Clinical and Molecular Biological Studies in Hirschsprung’s Disease.

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I hereby certify that the work presented herein is the result of original research, and is not being submitted for a higher degree to any other university or institution. All laboratory work was principally carried out by the author, with some assistance, as noted in the acknowledgements on page 4.

Geoffrey David Hain Croaker  
Date
Ethics Clearance.

The studies described in this thesis were approved by the ethics committee of the New Children’s Hospital at Westmead, and by the ethics committee at the Queen’s Medical Centre, Nottingham. (Studies in chapters on respiratory control and electrogastrography.) All involved families gave informed consent for the studies.
Dedication

To my family who tolerated my preoccupation and many absences.

And to the patients, who were (mostly) patient with me.

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Abstract:

HSCR has been felt to be a polygeneic disease on the basis of an incompletely penetrant sex modified transmission, which may be either autosomal dominant or recessive in different kindreds. During the 1990’s several of the genes involved in this transmission have come to light. Other genes remain to be discovered.

This thesis contributes to the understanding of the phenotype and genotype of polygeneic HSCR.

- A unique distribution of aganglionosis and sex distribution is shown for Ondine’s Curse, Hirschsprung disease (Haddad syndrome). Certain other features of this condition are defined.
- The distribution of associated abnormalities in Down Syndrome is shown to be affected by the presence of HSCR.
- A locus on chromosome 2 was defined which has since been shown to be the site of the SMADIP1 gene, mutated in some syndromic cases of HSCR.
- GDNF mutations are shown to be rare contributors to HSCR. One particular polymorphism (“R93W”) is demonstrated which probably contributes to the phenotype in association with RET mutations.
- The T366A polymorphism in GFRα1 shows a tendency to be under-represented in HSCR.
- The RET haplotype comprising polymorphisms in exons 11 and 15 is shown to be rarer in HSCR than in control populations.
- Consistent with the presence of mutations in genes widely expressed in the developing nervous system, more widespread subtle abnormalities are demonstrated in the autonomic system of HSCR patients than simply colonic aganglionosis.
- The outcome of DS/HSCR is shown to be dependent on not only length of aganglionosis, and the presence of other associated malformations, but also the child’s developmental capacity.

The above findings support the polygeneic nature of HSCR, and expand the range of genetic loci in the disease, as well as expanding the range of phenotype in the condition.
Publications and presentations directly arising from the thesis:


“Congenital Central Hypoventilation Syndrome and Hirschsprung’s Disease” Croaker GDH, Shi E, Simpson E, Cartmill T, Cass DT. Archives of Disease in Childhood, 78(4):316 - 322. (April 1998.)


Presentations:


"Late presentation of Hirschsprung's disease." Kumar R, Croaker GDH, Cass DT. PAPS meeting, Peking, May 1999


“Electrogastrogram (EGG) suggests diffuse disorder in Hirschsprung’s (HSCR) gut.” C Arndt, B Davies, GDH Croaker. Presented to surgical research club of BAPS, London, July 2001
Publications and presentations arising in conjunction with the thesis:

“Segregation of 9q susceptibility gene in RET-linked Hirschsprung Families” S Bolk, M Angrist, D Croaker, L Kruglyak, A Chakravarti. Poster at Annual Meeting of American Society of Human Genetics, San Francisco. October 1996. (Work contributing to a PhD project for S Bolk, Case Western Reserve University, Cleveland Ohio.)

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Abbreviations.

ACE Antegrade Continence Enema
ADHD Attention Deficit Hyperactivity Disorder
AS/AI Aortic Stenosis/Aortic Incompetence
ASD Atrial Septal Defect
BAER Brain Stem Auditory Evoked Response
BAPS British Association of Paediatric Surgeons
BSAER Brain Stem Auditory Evoked Response
BW Birth Weight
CA Colonic Atresia
CCHS Congenital Central Hypoventilation Syndrome. (Also Ondine’s Curse.)
CHD Congenital Heart Disease
CNS: Central nervous system
CSC Chronic Severe Constipation
CXR Chest X ray
DA Duodenal Atresia
DC Descending Colon
DGGE Denaturing Gradient Gel Electrophoresis.
DS: Down syndrome
ECE-1 Endothelin converting enzyme 1.
EDNRB: endothelin B receptor.
EGG ElectroGastroGram
ENS: Enteric nervous system
ET3: endothelin 3
FISH Fluorescence In Situ Hybridisation.
FMTC Familial Medullary Thyroid Carcinoma
GA General Anaesthetic
GDNF: Glial Derived Neurotrophic Factor.
GFRα1: GDNF family receptor alpha type 1. (Also types 2, 3, and 4 are known, and may be mentioned.)

GOR  Gastro-Oesophageal Reflux

HAEC:  Hirschsprung’s associated enterocolitis.

HSCR:  Hirschsprung’s disease.

DS/HSCR:  Down syndrome and Hirschsprung disease.

HVA  HomoVanillic Acid

ICU  Intensive Care Unit

IMV  Intermittent Mandatory Ventilation

IND  Intestinal Neuronal Dysplasia

ICAM1  Intercellular Adhesion Molecule 1

IET  Impedance Electrical Tomography. A method of measuring the gastric (or other hollow viscus) volume indirectly by passage of an electric current.

IVH  Intra Ventricular Haemorrhage

LICAM  Cell Adhesion molecule, the gene for which is mutated in X linked hydrocephalus.

LS  Long segment (aganglionosis)

LWF  Lethal White Foal (Horse model of aganglionosis.)

MASH-1  Mammalian AchaeteScute Homologous gene

Mb  Megabase. (One million DNA bases.)

MEN  Multiple Endocrine Neoplasia. (Types, 1, 2a and 2b)

MITF  Microphthalmia-Associated Transcription Factor. (Associated with Waardenberg type 2.)

MIS  Mullerian Inhibitory Substance

µl  microlitres

mM  millimoles

MRI  Magnetic Resonance Imaging

NEC  Necrotising EnteroColitis

NID  See IND

NR  Normal Range

NTN  Neurturin

OA/TOF  Oesophageal Atresia/Tracheo-Oesophageal Fistula

OC:  Ondine’s curse. (Also known as: CCHS: Congenital Central Hypoventilation Syndrome)
PAGE: PolyAcrylamide Gel Electrophoresis

PAX3 Paired Box 3. (Homeobox gene involved in Waardenburg type 1.)

PCR Polymerase Chain Reaction

PDA Patent Ductus Arteriosus

PDF Period Dominant Frequency (in EGG recordings)

PJ Proximal Jejunum

PPG Pulse PlethysmoGraphy

R93W Change from arginine to tryptophan at position 93 in GDNF. (This shorthand is generally used for amino acid changes due to mutation. [original amino acid-position number-new amino acid] The same letter before and after the number indicates a silent base pair substitution, e.g. R143R.)

REM Rapid Eye Movement (sleep)

RET: The full name of a membrane bound tyrosine kinase receptor. (Originally: “REarranged through Transfection)

RFLP Restriction Fragment Length Polymorphism

rnx Homeobox gene Hox11L2

RSB: Rectal suction biopsy

SD Standard Deviation

SIDS Sudden Infant Death Syndrome

SIP1 See SMADIP1

SLOS Smith Lemli Opitz Syndrome

SMADIP1 SMAD Interacting Protein 1. (Also known as SIP1, a HSCR gene on chromosome 2.)

SOX10 Sry related bOX 10. A gene for a transcription factor implicated in some HSCR.

SpO2 Pulse Oxygen Saturation

SS Short Segment (Aganglionosis)

SSCP: Single Stranded Conformational Polymorphism

taq Type of DNA polymerase enzyme used in PCR

TCA Total Colonic Aganglionosis

TGFβ Transforming Growth Factor Beta

TIA Total Intestinal Aganglionosis

TPN Total Parenteral Nutrition
UDT Undescended Testis
UTI Urinary Tract Infection.

VATER VATER association: Vertebral Ano-rectal Tracheo-Esophageal Renal association.

VMA Vanillyl Mandelic Acid
VSD Ventricular Septal Defect
VUR Vesico-Ureteric Reflux
WS Waardenburg Syndrome