal B, HER2-enriched, normal-like and basal-like. A decade later, the spectrum of copy number aberrations has further expanded the heterogeneous architecture of this disease with the identification of 10 integrative clusters (IntClusts). The referred classifications aim at explaining the diverse phenotypes and independent outcomes that impact clinical decision-making. However, intrinsic subtypes and IntClusts show limited overlap. In this context, novel methodologies in bioinformatics to analyse large-scale microarray data will contribute to further understanding the molecular subtypes. In this study, we focus on developing new approaches to cover multi-perspective, highly dimensional, and highly complex data analysis in breast cancer. Our goal is to review and reconcile the disease classification, underlying the differences across clinicopathological features and survival outcomes. For this purpose, we have explored the information processed by the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC); one of the largest of its type and depth, with over 2000 samples. A series of distinct approaches combining computer science, statistics, mathematics, and engineering have been applied in order to bring new insights to cancer biology. The translational strategy will facilitate a more efficient and effective incorporation of bioinformatics research into laboratory assays. Further applications of this knowledge are, therefore, critical in order to support novel implementations in the clinical setting; paving the way for future progress in medicine.

Keywords
Breast cancer, Intrinsic subtypes, Integrative clusters, IntClusts, Microarray, Gene expression, Copy number aberration, MicroRNA, METABRIC, Feature selection, Data mining, Ensemble learning, Prediction models, Classification