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Statins and other lipid-lowering therapy and pregnancy outcomes in homozygous familial hypercholesterolaemia – a retrospective review of 39 pregnancies.

By

Theunis Christoffel Botha

A research report submitted to the University of the Witwatersrand in fulfilment of the requirement for the degree of Master of Medicine.

Supervisor: Prof F J Raal

November 2018

Johannesburg

Declaration

I, Theunis Christoffel Botha, declare that this research report is my own work. It is being submitted for the degree of Master of Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.



……..

Theunis Christoffel Botha

Signed this 5th day of November 2018

Declaration: Student's contribution to article and agreement of co-authors

I, Theunis Christoffel Botha, student number 0504396R, declare that this Research Report is my own work and that I contributed adequately towards research findings published in the article stated below which are included in my Research Report .

Signature of Student  **Date:** 19 July 2018

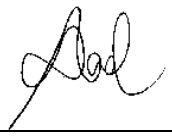
Name of Primary Supervisor: Professor F Raal

Signature of Primary Supervisor  **Date:** 19 July 2018

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of his/her Thesis/Dissertation/Research Report. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article 1: Title: Statins and other lipid-lowering therapy and pregnancy outcomes in homozygous familial hypercholesterolaemia – a retrospective review of 39 pregnancies.

Journal name, year, volume and page numbers:
In Press, Atherosclerosis, 2018, Volume and page numbers to follow

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Acknowledgements

Completing this MMed degree would not have been possible without the support of the following people:

Firstly, to Professor Raal, my supervisor, for stimulating an interest in the field of Lipidology, his support and encouragement to do research and to publish the research.

To Gill Pilcher who helped with finding records and data collection at CMJAH, for reading through countless draft versions and correcting my bad grammar.

To the team at Cape Town Lipid Clinic, Professor Blom and Dr Wolmarans, a huge thank you for assisting with ethics applications from Cape Town and sharing their knowledge and data.

And to my wife Arista: Thank you for your support through the late nights of writing and studying, running printing errands and proof reading. Thank you for your daily encouragement; this MMed would not have been completed without your help.

Table of content

Title page 1
Declaration 2
Declaration: Student's contribution to article and agreement of co-authors 3
Acknowledgements 4
Table of content 5
Abstract 6
1. Introduction 7
2. Patients and methods 8
3. Results 9
    3.1. Study population 9
    3.2. Pregnancy and pregnancy outcomes 10
    3.3. Lipid lowering treatment prior to and during pregnancy 10
    3.4. Lipid levels during pregnancy 11
    3.5. Delivery 12
    3.6. Breastfeeding 12
    3.7. Infant birth weight and early childhood development 13
4. Discussion 13
5. Conflict of interest 16
6. Funding 16
7. Authors' contributions 16
8. References 17
9. Tables 19
    9.1. Table 1: Clinical characteristics of cohort, arranged by maternal year of birth. 20
    9.2. Table 2: Demographics of cohort. 21
10. Supplementary Material 22
    10.1. Supplementary Table 1: LDL-receptor mutations of cohort. 23
    10.2. Supplementary Table 2: Pregnancy outcomes in 39 pregnancies, arranged by type of lipid lowering drug therapy. 24
    10.3. Supplementary Table 3: Pregnancy outcomes in 18 pregnancies exposed to statin therapy with or without other lipid lowering therapy at time of conception. 25
11. Appendices
    Appendix 1: Protocol 26
    Appendix 1.1: Patient information sheet 33
    Appendix 1.2: Patient consent form 38
    Appendix 1.3: Patient questionnaire for data collection 42
    Appendix 2: Change of Title 54
    Appendix 3: Ethics approval for the University of Witwatersrand HREC 56
    Appendix 4: Ethics approval from University of Cape Town HREC 58
    Appendix 5: Turnitin report 60
    Appendix 6: Acknowledgement of article acceptance for publication in the journal Atherosclerosis 73

Abstract

BACKGROUND:

Pregnancy in homozygous familial hypercholesterolaemia (HoFH) females is associated with further elevation of already markedly elevated low density lipoprotein cholesterol (LDL-C) levels, particularly if lipid-lowering therapy is discontinued, placing the mother and fetus at increased cardiovascular risk. Lipoprotein apheresis (LA) is the current recommended treatment for pregnant HoFH patients. However, this is costly, time consuming, and is not available in many countries. Alternative treatment strategies to control hypercholesterolaemia during pregnancy in HoFH patients are necessary. The aim of this study was to assess pregnancy outcomes in a cohort of female HoFH patients, many of whom received statins with or without other lipid lowering therapy.

METHODS:

This study was a retrospective review of 39 pregnancies from a cohort of 20 genotypically confirmed female HoFH patients. Demographic, clinical, laboratory and treatment information, particularly the use of medication during pregnancy, was extracted from hospital files. Either patients themselves or a close, surviving family member were contacted directly to obtain informed consent for anonymized data collection and additional information. We compared birth weights of statin exposed and statin unexposed infants with the Mann Whitney U test and utilized a one tailed t-test to compare lipid levels, prior to, and following pregnancy. We considered $p < 0.05$ significant

RESULTS:

No maternal cardiac complications or deaths occurred during the pregnancies or during the first year postpartum. Twenty-five pregnancies were exposed to lipid-lowering therapy, of which 18 were exposed to statin therapy, just prior to or during the pregnancy. Thirty-three (84%) pregnancies carried to term, 3 (8%) premature deliveries and 3 (8%) miscarriages were observed. Complications associated with pregnancy in these HoFH patients, did not differ from those reported during pregnancies of otherwise healthy woman.

CONCLUSION:

HoFH is a severe disease impacting significantly on life expectancy. However, for many females with HoFH, despite the high cardiovascular risk, pregnancy is not uncommon. In resource poor settings and when LA is not available, lipid lowering therapy, particularly statin therapy during pregnancy, appears to be safe for both mother and fetus and is an acceptable alternative for LDL-C reduction in these high risk patients.

KEY WORDS:
Homozygous familial hypercholesterolaemia, Pregnancy, Statins, Lipoprotein apheresis

1) Introduction

Homozygous familial hypercholesterolaemia (HoFH) is a rare but serious genetic disorder. HoFH is due to bi-allelic mutations in the low-density lipoprotein receptor (LDLR) gene resulting in marked LDLR dysfunction. The prevalence of HoFH is 1 in 160 000 to 1 000 000 worldwide but in founder populations, such as the Afrikaners in South Africa, the prevalence can be as high as 1 in 30 000 [1-3]. Patients with HoFH have elevated levels of low density lipoprotein cholesterol (LDL-C) from birth and often suffer from premature atherosclerotic cardiovascular disease and valvular and supra-valvular aortic stenosis which require surgical intervention [4,5].

Pregnancy in HoFH females is associated with further elevation of already markedly high LDL-C. In healthy patients, total cholesterol and LDL-C levels may increase by up to 40% during pregnancy [6]. This increase is exaggerated in HoFH patients, particularly if lipid-lowering therapy is discontinued, placing the mother at even higher cardiovascular risk [7]. Maternal hypercholesterolaemia during pregnancy have been linked to increased atherosclerotic lesions in the fetus [8,9]. Lipid management during pregnancy therefore has implications for both the mother and the unborn child.

HMG-CoA reductase inhibitors (statins) are currently the backbone of lipid-lowering treatment in patients with HoFH. Although statins only lower LDL-C modestly in most patients with HoFH, they reduce cardiovascular events and mortality [10-12]. The cholesterol absorption inhibitor ezetimibe reduces LDL-C further and is routinely used in patients with HoFH [13]. Other agents such as fibrates, niacin and bile acid sequestrants, as well as newer agents such as mipomersen, lomitapide and evolocumab have also been used as add-on treatment.

Statin use is not recommended during pregnancy due to teratogenicity. A report in 2004 increased fears regarding statin use during pregnancy [14]. The report showed malformations in 22 infants exposed to statins in the first trimester. Recently published studies however showed no teratogenic

effect associated with statin use after accounting for confounding variables [15]. Despite several studies there is currently no consensus regarding the safety of statins during pregnancy [16-18]. Little is known about the safety of newer agents such as mipomersen, lomitapide or PCSK9-inhibitor therapy with evolocumab or alirocumab during pregnancy in humans. Animal studies showed teratogenicity when ezetimibe was used in high dosages [19] and with Lomitapide at therapeutic dosages. Animal studies did not show any teratogenicity with the use of mipomersen however [20]. Studies that evaluated the safety of the PCSK9 inhibitor evolocumab showed no negative effect during pregnancy but did confirm placental transfer to the infant [21].
Lipoprotein apheresis (LA) is the recommended lipid lowering treatment for pregnant HoFH patients [22,23]. The benefits of LA are well established and include the ability to lower total cholesterol and LDL-C levels significantly and to prevent progression of atherosclerotic disease [24,25]. By down-regulating several cytokines, LA also has an anti-inflammatory response [26]. One study found that the use of LA could potentially improve a patient's overall quality of life as assessed by patient questionnaires [27].

However, LA is costly, time consuming and not available in all countries. Thus, we need alternative treatment options for pregnant HoFH patients in resource-poor settings such as South Africa where statins and ezetimibe are the main treatment modalities.

The aim of this study was to assess pregnancy outcomes in a cohort of female HoFH patients, many of whom received statins with or without other lipid lowering therapy.

2) Patients and methods

Data were collected retrospectively in January 2016 from a cohort of female HoFH patients who attended two specialised lipid clinics in South Africa (Charlotte Maxeke Academic Hospital,

Johannesburg or Groote Schuur Hospital, Cape Town) and who had reported a pregnancy prior to study commencement. All files from January 1970 till January 2016 were reviewed.

The study was approved by the Human Research Ethics Committees of the University of the Witwatersrand and the University of Cape Town. Demographic, clinical, laboratory and treatment information, particularly the use of medication during pregnancy, was extracted from hospital files. Either patients themselves or a close, surviving family member were contacted directly to obtain informed consent for anonymized data collection and additional information. We used Excel® 2016 Version 1611 (Microsoft Office 365®, Microsoft, Redmond, Washington, United States of America) for statistical analysis. Data were tested for normality. We compared birth weights of statin exposed and statin unexposed infants with the Mann Whitney U test and utilized a one tailed t-test to compare lipid levels, prior to, and following pregnancy. We considered $p < 0.05$ significant.

3) Results

3.1) Study population

Seventeen females were excluded as they were never pregnant and 45 were excluded as it was unclear if they were ever pregnant and was unable to be contacted. A total of 29 HoFH females qualified for the study. Of the 29 patients, 4 were lost to follow-up and could not be traced, 5 had died prior to study commencement and we could not trace any surviving family member to obtain further information. Twenty patients were therefore included in the study (Tables 1 and 2).

The majority of patients (n=12) were diagnosed, or visited the clinic for the first time, before the age of 18 years with the median age of first clinic visit being 9.3 years (range 1.0 - 53.5; interquartile range 4.1- 36.5 years). All patients had genotypically confirmed HoFH, either being true homozygotes with identical LDLR mutations or compound heterozygotes with two different LDLR mutations. The most common genotype was true homozygosity for the FH Afrikaner 1 mutation (c.681C > G / c.681C > G) (n= 9) followed by compound heterozygous FH Afrikaner 1

(c.681C > G) / FH Afrikaner 2 (c.1285G>A) mutations (n=3). (Supplementary table 1) This is in keeping with the high prevalence of these founder mutations in South Africa.

Mean (SD) total cholesterol and LDL-C levels were markedly elevated, 19.07 (3.57) mmol/L and 18.29 (4.02) mmol/L respectively, at first presentation to the clinics. Four patients had experienced cardiac events (either myocardial infarction, coronary artery disease requiring stenting or coronary artery bypass grafting) prior to their first pregnancy. The median age for first cardiac intervention was 30.5 years (range 13.6 – 50.8 years; interquartile range 19.9 – 41.5 years). No patients required valvular intervention (aortic valve replacement, aortic root surgery or aortic valve bypass surgery) prior to their first pregnancy. No maternal deaths occurred during pregnancy or the postpartum period.

3.2) Pregnancy and pregnancy outcomes

Thirty-nine pregnancies were reported. Patients started a family at a relatively young age (median age of primigravid patients was 24.3 years) and the majority (n=13; 65%) completed their families before age 30 years.

Overall pregnancy outcomes were favourable (i.e. a live infant born). There were 33 (85%) full-term pregnancies and only three premature deliveries (< 37 weeks). Premature deliveries were due to maternal pre-eclampsia at 33 weeks, chorioamnionitis at 35 weeks and due to underlying maternal cardiac disease at 36 weeks gestation. Two patients reported miscarriages at 10 and 13 weeks. One intrauterine death (six months) was attributed to intrauterine infection.

3.3) Lipid lowering treatment prior to and during pregnancy

Of 39 pregnancies a total of 18 were exposed to statins (Supplementary table 2 and 3). The majority of the women on statin therapy were using atorvastatin, either 40 or 80mg, daily.

During 12 of these pregnancies, patients were treated with statin monotherapy in the three months prior to conception. Eight pregnancies were exposed to statins during the first trimester until pregnancy was confirmed (approximately 4 - 8 weeks). Four patients resumed statin therapy in the second trimester between 12 and 16 weeks gestation. The remaining four resumed therapy within one month after delivery.

Five patients were treated with both statins and ezetimibe (10mg daily) in the three months prior to conception. In one case, the statin and ezetimibe combination was discontinued two months prior to planned conception and resumed during the third month of pregnancy. Two of the five pregnancies were exposed to statin and ezetimibe during the first trimester until pregnancy was confirmed and resumed in the second trimester.

One patient was treated with statin, ezetimibe and a PCSK9 inhibitor, evolocumab (420mg monthly), at the time of unplanned conception. All treatment was discontinued at 8 weeks gestation and resumed one week after delivery.

Five pregnancies were exposed to cholestyramine therapy during the first trimester. Cholestyramine was continued throughout three pregnancies. For two pregnancies it is unclear whether cholestyramine was continued for the full duration of the pregnancy.

Only one patient was treated with LA during pregnancy. LA was continued throughout her two pregnancies.

Fourteen pregnancies were not exposed to lipid-lowering therapy.

3.4) Lipid levels during pregnancy

Lipid levels were not routinely measured during pregnancy or following delivery. Only four pregnancies had lipid levels recorded in the three months prior to conception and again within three months following delivery. Two of the women were on atorvastatin 80mg daily which was stopped during the 1st month and recommenced in the 4th month of pregnancy. One woman was on rosuvastatin 40mg daily which was stopped during the 1st month and restarted during the 4th month

of pregnancy. One woman was on atorvastatin 80mg plus ezetimibe 10mg daily; both drugs were stopped 2 months prior to falling pregnant and restarted during the 3rd month. Total cholesterol levels for the four pregnancies increased significantly from 14.20 ± 1.69mmol/l to 17.86 ± 2.34mmol/l (p=0.03). LDL-C was available for three of the four pregnancies. Although LDL-C increased from 11.97 ± 1.90mmol/l to 14.73 ± 0.97mmol/l, this change was not significant (p=0.12).

3.5) Delivery

There were 15 (38%) caesarean sections, of which 11 were elective, and 4 were emergency procedures (Supplementary Table 2). There was no statistical difference in the elective caesarean rate between the statin exposed group and the group receiving no or other forms of treatment. (p=0.20). Most of the elective caesarean sections were due to the underlying maternal cardiac condition. Although only four patients had experienced cardiac events (either myocardial infarction, coronary artery disease requiring stenting or coronary artery bypass grafting) prior to their first pregnancy the other women whom had elective caesarean sections were considered high risk and therefor a caesarean section was performed. Another reason for observing more caesarean sections in the statin group might be the fact that there has been a global increase in caesarean section rates in the last 20 years [28]. Pregnancies exposed to statins were only observed after the year 2000 while pregnancies prior to the year 2000 were not exposed to statins. This might explain the increased number of elective caesarean sections observed in statin exposed pregnancies.

3.6) Breastfeeding

Twelve of the 20 patients chose to breastfeed (21 infants). Most patients breastfed for three to six months; three patients opted to breastfeed for nine months. Six patients (11 infants) continued breastfeeding despite restarting statin therapy after delivery, while three patients (four infants)

chose not to breastfeed while on statin therapy. No data regarding lipid lowering therapy were available for the other three patients (six infants).

3.7) Infant birth weight and early childhood development

Most infants had normal birth weights (2.5 – 4.0kg). Two infants had above normal birth weights (4.5kg and 4.2kg), neither of which had been exposed to lipid lowering therapy. Another two infants had below normal birth weights (2.4kg). The latter two pregnancies were exposed to statin therapy during the first trimester and both infants were born prematurely (Supplementary table 2). When we compared the birth weights of the pregnancies exposed to, and those not exposed to statins, there was no difference between the two groups (p=0.052).

One infant was born with a congenital squint which was surgically corrected. The pregnancy was not exposed to lipid modifying treatment neither during nor prior to pregnancy. Short legs were reported in one infant exposed to statin and ezetimibe therapy during the first trimester. However, this did not require surgical correction and did not impact negatively on the child. Unfortunately, no report of a formal assessment by a paediatrician could be obtained to confirm whether this was likely a congenital malformation but this is considered unlikely. Normal early child development was reported for all offspring. Children started school at the appropriate age and no learning difficulties were reported.

4) Discussion

The most significant finding of this study suggests that statin exposure might not be as detrimental as previously thought. In our cohort of 39 pregnancies, complications did not differ from those reported during pregnancies of healthy woman. The miscarriage rate (5.5%) in the 18 statin exposed pregnancies, was no higher than that of healthy populations (8 to 20%) [29]. Premature birth rate (11.1%) in the statin exposed pregnancies, also fell within the normal range (5 to 18%) [30,31].

Of the 18 statin exposed pregnancies in our study only two infants had low birth weights. There was no difference in birth weights between statin exposed and non-exposed pregnancies, no severe skeletal abnormalities or developmental delay. Early childhood development appeared to be unaffected by statin therapy prior to conception or during pregnancy. Methods for delivery were dependent on underlying cardiac or obstetrical circumstances. Normal vaginal delivery was the preferred choice for patients who completed pregnancies prior to cardiac intervention or valve surgery.

Although our study focused on HoFH patients, other studies from heterozygous familial hypercholesterolaemia (HeFH) patients in Norway have reported similar findings to our study. The HeFH women on lipid lowering therapy during pregnancy did not have any premature terminations or congenital malformations. There was also no difference in birth weights compared to the HeFH women not on lipid lowering therapy and to the general population [32].

Although one of the largest studies to report on pregnancy outcomes in HoFH patients, the relatively small study population and incomplete records are limitations of the study. This prevented us from being able to adequately compare different treatment groups and we are thus unable to comment on the effectiveness of different lipid lowering therapies during pregnancy. Lipid levels were not routinely measured before, during or after pregnancy and thus resulted in only 4 comparable samples. A larger number of samples would have been more useful in determining the effectiveness of statin usage during pregnancy. Other markers of progression of atherosclerotic disease (for example carotid intimal thickness) were not routinely measured or recorded and thus overall increase in risks for these patients could not be established. Unfortunately, due to the limited number of patients able to access LA in South Africa we were not able to compare our study population to a group receiving gold standard treatment - LA. Since this was a retrospective study, developmental and physical abnormalities reported were based on descriptions by patients and not scientifically measured and thus subtle congenital abnormalities might be under-reported by parents, recall bias may also influence results.

To date, studies involving HoFH pregnancies have been small and mainly limited to case reports [33]. Animal studies have shown placental transfer of statins and excretion of atorvastatin in breastmilk [34]. One case report found low concentrations of rosuvastatin in breastmilk in a patient with familial hypercholesterolaemia [35].

LDL-C and total cholesterol levels were not measured routinely in the offspring and it is thus unknown if maternal statin use during pregnancy had any effect on our study population. There are studies that show elevated maternal LDL-C and total cholesterol levels during early pregnancy results in elevated LDL-C levels and increased cardiovascular risks in their offspring in later life [36,37]. Animal studies have found that mice fed a high fat diet together with pravastatin during pregnancy resulted in lower total cholesterol and LDL-C levels in the offspring compared to mice on a high fat diet alone [38]. It would be interesting to see if this also holds true for HoFH patients and further studies are needed in this regard.

Due to possible teratogenic effects of statins on the fetus and infant, current guidelines recommend that patients discontinue statin therapy one month prior to conception and only resume once breastfeeding has ceased [23,39-41]. Lipoprotein apheresis is the recommended lipid lowering therapy for pregnant HoFH patients [22,23,40,42]. Lipoprotein apheresis is, however, not sustainable due to the high cost - particularly in developing countries [43,44].

Our findings agree with a systemic review by Avis et al, which concluded that statin therapy is safe in HeFH children and did not find any delay in sexual development or growth [45]. Current evidence shows that low LDL-C levels do not have significant adverse effects and cholesterol dependent hormone levels are unaffected [46]. Additionally, the infants of HoFH patients are obligate HeFH and likely already affected by atherosclerotic lesions at birth [8,9]. This suggests that the reinstatement of lipid lowering therapy during pregnancy could provide additional benefit for the infant.

The findings of our study add to the current pool of data and may assist health professionals when advising HoFH patients regarding statin use during pregnancy and breastfeeding. The findings may also be important for HoFH women making informed decisions regarding pregnancy and therapy. It is however important to note that the findings of our study cannot be extrapolated to the general population nor to HeFH patients.

We recommend that, where LA is not feasible, patients should discontinue all lipid lowering therapy a month prior to planned conception and reinstate lipid lowering therapy, statin plus ezetimibe, during the second trimester. This limits the possible teratogenic effect of statins during the first trimester while providing the mother with optimal care during a time of increased cholesterol production. Women's perceptions and preferences regarding statin use in pregnancy should also be considered when giving advice in these situations. We aim to manage pregnant HoFH patients as a multi-disciplinary team of lipid specialists, cardiologists and obstetricians.

5) Conflict of interest

TCB received a grant from Amgen to cover expenses for the research. GP, KW, DJB, FJR have received reimbursement for conducting clinical trials from Amgen, Regeneron Pharmaceuticals, Inc., and Sanofi Aventis; modest speaker fees or honoraria for lectures from AstraZeneca, Pfizer, Merck, Amgen, and Sanofi; and consultant/advisory board fees from Amgen, Sanofi Aventis and Regeneron Pharmaceuticals, Inc.

6) Funding

TCB received a grant from Amgen to cover expenses for the research.

7) Authors' contributions

TCB extracted data from the medical records for patients treated in Johannesburg, entered and analysed data and wrote the first draft of the article. KW extracted data from medical records for patients treated in Cape Town. All authors contributed equally to the revision of subsequent drafts, to the approval of the final version and agree to be accountable for the accuracy and integrity of the findings.

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9) Tables

Table 1: Clinical characteristics of cohort, arranged by maternal year of birth.

Table 2: Demographics of cohort.

Table 1: Clinical characteristics of cohort, arranged by maternal year of birth.

Year of birth of patient	Age at death	Age at diagnosis/ initial presentation	Total cholesterol (mmol/L) at initial presentation [a]	LDL-C (mmol/L) at initial presentation [a]	Mutations		Age at surgical interventions	Maternal age at first and subsequent pregnancies
					Allele 1	Allele 2		
1933	62	42	18.90	17.90	c.681C>G	c.681C>G	CABG at 50 and 59	20, 23, 25, 29
1936	70	39	21.80	21.00	c.681C>G	c.681C>G	Cholecystectomy at 28; CABG at 47; AV replacement and CABG at 58	22, 25, 26
1944	-	54	18.60	16.60	c.917C>T	c.1690A.C, 2458_2466del	-	30, 32, 38
1954	54	44	20.30	18.70	c.681C>G	c.523G>A	CABG at 41; AV surgery at 47	24, 26
1962	-	42	11.58	9.75	c.681C>G	c.1444G>A	Portacaval shunt at 18; CABG at 42	22, 26, 29
1968	44	6	24.60	23.73	c.681C>G	c.662A>G	-	37, 39
1968	34	9	16.06	-	c.681C>G	c.681C>G	CABG and AV repair at 30; CABG and AV replacement at 34	18, 23
1969	30	18	16.08	14.84	p.47_48del DG	p.47_48del DG	MI at 30	27
1969	-	8	23.50	22.49	c.681C>G	c.662A>G	Partial ileal bypass at 13; Coronary artery stent inserted at 21,33,43,45; CABG at 24; Carotid endarterectomy at 24 and 26; Cholecystectomy at 30; Bilateral ileal angioplasty at 42 and AV surgery at 44	34
1969	-	35	-	-	c.681C>G	c.1444G>A	-	27, 29
1970	38	1	17.70	-	c.681C>G	c.681C>G	Portacaval shunt at 4; Partial ileal bypass at 11; Aortic endarterectomy at 15 and MI at 16	26
1975	-	14	17.70	-	c.2054C>T	c.2054C>T	CABG at 34	25, 27, 32
1975	-	7	20.80	19.59	c.681C>G	c.681C>G	CABG at 18; Coronary artery stenting at 24; CABG at 31	25, 32
1977	-	1.5	18.40	16.70	c.681C>G	c.681C>G	AV replacement at 33	30
1980	-	4	22.58	21.68	c.681C>G	c.662A>G	-	32
1982	29	4	21.05	20.41	c.662A>G	c.523G>A	Portacaval shunt at 5; CABG and Aortic root repair at 14; MI at 23	23
1984	-	4	18.80	17.80	c.681C>G	c.681C>G	AV replacement at 27	21, 25
1986	-	18	-	-	c.2054C>T	c.2054C>T	-	23, 25
1989	-	10	12.20	11.20	c.681C>G	c.681C>G	-	17, 23
1993	-	4	22.60	21.90	c.681C>G	c.681C>G	-	21

[a] Patients already using lipid lowering therapy at initial presentation included.
CABG - Coronary artery bypass graft; MI - Myocardial infarction; AV - Aortic valve; LDL-C - Low density lipoprotein cholesterol;
All ages are reported in years

Table 2: Demographics of cohort.

		Number of patients	Median	IQR
Age at diagnosis		20	9.3 years	4.1 – 36.5 years
Age of death		8	40.6 years	33.4 – 55.8 years
Age at first cardiac intervention		11	30.5 years	19.9 – 41.5 years
Age at second cardiac intervention		6	29.6 years	24.4 – 52.1 years
Age at first valvular intervention		7	31.8 years	29.7 – 37.1 years
Maternal age at first pregnancy		20	24.3 years	21.9 – 28.3 years
Maternal age at second pregnancy		13	25.7 years	24.7 – 28.9 years
Maternal age at third pregnancy		5	29.4 years	25.8 – 31.7 years
		Number of patients	**Mean**	**SD**
Lipid levels at initial presentation [a]	Total cholesterol	18	19.07 mmol/L	± 3.57 mmol/L
	Triglycerides	18	1.17 mmol/L	± 0.47 mmol/L
	HDL-C	14	0.82 mmol/L	± 0.35 mmol/L
	LDL-C	15	17.20 mmol/L	± 5.11 mmol/L

HDL-C - High-density lipoprotein cholesterol; LDL-C - Low-density lipoprotein cholesterol;

IQR – interquartile range; SD – standard deviation;

[a] Some patients were already using lipid lowering therapy at initial presentation.

Supplementary Material

Supplementary Table 1: LDL-receptor mutations of cohort.

Supplementary Table 2: Pregnancy outcomes in 39 pregnancies, arranged by type of lipid lowering drug therapy.

Supplementary Table 3: Pregnancy outcomes in 18 pregnancies exposed to statin therapy with or without other lipid lowering therapy at time of conception.

Supplementary table 1: LDL-receptor mutations of cohort.

Mutation		Total number of patients	Percentage of cohort
Allele 1	**Allele 2**		
FH Afrikaner 1 (c.681C > G)	FH Afrikaner 1 (c.681C > G)	9	45%
FH Afrikaner 1 (c.681C > G)	FH Afrikaner 2 (c.1285G>A)	3	15%
FH Gujerat (c.2054C > T)	FH Gujerat (c.2054C > T)	2	10%
FH Afrikaner 1 (c.681C > G)	D461N (c.1444G > A)	2	10%
FH Afrikaner 1 (c.681C > G)	FH Afrikaner 3 (c.523G>A)	1	5%
FH Afrikaner 2 (c.1285G>A)	FH Afrikaner 3 (c.523G>A)	1	5%
FH Cape Town 2 (p.47_48del DG)	FH Cape Town 2 (p.47_48del DG)	1	5%
FH Amsterdam (c.917C>T)	c1690A>C, 2458_2466del	1	5%

Nomenclature as per LDLR Locus website - http://www.ucl.ac.uk/ldlr/current/

Supplementary table 2: Pregnancy outcomes in 39 pregnancies, arranged by type of lipid lowering drug therapy.

Maternal treatment during pregnancy	Gestational age [a]	Pregnancy outcome	Birth weight (kg) [b]	Pregnancy and intrapartum complications	Mode of delivery	Physical abnormalities in newborn	Early childhood development
Pravastatin (unknown dose)	10 weeks	Miscarriage	-	Spontaneous miscarriage	-	-	-
Atorvastatin 80mg	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Atorvastatin 80mg	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Atorvastatin 80mg	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Atorvastatin 80mg	33 weeks	Live infant	2.4	Pre-eclampsia at 30 weeks; maternal bleeding requiring transfusion	Emergency C/S	Nil	Normal
Atorvastatin 80mg	Term	Live infant	Normal	Oligohydramnios at 37 weeks	Emergency C/S	Nil	Normal
Atorvastatin 80mg	Term	Live infant	3.2	Fetal distress at 37 weeks	Emergency C/S	Nil	Normal
Atorvastatin 40mg	Term	Live infant	Normal	Nil	Elective C/S [c]	Nil	Normal
Atorvastatin 80mg	Term	Live infant	Normal	Nil	Elective C/S [c]	Nil	Normal
Rosuvastatin 40mg	Term	Live infant	2.6	Nil	Elective C/S [c]	Nil	Normal
Atorvastatin 80mg	Term	Live infant	2.9	Nil	Elective C/S [c]	Nil	Normal
Atorvastatin 80mg	Term	Live infant	3.8	Nil	Elective C/S [d]	Nil	Normal
Atorvastatin 80mg, Ezetimibe 10mg	Term	Live infant	Normal	Unknown	NVD	Nil	Normal
Atorvastatin 80mg, Ezetimibe 10mg	Term	Live infant	Normal	Unknown	NVD	Nil	Normal
Atorvastatin 40mg, Ezetimibe 10mg	Term	Live infant	3.5	Nil	NVD	Nil	Normal
Atorvastatin 80mg, Ezetimibe 10mg	Term	Live infant	Normal	Nil	Elective C/S [c]	Short legs, no intervention required	Normal
Atorvastatin 80mg, Ezetimibe 10mg	Term	Live infant	3.2	Nil	Elective C/S [e]	Nil	Normal
Atorvastatin 80mg, Ezetimibe 10mg, PCSK9 inhibitor (Evolocumab 420mg monthly)	35 weeks	Live infant	2.4	Chorioamnionitis at 35 weeks	Emergency C/S	Nil	Normal
LDL Apheresis	Term	Live infant	Unknown	Nil	NVD	Nil	Unknown
LDL Apheresis	Term	Live infant	Unknown	Nil	NVD	Nil	Unknown
Cholestyramine	24 weeks	Intrauterine death	-	Chorioamnionitis at 24 weeks	-	-	-
Cholestyramine	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Cholestyramine	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Cholestyramine	Term	Live infant	3.6	Nil	NVD	Nil	Normal
Cholestyramine	Term	Live infant	3.7	Hypertension during last trimester	Elective C/S [c]	Nil	Normal
Nil	13 weeks	Miscarriage	-	Spontaneous miscarriage	-	-	-
Nil	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Nil	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Nil	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Nil	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Nil	Term	Live infant	Normal	Nil	NVD	Nil	Normal
Nil	Term	Live infant	3.8	Nil	NVD	Nil	Normal
Nil	Term	Live infant	4.5	Nil	NVD	Nil	Normal
Nil	Term	Live infant	Unknown	Nil	NVD	Nil	Unknown
Nil	Term	Live infant	Unknown	Nil	NVD	Nil	Normal
Nil	Term	Live infant	4.2	Fetal distress with prolonged delivery	Vacuum extraction	Nil	Normal
Nil	36 weeks	Live infant	Normal	Nil	Elective C/S [c]	Nil	Normal
Nil	Term	Live infant	Normal	Nil	Elective C/S [c]	Congenital Squint	Normal
Nil	Term	Live infant	Normal	Nil	Elective C/S [c]	Nil	Normal

[a] Term regarded as between 37 and 42 weeks gestation; [b] Normal birth weight regarded as 2.5 – 4.0kg but exact birth weights not known for all infants; [c] Underlying cardiac condition; [d] Previous caesarian section; [e] Patient preference; Statin - HMG CoA reductase inhibitor; NVD - Normal vaginal delivery; C/S - Caesarian section; PCSK9 inhibitor - Proprotein convertase subtilisin/kexin type 9 inhibitor

Supplementary table 3: Pregnancy outcomes in 18 pregnancies exposed to statin therapy with or without other lipid lowering therapy at time of conception

Lipid lowering therapy	Statin therapy		Ezetimibe therapy		Gestational age [a]	Birth weight (kg) [b]	Pregnancy and intrapartum complications	Mode of delivery	Physical abnormalities in newborn	Early childhood development
	Month of pregnancy discontinued	Month of pregnancy resumed	Month of pregnancy discontinued	Month of pregnancy resumed						
Pravastatin (unknown dose)	Continued throughout		-	-	10 weeks	-	Spontaneous miscarriage	-	-	-
Atorvastatin 80mg	First month	Third month	-	-	Term	3.2	Fetal distress at 37 weeks	Emergency C/S	Nil	Normal
Atorvastatin 80mg	First month	Fourth month	-	-	Term	3.8	Nil	Elective C/S [e]	Nil	Normal
Rosuvastatin 40mg	First month	Fourth month	-	-	Term	2.6	Nil	Elective C/S [c]	Nil	Normal
Atorvastatin 80mg	First month	Fourth month	-	-	Term	2.9	Nil	Elective C/S [c]	Nil	Normal
Atorvastatin 80mg	First month	First week postpartum	-	-	33 weeks	2.4	Pre-eclampsia at 30 weeks; maternal bleeding requiring transfusion	Emergency C/S	Nil	Normal
Atorvastatin 80mg	First month	First month postpartum	-	-	Term	Normal	Nil	NVD	Nil	Normal
Atorvastatin 80mg	First month	First month postpartum	-	-	Term	Normal	Nil	NVD	Nil	Normal
Atorvastatin 80mg	First month	First month postpartum	-	-	Term	Normal	Oligohydramnios at 37 weeks	Emergency C/S	Nil	Normal
Atorvastatin 80mg	Sixth month	First month postpartum	-	-	Term	Normal	Nil	NVD	Nil	Normal
Atorvastatin 40mg	Unknown	Unknown	-	-	Term	Normal	Nil	Elective C/S [c]	Nil	Normal
Atorvastatin 80mg	Unknown	Unknown	-	-	Term	Normal	Nil	Elective C/S [c]	Nil	Normal
Atorvastatin 80mg Ezetimibe 10mg	Two months prior to planned conception	Third month	Two months prior to planned conception	Third month	Term	3.2	Nil	Elective C/S [d]	Nil	Normal
Atorvastatin 40mg Ezetimibe 10mg	First month	Fifth month	First month	Fifth month	Term	3.5	Nil	NVD	Nil	Normal
Atorvastatin 80mg Ezetimibe 10mg	First month	Unknown	First month	Unknown	Term	Normal	Nil	Elective C/S [c]	Short legs, no intervention required	Normal
Atorvastatin 80mg Ezetimibe 10mg	Unknown	Unknown	Unknown	Unknown	Term	Normal	Unknown	NVD	Nil	Normal
Atorvastatin 80mg Ezetimibe 10mg	Unknown	Unknown	Unknown	Unknown	Term	Normal	Unknown	NVD	Nil	Normal
Atorvastatin 80mg Ezetimibe 10mg, PCSK9 inhibitor (Evolocumab 420mg monthly)	Second month	First week postpartum	Second month	First week postpartum	35 weeks	2.4	Chorioamnionitis at 35 weeks	Emergency C/S	Nil	Normal

[a] Term regarded as between 37 and 42 weeks gestation; [b] Normal birth weight regarded as 2.5 – 4.0kg but exact birth weights not known for all infants; [c] Underlying cardiac condition; [d] Patient preference; [e] Previous caesarian section; Statin - HMG CoA reductase inhibitor; NVD - Normal vaginal delivery; C/S - Caesarian section;
PCSK9 inhibitor - Proprotein convertase subtilisin/kexin type 9 inhibitor

Appendices

Appendix 1: Protocol

Title:

Statin therapy and pregnancy outcomes in a cohort of homozygous familial hypercholesterolaemia patients.

Introduction

Background

Homozygous familial hypercholesteroleamia (HoFH) is a rare genetic disorder. Most HoFH patients have two mutant alleles in the gene coding for the low density lipoprotein receptor (LDLR) while heterozygous patients only has one mutant allele. The mutation results in the absence or malfunction of the LDL receptors and in turn results in decreased hepatic uptake of low density lipoprotein. To date more than a 1600 different mutations in the LDLR gene have been identified(1). More recently identified mutations in other genes coding for apolipoprotein B (ApoB), low density lipoprotein receptor adaptor protein 1 (LDLRAP1) and proprotein convertase subtilisin/kexin type 9 (PCSK9) can also result in a HoFH phenotype (2). The prevalence of HoFH is about 1 in 160 000 (3) but certain populations like Afrikaners in South Africa can have a prevalence as high as 1 in 30 000 due to founders effect (4).

Consequences

Patients with this genetic abnormality suffer from elevated levels of low density lipoprotein cholesterol (LDL-C) from birth which predispose them to atherosclerosis and coronary artery disease at a young age. Atherosclerosis of the coronary arteries, aortic root, carotids, descending aorta, ileo-femoral and renal arteries are commonly seen early in life in HoFH patients (5) and often requires surgical intervention. Aortic valve stenosis is also a known complication in HoFH patients (6). If left untreated, HoFH patients have a life expectancy of about thirty years but with good treatment and lipid control they can have a long and successful reproductive life (2).

Mortality from complications due to hypercholesterolaemia in HoFH patients is high. HoFH females are especially at high risk as they are exposed to periods during their life where lipid lowering treatment is not optimal. One such period is during pregnancy. There is a natural increase in cholesterol levels during normal pregnancy. A review by Avis et al reported increases up to 40% in both total cholesterol and LDL-C levels in normal pregnancies. This increase is exaggerated in

patients with familial hypercholesterolaemia, both homozygotes and heterozygotes (7). A study by Amundsen et al showed that the LDL-C level increased by 29.6% in familial hypercholesterolemia patients. Although this increase in LDL-C level was not statistically significant compared to the control group (34.2%), the absolute level was much higher (8). This period of increased levels of total and LDL cholesterol in the pregnant patient can potentially increase her risk for atherosclerosis and worsening of existing disease.

Maternal hypercholesterolaemia does not only impact the mother but can also affect the offspring. It was previously postulated that high LDL-C levels and increased atherosclerotic plague formation can decrease placental blood flow and thus lead to foetal compromise. However, the study by Amundsen et al did not show differences in pregnancy outcomes in birth weight and gestational age of offspring between hypercholesterolaemic women and normocholesterolaemic women. (8). In the late 1990's Napoli studied aortas of spontaneously aborted foetuses and premature infants that died within 12 hours following birth. They found that foetuses born to mothers with hypercholesterolaemia had a greater number and larger atherosclerotic lesions than foetuses born to normocholesterolaemic mothers. (9, 10). In view of this it can be extrapolated that good lipid control is essential in HoFH patients to provide maximum benefit to both mother and the unborn child.

Current Practise

Currently HoFH is treated with a low fat diet and lipid lowering drugs with or without LDL apheresis if available. Mainstay treatment is with HMG CoA reductase inhibitors, commonly referred to as statins. Statins are effective in lowering cholesterol levels (11, 12) and show major benefits for HoFH patients by lowering their cardiovascular risk and mortality (13). Adding agents such as ezetimibe can provide further lowering of LDL-C (14) while fibrates, niacin and bile acid sequestrates can also be used as add-on treatment.

Statin therapy is regarded as a category X drug and thus not recommended to be used in pregnancy. Toxic doses of statins in animal studies showed an increased incidence of skeletal malformations, developmental delay and increased fetal reabsorption rates (15, 16). Publications in 2004 by Edison et al on malformations seen in 22 infants exposed to first trimester statin use increased fears of using statins in pregnancy (17). Many studies have been done to determine if there is an increased risk of adverse pregnancy outcome when taking a statin while pregnant. Currently there is no consensus as to the safety of statin use during pregnancy. (18-20). A recently published cohort study by Bateman et al showed no statistical significant teratogenic effect due to statin use after adjusting for confounding variables. One such confounding variable was pre-existing maternal diabetes. The

relative risk for having a child with a malformation when exposed to first trimester statins was 1.34 after adjusting for diabetes and even lower (1.04) when other confounding variables like hypertension, obesity, dyslipidaemia, maternal age, smoking and alcohol use were taken into account (21).

As mentioned earlier many HoFH patients require surgical procedures such as aortic valve replacement or repair to treat complications of hypercholesterolaemia early in life. This mean that they might also be taking other teratogenic treatment such as warfarin or angiotensin converting enzyme inhibitors when they fall pregnant. It is sometimes very difficult to assess the confounding effect of these other drugs when looking at studies where fetal abnormalities are observed.

The Canadian Cardiovascular Society (CCS) (22), the European Atherosclerosis Society (EAS)(2) and the National Lipid Association expert panel on FH (23), recommend the discontinuation of statin therapy at least one month prior to conception and to resume statins only subsequent breastfeeding. Other lipid lowering drugs such as fibrates, niacin, ezetimibe and cholestyramine (a bile acid sequestrant) are also contraindicated in pregnancy (category B). Colesevelam is the only bile acid sequestrant that can be used in pregnancy (category B) but is not available in South Africa. The current recommendation for treating pregnant HoFH patients is lipoprotein apheresis. The major problem however is that lipoprotein apheresis is costly and time consuming and not widely available in developing countries. Alternative treatment options for pregnant HoFH patients are thus necessary in resource poor settings to optimise lipid control during pregnancy for these patients.

Limited data are available on alternative treatment options in pregnant HoFH patients and this study aims to provide some information on this.

Reasearch Question:

Does statin use in pregnant HoFH patients have an adverse effect on pregnancy?

Objectives:

1) To determine pregnancy outcomes of female HoFH patients attending the Lipid clinic at Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) and Groote Schuur Hospital (GSH).
2) To review the lipid lowering treatment in pregnant HoFH patients at these clinics and compare it with international guidelines.
3) To relate these pregnancy outcomes with lipid lowering treatment used during pregnancy.

Study design

This is a retrospective study. Data regarding lipid lowering therapy and pregnancy outcomes will be assessed in a cohort of HoFH patients who attended the lipid clinic at CMJAH and the lipid clinic at GSH in the Western Cape.

Population

All HoFH female patients known to the lipid clinic at CMJAH and GSH who completed a pregnancy prior to January 2015 will be entered into the study. I anticipate to find ~25 eligible patients.

Exclusion criteria

HoFH females known to the clinics with no prior pregnancies.

Data collection

The hospital records of eligible HoFH patients will be reviewed. Data regarding the demographics, surgical procedures, comorbidities and lipid profiles will be collected.

Data unavailable from the patients' hospital record such as details pertaining to the pregnancy, delivery and the development of the offspring will be collected by personal or telephonic interviews with the HoFH patients.

In the case of deceased HoFH individuals or HoFH individuals unable to provide the information a close family member will be contacted to collect the necessary information where possible.

See appendix 1 for the data collection sheet.

Consent

Consent will be obtained from the patient to collect data from their clinic files. Written consent will be obtained where possible. If a patient is unable to provide written consent, verbal consent will be obtained. In the case of deceased HoFH individuals or HoFH individuals unable to provide consent a close family member will be contacted to give written or verbal consent.

Ethics

Written permission will be requested from the Faculty of Health Sciences Ethics committee to approve the study.

Written permission to access patient files from the lipid clinic at CMJAH and GSH will be obtained from Professor F Raal (Director of the Carbohydrate and Lipid Metabolism Research Unit) and Professor D Blom (Head of Lipidology Division) respectively.

Patient identity will be protected. Each patient will be assigned a number starting at 0001 and all pregnancies related to the patient will be assigned an alphabet number following the patient number. For example 0001A and 0001B for the first and second pregnancy of patient 0001.
A list of these numbers and the corresponding patient will be kept separate from the collected data.

Analysis

This study will be a descriptive analysis. The demographics and the pregnancy outcomes of the cohort will be determined and described. Possible confounders and treatment during pregnancy will be assessed and compared to the current available literature.

Limitations of the study

A very small number of patients will be included in the study due to the low prevalence of HoFH.
Data collection will be retrospective. Data may be incomplete due to poor clinical records or inability of patients to recall details of events.
Pregnancy outcomes will be assessed based on patient recall of events. Outcomes will not formally be assessed by a medical professional.

Funding

All expenses will be self-funded which include printing expenses and costs involved in personal and telephonic interviews.

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Appendices to protocol

Appendix 1.1:

Patient information sheet

UNIVERSITY OF THE WITWATERSRAND HUMAN RESEARCH ETHICS COMMITTEE

INFORMATION LEAFLET

STUDY TITLE:

STATIN THERAPY AND PREGNANCY OUTCOMES IN A COHORT OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA PATIENTS

INVESTIGATOR:

DR THEUNIS CHRISTOFFEL BOTHA

INSTITUTION:

UNIVERSITY OF WITWATERSRAND, DEPARTMENT OF MEDICINE

DAYTIME AND AFTER HOURS

TELEPHONE NUMBER(S):

0721386053

To the potential Participant: *This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. You may take home* ***an unsigned copy of this consent*** *form to think about or discuss with family or friends* ***before making your decision.***

INTRODUCTION:

Good day, my name is Theunis Botha, I am a Medical registrar at the University of the Witwatersrand. I would like to invite you to consider participating in a research study, entitled: Statin therapy and pregnancy outcomes in a cohort of homozygous familial hypercholesterolaemia patients.

1. Before agreeing to participate, it is important that you read and understand the following explanation of the purpose of the study and the study procedures, the benefits and the risks. You have the right to withdraw from the study at any time and this will not impact on your medical care in any way.
2. This information leaflet is to help you to decide if you would like to participate. You need to understand what is involved before you agree to take part in this study.
3. If you have any questions, do not hesitate to ask me.
4. You should not agree to take part unless you are satisfied that you understand what this study is about and what taking part in this study involves.
5. If you decide to take part in this study, you will be asked to sign this document to confirm that you understand the study. You will be given a copy to keep.

PURPOSE OF THE STUDY:

- You, or a member of your family, have been diagnosed as suffering from homozygous familial hypercholesterolaemia (HoFH) and I would like you to consider taking part in this study.
- Little is known about the safety and outcome of pregnancy in patients with HoFH. This means that it is difficult to give women with HoFH, who are considering starting a family, good advice.
- The purpose of this study is to determine whether pregnancy has negative effects in mothers with HoFH and to assess whether children born to mothers with HoFH show any abnormalities as a consequence of high cholesterol in the mother or the use of cholesterol-lowering medication during pregnancy.
- This study will involve assessing your clinical condition and medication use before, after and during pregnancy. Additionally we will assess the outcome of all pregnancies and the health of all children born.

LENGTH OF THE STUDY AND NUMBER OF PARTICIPANTS:

- The study will be performed in South Africa.
- Approximately 25 participants at the University of the Witwatersrand and the University of Cape Town will participate in this study.
- The amount of time required for your participation in this study will be a once off telephonic interview or meeting to fill in a questionnaire. This will take approximately 30 min.

PROCEDURES:

- If you agree to take part in this study, you will be asked to complete a questionnaire. The questionnaire will consist of questions regarding your medical and surgical history or that of your family member with HoFH. It will also contain questions about the treatment you or your

family member took while pregnant. The last section contains questions about the delivery and the children's health and development.

RISKS:

- The study does not pose any risks to your health. All your answers will be kept confidential and the study results will not influence your treatment or care.
- You may feel sad or distressed when answering questions about pregnancies that did not have a good outcome. You can choose not to answer any question that you find distressing.

BENEFITS:

- Your participation in this study will contribute to medical knowledge that may help other patients that, like you, or your family member, have homozygous familial hypercholesterolaemia.

RIGHTS AS A PARTICIPANT IN THIS STUDY:

- Your participation in this study is entirely voluntary and you can decline to participate, or stop at any time, without stating any reason. Your withdrawal will not affect your current medical care or access to medical care.

REIMBURSEMENT FOR STUDY PARTICIPATION:

- You will not be paid to participate in this study. Taking part in the study will not cost you anything. We will attempt to schedule your interview on a day when you are already attending the clinic so that you do not have to make an extra trip to the hospital.

ETHICAL APPROVAL:

- This clinical study protocol has been submitted to the University of the Witwatersrand, **Human Research Ethics Committee (HREC)** and written approval has been granted by that committee.
- The study has been structured in accordance with the **Declaration of Helsinki** (last updated: October 2008), which deals with the recommendations guiding doctors in biomedical research involving human participants. A copy may be obtained from me should you wish to review it.
- If you want any information regarding your **rights as a research participant, or complaints regarding this research study**, you may contact Prof. Cleaton-Jones, Chairperson of the University of the Witwatersrand, Human Research Ethics Committee (HREC), which is an independent committee established to help protect the rights of research participants at (011) 717 2301.

CONFIDENTIALITY:

- All information obtained during the course of this study, including hospital records, personal data and research data will be kept strictly confidential. Data that may be reported in scientific journals will not include any information that identifies you as a participant in this study.

- The information might also be inspected by the University of the Witwatersrand, Human Research Ethics Committee (HREC).
- These records will be utilised by them only in connection with carrying out their obligations relating to this study

PARTICIPANT QUESTIONS:

If you have any questions you can ask me directly or phone me on the following number: 0721386053

Appendix 1.2:
Patient consent form

UNIVERSITY OF THE WITWATERSRAND HUMAN RESEARCH ETHICS COMMITTEE

INFORMED CONSENT FORM

STUDY NUMBER:

STUDY TITLE:

STATIN THERAPY AND PREGNANCY OUTCOMES IN A COHORT OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA PATIENTS

INVESTIGATOR:

DR THEUNIS CHRISTOFFEL BOTHA

INSTITUTION:

UNIVERSITY OF WITWATERSRAND, DEPARTMENT OF MEDICINE

DAYTIME AND AFTER HOURS

TELEPHONE NUMBER(S):

0721386053

To the potential Participant: *This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. You may take home* ***an unsigned copy of this consent*** *form to think about or discuss with family or friends* ***before making your decision.***

INFORMED CONSENT:

- I hereby confirm that I have been informed by the study doctor Dr Theunis C Botha about the nature, conduct, benefits and risks of the study: Statin therapy and pregnancy outcomes in a cohort of homozygous familial hypercholesterolaemia patients.
- I have also received, read and understood the above written information (Participant Information Leaflet and Informed Consent) regarding the study.
- I am aware that the results of the study, including personal details regarding gender, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerised system.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

PARTICIPANT:

Printed Name Signature / Mark or Thumbprint Date and Time

I, Dr Theunis C. Botha, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY DOCTOR:

Printed Name **Signature** **Date and Time**

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT……**(DESIGNATION):**

Printed Name **Signature** **Date and Time**

WITNESS (If applicable)**:**

Printed Name Signature Date and Time

VERBAL PARTICIPANT INFORMED CONSENT:

- I, the undersigned Dr Theunis C. Botha have read and have explained fully to the participant, named .....……. and/or his/her relative/friend/legal representative, ...……, the participant information leaflet.
- The participant and/or his/her relative/friend/legal representative indicated that he/she understands that the participant will be free to decline participation or withdraw from the study at any time for any reason and without jeopardising his/her subsequent treatment.

I hereby certify that, the participant and/or his/her relative/friend/legal representative, acting on his/her behalf, has agreed to participate in this study.

PARTICIPANT:

Printed Name **Mark or Thumbprint (if applicable)** **Date and Time**

STUDY DOCTOR:

Printed Name **Signature** **Date and Time**

TRANSLATOR / OTHER PERSON EXPLAINING INFORMED CONSENT :……..…**(DESIGNATION)**

Printed Name **Signature** **Date and Time**

PARENT/LEGALGUARDIAN/LEGALREPRESENTATIVE/FRIEND:

……**(RELATIONSHIP)**

Printed Name **Signature / Mark or Thumbprint** **Date and Time**

WITNESS:

Printed Name **Signature** **Date and Time**

Appendix 1.3:

Patient questionnaire for data collection

Patient Questionnaire

Study:

Statin therapy and pregnancy outcomes in a cohort of Homozygous Familial Hypercholesterolemia patients.

Where applicable:

Record all dates in the format DDMMYYYY.
Mark with a cross the appropriate tick box.
If the date of procedures etc are not known and only the age when it was done then estimate the date based on the birth date.
Where additional details are required, duration and time must be recorded.

Study participant details

(Obtained from patient's hospital file) Date questionnaire completed: _____

Participant number (eg 0001): _____

Age of patient: _____

Date of death: _____

Date when diagnosis was made: _____

HoFH diagnosis: Yes No N/A

□ □ □

Confirmed by genetic screening: □ □ □

mutations identified:

LDLR □ □ □

Apo B □ □ □

PCSK9 □ □ □

Other □ □ □

of other mutations: _____

_____

Details of LDLR mutations:

Afrikaner FH 1/1 □
Afrikaner FH 1/2 □
Afrikaner FH 1/3 □
Afrikaner FH 2/2 □
Afrikaner FH 2/3 □
Afrikaner FH 3/3 □
FH 664/664 □
Other □
Details:

_____
_____
_____
_____
_____

Clinical only:

Genetic

Details

Consent

	Yes	No	N/A
Consent obtained from:			
HoFH patient:	□	□	□
Child of HoFH patient:	□	□	□
Relative of HoFH patient:	□	□	□
Relationship to HoFH patient: _____			
Other:	□	□	□

Consent type

Written □

Verbal □

Relationship to HoFH patient: _____

Untreated Lipid Levels

Date taken: _____

Unavailable: □

Total cholesterol: _____mmol/L

Triglycerides: _____mmol/L

HDL: _____mmol/L

LDL: _____mmol/L

Cardiac History and Cardiac / Vascular procedures

	Yes	No	Unknown
Myocardial Infarction:	□	□	□
Date(s): _____			
Coronary Artery Angioplasty or Stenting:	□	□	□
Date(s): _____			
_____			
_____			
_____			
Coronary Artery Bypass Grafting:	□	□	□
Date(s): _____			
_____			
_____			
_____			
Cerebrovascular Accident:	□	□	□
Date(s): _____			
Peripheral Vascular Disease:	□	□	□
Date(s): _____			
Peripheral Vascular Bypass:	□	□	□
Date(s): _____			
Aortic Valve Replacement or repair:	□	□	□
Date(s): _____			

Aortic Root Replacement or repair: □ □ □

Date(s): _____

Carotid Endarterectomy: □ □ □

Date(s): _____

Other cardiac / vascular surgeries / procedures:

_____

_____

Major Non-cardiac Procedures

		Yes	No	Unknown
Plasma exchange:		□	□	□
	Date started:	_____		
	Frequency:	_____		
	Date Stopped:	_____		
LDL Apheresis:		□	□	□
	Date started:	_____		
	Frequency:	_____		
	Date Stopped:	_____		
Portacaval Shunt:		□	□	□
	Date(s):	_____		
Partial Ileal bypass:		□	□	□
	Date(s):	_____		

Other non-cardiac surgery / procedures not mention above

Details:

_____

_____

_____

Non-cardiac History and Concomitant Disease

	Yes	No	Unknown	Date of diagnosis:
Hypertension:	□	□	□	_____
Diabetes Mellitus:	□	□	□	_____
Hyperthyroidism:	□	□	□	_____

Hypothyroidism: □ □ □ _____

Epilepsy: □ □ □ _____

Psychiatric disorders: □ □ □ _____

Details: _____ _____

Autoimmune Disease: □ □ □ _____

Details: _____ _____

Other diseases not mentioned above

Details:

_____

_____

_____

Pregnancy details and outcomes

Total number of pregnancies: One □ Two □ Three □ Four □

Please complete one questionnaire per pregnancy.

These are details of pregnancy number: One □ Two □ Three □ Four □

Pregnancy / child number (eg 0001A): _____ Male / Female

Details obtained from:	Yes	No	N/A
HoFH patient:	□	□	□
Child of HoFH patient:	□	□	□
Relative of HoFH patient:	□	□	□
Relationship to HoFH patient:	_____		
Other:		□	□ □
Relationship to HoFH patient:	_____		
	_____		

Date of birth: _____

Gestational age:	Yes	No	Unknown	Duration in weeks:	Premature (< 37 weeks): □ □ □
				_____	
Full term (37 – 40 weeks):	□	□	□	_____	
Post dates (> 40 weeks):	□	□	□	_____	

Calculated date of conception: _____
(DoB – gestational age)

Lipid levels of HoFH patient:

Lipid levels within 3 months **prior to conception** (mmol/L) Date: _____		Lipid levels within 3 months **following delivery** (mmol/L) Date: _____	
Unavailable □		Unavailable □	
Total cholesterol		Total cholesterol	
Triglycerides		Triglycerides	
HDL		HDL	
LDL		LDL	

Outcome of pregnancy:	Yes	No	Unknown
Live birth:	□	□	□
Miscarriage:	□	□	□
Ectopic:	□	□	□
Intrauterine death:	□	□	□
Early neonatal death (1-7days):	□	□	□
Late neonatal death (7-28days):	□	□	□

Maternal age at time of pregnancy: _____

Paternal age at time of pregnancy: _____

	Yes	No	Unknown
Smoking during pregnancy:	□	□	□
Alcohol use during pregnancy:	□	□	□
Infertility treatment or procedure used:	□	□	□

Details: _____

Low fat diet followed during pregnancy: □ □ □

Other diet followed during pregnancy: □ □ □

Details: _____

Supplements taken during pregnancy:				Details:
Omega 3:	□	□	□	_____
Vitamins:	□	□	□	_____
Folate:	□	□	□	_____

Iron:	□	□	□	_____
Other:	□	□	□	_____

Maternal medication and lipid lowering procedures **PRIOR** to pregnancy (within 3 months of pregnancy)

	Yes	No	Unknown	Name	Daily dose
Statin:	□	□	□	_____	_____
Ezetimibe:	□	□	□	_____	_____
Fibrates:	□	□	□	_____	_____
Niacin:	□	□	□	_____	_____
BAS[1]:	□	□	□	_____	_____
LDL Apheresis:	□	□	□	Details: _____	
PE[2]:	□	□	□	Details: _____	

[1]Bile acid sequestrants, [2]Plasma Exchange

Other medications used PRIOR pregnancy

Name	Daily dose
_____	_____
_____	_____
_____	_____
_____	_____

Maternal medications and lipid lowering procedures used DURING pregnancy

Statins

Name: _____ Daily dose: _____

	Yes	No	Unknown
Continued throughout (whole) pregnancy:	□	□	□
	Prior	During	Month
Discontinued in which month prior or during pregnancy:	□	□	_____
	During	After	Month
Restarted in which month during or after pregnancy:	□	□	_____

Ezetimibe

Name: _____ Daily dose: _____

	Yes	No	Unknown
Continued throughout (whole) pregnancy:	□	□	□
	Prior	During	Month
Discontinued in which month prior or during pregnancy:	□	□	_____
	During	After	Month
Restarted in which month during or after pregnancy:	□	□	_____

Fibrates

Name: _____ Daily dose: _____

	Yes	No	Unknown
Continued throughout (whole) pregnancy:	□	□	□
	Prior	During	Month
Discontinued in which month prior or during pregnancy:	□	□	_____
	During	After	Month
Restarted in which month during or after pregnancy:	□	□	_____

Niacin

Name: _____ Daily dose: _____

	Yes	No	Unknown
Continued throughout (whole) pregnancy:	□	□	□
	Prior	During	Month
Discontinued in which month prior or during pregnancy:	□	□	_____
	During	After	Month
Restarted in which month during or after pregnancy:	□	□	_____

Bile acid sequestrants

Name: _____ Daily dose: _____

	Yes	No	Unknown
Continued throughout (whole) pregnancy:	□	□	□
	Prior	During	Month
Discontinued in which month prior or during pregnancy:	□	□	_____
	During	After	Month
Restarted in which month during or after pregnancy:	□	□	_____

LDL Apheresis

	Yes	No	Unknown
Continued throughout (whole) pregnancy:	□	□	□
	Prior	During	Month
Discontinued in which month prior or during pregnancy:	□	□	_____
	During	After	Month
Restarted in which month during or after pregnancy:	□	□	_____

Details: _____

Plasma Exchange

	Yes	No	Unknown
Continued throughout (whole) pregnancy:	□	□	□
	Prior	During	Month
Discontinued in which month prior or during pregnancy:	□	□	_____
	During	After	Month
Restarted in which month during or after pregnancy:	□	□	_____

Details: _____

Other medications used DURING pregnancy

Name	Daily dose	Details if discontinued or changed:
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Complications DURING pregnancy

	Yes	No	Unknown	Details:
Infections:	□	□	□	_____
Bleeding:	□	□	□	_____
Trauma:	□	□	□	_____
Hypertension:	□	□	□	_____
Pre-eclampsia:	□	□	□	_____
Eclampsia:	□	□	□	_____
HELLP syndrome:	□	□	□	_____
Gestational diabetes:	□	□	□	_____
Other:	□	□	□	_____

Details of delivery

Mode of delivery:	Yes	No	Unknown	Details / Reason:
Normal vaginal delivery:	□	□	□	_____
Planned C/S:	□	□	□	_____
Emergency C/S:	□	□	□	_____

Timing of delivery:	Yes	No	Unknown	Duration in weeks:	Premature (< 37 weeks):
	□	□	□	_____	
Full term (37 – 40 weeks):	□	□	□	_____	
Post dates (> 40 weeks):	□	□	□	_____	

Intrapartum complications:	Yes	No	Unknown	Details:
Severe Bleeding:	□	□	□	_____
Maternal transfusion needed:	□	□	□	_____
Maternal hypotension:	□	□	□	_____
Fetal distress:	□	□	□	_____
Prolonged delivery:	□	□	□	_____
Instrument delivery:	□	□	□	_____
Other:	□	□	□	_____

Condition of newborn

Weight of child:	Yes	No	Unknown	
Small (<2.5kg):	□	□	□	
Normal (2.5 – 4kg):	□	□	□	
Large (>4kg):	□	□	□	
	Yes	No	Unknown	Duration in hours, days, weeks:
Resuscitation required:	□	□	□	_____
Ventilation required:	□	□	□	_____
Incubator required:	□	□	□	_____
ICU required:	□	□	□	_____
Phototherapy required:	□	□	□	_____
Blood transfusions required:	□	□	□	_____

Physical abnormalities

	Yes	No	Unknown	Details:
Brain or neural abnormalities:	□	□	□	_____
Abdominal abnormalities:	□	□	□	_____
Limb abnormalities:	□	□	□	_____
Facial abnormalities:	□	□	□	_____
Cardiac abnormality:	□	□	□	_____
Surgery needed:	□	□	□	_____
Other problems:	□	□	□	_____

Breast feeding and early child development (birth to 6 years)

	Yes	No	Unknown	Duration (months):
Breast feeding:	□	□	□	_____
Developmental milestones achieved at appropriate ages:				Details:
Babbling (± 6 months):	□	□	□	_____

Sitting unaided (± 6 months):	□	□	□	_____
Standing up (± 8 months):	□	□	□	_____
Crawling (± 9 months):	□	□	□	_____
Walking unaided (± 14 months):	□	□	□	_____
Talking (± 18 months):	□	□	□	_____

Late child development (> 6 years)

	Yes	No	Unknown	
Age child started on a statin:				
0 – 5 years:	□	□	□	
5 – 10 years:	□	□	□	
10 – 15 years:	□	□	□	
15 – 20 years:	□	□	□	
				Details:
Normal school going age (6-7 years):	□	□	□	_____
Special school needs:	□	□	□	_____
Schooling years repeated:	□	□	□	_____

	Yes	No	Unknown	Details:
Disabilities:	□	□	□	_____
Childhood disorders:				
ADHD:	□	□	□	_____
Psychiatric disorders:	□	□	□	_____
Autism:	□	□	□	_____
Other:	□	□	□	_____

Highest level of education completed:	Yes	No	Unknown
General Education (Grade 0 – 9):	□	□	□
Further Education (Grade 10 – 12):	□	□	□
Tertiary Education (Studies after Grade 12):	□	□	□

Appendix 2:
Change of Title

UNIVERSITY OF THE
WITWATERSRAND,

Private Bag 3 Wits, 2050
Fax: 027117172119
Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

12 June 2018
Person No: 0504396R
TAA

Dr TC Botha
Postnet Suite 150
Private Bag X10016
Edenvale
1610

Dear Dr Botha

Master of Medicine: Change of title of research

I am pleased to inform you that the following change in the title of your Research Report for the degree of **Master of Medicine** has been approved:

From:	**Statin therapy and pregnancy outcomes in a cohort of homozygous familial hypercholesterolaemia patients**
To:	**Statins and other lipid-lowering therapy and pregnancy outcomes homozygous familial hypercholesterolaemia - a retrospective review of 39 pregnancies**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

Appendix 3:

Ethics approval for the University of Witwatersrand HREC

R14/49 Dr Theunis Christoffel Botha

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150724

NAME: Dr Theunis Christoffel Botha
(Principal Investigator)

DEPARTMENT: Internal Medicine
Charlotte Maxeke Johannesburg Academic Hospital
Carbohydrate and Metabolism Research Unit Groote Schuur Hospital, Lipidology Division

PROJECT TITLE: Statin Therapy and Pregnancy Outcomes in a Cohort of Homozygous Familial Hypercholesterolaemia Patients

DATE CONSIDERED: 31 July 2015

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Prof Frederick Raal

APPROVED BY: _____
Professor P Cleaton-Jones, Chairperson, HREC (Medical)

DATE OF APPROVAL: 28/08/2015

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Secretary in Room 10004, 10th floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report.**

_____ _____
Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 4:

Ethics approval from University of Cape Town HREC

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
Email: sumayah.ariefdien(auct.ac.za
Website: www.health.uctac.zaghs/research/humanethics/forms

07 July 2015

HREC REF: 475/2015

A/Prof D Blom

Division of Lipidology

5th Floor

Chris Barnard Building-FHS

Dear A/Prof Blom

PROJECT TITLE: STATIN THERAPY AND PREGNANCY OUTCOMES IN A COHORT OF HOMOZYGOUS FAMILIAL HYPERCHOLESTEROLAEMIA PATIENTS (MMed Candidate – T Botha)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has formally approved the above-mentioned study. Approval is granted for one year until the 30th July 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We *acknowledge that the following student:- Theunis Botha is also involved in this project.* Please quote the HREC reference no in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely



PROFESSOR M BLOCKMAN CHAIRPERSON, FHS HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637. Institutional Review Board (IRB) number: IRB00001938

Hrec/ref : 475/2015

Appendix 5:
Turnitin report

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by Theunis Botha

Submission date: 18-Jul-2018 11:46AM (UTC+0200)
Submission ID: 983424416
File name: 7e7b85-1e9b-4246-ac8e-478f8b5732fb_turnitin_report_TC_Botha.docx (20.26K) **Word count:** 2959
Character count: 16831

1) Introduction

Homozygous familial hypercholesterolaemia (HoFH) is a rare but serious genetic disorder. HoFH is due to bi-allelic mutations in the low-density lipoprotein receptor (LDLR) gene resulting in marked LDLR dysfunction. The prevalence of HoFH is 1 in 160 000 to 1 000 000 worldwide but in founder populations, such as the Afrikaner in South Africa, the prevalence can be as high as 1 in 30 000[1-3]. Patients with HoFH have elevated levels of low density lipoprotein cholesterol (LDL-C) from birth and often suffer from premature atherosclerotic cardiovascular disease and valvular and supra-valvular aortic stenosis which require surgical intervention[4,5].

Pregnancy in HoFH females is associated with further elevation of already markedly high LDL-C. In healthy patients, total cholesterol and LDL-C levels may increase by up to 40% during pregnancy[6]. This increase is exaggerated in HoFH patients, particularly if lipid-lowering therapy is discontinued, placing the mother at even higher cardiovascular risk[7]. Maternal hypercholesterolaemia during pregnancy have been linked to increased atherosclerotic lesions in the fetus[8,9]. Lipid management during pregnancy therefore has implications for both the mother and the unborn child.

HMG-CoA reductase inhibitors (statins) are currently the backbone of lipid-lowering treatment in patients with HoFH. Although statins only lower LDL-C modestly in most patients with HoFH, they reduce cardiovascular events and mortality[10-12]. The cholesterol absorption inhibitor ezetimibe reduces LDL-C further and is routinely used in patients with HoFH[13]. Other agents such as fibrates, niacin and bile acid sequestrants, as well as newer agents such as mipomersen, lomitapide and evolocumab have also been used as add-on treatment.

Statin use is not recommended during pregnancy due to teratogenicity. A report in 2004 increased fears regarding statin use during pregnancy[14]. The report showed malformations in 22 infants exposed to statins in the first trimester. Recently published studies however showed no teratogenic effect associated with statin use after accounting for confounding variables[15]. Despite several studies there is currently no consensus regarding the safety of statins during pregnancy[16-18].

Little is known about the safety of newer agents such as mipomersen, lomitapide or PCSK9-inhibitor therapy with evolocumab or alirocumab during pregnancy in humans. Animal studies showed teratogenicity when ezetimibe was used in high dosages[19]. Lomitapide was found to be teratogenic at therapeutic dosages. Animal studies did not show any teratogenicity with the use of mipomersen however[20]. Studies that evaluated the safety of the PCSK9 inhibitor evolocumab showed no negative effect during pregnancy but did confirm placental transfer to the infant[21].

Lipoprotein apheresis (LA) is the recommended lipid lowering treatment for pregnant HoFH patients[22,23]. The benefits of LA are well established and include the ability to lower total cholesterol and LDL-C levels significantly and to prevent progression of atherosclerotic disease[24,25]. By down-regulating several cytokines, LA also has an anti-inflammatory response[26]. One study found that the use of LA could potentially improve a patient's overall quality of life as assessed by patient questionnaires[27].

However, LA is costly, time consuming and not available in all countries. Thus, we need alternative treatment options for pregnant HoFH patients in resource-poor settings.

The aim of this study was to assess pregnancy outcomes in a cohort of female HoFH patients, many of whom received statins with or without other lipid lowering therapy.

2) Patients and methods

Data was collected retrospectively in January 2016 from a cohort of female HoFH patients who attended two specialised lipid clinics in South Africa (Charlotte Maxeke Academic Hospital, Johannesburg or Groote Schuur Hospital, Cape Town) and who had reported a pregnancy prior to study commencement. The study was approved by the Human Research Ethics Committees of the University of the Witwatersrand and the University of Cape Town. Demographic, clinical, laboratory and treatment information, particularly the use of medication during pregnancy, was extracted from hospital files. Either patients themselves or a close, surviving family member were contacted directly to obtain informed consent for anonymized data collection and additional information. We used Microsoft Office 365® Excel® 2016 (Version 1611) for statistical analysis. We compared birth weights of statin exposed and statin unexposed infants with the Mann Whitney U test and utilized a one tailed t-test to compare lipid levels, prior to, and following pregnancy. We considered $p < 0.05$ significant.

3) Results

3.1) Study population

We had medical records of 29 female HoFH patients with pregnancies. Of the 29 patients, 4 were lost to follow-up and could not be traced, 5 had died prior to study commencement and we could not trace any surviving family member to obtain further information. Twenty patients were therefore included in the study (Tables 1 and 2).

The majority of patients (n=12) were diagnosed, or visited the clinic for the first time, before the age of 18 years with the median age of first clinic visit being 9.3 years (range 1.0 - 53.5; interquartile range 4.1-36.5 years). All patients had genotypically confirmed HoFH, either being true homozygotes with identical LDLR mutations or compound heterozygotes with two different LDLR mutations. The most common genotype was true homozygosity for the FH Afrikaner 1 mutation (c.681C > G / c.681C > G) (n= 9) followed by compound heterozygous FH Afrikaner 1 (c.681C > G) / FH Afrikaner 2 (c.1285G>A) mutations (n=3). (Supplementary table 1) This is in keeping with the high prevalence of these founder mutations in South Africa.

Mean (SD) total cholesterol and LDL-C levels were markedly elevated, 19.07 (3.57) mmol/L and 18.29 (4.02) mmol/L respectively, at first presentation to the clinics. Four patients had experienced cardiac events (either myocardial infarction, coronary artery disease requiring stenting or coronary artery bypass grafting) prior to their first pregnancy. The median age for first cardiac intervention was 30.5 years (range 13.6 – 50.8 years; interquartile range 19.9 – 41.5 years). No patients required valvular intervention (aortic valve replacement, aortic root surgery or aortic valve bypass surgery) prior to their first pregnancy. No maternal deaths occurred during pregnancy or the postpartum period.

3.2) Pregnancy and pregnancy outcomes

Thirty nine pregnancies were reported. Patients started a family at a relatively young age (median age of primigravid patients was 24.3 years) and the majority (n=13; 65%) completed their families before age 30 years.

Overall pregnancy outcomes were favourable (i.e. a live infant born). There were 33 (85%) full-term pregnancies and only three premature deliveries (< 37 weeks). Premature deliveries were due to maternal pre-eclampsia at 33 weeks, chorioamnionitis at 35 weeks and due to underlying maternal cardiac disease at 36 weeks gestation. Two patients reported miscarriages at 10 and 13 weeks. One intrauterine death (six months) was attributed to intrauterine infection.

3.3) Lipid lowering treatment prior to and during pregnancy

Of 39 pregnancies a total of 18 were exposed to statins (Supplementary table 2 and 3). The majority of the women on statin therapy were using atorvastatin, either 40 or 80mg, daily.

During 12 of these pregnancies, patients were treated with statin monotherapy in the three months prior to conception. Eight pregnancies were exposed to statins during the first trimester until pregnancy was confirmed (approximately 4 - 8 weeks). Four patients resumed statin therapy in the second trimester between 12 and 16 weeks gestation. The remaining four resumed therapy within one month after delivery.

Five patients were treated with both statins and ezetimibe (10mg daily) in the three months prior to conception. In one case, the statin and ezetimibe combination was discontinued two months prior to planned conception and resumed during the third month of pregnancy. Two of the five pregnancies were exposed to statin and ezetimibe during the first trimester until pregnancy was confirmed and resumed in the second trimester.

One patient was treated with statin, ezetimibe and a PCSK9 inhibitor, evolocumab (420mg monthly), at the time of unplanned conception. All treatment was discontinued at 8 weeks gestation and resumed one week after delivery.

Five pregnancies were exposed to cholestyramine therapy during the first trimester. Cholestyramine was continued throughout three pregnancies. For two pregnancies it is unclear whether cholestyramine was continued for the full duration of the pregnancy.

Only one patient was treated with LA during pregnancy. LA was continued throughout her two pregnancies.

Fourteen pregnancies were not exposed to lipid-lowering therapy.

3.4) Lipid levels during pregnancy

Lipid levels were not routinely measured during pregnancy or following delivery. Only four pregnancies had lipid levels recorded in the three months prior to conception and again within three months following delivery. Two of the women were on atorvastatin 80mg daily which was stopped during the 1st month and recommenced in the 4th month of pregnancy. One woman was on rosuvastatin 40mg

daily which was stopped during the 1st month and restarted during the 4th month of pregnancy. One woman was on atorvastatin 80mg plus ezetimibe 10mg daily; both drugs were stopped 2 months prior to falling pregnant and restarted during the 3rd month. Total cholesterol levels for the four pregnancies increased significantly from 14.20 ± 1.69mmol/l to 17.86 ± 2.34mmol/l (p=0.03). LDL-C was available for three of the four pregnancies. Although LDL-C increased from 11.97 ± 1.90mmol/l to 14.73 ± 0.97mmol/l, this change was not significant (p=0.12).

3.5) Delivery

Methods for delivery were dependent on underlying cardiac or obstetrical circumstances. Normal vaginal delivery was the preferred choice for patients who completed pregnancies prior to cardiac intervention or valve surgery. There were 15 (38%) caesarean sections, of which 11 were elective, and 4 were emergency procedures (Supplementary Table 2). There was no statistical difference in the elective caesarean rate between the statin exposed group and the group receiving no or other forms of treatment. (p>0.1). Most of the elective caesarean sections where due to the underlying maternal cardiac condition. Although only four patients had experienced cardiac events (either myocardial infarction, coronary artery disease requiring stenting or coronary artery bypass grafting) prior to their first pregnancy the other women whom had elective caesarean sections were considered high risk and therefor a caesarean section was performed. Another reason for observing more caesarean sections in the statin group might be the fact that there has been a global increase in caesarean section rates in the last 20 years[28]. Pregnancies exposed to statins were only observed after the year 2000 while pregnancies prior to the year 2000 were not exposed to statins. This might explain the increased number of elective caesarean sections observed in statin exposed pregnancies.

3.6) Breastfeeding

Twelve of the 20 patients chose to breastfeed (21 infants). Most patients breastfed for three to six months; three patients opted to breastfeed for nine months. Six patients (11 infants) continued breastfeeding despite restarting statin therapy after delivery, while three patients (four infants) chose not to breastfeed while on statin therapy. No data regarding lipid lowering therapy were available for the other three patients (six infants).

3.7) Infant birth weight and early childhood development

Most infants had normal birth weights (2.5 – 4.0kg). Two infants had above normal birth weights (4.5kg and 4.2kg), neither of which had been exposed to lipid lowering therapy. Another two infants had below normal birth weights (2.4kg). The latter two pregnancies were exposed to statin therapy during the first trimester and both infants were born prematurely (Supplementary table 2). When we compared the birth weights of the pregnancies exposed to, and those not exposed to statins, there was no difference between the two groups (p>0.1).

One infant was born with a congenital squint which was surgically corrected. The pregnancy was not exposed to lipid modifying treatment neither during nor prior to pregnancy. Short legs were reported in

one infant exposed to statin and ezetimibe therapy during the first trimester. However, this did not require surgical correction and did not impact negatively on the child. Unfortunately, no report of a formal assessment by a paediatrician could be obtained to confirm whether this was likely a congenital malformation but this is considered unlikely.

Normal early child development was reported for all offspring. Children started school at the appropriate age and no learning difficulties were reported.

4) Discussion

The most significant finding of this study suggests that statin exposure might not be as detrimental as previously thought. In our cohort of 39 pregnancies, complications did not differ from those reported during pregnancies of healthy woman. The miscarriage rate (5.5%) in the 18 statin exposed pregnancies, was no higher than that of healthy populations (8 to 20%)[29]. Premature birth rate (11.1%) in the statin exposed pregnancies, also fell within the normal range (5 to 18%)[30,31].

Of the 18 statin exposed pregnancies in our study only two infants had low birth weights. There was no difference in birth weights between statin exposed and non-exposed pregnancies, no severe skeletal abnormalities or developmental delay. Early childhood development appeared to be unaffected by statin therapy prior to conception or during pregnancy.

Although our study focused on HoFH patients, other studies from heterozygous familial hypercholesterolaemia (HeFH) patients in Norway have reported similar findings to our study. The HeFH women on lipid lowering therapy during pregnancy did not have any premature terminations or congenital malformations. There was also no difference in birth weights compared to the HeFH women not on lipid lowering therapy and to the general population[32].

Although one of the largest studies to report on pregnancy outcomes in HoFH patients, the relatively small study population and incomplete records are limitations of the study. This prevented us from being able to adequately compare different treatment groups and we are thus unable to comment on the effectiveness of different lipid lowering therapies during pregnancy. Lipid levels were not routinely measured before, during or after pregnancy and thus resulted in only 4 comparable samples. A larger number of samples would have been more useful in determining the effectiveness of statin usage during pregnancy. Other markers of progression of atherosclerotic disease (for example carotid intimal thickness) were not routinely measured or recorded and thus overall increase in risks for these patients could not be established. Unfortunately, due to the limited number of patients able to access LA in South Africa we were not able to compare our study population to a "control" group receiving LA. Since this was a retrospective study, developmental and physical abnormalities reported were based on descriptions by patients and not scientifically measured and thus subtle congenital abnormalities might be under-reported by parents.

To date, studies involving HoFH pregnancies have been small and mainly limited to case reports[33]. Animal studies have shown placental transfer of statins and excretion of atorvastatin in breastmilk[34].

One case report found low concentrations of rosuvastatin in breastmilk in a patient with familial hypercholesterolaemia[35].

LDL-C and total cholesterol levels were not measured routinely in the offspring and it is thus unknown if maternal statin use during pregnancy had any effect on our study population. There are studies that show elevated maternal LDL-C and total cholesterol levels during early pregnancy results in elevated LDL-C levels and increased cardiovascular risks in their offspring in later life[36,37]. Animal studies have found that mice fed a high fat diet together with pravastatin during pregnancy resulted in lower total cholesterol and LDL-C levels in the offspring compared to mice on a high fat diet alone[38]. It would be interesting to see if this also holds true for HoFH patients and further studies are needed in this regard.

Due to possible teratogenic effects of statins on the fetus and infant, current guidelines recommend that patients discontinue statin therapy one month prior to conception and only resume once breastfeeding has ceased[23,39-41]. LA is the recommended lipid lowering therapy for pregnant HoFH patients[22,23,40,42]. LA is, however, not sustainable due to the high cost - particularly in developing countries[43,44].

Our findings agree with a systemic review by Avis et al, which concluded that statin therapy is safe in HeFH children and didn't find any delay in sexual development or growth[45]. Current evidence shows that low LDL-C levels do not have significant adverse effects and cholesterol dependent hormone levels are unaffected[46]. Additionally, the infants of HoFH patients are obligate HeFH and likely already affected by atherosclerotic lesions at birth[8,9]. This suggests that the reinstatement of lipid lowering therapy during pregnancy could provide additional benefit for the infant.

The findings of our study add to the current pool of data and may assist health professionals when advising HoFH patients regarding statin use during pregnancy and breastfeeding. The findings may also be important for HoFH women making informed decisions regarding pregnancy and therapy.

It is however important to note that the findings of our study cannot be extrapolated to the general population nor to HeFH patients.

We recommend that, where LA is not feasible, patients should discontinue all lipid lowering therapy a month prior to planned conception and reinstate lipid lowering therapy, statin plus ezetimibe, during the second trimester. This limits the possible teratogenic effect of statins during the first trimester while providing the mother with optimal care during a time of increased cholesterol production. Women's perceptions and preferences regarding statin use in pregnancy should also be considered when giving advice in these situations. We aim to manage pregnant HoFH patients as a multi-disciplinary team of lipid specialists, cardiologists and obstetricians.

5) Conflict of interest

TCB received a grant from Amgen to cover expenses for the research. GP, KW, DJB, FJR have received reimbursement for conducting clinical trials from Amgen, Regeneron Pharmaceuticals, Inc., and Sanofi Aventis; modest speaker fees or honoraria for lectures from AstraZeneca, Pfizer, Merck, Amgen, and

Sanofi; and consultant/advisory board fees from Amgen, Sanofi Aventis and Regeneron Pharmaceuticals, Inc.

6) Funding

TCB received a grant from Amgen to cover expenses for the research.

7) Authors' contributions

TCB extracted data from the medical records for patients treated in Johannesburg, entered and analysed data and wrote the first draft of the article. KW extracted data from medical records for patients treated in Cape Town. All authors contributed equally to the revision of subsequent drafts, to the approval of the final version and agree to be accountable for the accuracy and integrity of the findings.

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ORIGINALITY REPORT

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SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Theunis C. Botha, Frederick J. Raal, Dirk J. Blom, Gillian J. Pilcher, Karen Wolmarans. "Statin and Other Lipid-Lowering Therapy and Pregnancy Outcomes in Homozygous Familial Hypercholesterolaemia", Atherosclerosis Supplements, 2018 Publication

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2 Aurélie Thedrez, Dirk J. Blom, Stéphane Ramin-Mangata, Valentin Blanchard et al. "Homozygous Familial Hypercholesterolemia Patients With Identical Mutations Variably Express the LDLR (Low-Density Lipoprotein Receptor)Highlights", Arteriosclerosis, Thrombosis, and Vascular Biology, 2018
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4 Raal, Frederick J., Barbara Sjouke, G. Kees Hovingh, and Barton F. Isaac. "Phenotype diversity among patients with homozygous familial hypercholesterolemia: A cohort study", Atherosclerosis, 2016.
Publication

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5	Gilbert R Thompson, Dirk J Blom, A David Marais, Mary Seed, Gillian J Pilcher, Frederick J Raal. "Survival in homozygous familial hypercholesterolaemia is determined by the on-treatment level of serum cholesterol", European Heart Journal, 2018 Publication	1%
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Publication

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Appendix 6:

Acknowledgement of article acceptance for publication in the journal Atherosclerosis

Simona Negrini <eesserver@eesmail.elsevier.com> Tue, May 22, 2018 at 12:21 PM

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Ref.: Ms. No. ATH-D-18-00379R1
FH Special issue: Statins and other lipid-lowering therapy and pregnancy outcomes in homozygous familial hypercholesterolaemia - a retrospective review of 39 pregnancies.
Atherosclerosis

Dear Dr. Botha,

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Thank you for submitting your paper to this Journal.

Yours sincerely,

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Article available online at:

https://doi.org/10.1016/j.atherosclerosis.2018.05.038