

SCREENING FOR SPECIFIC MUTATIONS IN MALIGNANT HYPERTHERMIA SUSCEPTIBLE FAMILIES

by

YOLANDÉ HAVENGA

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Department of Human Genetics and Developmental Biology,
Faculty of Medicine, University of Pretoria

Project supervisor: **Dr. A. OLCKERS**

Department of Human Genetics and Developmental Biology,
Faculty of Medicine, University of Pretoria

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
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TO MY PARENTS

ABSTRACT

Malignant hyperthermia (MH) is an autosomal dominant pharmacogenetic disorder (Deufel *et al.*, 1992). It is potentially fatal and one of the leading causes of death due to anaesthesia. An MH episode is triggered when MH susceptible individuals are exposed to certain inhalation anaesthetic agents and depolarising skeletal muscle relaxants (MacLennan, 1992).

To date, twenty-three mutations have been reported in the skeletal muscle ryanodine receptor gene (RYR1), located on chromosome 19q13.1, and twenty-two of these mutations have been associated with MH (Brandt *et al.*, 1999). The I403M mutation has only been associated with central core disease (CCD). CCD and MH are phenotypically distinct allelic variants as mutations in the same gene are responsible for both these disorders (Quane *et al.*, 1993).

Seventeen MH families, four South African and thirteen North American families, were included in this study. DNA from selected MH individuals were screened for nine of the reported mutations (Cys35Arg, Arg163Cys, Gly248Arg, Gly341Arg, Ile403Met, Tyr522Ser, Arg614Cys, Gly2435Arg and Arg2436His). The interaction between polymorphisms and mutations, which might modify or contribute to the MH phenotype, is currently still unknown. For this reason the DNA from selected individuals were screened for the presence of all of the nine mutations investigated in this study.

Two mutations, Arg614Cys and Gly2435Arg, were observed in the group of MH families included in this study. The Arg614Cys mutation was identified in a large South African MH family (MH105). A total of 39 individuals of South African family MH105 were subsequently screened for the presence of this mutation. Twelve individuals, three of whom have not been tested via the *in vitro* contracture test (IVCT), were positive for the mutation. Two individuals from this family displayed phenotype-genotype discordance. The Gly2435Arg mutation was identified in a North American MH family (US2). This small family of five individuals included three individuals diagnosed via the North American IVCT protocol. The three phenotyped individuals included two MH positive and one MH negative individual. The Gly2435Arg mutation segregated with the MH phenotype observed in this North American family. This is the first report of the Gly2435Arg mutation in the North American MH population. The identification of the Arg614Cys and

Gly2435Arg mutations contributed towards establishing a molecular diagnostic service for individuals within these particular MH families.

None of the other seven mutations investigated were detected in the group of families included in this study. The DNA alterations are therefore not present as polymorphisms in these families, and can thus not contribute to the MH phenotype segregating in any of the families. If the families investigated in this study are truly representative of the South African and North American populations it is possible that these seven mutations are absent in these two populations. These mutations might, therefore, be population specific or even family specific.

OPSOMMING

Maligne hipertermie (MH) is 'n outosomale dominante farmakogenetiese toestand (Deufel *et al.*, 1992). Dit is 'n potensieel fatale toestand en een van die hoof oorsake van sterftes as gevolg van narkose. 'n MH aanval word veroorsaak wanneer MH vatbare individue blootgestel word aan sekere inhalasie narkosegasse en depolariserende skeletspier verslappers (MacLennan, 1992).

Tot op hede is drie-en-twintig mutasies gerapporteer in die skeletale ryanodien reseptor geen (RYR1), gelokaliseer op chromosoom 19q13.1, waarvan twee-en-twintig geassosieer word met MH (Brandt *et al.*, 1999). Die Ile403Met mutasie is slegs geassosieer met "central core" siekte (CCS). CCS en MH is unieke fenotipiese alleliese variante aangesien mutasies in dieselfde geen verantwoordelik is vir beide hierdie toestande (Quane *et al.*, 1993).

Sewentien MH families, vier Suid-Afrikaanse en dertien Noord-Amerikaanse families, was ingesluit in hierdie studie. DNA van geselekteerde MH individue is ondersoek vir nege van die gerapporteerde mutasies (Cys35Arg, Arg163Cys, Gly248Arg, Gly341Arg, Ile403Met, Tyr522Ser, Arg614Cys, Gly2435Arg en Arg2436His). Die interaksie tussen polimorfismes en mutasies, wat moontlik die MH fenotipe mag wysig of daartoe mag bydra, is tans steeds onbekend. Om hierdie rede is die DNA van geselekteerde individue ondersoek vir die teenwoordigheid van al nege die mutasies wat ingesluit was in die studie.

Twee mutasies, Arg614Cys en Gly2435Arg, is waargeneem in die groep MH families wat ingesluit was in hierdie studie. Die Arg614Cys mutasie is geïdentifiseer in 'n uitgebreide Suid-Afrikaanse MH familie (MH105). 'n Totaal van 39 individue van Suid-Afrikaanse familie MH105 is gevolglik ondersoek vir die teenwoordigheid van die mutasie. Twaalf individue, drie wie nog nie voorheen getoets was via die *in vitro* kontraktsie toets (IVKT) nie, is positief vir die mutasie. Twee individue van hierdie familie toon nie fenotiepe-genotiepe korrelasie nie. Die Gly2435Arg mutasie is geïdentifiseer in 'n Noord-Amerikaanse MH familie (US2). Die klein familie van vyf individue sluit drie individue in wat voorheen gediagnoseer was via die Noord-Amerikaanse IVKT protokol. Die drie gefenotipeerde individue sluit twee MH positiewe en een MH negatiewe individu in. Die Gly2435Arg mutasie segregeer saam met die MH fenotipe wat waargeneem is in die Noord-Amerikaanse familie. Hierdie is die eerste berig van die Gly2435Arg mutasie in die

Noord-Amerikaanse MH populsie. Die identifikasie van die Arg614Cys en Gly2435Arg mutasies dra by tot die vestiging van 'n molekulêre diagnostiese diens vir individue binne hierdie bepaalde families.

Geen van die ander sewe mutasies wat ondersoek is, is geïdentifiseer in die groep families wat in hierdie studie ingesluit was nie. Hierdie DNA veranderinge is dus nie teenwoordig as polimorfismes in hierdie families nie en kan dus nie bydrae tot die MH fenotipe wat segregeer in enige van hierdie families nie. Indien die families wat in hierdie studie ondersoek is werklik verteenwoordigend is van die Suid-Afrikaanse en Noord-Amerikaanse populasies, is dit moontlik dat hierdie sewe mutasies afwesig is in die twee populasies. Hierdie mutasies mag dus populasie spesifiek of selfs familie spesifiek wees.

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LIST OF ABBREVIATIONS AND SYMBOLS

α	alpha
α_1	alpha 1 sub-unit of the DHPR, expressed in skeletal muscle
α_2	alpha 2 sub-unit of the DHPR, expressed in skeletal muscle
α_2/δ	alpha 2 gamma sub-unit complex of the skeletal muscle dihydropyridine receptor
$\alpha^{32}\text{P-dCTP}$	dCTP labelled in the α position with ^{32}P isotope
$\alpha^{35}\text{S-dATP}$	dATP labelled in the α position with ^{35}S isotope
a	adenine
A or A	adenine (in DNA sequence, indicating exon sequence)
A or A	alanine (in amino acid sequence)
A_{260}	absorbency at 260 nm
A_{260}/A_{280}	ratio of absorbency measured at 260 nm and 280 nm
<i>Aci I</i>	restriction endonuclease isolated from <i>Arthrobacter citreus</i> , with recognition site 5'-...C↓CGC...-3'
ACRS	amplification created restriction site
ADP	adenosine diphosphate
Ala	alanine
<i>Alw NI</i>	restriction endonuclease isolated from <i>Acinetobacter Iwoffii N</i> , with recognition site 5'-...CAGNNN↓CTG...-3'
AMP	adenosine monophosphate
APS	ammonium persulphate: $(\text{NH}_4)_2\text{S}_2\text{O}_8$
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATG	initiator codon
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
β	beta
β_1	beta sub-unit of the DHPR, expressed in skeletal muscle
BESS T	base excision sequence scanning mutation detection and localisation
boric acid	boracic acid: H_3BO_3
bp	base pair
BPB	bromophenol blue: $\text{C}_{14}\text{H}_{10}\text{Br}_4\text{O}_5\text{S}$
BSA	bovine serum albumin
<i>Bsh 1236I</i>	restriction endonuclease isolated from <i>Bacillus sphaericus</i> RFL1236, with recognition site 5'-...CG↓CG...-3'
<i>BsmA I</i>	restriction endonuclease isolated from <i>Bacillus stearothermophilus</i> , with recognition site 5'-...GTCTC(N) ₁ ↓...-3'
<i>Bsr BI</i>	restriction endonuclease isolated from <i>Bacillus stearothermophilus cpw 193</i> , with recognition site 5'-...GAG↓CGG...-3'
<i>Bst UI</i>	restriction endonuclease isolated from <i>Bacillus stearothermophilus U</i> , with recognition site 5'-...CG↓CG...-3'
c	cytosine
C or C	cytosine (in DNA sequence, indicating exon sequence)
C or C	cysteine (in amino acid sequence)
°C	degrees centigrade
%C	percentage crosslinking monomer
ca.	circa: approximately
Ca^{2+}	calcium ions
Ca^{2+} -ATPase	calcium adenosine triphosphatase
Ca^{2+} -release	calcium release
Ca^{2+} -channel	calcium channel
CACNL1A3	α_1 sub-unit of the skeletal muscle dihydropyridine receptor

List of abbreviations and symbols (continued ...)

CACNL2A	α_2/δ sub-unit of the skeletal muscle dihydropyridine receptor
CACNLB1	β sub-unit of the dihydropyridine receptor
CACNLG	γ sub-unit of the dihydropyridine receptor
caffeine	3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione: $C_8H_{10}N_4O_2$
CAG	3' acceptor site of splice junction boundaries
CaM	calmodulin
CCAAT	regulatory DNA sequence in the 5' region of eukaryotic genes; transcription factors binding site
CCD	central core disease
cDNA	complementary DNA
CHCT	caffeine halothane contracture test
Ci	curie: quantity of any radioactive nuclide in which there are 3.7×10^{10} disintegrations per second
CK	creatine kinase
cm	centimetre
cM	centimorgan
CO ₂	carbon dioxide
COOH	carboxyl group, indicating the C-terminal of a protein molecule
CpG	indicates a C covalently linked to a neighboring G on the same DNA stand
CPK	creatine phosphokinase
CsCl	cesium chloride
C-terminal	carboxyl terminal
Cys	cysteine
δ	delta
ΔG	free energy: indicating nucleic acid duplex stability
D or D	aspartic acid
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
ddA	dideoxyadenosine
ddATP	2',3'-dideoxyadenosine 5'-triphosphate
ddC	dideoxycytidine
ddCTP	2',3'-dideoxycytidine 5'-triphosphate
<i>Dde I</i>	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Dde I</i> gene from <i>Desulfovibrio desulfuricans</i> , with recognition site 5'-...C↓TNAG...-3'
ddG	dideoxyguanosine
ddGTP	2',3'-dideoxyguanosine 5'-triphosphate
ddH ₂ O	double distilled water
ddN	dideoxynucleotide
ddNTP	2',3'-dideoxynucleotide
ddNTPs	2',3'-dideoxynucleotides
ddT	dideoxythymidine
ddTTP	2',3'-dideoxythymidine 5'-triphosphate
7-deaza-dGTP	7-deaza-2'-deoxyguanosine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
DHP	dihydropyridine
DHPR	dihydropyridine receptor
DHPR β	dihydropyridine receptor β sub-unit
DHPR γ	dihydropyridine receptor γ sub-unit
DMD	Duchenne muscular dystrophy
<i>D. melanogaster</i>	<i>Drosophila melanogaster</i>
DNA	deoxyribonucleic acid
dNTP	2'-deoxynucleotide
dsDNA	double stranded DNA
DTT	dithiothreitol: threo-1,4-dimercapto-2,3-butanediol: $C_4H_{10}O_2S_2$



List of abbreviations and symbols (continued ...)

dTTP	2'-deoxythymidine 5'-triphosphate
dUTP	2'-deoxyuracil 5'-triphosphate
E or E	glutamic acid
EC-coupling	excitation-contraction coupling
EDTA	ethylenediamine tetra-acetic acid: C ₁₀ H ₁₆ N ₂ O ₈
EF	EF hand domain
EMG	electromyograph
EtBr	ethidium bromide: C ₂₁ H ₂₀ BrN ₃
EtOH	ethanol: CH ₃ CH ₂ OH
F or F	phenylalanine
FFA	free fatty acids
FKBP12	FK-506 binding protein
FMC	FMC Corporation
Formamide	carbamide: CH ₃ NO
γ	gamma
γ ³² P-dATP	dATP labelled in the γ position with ³² P isotope
γ ³² P-dCTP	dCTP labelled in the γ position with ³² P isotope
g	guanine
g	gram
G or G	guanine (in DNA sequence, indicating exon sequence)
G or G	glycine (in amino acid sequence)
GC	indicates a G on one strand that is hydrogen-bonded to a C on the opposite strand
g/v	gram per volume
gDNA	genomic DNA
Genbank	GenBank ^{®1} : United States repository of DNA sequence information
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GPI	glucose phosphate isomerase
GT	5' donor site of splice junction boundaries
H or H	histidine
<i>Hae III</i>	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Hae III</i> gene from <i>Haemophilus aegyptius</i> , with recognition site 5'-...GG↓CC...-3'
halothane	2-bromo-2-chloro-1,1,1-trifluoroethane: C ₂ HBrClF ₃
HCl	hydrochloric acid
HCO ₃	bicarbonate
<i>Hga I</i>	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Hga I</i> gene from <i>Haemophilus gallinarum</i> , with recognition site 5'-...GACGC(N) ₅ ↓...-3'
Hg	mercury
His	histidine
HLA	human leukocyte antigen
H ₂ O	water
I or I	isoleucine
ICU	intensive care unit
Ile	isoleucine
IU	international units
IU.l ⁻¹	international units per litre
IV	<i>intra venous</i>
IVCT	<i>in vitro</i> contracture test
K or K	lysine
kb	kilo base pair

¹ GenBank[®] is a registered trademark of the National Institutes of Health, U.S.A.

List of abbreviations and symbols (continued ...)

kcal.mol ⁻¹	kilo calorie per mole
KCl	potassium chloride
kDa	kilo dalton
kinase	polynucleotide kinase: ATP: 5'-dephosphopolynucleotide 5'-phospho-transferase
L or L	leucine
Leu	leucine
LDH	lactate dehydrogenase
2X loading buffer	95% formamide, 10 mM EDTA, 0.1% XC and 0.1% BPB [pH 11.0]
10X loading buffer	0.25% bromophenol blue (BPB), 0.25% xylene cyanol FF (XC), 0.4% Orange G (OG) and 50% glycerol
Ltd.	limited
Lys	lysine
lysis buffer	0.32 M sucrose; 10 mM Tris-HCl [pH 8.0]; 5 mM MgCl ₂ ; 1% Triton X-100 ^{®1}
μ	micro: 10 ⁻⁶
μCi	micro Curie
μg	microgram
μg.l ⁻¹	microgram per litre
μg.μl ⁻¹	microgram per microlitre
μg.ml ⁻¹	microgram per millilitre
μl	microlitre
μM	micromolar
m	milli: 10 ⁻³
M	molar: moles per litre
M or M	methionine
M' – M''	transmembrane regions
M1 - M10	transmembrane regions, numbers 1 to 10
<i>Mbo</i> I	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Mbo</i> I gene from <i>Moraxella bovis</i> , with recognition site 5'-...↓GATC...-3'
5meC	5-methylcytosine
mEq	milliequivalents
mEq.kg ⁻¹	milliequivalents per kilogram
mEq.l ⁻¹	milliequivalents per litre
Met	methionine
MeV	mega electron volt
mg	milligram
mg.kg ⁻¹	milligram per kilogram
Mg ²⁺	magnesium ion
MgCl ₂	magnesium chloride
MH	malignant hyperthermia
MHE	MH equivocal
MHEc	MH equivocal: positive only for caffeine
MHEh	MH equivocal: positive only for halothane
MHN	MH normal
MHS	MH susceptible
min	minutes
ml	millilitre
mm	millimetre
mmHg	millimetre mercury
mM	millimolar
MMR	masseter muscle rigidity

¹ Triton X-100[®] is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.

List of abbreviations and symbols (continued ...)

MMS	masseter muscle spasm
<i>Mnl I</i>	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Mnl I</i> gene from <i>Moraxella nonliquefaciens</i> , with recognition site 5'-...CCTC(N) ₇ ↓...-3'
mRNA	messenger RNA
<i>Msp I</i>	restriction endonuclease isolated from an <i>E. coli</i> strain that carries the cloned <i>Msp1</i> gene from <i>Moraxella</i> species, with recognition site 5'-...C↓CGG...-3'
mV	millivolt
n	nano: 10 ⁻⁹
N	A or C or G or T nucleotide
N or N	asparagine
Na	sodium
NA	not available
NaCH	sodium channel
NaCl	sodium chloride
NaClO ₄	sodium perchlorate
Na ₂ EDTA	disodium EDTA: C ₁₀ H ₁₄ N ₂ Na ₂ O ₈ .2H ₂ O
NaOH	sodium hydroxide
ng	nanogram
NH ₂	amino group, indicating the N-terminal of a protein molecule
nm	nanometer
nM	nanomolar
NR	no response at
NT	not tested with the <i>in vitro</i> contracture test
N-terminal	amino terminal
O ₂	oxygen
OD	optical density
OG	orange G: C ₁₆ H ₁₀ N ₂ O ₇ S ₂ Na ₂
OI	osteogenesis imperfecta
p	short arm of chromosome
p	pico: 10 ⁻¹²
P or P	proline
³² P	phosphorus isotope: maximum β emission energy 1.71 MeV: half-life 14.3 days
P _a CO ₂	partial pressure of arterial CO ₂
PAGE	polyacrylamide gel electrophoresis
pBR322	<i>E. coli</i> plasmid cloning vector
pBR322/ <i>Hae III</i>	pBR322 vector DNA digestion with restriction endonuclease <i>Hae III</i>
pCO ₂	carbon dioxide partial pressure
PCR	polymerase chain reaction
P _{ET} CO ₂	partial pressure of end-tidal CO ₂
pH	indicates acidity: numerically equal to the negative logarithm of H ⁺ concentration expressed in molarity
Phe	phenylalanine
P _i	inorganic phosphate
PIC	polymorphism information content
PLA ₂	phospholipase A ₂
pmol	pico mole
pmol.μl ⁻¹	pico mole per microlitre
PMRS	PCR-modified restriction site
Pro	proline
q	long arm of chromosome
Q or Q	glutamine
R or R	arginine
RFLP	restriction fragment length polymorphism

List of abbreviations and symbols (continued ...)

RNA	ribonucleic acid
rpm	revolutions per minute
RT	room temperature
<i>Rsa</i> 1	restriction endonuclease isolated from <i>Rhodopseudomonas sphaeroides</i> , with recognition site 5'-...GT↓AC...-3'
RYR	ryanodine receptor
RYR1	RYR gene expressed in skeletal muscle
RYR2	RYR gene expressed in cardiac muscle and brain
RYR3	RYR gene expressed by the β4 gene
RYRs	ryanodine receptors
S or S	serine
³⁵ S	sulphur isotope: maximum β emission energy 0.167 MeV: half-life 87.4 days
S1 - S6	transmembrane segments numbers 1 to 6
SA	South African
SAP	shrimp alkaline phosphatase
<i>Sau</i> 3AI	restriction endonuclease isolated from <i>Staphylococcus aureus</i> 3A, with recognition site 5'-...↓GATC...-3'
SCN4A	α sub-unit of the skeletal muscle sodium channel
SDS	sodium dodecyl sulphate: C ₁₂ H ₂₅ NaSO ₄
SDS-EDTA	0.5% SDS; 50 mM EDTA
sec	seconds
Sequenase	Sequenase [®] 1 Version 2.0 T7 DNA Polymerase
Sequenase buffer	200 mM Tris-HCl [pH 7.5]; 100 mM MgCl ₂ ; 250 mM NaCl
Ser	serine
SLS	single lane sequencing
Sp1	GC box sequences usually clustered near the initiation site of a gene
SR	sarcoplasmic reticulum
SSCP	single stranded conformational polymorphism
ssDNA	single stranded DNA
stop buffer	95% formamide; 0.05% xylene cyanol FF; 0.05% bromophenol blue; 20 mM EDTA
STRP	short tandem repeat polymorphism
suspension buffer	50 mM Tris-HCl [pH 8.0]; 100 mM EDTA; 150 mM NaCl
t	thymine
T or T	thymine (in DNA sequence, indicating exon sequence)
T or T	threonine (in amino acid sequence)
T _a	annealing temperature
T _c	calculated annealing temperature
T _m	melting temperature
TAG	termination codon
Taq DNA Polymerase	deoxynucleosidetriphosphate: DNA deoxynucleotidyltransferase from <i>Thermus aquaticus</i>
TATA	conserved non-coding DNA sequence in the 5' region of most eukaryotic genes
TBE	89.15 mM Tris [®] 2 [pH 8.0], 88.95 mM boric acid, 2.498 mM Na ₂ EDTA
TE	10 mM Tris-HCl [pH 7.5]; 1 mM EDTA
TEMED	N,N,N',N'-tetramethylethylenediamine: C ₆ H ₁₆ N ₂
temp	temperature
Thr	threonine
Tris	Tris [®] : tris(hydroxymethyl)aminomethan: 2-Amino-2-(hydroxymethyl)-1,3-propanediol: C ₄ H ₁₁ NO ₃

¹ Sequenase[®] is a registered trademark of United States Biochemical Corporation, Cleveland, Ohio, U.S.A.

² Tris[®] is a registered trademark of Rohm & Haas Company, Philadelphia, PA, U.S.A.

List of abbreviations and symbols (continued ...)

Tris-HCl	2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride: $C_4H_{11}NO_3 \cdot H_2O$
Triton X-100	Triton X-100 [®] : octylphenolpoly(ethylene-glycolether) _n : $C_{34}H_{62}O_{11}$, for $n = 10$
Trp	tryptophan
T-tubule	transverse tubule
Tyr	tyrosine
U	uracil
U	units
U.ml ⁻¹	units per millilitre
UK	United Kingdom
Urea	H_2NCONH_2
US	United States
USA	United States of America
USB	United States Biochemical
UV	ultraviolet
V	volts
V or v	valine
Val	valine
W	watts
W or w	tryptophan
XC	xylene cyanole FF: $C_{25}H_{27}N_2O_6S_2Na$
xg	gravitational acceleration
Y or y	tyrosine
□ / ○	male/female: tested normal for malignant hyperthermia susceptibility with the IVCT
■ / ●	male/female: tested susceptible to malignant hyperthermia with the IVCT
▣ / ◐	male/female: never tested, malignant hyperthermia status unknown
▤ / ◑	male/female: obligate malignant hyperthermia susceptible carrier
▥ / ◒	male/female: malignant hyperthermia equivocal, positive for halothane
◻ / ◓	male/female: deceased
◇	sex unknown
—/—	divorced
•	spontaneous abortion
↗	proband
↓	indicates restriction enzyme cutting site

PLEASE NOTE:

Some of the product names, patents and registered designs mentioned in this thesis are registered trademarks or proprietary names even though it was not always indicated in the text. Where a name appears in the text without a designation that it is proprietary, it is not to be construed as a presentation by the author that it is in the public domain.

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